STEVE LACROIX

INFLUENCES DE LA RÉPONSE IMMUNITAIRE SUR L'ACTIVITÉ NEURONALE ET NEUROENDOCRINIENNE: RÔLES ET SITES D'ACTION DES PROSTAGLANDINES

Thèse
présentée
à la faculté des études supérieures
de l'Université Laval
pour l'obtention
du grade de Philosophiae Doctor (Ph.D.)

Programme de physiologie-endocrinologie
FACULTÉ DE MÉDECINE
UNIVERSITÉ LAVAL

SEPTEMBRE 1998

À mes parents

pour votre amour, votre aide, votre compréhension, votre patience et tout l'intérêt que vous avez su manifester envers mes nombreux projets de vie et de carrière.

À mes amis(es)

pour vos encouragements et l'aide apportée tout au long de mes études graduées.

À vous, je dédie cette thèse

à titre de modestes remerciements.

RÉSUMÉ COURT

L'administration d'un inhibiteur de la synthèse des prostaglandines (PGs) atténue les effets stimulateurs de la lipopolysaccharide (LPS) sur l'activité cellulaire cérébrale et la transcription du facteur de libération des corticotrophines (CRF) et son récepteur de type 1 (R-CRF_1). Nos résultats montrent également que l'injection intracérébroventriculaire des PGE_2 induit l'expression du gène *c-fos* dans les structures activées en réponse à la LPS et stimule la transcription du CRF et du R-CRF_1. Ainsi, la production centrale des PGE_2 constituerait une étape cruciale dans la médiation des effets des cytokines sur l'activité de l'axe corticotrope. L'induction d'un stress inflammatoire stimule la transcription du gène encodant l'enzyme limitante à la synthèse des PGs dans la microvasculature cérébrale; un effet vraisemblablement médié par l'interleukine-1ß systémique. Toutefois, puisque le récepteur CD14 de la LPS est exprimé dans le cerveau suite à un challenge immunitaire, la LPS pourrait aussi influencer directement les fonctions neuronales/neuroendocriniennes.

Steve Lacroix Date Serge Rivest, Ph. D. Date

RÉSUMÉ LONG

Les mécanismes cellulaires sollicités par les médiateurs inflammatoires afin d'induire l'activité des neurones synthétisant le facteur de libération des corticotrophines (CRF), et par conséquent l'activation de l'axe hypothalamo-hypophyso-surrénalien (HPA), sont mal connus. Nos travaux avaient pour objectif de clarifier l'hypothèse voulant que les prostaglandines (PGs) puissent médier les effets des cytokines pro-inflammatoires sur l'activation des neurones endocriniens. Le modèle d'infection induit par l'endotoxine lipopolysaccharide (LPS) fut employé afin de mimer la réponse immunitaire de type aigu et d'induire la relâche des cytokines.

Nos résultats démontrent que l'administration d'un inhibiteur de la synthèse des PGs atténue les effets de la LPS sur l'activité cellulaire dans le cerveau et la transcription du CRF et de son récepteur de type 1 (R-CRF_1) dans les noyaux paraventriculaires de l'hypothalamus (PVH). Afin d'identifier le type de PG responsable, nous avons étudié les effets d'une administration centrale des PGs de type E2 (PGE_2). Nos résultats montrent que l'injection intracérébroventriculaire (i.c.v.) des PGE_2 induit l'expression du gène c-*fos* dans les structures activées en réponse à l'injection de LPS. De plus, l'administration i.c.v. des PGE_2 stimule la transcription du CRF et du R-CRF_1 dans les noyaux PVH. Ainsi, la production centrale des PGE_2 constituerait une étape cruciale dans la médiation des effets des cytokines et/ou de la LPS sur l'activité de l'axe corticotrope. Dans le but d'identifier les populations cellulaires responsables de la production centrale des PGs, nous avons analysé par hybridation *in situ* la distribution du transcrit encodant l'enzyme limitante de la synthèse des PGs, COX-2, dans différents modèles d'inflammation. Nos observations démontrent qu'un stress inflammatoire stimule la transcription du gène COX-2 dans la microvasculature cérébrale, et que l'interleukine-1ß systémique pourrait médier ces effets.

La deuxième partie de nos recherches consistait à vérifier si la LPS peut agir directement sur certaines populations cellulaires du cerveau afin d'influencer les fonctions neuronales et neuroendocriniennes. Nos études ont montré que le récepteur CD14 de la LPS est exprimé dans les structures étant en contact direct avec la circulation sanguine, en plus de certaines cellules parenchymales lors d'une septicémie sévère.

Steve Lacroix Date Serge Rivest, Ph. D. Date

REMERCIEMENTS

Cette thèse de doctorat est le résultat de plusieurs expériences réalisées dans les laboratoires d'endocrinologie moléculaire du Centre de recherche du Centre hospitalier de l'Université Laval. La réalisation de l'ensemble de ces travaux a nécessité la collaboration de plusieurs personnes qu'il me serait difficile de citer nommément sans en oublier quelques-unes. Je leur adresse par l'occasion mes sincères remerciements.

D'abord et avant tout, je tiens à remercier très sincèrement mon directeur de recherche, le Dr. Serge Rivest, professeur adjoint au département d'anatomie-physiologie à la faculté de médecine de l'Université Laval. Je saisis cette opportunité afin de pouvoir lui exprimer ma reconnaissance pour l'excellent encadrement scientifique qu'il a su me procurer, ses encouragements, sa très grande disponibilité et son amour pour la science qu'il a su m'inculquer. J'aimerais aussi le remercier pour la confiance qu'il m'a témoignée et de l'aide apportée depuis mes débuts en recherche. De plus, je m'en voudrais de ne pas souligner les nombreux congrès scientifiques internationaux auxquels le Dr. Rivest m'a permis de participer dans le but de parfaire ma formation scientifique. Outre l'aspect scientifique, j'ai aussi eu la chance de développer une très grande amitié avec le Dr. Rivest qui a toujours su me consacrer temps et conseils malgré la lourde charge de travail à laquelle les chercheurs sont soumis. Le premier contact avec le monde de la recherche est souvent déterminant dans le choix de carrière d'un jeune étudiant aux études graduées, le mien aura été inoubliable.

Il m'est difficile de passer sous silence l'aide de Francesca Cicchetti, étudiante au doctorat en neurobiologie, qui a su me consacrer de son temps précieux afin de parfaire ma maîtrise de la langue de Shakespeare. J'espère que, tout comme moi, nos multiples conversations et notre grand esprit de compétition auront contribué à te motiver et surtout, à t'aider à franchir avec succès les nombreux obstacles et déceptions qui truffent la vie en recherche. Je suis également très reconnaissant envers mon collègue de travail Luc Vallières. Nos innombrables discussions scientifiques ont sans aucun doute grandement contribué à améliorer ma formation académique. Quant à nos discussions palpitantes à l'extérieur du milieu de travail, elles ont aussi été très constructives. À vous deux mes amis, je souhaite énormément de succès dans vos carrières respectives et vos projets futurs.

Je rends également un vibrant hommage à Nataly Laflamme, sans qui, l'apprentissage technique aurait été extrêmement laborieux. Nataly a su consacrer beaucoup de son temps et de son énergie afin de m'enseigner les rudiments de la vie en laboratoire, en plus de contribuer à l'avancement de mes travaux. Je tiens également à remercier tous les étudiants et étudiantes du centre de recherche. L'ambiance au travail et la vie sociale qui s'y rattache sont souvent des conditions sine qua non à notre amour pour une profession; ce fut un grand plaisir pour moi de travailler en votre compagnie au cours des quatre dernières années. Un gros merci à Marc Auger et à toute l'équipe de photographie/illustration médicale pour leur professionnalisme hors du commun.

Mes remerciements s'adressent aussi au Dr. Guy Drolet, professeur adjoint au Département de médecine, qui a accepté de faire la pré-lecture de cette thèse. Merci également au Dr. Drolet pour l'aide apportée au cours des nombreuses demandes de bourses auprès des organismes subventionnaires. Qu'il soit assuré de ma plus profonde reconnaissance.

J'adresse ma plus vive gratitude au Dr. Paul Sawchenko, professeur et directeur du laboratoire de "Neuronal structures and functions" au Salk Institute à San Diego qui, malgré un horaire très chargé, a tout de même accepté de reviser cette thèse et d'agir comme examinateur externe. Merci au Dr. Martin Olivier, professeur adjoint au Département d'infectiologie qui, lui aussi, a accepté de reviser cette thèse et d'agir à titre d'examinateur interne. Je remercie également tous mes professeurs et plus particulièrement mon ancien directeur de programme, le Dr. Denis Richard, pour leur aide et leur contribution à mon développement.

Mes études doctorales ont été subventionnées par le Conseil de recherche en sciences naturelles et en génie du Canada (CRSNG). Quant à mes travaux de maîtrise, ceux-ci ont été entièrement financés par le Dr. Rivest. J'exprime toute ma gratitude à ces organismes et au Dr. Rivest sans qui l'obtention de ces bourses d'études aurait été rendue très difficile.

Finalement, je tiens à manifester mon affection la plus sincère envers deux personnes extraordinaires sans qui rien n'aurait été possible, mes parents Jean-Paul et Marielle. Merci également à ma compagne Nadia Fortin pour son amour sans borne et tout l'intérêt qu'elle a su manifester envers mon travail. Que tous les autres membres de ma famille, mes amis(es) et ceux qui ont contribué, de près ou de loin, à l'aboutissement de cette thèse, trouvent ici le témoignage de ma profonde reconnaissance.

AVANT-PROPOS

Cette thèse comprend l'ensemble des travaux réalisés au cours de ma maîtrise et de mes études doctorales effectuées sous la supervision du Dr. Serge Rivest. Elle a été présentée sous forme d'un recueil d'articles scientifiques dont la plupart ont déjà été publiés dans des journaux scientifiques internationaux avec comité de pairs. Précisons que les résultats rapportés dans cette thèse ont aussi fait l'objet de nombreuses présentations dans des congrès d'envergure nationale ou internationale. Comme vous pourrez le constater, chacun des chapitres deux à six correspond à un article en particulier; ceux-ci ont été rédigés en anglais. Cependant, l'introduction et la conclusion générale furent composées en français. De plus, un résumé dans la langue de Molière fut inséré au début de chaque article inclus dans la présente thèse.

Voici donc la liste complète des articles inclus dans cette thèse:

1) LACROIX, S and RIVEST, S. Functional circuitry in the brain of immune-challenged rats: Partial involvement of prostaglandins. *The Journal of Comparative Neurology*, 387: 307-324, 1997.

2) LACROIX, S and RIVEST, S. Role of cyclo-oxygenase pathways in mediating the stimulatory influence of immune challenge on the transcription of a specific CRF receptor subtype in the rat brain. *Journal of Chemical Neuroanatomy*, 10: 53-71, 1996.

3) LACROIX, S, VALLIÈRES, L and RIVEST, S. C-*fos* mRNA pattern and CRF neuronal activity throughout the brain of rats injected centrally with a prostaglandin of E2 type, *Journal of Neuroimmunology*, 70: 163-179, 1996.

4) LACROIX, S and RIVEST, S. Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzyme (COX-1 and 2) in the rat brain. *Journal of Neurochemistry*, 70: 452-466, 1998.

5) LACROIX, S, FEINSTEIN, D and RIVEST, S. The bacterial endotoxin lipopolysaccharide has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations. *Brain Pathology (sous presse)*, 1998.

TABLE DES MATIÈRES

Page

Page

CHAPITRE 1 (suite).

Page

CHAPITRE 2 (suite).

Page

Page

Page

Page

CHAPITRE 7 (suite).

LISTE DES ABRÉVIATIONS

AA:	Acide arachidonique
ACTH*:	Hormone adrénocorticotrope
AP*:	Area postrema
ARC*:	Noyaux arqués
AVP*:	Vasopressine
CD14m:	Forme membranaire du récepteur CD14
CRF*:	Facteur de libération des corticotrophines
COX:	Cyclooxygénase
DAINS:	Drogues anti-inflammatoires non-stéroïdiennes
EM:	Éminence médiane
FSH*:	Hormone folliculo-stimulante
GH*:	Hormone de croissance
gp:	Glycoprotéine
HISH:	Hybridation *in situ* histochimique
hn:	Hétéronucléaire
HPA*:	Axe hypothalamo-hypophyso-surrénalien
i.c.v.:	Intracérébroventriculaire

IEGs*:	Gènes de réponse précoce
IL:	Interleukine
i.m.:	Intramusculaire
i.p.:	Intrapéritonéale
ir:	Immunoréactif
i.v.:	Intraveineuse
LBP*:	Protéine de liaison de la lipopolysaccharide
LC:	Locus coeruleus
LDT*:	Noyau tegmental latérodorsal
LH*:	Hormone lutéinisante
LHRH ou GnRH*:	Hormone de libération des hormones gonadotropes
LPS:	Lipopolysaccharide
MPOA*:	Région médiane préoptique
OCVs:	Organes circumventriculaires
OT*:	Oxytocine
OVLT:	Organe vasculaire de la lame terminale
PB:	Noyaux parabrachiaux
PCPA:	parachlorophénylalanine

PGs:	Prostaglandines
POMC:	Pro-opio-mélano-cortine
PVH*:	Noyaux paraventriculaires de l'hypothalamus
R-CRF$_1$:	Récepteur de type 1 du facteur de libération des corticotrophines
SFO*:	Organe subfornical
SNC:	Système nerveux central
TNF-α*:	Facteur nécrosant des tumeurs-alpha
TNFR*:	Récepteur du facteur nécrosant des tumeurs-alpha
TSH*:	Hormone thyréotrope
VLM:	Région ventro-latérale médullaire

P.S.: Les abréviations marquées d'une étoile (*) proviennent directement de la langue anglaise. Dû à l'utilisation courante de celles-ci dans la littérature française, ces abréviations non pas été définies comme les autres à partir de la forme française des termes.

LISTE DES TABLEAUX

Page

Page

CHAPITRE 6.

LISTE DES FIGURES

Page

CHAPITRE 1.

CHAPITRE 2.

Page

CHAPITRE 2 (suite).

Page

CHAPITRE 3.

Page

CHAPITRE 3 (suite).

CHAPITRE 4.

Page

CHAPITRE 4 (suite).

CHAPITRE 5.

Page

CHAPITRE 5 (suite).

CHAPITRE 6.

Page

CHAPITRE 6 (suite).

CHAPITRE 7.

Page

CHAPITRE 7 (suite).

CHAPITRE 1. INTRODUCTION GÉNÉRALE

1.1 Communication bilatérale entre les systèmes immunitaire et neuroendocrinien

Jusqu'au milieu des années 70, les immunologistes croyaient que les protéines synthétisées par les différentes cellules immunitaires activées, suite à la présentation d'un corps étranger, étaient confinées à la périphérie. Ainsi, on croyait que ces molécules étaient relarguées en systémie dans l'unique but de moduler localement la réponse de l'organisme face à une infection. Les neurobiologistes, quant à eux, considéraient le cerveau comme un organe privilégié totalement isolé des influences immunitaires périphériques. Les neuropeptides, neurotransmetteurs, neurotrophines et autres médiateurs centraux étaient essentiellement reconnus pour leur rôle joué au cours du développement du système nerveux central (SNC) et la régulation des diverses fonctions neuronales. Toutefois, bien que longtemps considérés virtuellement indépendants l'un de l'autre, il est maintenant bien admis que les axes immunitaires et neuroendocriniens communiquent via des ligands et des récepteurs communs. Il est dorénavant bien établi que les récepteurs de nombreux neurotransmetteurs, neuropeptides et neurohormones sont localisés sur certains types de cellules immunocompétentes comme les lymphocytes ou les macrophages (Blalock, 1989; Johnson *et al.*, 1982). Fait intéressant, il a été démontré que des terminaisons nerveuses noradrénergiques innervent directement les organes lymphoïdes primaires et secondaires, suggérant par le fait même que la prolifération, la différenciation et la migration des cellules immunocompétentes puissent être grandement influencées par le cerveau (Calvo and Forteza-Vila, 1969; Felten *et al.*, 1987; Felten *et al.*, 1985; Nance *et al.*, 1987). De plus, de nombreux travaux ont révélé la présence de neuropeptides au niveau des fibres terminales innervant les divers tissus lymphoïdes (Felten *et al.*, 1985; Fink and Weihe, 1988; Fried *et al.*, 1986; Kurkowski *et al.*, 1990; Ottaway and Greenberg, 1984; Stead *et al.*, 1987; Weihe *et al.*, 1989). L'hypothèse d'une interaction directe entre le SNC et le système immunitaire a atteint son paroxysme avec les études de Jankovic qui confirmèrent, hors de tout doute, que les peptides neuroendocriniens peuvent eux aussi influencer les cellules du système immunitaire. Dans ce concept, l'hypothalamus, une région du cerveau contenant plusieurs peptides qui modulent les fonctions endocriniennes, est considéré comme une région déterminante pour la communication entre les axes immunitaire et endocrinien. L'importance de cette région, dans la régulation de la réponse immunitaire, fut d'ailleurs confirmée par des travaux démontrant que des lésions de certaines régions de l'hypothalamus pouvaient provoquer une activation ou une inhibition des fonctions immunitaires (Cross *et al.*, 1984; Jankovic and Isakovic, 1973; Jankovik, 1989). De plus, puisque la grande majorité des

actions neuroendocriniennes de l'hypothalamus sur la périphérie ont été reconnues pour s'effectuer via la modulation de l'hypophyse, le rôle joué par la glande hypophysaire dans le contrôle du système immunitaire fut lui aussi l'objet de nombreuses recherches. L'hypothalamus exerce son influence sur l'adénohypophyse grâce à la sécrétion de "facteurs de libération" acheminés par un système vasculaire porte veineux hypothalamo-hypophysaire, tandis que, la relation entre l'hypothalamus et la neurohypophyse s'effectue directement grâce aux prolongements des terminaisons nerveuses hypothalamiques au niveau de l'hypophyse. Il a été démontré d'une part, que les effets facilitateurs ou inhibiteurs des lésions hypothalamiques sur les fonctions immunitaires sont complètement renversés chez des animaux préalablement hypophysectomisés et, d'autre part, qu'une lésion de l'hypophyse peut elle aussi stimuler ou inhiber certaines fonctions immunitaires (Khansari *et al.*, 1990). Ainsi, le SNC peut agir directement sur les cellules immunitaires: 1} via l'action des neurotransmetteurs et neuropeptides relâchés par les terminaisons nerveuses situées à proximité des cellules immunocompétentes (ex.: dans les organes lymphoïdes primaires et secondaires) et; 2} via l'action des hormones hypophysaires circulantes (ex.: hormone adrénocorticotrope [ACTH], hormone de croissance [GH], hormone thyréotrope [TSH], etc) (Blalock, 1989; Gilbert and Payan, 1991).

Toutefois, bien que les résultats discutés précédemment démontrent l'influence du SNC sur la régulation du système immunitaire, de nombreuses évidences ont confirmé plus récemment que la communication entre les deux systèmes n'est pas unidirectionnelle, mais plutôt bidirectionnelle. Ainsi, il est admis que certains facteurs produits lors d'une activation immunitaire communiquent avec le cerveau afin de permettre la coordination appropriée des changements comportementaux, endocriniens et autonomiques nécessaires au rétablissement de l'homéostasie. Parmi les candidats les plus intéressants, on retrouve notamment: les cytokines, les prostaglandines, les leucotriènes, les facteurs de coagulation et plusieurs autres médiateurs inflammatoires. Toutefois, la capacité des cytokines à rejoindre la circulation générale, dans le but de stimuler le relâchement des hormones hypophysaires et neuropeptides centraux, a suscité un grand engouement au cours de la dernière décennie. En effet, en plus d'induire l'activité immunologique au site d'infection et d'orchestrer la cascade d'événements cellulaires menant à la destruction des pathogènes, les cytokines synthétisées par les macrophages et les lymphocytes activés en présence d'un antigène (Dinarello, 1989; Rabin *et al.*, 1990) sont maintenant reconnues pour leur capacité d'agir sur le système neuroendocrinien. De nombreux travaux démontrent que l'administration *in vivo* des cytokines pro-inflammatoires module la sécrétion de l'ACTH, la GH , la TSH, l'hormone lutéinisante (LH), l'hormone folliculo-stimulante (FSH) et la prolactine (Berkenbosch *et al.*,

1987; Bernton *et al.*, 1987; Dubuis *et al.*, 1988; Payne *et al.*, 1992; Rettori *et al.*, 1987; Rivier and Vale, 1989; Sapolsky *et al.*, 1987; Sharp *et al.*, 1989; Spangelo *et al.*, 1989; Uehara *et al.*, 1987). Bien que l'hypothèse d'une action directe des cytokines sur les cellules hypophysaires soit très attrayante, il semble de plus en plus évident que les cytokines puissent aussi agir indirectement via une circuiterie neuronale beaucoup plus complexe qui serait responsable du contrôle de l'axe hypothalamo hypophysaire.

1.1.1 Axe hypothalamo-hypophyso-surrénalien

La grande majorité des influences du SNC sur le système immunitaire s'effectuent via la régulation de l'axe HPA, justifiant par le fait même l'attention particulière donnée à celle-ci au cours des dernières années. Les résultats accumulés jusqu'à présent ont permis de démontrer que, comme illustré à la figure 1, les cellules immunitaires périphériques activées (ex.: principalement les monocytes et les macrophages) libéraient des signaux, telles les cytokines, qui stimuleraient l'activité de l'axe HPA. Bien que cette capacité de la réponse immunitaire à activer l'axe HPA ait été contestée par la suite (Stenzel-Poore *et al.*, 1993), plusieurs études ont montré que l'augmentation des cytokines sanguines pouvait induire le relâchement du facteur de libération des corticotrophines (CRF) (Barbanel *et al.*, 1990; Berkenbosch *et al.*, 1987; Navarra *et al.*, 1991; Rivest *et al.*, 1992; Sapolsky *et al.*, 1987; Suda *et al.*, 1990; Tsagarakis *et al.*, 1989) et de l'ACTH (Bateman *et al.*, 1989; Besedovsky *et al.*, 1991; Rivier, 1993). Rappelons que le CRF neuroendocrinien est produit par un groupe spécifique de neurones de la division parvocellulaire des noyaux paraventriculaires de l'hypothalamus (PVH; voir figure 2) (Antoni, 1986; Sawchenko and Swanson, 1989) et qu'une fois synthétisé dans le périkaryon, le neuropeptide est transporté par voie axonale puis stocké dans les vésicules terminales des terminaisons nerveuses CRFergiques regroupées dans la région de l'éminence médiane (EM) (Kawano *et al.*, 1988; Lennard *et al.*, 1993; Swanson *et al.*, 1983). Suite à sa libération dans le système vasculaire porte veineux hypothalamo-hypophysaire, le CRF est acheminé aux cellules corticotropes de l'adénohypophyse où il pourra stimuler la synthèse du peptide pro-opio-mélano-cortine (POMC) qui, une fois clivé, va engendrer la libération d'une série de messagers dont l'ACTH (Whitnall, 1993). Une fois sécrétée dans le sang, l'ACTH pourra agir sur son récepteur à la surface membranaire des cellules fasciculées et réticulées des corticosurrénales afin d'induire la libération d'hormones stéroïdes: les glucocorticoïdes.

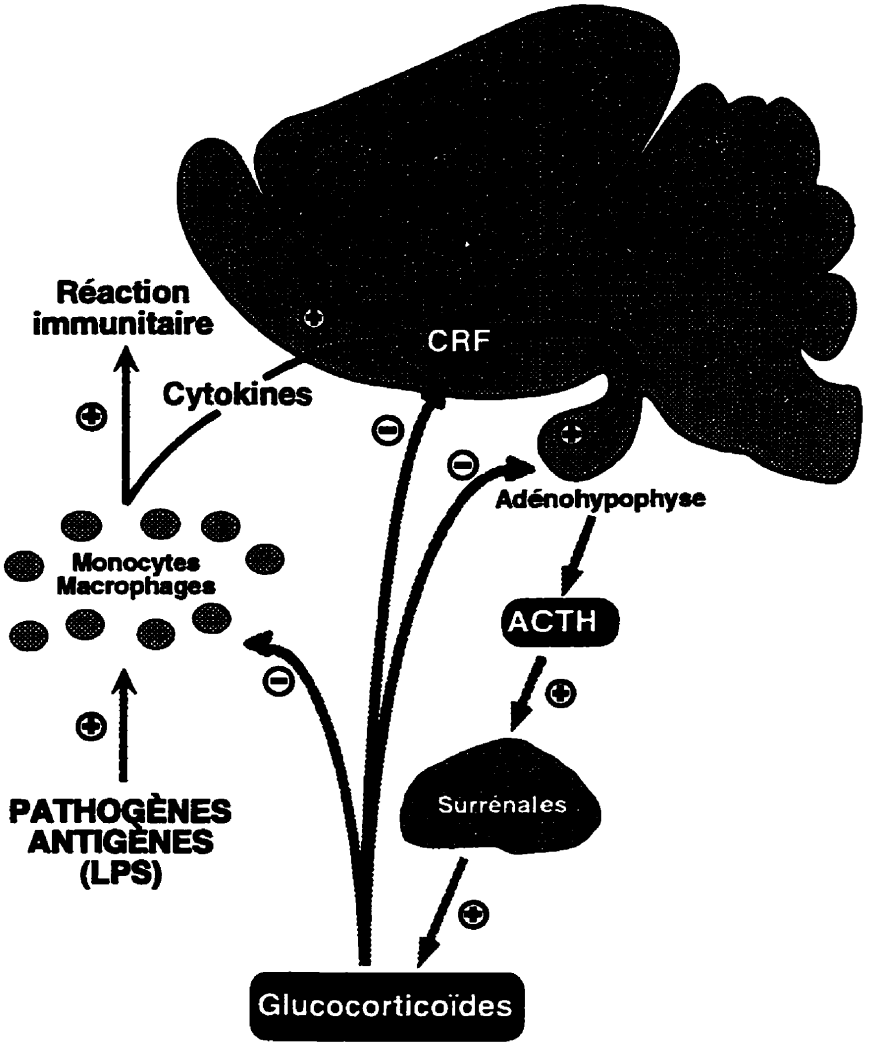


Figure 1. Axe hypothalamo-hypophyso-surrénalien. Abréviations: (LPS) lipopolysaccharide; (CRF) facteur de libération des corticotrophines; (ACTH) hormone adrénocorticotrope.

En plus de réguler par rétroaction négative l'activité de l'axe HPA, au niveau de l'adénohypohyse et de l'hypothalamus (Keller-Wood and Dallman, 1984; Levin *et al.*, 1988; Saphier and Feldman, 1988; Sapolsky *et al.*, 1990), les glucocorticoïdes s'avèrent essentiels afin d'assurer un contrôle approprié de la réponse immunitaire (Munck *et al.*, 1984). En effet, il a été démontré que les glucocorticoïdes peuvent inhiber la prolifération et la distribution des monocytes, macrophages et lymphocytes, en plus de réguler la synthèse et la sécrétion des cytokines (Dupont *et al.*, 1985; Fauci and Dale, 1974; Gillis *et al.*, 1979; Kelso and Munck, 1984; Snyder and Unanue, 1982; Werb, 1978). De nombreuses évidences directes et indirectes ont d'ailleurs confirmé que ces stéroïdes surrénaliens possèdent la

capacité de rétrocontrôler négativement la réaction inflammatoire. Par exemple, il a été démontré que des rats surrénalectomisés (Harbuz *et al.*, 1993) ou hypophysectomisés (Neidhart and Fluckiger, 1992) ainsi que des rats Lewis génétiquement déficients en CRF et non-répondants au CRF exogène (Sternberg *et al.*, 1989) ont une susceptibilité augmentée de développer de l'arthrite. À l'opposé, des rats Fischer qui sont hyper-répondants au CRF semblent moins susceptibles aux diverses maladies inflammatoires (Calogero *et al.*, 1992; Sternberg *et al.*, 1990; Zelazowski *et al.*, 1992). Les glucocorticoïdes sont présentement reconnus comme étant les plus puissants agents anti-inflammatoires. Encore aujourd'hui, ceux-ci sont prescrits fréquemment par les cliniciens afin de réduire la réponse immunitaire, inflammatoire et allergique. En effet, les glucocorticoïdes s'avèrent efficaces dans le traitement d'une multitude de maladies inflammatoires incluant l'arthrite rhumatoïde, l'asthme, le psoriasis, de même que la sclérose en plaques. L'importance de la relâche des glucocorticoïdes lors d'une infection est d'ailleurs bien illustrée par l'observation d'une mortalité élevée chez des animaux surrénalectomisés (Edwards *et al.*, 1991; Mason *et al.*, 1990; Sternberg *et al.*, 1989) ou chez certains patients non-traités souffrant de la maladie d'Addison (Orth *et al.*, 1992). Ainsi, une réponse inappropriée des glandes surrénales pourrait être à l'origine d'une réponse immunitaire inadéquate retrouvée dans certaines pathologies systémiques (ex.: arthrite, hypertension), désordres affectifs (ex.: dépression) et maladies neurodégénératives (maladie d'Alzheimer); d'où la nécessité d'identifier et de caractériser les mécanismes par lesquels les systèmes immunitaire et neuroendocrinien interagissent.

Bien que la figure 1 suggère que les influences des cytokines d'origine systémique sur l'activité de l'axe HPA passent nécessairement par la stimulation de mécanismes CRF-dépendants, de nombreuses études, pour la plupart réalisées *in vitro*, ont tenté de démontrer que les cytokines circulantes possèdent aussi la capacité d'agir directement sur les cellules neurosécrétrices de l'hypophyse. Toutefois, les résultats obtenus demeurent encore controversés, et ce, malgré le fait que les récepteurs de plusieurs cytokines aient été localisés sur certains types de cellules de l'hypophyse antérieure (Cunningham *et al.*, 1992; Haour *et al.*, 1990; Kobayashi *et al.*, 1997; Ohmichi *et al.*, 1992). En effet, une sécrétion d'ACTH a été observée suite au traitement avec de l'IL-1 ou d'autres cytokines de cellules tumorales hypophysaires en culture qui provenaient de patients atteints de la maladie de Cushing (Malarkey and Zvara, 1989). Cependant, il semblerait que ces mêmes cytokines soient incapables de provoquer la relâche d'ACTH sur des cultures primaires réalisées à partir de tissus hypophysaires normaux (Berkenbosch *et al.*, 1987; Sapolsky *et al.*, 1987; Uehara *et al.*, 1987), bien que certaines équipes ont prouvé le contraire (Beach *et al.*, 1989; Bernton *et*

al., 1987; Kehrer *et al.*, 1988; McGillis *et al.*, 1988; Milenkovic *et al.*, 1989; Sharp *et al.*, 1989). Cette controverse suggère la possibilité que les cellules hypophysaires ne répondent qu'à une exposition à long terme aux cytokines. C'est d'ailleurs ce que deux groupes distincts ont rapporté à peu près au même moment. Selon leurs travaux, une période d'incubation de 15 à 24 heures avec les cytokines serait nécessaire avant d'observer la sécrétion d'ACTH; des temps d'incubation plus courts s'étant avérés inefficaces (Kehrer *et al.*, 1988; Suda *et al.*, 1989). D'autres chercheurs affirment plutôt que c'est la destruction ou la préservation des structures intercellulaires de l'hypophyse qui serait responsable des résultats contradictoires obtenus jusqu'à présent. Ainsi, lorsque ces structures sont entièrement sauvegardées, les cellules corticotropes pourraient libérer l'ACTH en présence d'interleukines (Cambronero *et al.*, 1992). Par contre, ces résultats n'ont pas été corroborés par un autre groupe ayant utilisé des cellules hypophysaires dont les structures intercellulaires ont été soumises à un minimum de stress (Parsadaniantz *et al.*, 1993).

À l'heure actuelle, l'hypothèse que les cytokines systémiques puissent agir directement sur l'hypophyse est en perte de popularité et il semble de plus en plus évident que la présence du CRF endogène soit une condition sine qua non à l'observation des effets stimulateurs de ces médiateurs sur l'axe HPA. En effet, il a été démontré que l'immunoneutralisation du CRF a pour conséquence de bloquer la sécrétion d'ACTH induite suite à une injection des cytokines pro-inflammatoires (Berkenbosch *et al.*, 1987; Bernardini *et al.*, 1990; Naitoh *et al.*, 1988; Rivier, 1993; Sapolsky *et al.*, 1987; Uehara *et al.*, 1987). Berkenbosch et son groupe ont rapporté, chez des rats traités avec de la colchicine (la colchicine est un inhibiteur du transport axonal utilisé dans ce cas précis afin d'éviter le renouvellement des réserves de CRF), que l'administration d'IL-1 provoque une déplétion en CRF des vésicules neurosécrétoires situées au niveau de la région externe de l'EM (Berkenbosch *et al.*, 1987). De plus, de nombreux travaux ont montré que l'injection intraveineuse (i.v.) des cytokines pro-inflammatoires est capable d'induire la transcription du gène encodant le CRF dans les noyaux PVH (Ericsson *et al.*, 1994; Harbuz *et al.*, 1992; Rivest and Rivier, 1994), d'augmenter la concentration de CRF dans la circulation porte hypothalamo-hypophysaire (Sapolsky *et al.*, 1987; Watanobe and Takebe, 1992) et d'élever la concentration plasmatique d'ACTH (Berkenbosch *et al.*, 1987; Bernardini *et al.*, 1990; Naitoh *et al.*, 1988; Sapolsky *et al.*, 1987; Uehara *et al.*, 1987). D'autres équipes ont rapporté que les cytokines entraînent une élévation rapide de l'activité électrique des neurones CRFergiques situés dans les noyaux PVH (Saphier and Ovadian, 1990). Ainsi, l'ensemble de ces résultats suggèrent que les neurones CRFergiques des noyaux PVH sont largement responsables de l'activation de l'axe HPA et ce à tout le moins lors d'un traitement

systémique avec des cytokines pro-inflammatoires. Toutefois, il ne faudrait pas négliger l'apport de certains autres facteurs hypothalamiques comme la vasopressine (AVP) et l'oxytocine (OT), deux puissants agents sécrétagogues de l'ACTH pouvant agir en synergie avec le CRF (Antoni *et al.*, 1983; Rivier and Vale, 1985; Rivier and Plotsky, 1986; Yasin *et al.*, 1994). La figure 2 illustre la relation entre la nature neurochimique des sous-groupes de neurones des noyaux PVH et leurs rôles en fonction de leurs efférences. Les groupes cellulaires qui nous intéressent davantage sont les neurones AVP et OT situés dans la division magnocellulaires des noyaux PVH, ceux-ci projettent directement vers le lobe postérieur de la neurohypophyse, et les neurones CRFergiques endocriniens projetant à l'EM.

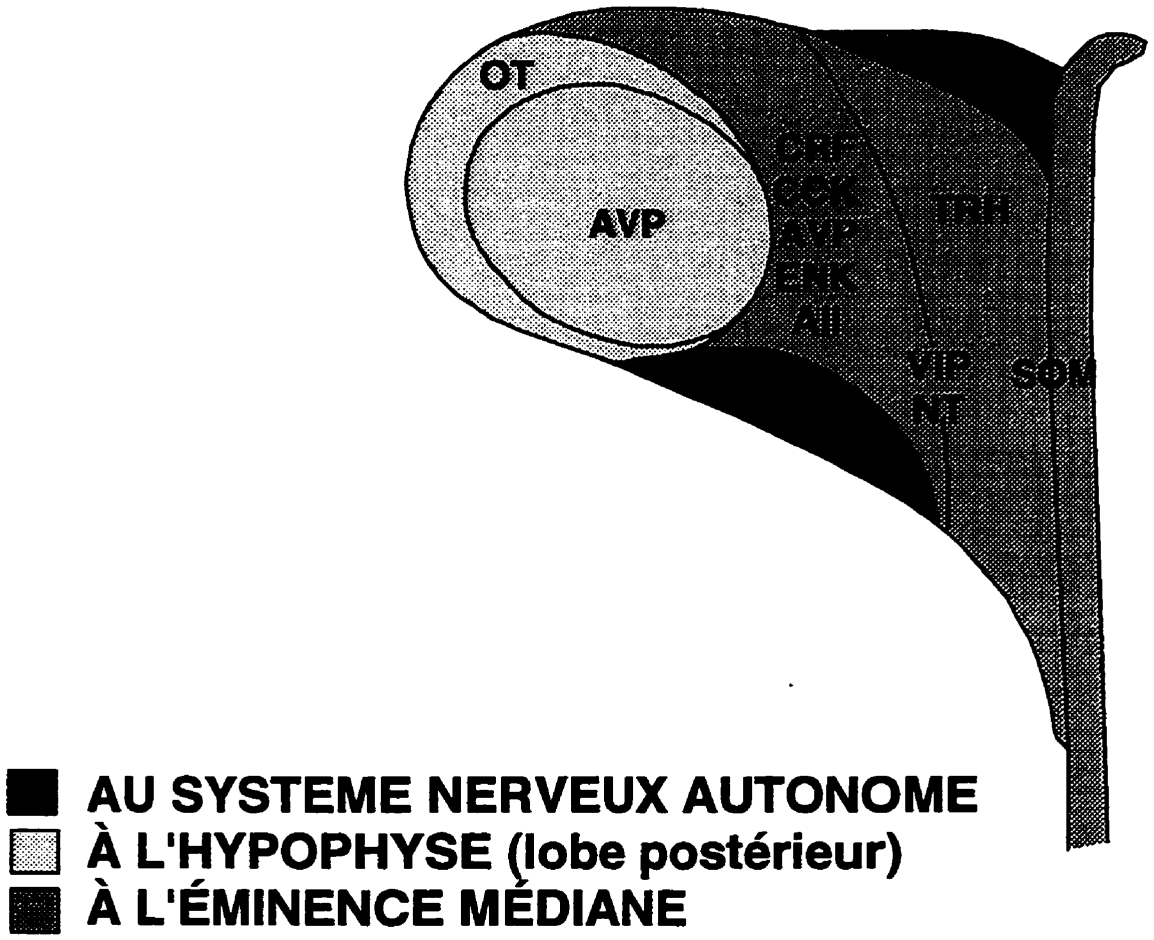


Figure 2. Subdivisions fonctionnelles des noyaux paraventriculaires de l'hypothalamus: neuropeptides & efférences. Abréviations: Angiotensine II (AII), vasopressine (AVP), corticolibérine (CRF), cholécystokinine (CCK), Enképhaline (ENK), neurotensine (NT), oxytocine (OT), somatostatine (SOM), thyrolibérine (TRH), polypeptide intestinal vasoactif (VIP) [figure tirée de: J. Z. Kiss. 1988. Dynamism of chemoarchitecture in the hypothalamic paraventricular nucleus. *Brain Res. Bull.* 20: 699-708].

Les actions du CRF sont médiés par des récepteurs membranaires appartenant à la grande famille des récepteurs couplés à une protéine G. Tout récemment, plusieurs groupes ont cloné à quelques mois d'intervalle, les différents types de récepteur du CRF chez le rat: récepteurs du CRF de type 1 (R-CRF$_1$), 2α et ß (Chang *et al.*, 1993; Chen *et al.*, 1993; Kishimoto *et al.*, 1995; Lovenberg *et al.*, 1995; Perrin *et al.*, 1995; Perrin *et al.*, 1993; Stenzel *et al.*, 1995). Bien qu'on ait décelé la présence respective des récepteurs de type 2α et ß dans quelques structures du système limbique et les plexus choroïdiens, ceux-ci n'ont pas été détectés dans l'EM ainsi que l'hypophyse antérieure (Chalmers *et al.*, 1995). Cette distribution très sélective des ARNm des récepteurs du CRF de type 2 dans le SNC nous permet de croire que ces récepteurs pourraient jouer un rôle crucial dans la modulation des effets comportementales et autonomiques, mais non pas neuroendocriniens, du CRF. Le R-CRF$_1$ a quant à lui été retrouvé dans plusieurs régions du SNC incluant l'adénohypophyse, où les cellules exprimant l'ARNm du R-CRF$_1$ se sont avérées immunoréactives au peptide ACTH (Potter *et al.*, 1994). Peu ou pas de cellules positives pour le R-CRF$_1$ ont été détectées dans les noyaux PVH et l'EM des animaux témoins (Luo *et al.*, 1994; Potter *et al.*, 1994; Rivest *et al.*, 1995). Toutefois, les animaux soumis à un stress inflammatoire ont tous montré des niveaux très élevés d'ARNm du R-CRF$_1$ dans les noyaux PVH ainsi que dans les noyaux SON (Rivest *et al.*, 1995). Ces résultats suggèrent donc que le CRF neuroendocrinien régule l'activité de l'axe HPA par l'entremise du R-CRF$_1$. De plus, le fait que le transcrit du R-CRF$_1$ ait été localisé dans des neurones immunoréactifs au CRF des noyaux PVH soulève la possibilité que le CRF, en soi, puisse contrôler l'activité des motoneurones CRFergiques au cours de la réponse immunitaire (Rivest *et al.*, 1995). Il est intéressant de constater que l'injection intracérébroventriculaire (i.c.v.) de CRF exogène provoque une induction significative des niveaux d'ARNm du R-CRF$_1$ dans les noyaux PVH uniquement, particulièrement dans la division parvocellulaire (Mansi *et al.*, 1996). Ce traitement est aussi capable de stimuler l'activité transcriptionnelle du CRF dans les noyaux PVH et d'entraîner la relâche du neuropeptide dans le système infundibulaire, un phénomène renversé par l'injection d'un antagoniste du récepteur du CRF (Arnold *et al.*, 1992; Mansi *et al.*, 1996; Parkes *et al.*, 1993). Watanobe et ses collègues ont montré, à l'aide de la technique de "push-pull", que l'injection i.v. d'IL-1ß entraîne une augmentation rapide des niveaux de CRF dans les noyaux PVH (Watanobe and Takebe, 1994). Ainsi, à la lueur de ces travaux et ceux du groupe de McCann (Ono *et al.*, 1985), il semble que le CRF d'origine centrale possède la capacité d'auto-réguler positivement sa propre biosynthèse, et probablement l'activité de l'axe HPA, dans certains neurones CRFergiques des noyaux PVH via son récepteur de type 1. Cette hypothèse est appuyée par diverses études neuroanatomiques ayant suggéré la présence potentielle de synapses axo-somatiques entre

des terminaisons nerveuses CRFergiques et les corps cellulaires de neurones CRFergiques de la division parvocellulaire des noyaux PVH (Champagne *et al.*, 1998; Moga and Saper, 1994; Silverman *et al.*, 1989; Swanson *et al.*, 1987).

1.1.2 Principaux médiateurs immunologiques impliqués dans la régulation l'axe HPA

Jusqu'à ce jour, on dénombre une multitude de cytokines et médiateurs inflammatoires capables d'influencer le système neuroendocrinien corticotrope. Que ce soit des cytokines (ex.: IL-1, IL-2, IL-6, TNF-α, interférons α et γ), des facteurs de croissance (ex.: facteur de croissance épidermique, facteur d'angiogénèse tumoral) ou encore des médiateurs lipidiques (ex.: prostanoïdes, facteur activateur des plaquettes), tous ont été soupçonnés, tôt ou tard, pour jouer un rôle dans la régulation de l'axe HPA. Cependant, il semble que les cytokines pro-inflammatoires telles que l'IL-1, l'IL-6 et le TNF-α soient principalement responsables de la stimulation de l'activité de cet axe. En effet, l'administration d'anticorps dirigés contre ces cytokines ou d'agents capables d'inhiber leur synthèse et leur relâchement, ou d'antagonistes des récepteurs cytokinergiques prévient ou réduit les divers changements neuroendocriniens associés à l'infection induite par un pathogène (Besedovsky and Del Rey, 1996; Dinarello and Thompson, 1991; Dunn, 1993; Perlstein *et al.*, 1993; Rivier *et al.*, 1989; Rothwell and Luheshi, 1994; Schotanus *et al.*, 1993). De plus, comme mentionné précédemment, l'injection périphérique d'IL-1, d'IL-6 ou de TNF-α mime les effets de l'infection sur l'activation de l'axe HPA (Berkenbosch *et al.*, 1987; Bernardini *et al.*, 1990; Naitoh *et al.*, 1988; Sapolsky *et al.*, 1987; Suda *et al.*, 1990; Uehara *et al.*, 1987; Watanobe and Takebe, 1992), en plus de reproduire la plupart des symptômes normalement observés lors de ce genre de situation pathologique: fièvre, anorexie, dépression, diminution de l'activité locomotrice, hyperalgésie, etc (Dinarello, 1984; Dinarello and Thompson, 1991; Dunn, 1993; Ertel *et al.*, 1992; Hart, 1988; Kent *et al.*, 1992; Kushner, 1982; Maier *et al.*, 1993). Les cytokines pro-inflammatoires sont produites et relâchées en cascade par les monocytes/macrophages et les lymphocytes systémiques lors de la stimulation de la première phase d'une réaction immunitaire (Nathan, 1987). Ainsi, il est maintenant bien connu que lorsque l'hôte est infecté par un antigène quelconque, les macrophages et les lymphocytes sont appelés au site d'infection afin de déclencher la réponse immunitaire de type aigu. C'est à ce moment même qu'il y a largage des cytokines et début de la réponse inflammatoire (Dinarello, 1989; Rabin *et al.*, 1990).

L'activation de la synthèse des prostaglandines (PGs) par les voies de la cyclooxygénase est, elle aussi, maintenant reconnue comme une étape cruciale dans la cascade des événements menant à l'activation finale de l'axe corticotrope lors d'une réponse immunitaire/inflammatoire. En effet, plusieurs résultats démontrent qu'un prétraitement avec des inhibiteurs de la cyclooxygénase, l'enzyme limitante dans la synthèse des PGs, bloque les effets des cytokines pro-inflammatoires sur la sécrétion du CRF et l'activation subséquente de l'axe HPA (Bernardini *et al.*, 1990; Katsuura *et al.*, 1990; Katsuura *et al.*, 1988; Lyson and McCann, 1992; McCoy *et al.*, 1994; Morimoto *et al.*, 1989; Murakami and Watanabe, 1989; Navarra *et al.*, 1991; Sharp and Matta, 1993; Watanabe *et al.*, 1990). De plus, d'autres évidences semblent suggérer que les prostaglandines produites centralement puissent jouer un rôle d'intermédiaire entre les cytokines pro-inflammatoires et l'activation de l'axe HPA. Dans les sections qui vont suivre, il sera donc question du rôle des cytokines pro-inflammatoires et des prostaglandines dans la régulation des fonctions neuroendocriniennes. Précisons toutefois, qu'une attention plus particulière sera attribuée au contrôle de l'axe corticotrope par ces médiateurs de la réponse inflammatoire/immunitaire.

1.1.2.1 Interleukine-1

Le nom d'IL-1 a été attribué à deux protéines distinctes, les interleukines-1α et ß, provenant de gènes différents, mais capables de reconnaître les mêmes récepteurs membranaires. Jusqu'à maintenant, deux sous-types de récepteur de l'IL-1 ont été identifiés et clonés. Cependant, seul le récepteur de type 1 de l'IL-1 semble être responsable de médier les effets biologiques des IL-1α et ß (Sims *et al.*, 1994). Bien que la production d'IL-1 soit généralement considérée comme une conséquence de la réaction inflammatoire, cette cytokine peut aussi agir sur différents types cellulaires et ainsi produire une multitude d'effets biologiques (Oppenheim and Gery, 1982; Oppenheim *et al.*, 1986). En effet, des évidences récentes suggèrent que l'augmentation des taux d'IL-1 puisse moduler certains événements tels que: la formation osseuse, le cycle menstruel, la coagulation sanguine, la résorption des cartilages, etc (Dinarello, 1991; Dodds *et al.*, 1994; Le and Vilcek, 1987; Lynch *et al.*, 1994).

Une attention particulière a été portée au cours des dernières années quant au rôle joué par l'IL-1 dans la stimulation de l'axe corticotrope durant la réponse immunitaire. Ainsi, plusieurs travaux ont rapporté que l'utilisation de différents modèles infectieux ou inflammatoires chez les animaux de laboratoire a pour effet de provoquer une augmentation

dramatique de la production d'IL-1 par les cellules immunitaires activées (Berkenbosch *et al.*, 1991; Bristow *et al.*, 1991; DeRijk and Berkenbosch, 1992; Givalois *et al.*, 1994). Il est intéressant de constater que l'IL-1 est maintenant considérée comme l'un des plus puissants agents sécrétagogues de la relâche de l'ACTH et des glucocorticoïdes (Besedovsky and Del Rey, 1996; Rivest, 1995; Schöbitz *et al.*, 1994), un effet dépendant de la sécrétion du CRF neuroendocrinien (Berkenbosch *et al.*, 1987; Sapolsky *et al.*, 1987; Uehara *et al.*, 1987). L'injection d'IL-1ß induit l'expression du gène de réponse précoce c-*fos* (marqueur de l'activité neuronale; l'utilisation des gènes de réponses précoces comme outil de travail en neuroscience sera discuté plus abondamment à la section 1.3) dans les neurones CRFergiques des noyaux PVH (Ericsson *et al.*, 1994; Rivest and Rivier, 1994; Rivest *et al.*, 1992). De plus, tel que mentionné auparavant, l'administration d'IL-1ß augmente les niveaux d'ARNm du CRF dans les noyaux PVH (Brady *et al.*, 1994; Ericsson *et al.*, 1994; Rivest and Rivier, 1994; Suda *et al.*, 1990), en plus de stimuler la relâche du neuropeptide par les axones neurosécréteurs CRFergiques regroupés au niveau de l'EM (Berkenbosch *et al.*, 1987; Sapolsky *et al.*, 1987; Uehara *et al.*, 1987). Toutefois, le mécanisme et les sites d'action empruntés par l'IL-1 d'origine systémique dans la modulation de la biosynthèse du CRF sont encore mal compris. En effet, étant donné la présence de la barrière hémato-encéphalique empêchant la pénétration des macromolécules systémiques dans le SNC, plusieurs questions demeurent sans réponses quant à la façon dont l'IL-1 ou les autres cytokines périphériques peuvent influencer les structures hypothalamiques responsables de l'activation de l'axe HPA. Est-ce que l'IL-1α et ß agissent en initiant la sécrétion du CRF par les terminaisons nerveuses situées dans l'EM ou, est-ce que celles-ci peuvent atteindre directement les corps cellulaires des neurones CRFergiques des noyaux PVH? Bien que certaines études aient démontré que l'infusion d'IL-1ß dans l'EM entraîne une sécrétion rapide d'ACTH (Sharp *et al.*, 1989), la théorie voulant que les cytokines passent la barrière hémato-encéphalique, demeure incertaine et controversée. En effet, certains résultats supportent la possibilité que les cytokines puissent pénétrer le cerveau (Banks *et al.*, 1989; Hashimoto *et al.*, 1991) alors que d'autres la rejette (Coceani *et al.*, 1988; Dunn, 1988). De toute évidence, si l'IL-1α et ß peuvent pénétrer la barrière hémato-encéphalique, ce sera en très petite quantité. Les cytokines pourraient toutefois atteindre le SNC via des sites dépourvus de barrière hémato-encéphalique; c'est le cas notamment des organes circumventriculaires (OCVs) tels que: l'organe vasculaire de la lame terminale (OVLT), l'organe subfornicale (SFO), l'EM et l'area postrema (AP) (Partridge, 1983). Cependant, l'utilisation de la technique d'hybridation *in situ* n'a pas permis de détecter la présence de l'ARNm encodant le récepteur de type 1 de l'IL-1 dans les cellules parenchymales des OCVs du rat, à l'exception de l'AP, dans des conditions de base ou suite à l'injection i.v. d'IL-1ß

(Ericsson *et al.*, 1995). Bien que ces résultats suggèrent que l'IL-1 ne puisse pas agir directement sur les éléments du parenchyme de la majorité des OCVs, il semble néanmoins envisageable que l'IL-1 d'origine systémique soit en mesure de lier son récepteur de type 1 localisé au niveau de l'AP, l'endothelium de la microvasculature cérébrale ou des autres structures non-parenchymales du cerveau (Ericsson *et al.*, 1995; Van Dam *et al.*, 1996), afin de stimuler la production de médiateurs secondaires capables de réguler l'activité des neurone CRFergiques.

Une autre hypothèse voudrait que certaines cellules du SNC relâchent elles aussi des cytokines. Même si plusieurs travaux confirment l'absence des cytokines dans le cerveau dans des conditions normales (Ban *et al.*, 1992; Buttini *et al.*, 1994; Higgins and Olschowka, 1991; Nadeau and Rivest, 1998; Quan *et al.*, 1998; Vallières and Rivest, 1997), la présence d'IL-1ß et d'une multitude d'autres médiateurs inflammatoires a été rapportée dans le SNC en réponse à diverses conditions de stress: infection centrale (ex.: malaria, sida, méningite, etc.), traumatisme, tumeur, sclérose en plaques, ischémie, maladie d'Alzheimer, syndrome de Down, lésion cérébrale, etc (Hopkins and Rothwell, 1995). Le rôle exact joué par les cytokines d'origine centrale dans de telles situations reste encore obscur. Toutefois, les résultats préliminaires semblent leur attribuer des fonctions régulatrices importantes afin de rétablir l'homéostasie en promouvant la croissance et la différenciation cellulaire, et en modulant la défense et la réparation des tissus.

C'est afin de mieux comprendre le rôle de l'IL-1 produite localement dans le SNC que plusieurs groupes ont entrepris une étude exhaustive des sites et des types cellulaires exprimant cette cytokine. Ainsi, chez des rats soumis à un challenge immunitaire, l'ARNm encodant l'IL-1 et la protéine elle-même ont été détectés dans les cellules des méninges, des plexus choroïdiens, dans les vaisseaux sanguins du cerveau et les cellules parenchymales de nombreuses régions incluant les OCVs et les noyaux PVH (Quan *et al.*, 1998; Van Dam *et al.*, 1992). La combinaison de l'hybridation *in situ* à l'immunohistochimie de même que le double marquage immunohistochimique ont révélé que la grande majorité de ces cellules sont d'origine myéloïde, c'est-à-dire: des cellules microgliales, des macrophages et des cellules microgliales associées à la microvasculature cérébrale (Buttini and Boddeke, 1995; Van Dam *et al.*, 1992). Ces observations confirment donc les données recueillies par divers groupes de recherche il y a tout près de dix ans déjà. En effet, ceux-ci avaient décelé, grâce à l'utilisation de cultures primaires constituées de macrophages du cerveau, que les cellules microgliales synthétisent les ARNm encodant l'IL-1α et ß et les protéines correspondantes

suite à un traitement avec une endotoxine bactérienne (Giulian *et al.*, 1986; Hetier *et al.*, 1988). L'existence de l'IL-1(α et ß) dans les astrocytes en réponse à un stimulus semblable fut aussi rapportée (Fontana *et al.*, 1982; Fontana *et al.*, 1984). Bien que controversées, des études neuroanatomiques ont démontré que les neurones localisés dans certaines régions du cerveau, incluant l'hypothalamus, sont eux aussi capables de synthétiser et de sécréter différentes cytokines (Bandtlow *et al.*, 1990; Busbridge and Grossman, 1991; Koenig, 1991). En 1988, Breder et ses collaborateurs ont rapporté la présence d'IL-1ß dans les fibres nerveuses des noyaux PVH, des noyaux arqués (ARC) et de l'EM (Breder *et al.*, 1988). Plus récemment, il a été possible d'identifier, en utilisant un anticorps spécifique à l'IL-1ß, plusieurs corps cellulaires de neurones positifs à l'IL-1ß dans la subdivision magnocellulaire des noyaux PVH (Lechan *et al.*, 1990). Il semble ainsi de plus en plus évident que la machinerie de synthèse de diverses cytokines est présente dans certaines structures sélectives du cerveau. De plus, il est fort probable que ces structures aient un rôle déterminant à jouer dans le contrôle des fonctions neuroendocriniennes via la biosynthèse de médiateurs centraux comme l'IL-1 par exemple. Les recherches réalisées *in vitro* ont d'ailleurs montré qu'un traitement de fragments hypothalamiques avec l'IL-1ß a pour effet de provoquer la libération du CRF (Navarra *et al.*, 1991; Tsagarakis *et al.*, 1989). L'injection i.c.v. et intrahypothalamique d'IL-1 ont toutes deux été rapportées capables de stimuler la sécrétion d'ACTH par l'hypophyse via la sécrétion du CRF dans le système infundibulaire (Barbanel *et al.*, 1990; Rivier and Rivest, 1993). D'autres études ont montré qu'une lésion bilatérale des noyaux PVH inhibe de façon significative l'augmentation des niveaux plasmatiques d'ACTH et de corticostérone observée chez des rats traités avec l'IL-1ß dans le ventricule latéral du cerveau (Rivest and Rivier, 1991). Finalement, Lechan et son groupe ont démontré que l'administration centrale, mais pas systémique, d'un antagoniste du récepteur de l'IL-1 a pour effet de bloquer complètement la stimulation de la transcription du CRF observée dans les noyaux PVH suite à une infection bactérienne (Kakucska *et al.*, 1993). En plus de confirmer que les neurones CRFergiques des noyaux PVH sont largement responsables de l'activation de l'axe HPA, ces résultats démontrent que l'IL-1 d'origine centrale joue un rôle déterminant dans les étapes menant à la biosynthèse du CRF neuroendocrinien au cours d'un challenge immunitaire. Toutefois, la question demeure entière quant à savoir si c'est l'IL-1 produite localement dans les noyaux PVH qui est responsable de la stimulation de l'axe corticotrope. En effet, il est possible que certains groupes de cellules extrahypothalamiques activées par l'IL-1 puissent réguler le système neuroendocrinien via leurs projections afférentes directes ou indirectes aux neurones CRFergiques.

1.1.2.2 Facteur nécrosant des tumeurs-alpha

Le TNF-α, aussi connu sous le nom de cachectine, est une cytokine pléiotrophique. Parmi les nombreux effets du TNF-α, on retrouve entre autre son habilité à tuer directement certaines cellules tumorales, expliquant du même coup l'appellation de facteur nécrosant des tumeurs (Carswell *et al.*, 1975). Le TNF-α est considéré comme une cytokine pro-inflammatoire jouant un rôle déterminant dans l'attraction des cellules immunitaires au site d'infection, l'activation de celles-ci et l'initiation subséquente de la relâche en cascade des autres cytokines et facteurs responsables du contrôle de la réponse immunitaire et inflammatoire. Ainsi, il n'est pas surprenant de constater que, lors d'un stress immunitaire, le TNF-α est reconnu comme étant la première cytokine libérée par les cellules phagocytaires activées (Givalois *et al.*, 1994). Les divers effets du TNF sont obtenus suite à la liaison de la cytokine à deux types de récepteurs distincts, le récepteur du TNF de type I (TNFR-I) ou p55 et le récepteur du TNF de type II (TNFR-II) ou p75. L'injection i.v. du TNF-α est capable de reproduire la grande majorité des symptômes observés lors d'une infection par un agent pathogène (hypotension, hypoglycémie, acidose, nécrose hépatique, perte de poids, etc.); expliquant du même coup pourquoi tant d'emphase a été consacrée à la toxicité du TNF recombinant et au rôle joué par le TNF endogène en tant que médiateur des effets toxiques des endotoxines bactériennes comme la lipopolysaccharide (LPS) (Vieira *et al.*, 1996). Toutefois, des études subséquentes ont aussi démontré que le TNF est essentiel au rétablissement de l'homéostasie lors de différents types d'infections (Marino *et al.*, 1997). En effet, Vieira et ses collaborateurs ont rapporté tout récemment la présence de lésions inflammatoires beaucoup plus larges et nombreuses chez des souris déficientes en récepteur du TNF de type I que chez des souris sauvages infectées avec un parasite (Vieira *et al.*, 1996). De plus, l'administration d'un anticorps dirigé contre le TNF-α a pour conséquence de rendre plusieurs types d'infections bactériennes létales (Havell, 1989; Nakane *et al.*, 1988). Enfin, Nakano et son groupe ont démontré que l'injection de TNF-α protège les souris de la mort à la suite d'une infection par un agent pathogène (Nakano *et al.*, 1990). Bien que plusieurs des effets bénéfiques du TNF-α soient attribuables à sa capacité de stimuler les mécanismes cytotoxiques des cellules phagocytaires, de nombreux travaux ont confirmé l'importance capitale de l'activation de l'axe HPA et de l'atténuation subséquente de la réponse inflammatoire dans le rôle protecteur du TNF-α.

Plusieurs groupes ont rapporté que l'injection i.v. de TNF-α provoque une sécrétion rapide du CRF dans l'EM (Watanobe and Takebe, 1992) et l'élévation des niveaux d'ACTH

et de corticostérone dans le plasma (Bernardini *et al.*, 1990; Besedovsky *et al.*, 1991; Sharp *et al.*, 1989). De plus, les études menées par Bernardini et ses collègues ont confirmé que cette activation rapide de l'axe HPA dépend entièrement de la sécrétion du CRF neuroendocrinien (Bernardini *et al.*, 1990). Dans cette perspective, les noyaux PVH semblent jouer un rôle déterminant puisque la lésion complète de ceux-ci a pour conséquence d'abolir la sécrétion d'ACTH en réponse à l'infusion de TNF-α (Kovacs and Elenkov, 1995). Quant à la stimulation directe des fibres terminales CRFergiques regroupées dans l'EM, cette hypothèse semble plutôt improbable puisqu'il a été rapporté qu'une injection de TNF-α dans un site adjacent à l'EM n'entraîne pas la sécrétion du CRF neuroendocrinien (Sharp *et al.*, 1989). Par conséquent, ces résulats suggèrent que les actions du TNF-α sur la régulation de l'axe HPA passent par la stimulation des corps cellulaires des neurones sécréteurs du CRF localisés dans les noyaux PVH. Ainsi, l'administration i.v. de TNF-α entraîne l'activation des neurones CRFergiques des noyaux PVH, en plus de provoquer l'expression du transcrit primaire encodant le neuropeptide CRF uniquement dans cette structure hypothalamique (S. Nadeau et S. Rivest, papier en préparation). Toutefois, la présence du gène de réponse précoce c-*fos* dans plusieurs autres régions du cerveau, incluant les OCVs et les plexus choroïdiens, laisse entrevoir la possibilité que le TNF-α relargué dans le sang au cours d'une inflammation puisse stimuler la biosynthèse du CRF neuroendocrinien via une circuiterie relativement complexe (S. Nadeau et S. Rivest, papier en préparation). Les ARNm encodant les récepteurs p55 et p75 du TNF-α ont été détecté dans les cellules endothéliales de la microvasculature cérébrale dans des conditions basales (Bebo and Linthicum, 1995). De plus, grâce à la cytométrie de flux, Lucas et ses collaborateurs ont découvert que les niveaux d'expression du récepteur p75 sont fortement induits dans l'endothelium du SNC suite à une infection (Lucas *et al.*, 1997). Des résultats préliminaires obtenus par hybridation *in situ* ont révélé la présence de l'ARNm du récepteur p75 dans les noyaux PVH et les OCVs de rats (S. Nadeau et S. Rivest, papier en préparation). Ces mêmes travaux ont aussi rapporté une augmentation des niveaux de transcrits du récepteur p75 dans les OCVs à la suite d'un stress immunitaire. À la lumière de ces résultats, il est possible d'envisager que le TNF-α d'origine périphérique rejoigne les structures situées à l'extérieur de la barrière hémato-encéphalique afin de moduler certains mécanismes responsables de la stimulation de l'axe corticotrope. Il est intéressant de noter que l'administration systémique d'un antisérum contre le TNF-α réduit ou prévient l'augmentation des niveaux plasmatiques d'ACTH durant la réponse immunitaire (Ebisui *et al.*, 1994; Perlstein *et al.*, 1993; Turnbull and Rivier, 1998).

Récemment, des travaux ont démontré qu'une inhibition de l'action du TNF-α à l'intérieur du SNC, grâce à une immunoneutralisation intracérébrale passive ou encore suite à l'administration centrale de la forme soluble du récepteur du TNF, a pour effet d'entraîner une baisse dramatique des hauts niveaux d'ACTH normalement observés durant une inflammation locale (Turnbull *et al.*, 1997; Wooley *et al.*, 1993). Bien que par la suite certains aient également obtenu des résultats similaires avec un modèle d'infection bactérienne (Molher *et al.*, 1993; Turnbull and Rivier, 1998), des analyses plus approfondies ont montré que les effets observés seraient plutôt attribuables à l'augmentation des niveaux systémiques d'antisérum dirigé contre le TNF-α grâce au phénomène de "bulk flow" (facilite le passage de diverses substances contenues dans le liquide céphalo-rachidien vers le sang afin d'être éliminées). En effet, il semble que l'administration centrale d'antisérum contre le TNF-α immédiatement avant l'induction d'un stress immunitaire, n'altère pas de façon significative les taux d'ACTH dans le plasma (Turnbull and Rivier, 1998). Curieusement, des quantités très faibles d'anticorps anti-TNF-α ont été mesurées dans la circulation sanguine dans ce cas bien précis. Quoiqu'il en soit, l'injection i.c.v. de TNF-α recombinant stimule l'activité transcriptionnelle du CRF dans les noyaux PVH (S. Nadeau et S. Rivest, papier en préparation), en plus de provoquer une hausse des concentrations d'ACTH plasmatiques (Turnbull *et al.*, 1997). La présence du TNF-α a été détectée dans le liquide céphalo-rachidien d'animaux souffrant d'une méningite d'origine bactérienne (Mustafa *et al.*, 1989). De plus, des niveaux élevés d'ARNm encodant la cytokine pro-inflammatoire TNF-α ont été rapidement décelés dans les OCVs, les plexus choroïdiens et les méninges de rat et souris soumis à une endotoxinémie systémique (Breder *et al.*, 1994; Nadeau and Rivest, 1998). Plusieurs cellules exprimant le gène du TNF-α ont été identifiées dans les régions adjacentes aux OCVs quelques heures après l'infection. Les procédures de double marquage réalisées, grâce à la combinaison des techniques d'immunohistochimie et d'hybridation *in situ*, ont révélé que la grande majorité des cellules positives sont en réalité des cellules microgliales parenchymales (Nadeau and Rivest, 1998). D'autres travaux semblent montrer que les astrocytes et les neurones peuvent également synthétiser du TNF-α lors d'une endotoxinémie (Breder *et al.*, 1994; Chung and Benveniste, 1990; Sawada *et al.*, 1989). Ainsi, il demeure possible que le TNF-α produit centralement puisse participer directement à la régulation de la réponse neuroendocrinienne suite à une infection et une inflammation. Toutefois, à la lumière des nombreux résultats décrits dans cette section, il est aussi probable que le TNF-α d'origine centrale agisse comme facteur autocrinien ou paracrinien, afin de stimuler la biosynthèse de neuromédiateurs qui eux, pourront à leur tour moduler l'activité de l'axe corticotrope.

1.1.2.3 Interleukine-6

Plusieurs des effets pléiotrophiques de l'IL-1 et du TNF-α semblent être médiés par l'IL-6 (Shalaby *et al.*, 1989). En effet, il a été démontré que ces trois cytokines pro-inflammatoires agissent en synergie dans une multitude de processus tels que l'induction de la prolifération cellulaire, la biosynthèse des protéines de la phase aiguë par le hépatocytes, la neuroprotection et même l'activation de l'axe HPA (Bruce *et al.*, 1996; Conti *et al.*, 1995; Hirota *et al.*, 1996; Perlstein *et al.*, 1991; Perlstein *et al.*, 1993; Tritarelli *et al.*, 1994). De plus, l'IL-1 et le TNF-α ont été reconnues comme des stimulateurs importants de la production de l'IL-6 dans la systémie et les organes périphériques (Akira *et al.*, 1990; Besedovsky and del Rey, 1992; Kasid *et al.*, 1989; Shalaby *et al.*, 1989). Lors de l'induction d'une réponse immunitaire, l'IL-1ß et le TNF-α sont toujours les deux premières cytokines à apparaître en concentration mesurable dans la circulation sanguine (Givalois *et al.*, 1994), un résultat corroboré par des études *in vitro* (De Groote *et al.*, 1992; DeForge and Remick, 1991). Il n'est donc pas étonnant de constater que l'administration d'un antisérum dirigé contre l'IL-1ß, le TNF-α ou l'infusion d'un antagoniste du récepteur de l'IL-1 a pour effet d'atténuer de façon significative l'augmentation des niveaux d'IL-6 plasmatiques lors d'une septicémie (Fong *et al.*, 1989; Lemay *et al.*, 1990; Luheshi *et al.*, 1996). Ainsi, ces résultats suggèrent que certaines cytokines pro-inflammatoires, comme l'IL-1 et le TNF-α, peuvent agir en synergie afin d'induire la biosynthèse de l'IL-6 par les cellules myéloïdes de la systémie.

L'IL-6 est une protéine multifonctionnelle exerçant son activité grâce à la liaison d'un complexe formé de deux glycoprotéines (gp) membranaires; le récepteur de l'IL-6 et le gp130 responsable de la modulation des signaux de transduction (Hibi *et al.*, 1990). Cette cytokine joue un rôle crucial au cours de diverses circonstances incluant la défense de l'hôte, la réponse immunitaire, la réponse hépatique de phase aiguë, l'hématopoïèse ainsi que l'induction de la fièvre (Castell *et al.*, 1989; Kishimoto, 1989). En effet, plusieurs évidences semblent supporter l'hypothèse que la fièvre induite lors d'une infection bactérienne est causée par la production d'IL-6 sous l'influence de l'IL-1ß (Chai *et al.*, 1996; Klir *et al.*, 1994). Le rôle de l'IL-6 dans la modulation de l'axe HPA reste toutefois mal compris. Cette cytokine peut induire la production d'ACTH et des glucocorticoïdes en stimulant la relâche du CRF dans le système porte veineux hypothalamo-hypophysaire (Naitoh *et al.*, 1988). Par contre, contrairement à l'IL-1ß, l'IL-6 n'induit pas l'activité transcriptionnelle du CRF dans les noyaux PVH (Harbuz *et al.*, 1992; Vallières *et al.*, 1997). Cette incapacité de l'IL-6 à

stimuler l'expression du CRF neuroendocrinien peut néanmoins être expliquée par le fait que le récepteur de l'IL-6 n'est pas présent dans les noyaux PVH dans des conditions basales (Vallières and Rivest, 1997). Il est intéressant de noter qu'une injection i.v. d'IL-6 réalisée six heures après l'induction d'une réponse immunitaire systémique a pour effet d'activer la transcription du gène encodant le CRF (Vallières and Rivest, 1998). Il est possible que la synthèse du récepteur de l'IL-6 soit fortement stimulée dans le noyaux PVH au cours d'un stress immunitaire, permettant ainsi à la cytokine d'activer les neurones responsables du contrôle de l'axe corticotrope. Des études effectuées chez des souris déficientes pour le gène qui encode l'IL-6 ont d'ailleurs supporté ces résultats; une baisse significative des niveaux d'ARN hétéronucléaire (hn) du CRF et une diminution très importante de l'activité cellulaire ont été observées dans les noyaux PVH de souris déficientes en IL-6 à la suite d'une injection systémique de l'endotoxine LPS (Vallières and Rivest, 1998). De plus, il a été rapporté que l'administration d'anticorps spécifiques à l'IL-6 atténue la sécrétion d'ACTH normalement observée suite à l'injection d'IL-1ß (Neta *et al.*, 1992) ou un stress immunitaire (Perlstein *et al.*, 1993). Par conséquent, il est possible d'affirmer que l'IL-6 apporte une contribution non-négligeable à l'activation de l'axe HPA au cours de la réponse inflammatoire. Toutefois, le rôle de cette cytokine consisterait à prolonger l'activité de l'axe corticotrope plutôt que de l'induire. Ce mécanisme pourrait être essentiel à la restauration de l'homéostasie corporelle durant l'infection et l'inflammation.

La présence du récepteur de l'IL-6 dans la zone externe de l'EM (Vallières and Rivest, 1997) supporte l'hypothèse que cette cytokine pro-inflammatoire puisse agir directement sur les fibres terminales des neurones CRFergiques afin d'induire la sécrétion de l'ACTH et des glucocorticoïdes. Ce mécanisme a d'ailleurs été supporté par des études *in vitro* montrant que l'IL-6 peut stimuler la sécrétion du CRF à partir de l'hypothalamus médiobasal qui inclut l'EM (Lyson and McCann, 1992). Toutefois, une lésion complète des noyaux PVH est capable de prévenir l'élévation des niveaux plasmatiques d'ACTH chez des rats recevant une injection i.v. d'IL-6 (Kovacs and Elenkov, 1995). Nous avons aussi observé une forte stimulation transcriptionnelle du gène encodant le récepteur de l'IL-6 dans les noyaux PVH de rats au cours d'une endotoxinémie (Vallières and Rivest, 1997). Par conséquent, il est probable que l'EM et les cellules parenchymales des noyaux PVH soient les cibles respectives de l'IL-6 d'origine systémique et centrale.

Cette vision est néanmoins assez simplifiée puisque l'injection i.v. d'IL-6 a pour conséquence de stimuler l'activité cellulaire (révélée par la détection du gène de réponse précoce c-*fos*) dans plusieurs structures spécifiques du SNC, dont les OCVs (Vallières *et al.*, 1997). Des niveaux très élevés d'ARNm encodant le récepteur de l'IL-6 et le gp130 ont été détectés dans ces mêmes régions ainsi que dans quelques autres structures, incluant la microvasculature cérébrale et les noyaux PVH (Vallières and Rivest, 1997). En plus d'être sensibles à l'IL-6 systémique, les OCVs ont la capacité de produire cette cytokine localement suite à une infection bactérienne (Vallières and Rivest, 1997). Il est donc possible que l'IL-6 agisse directement sur différentes populations cellulaires des OCVs et les vaisseaux sanguins du cerveau afin de moduler l'activité neuronale, la sécrétion du CRF et par conséquent celle de l'ACTH. Il est important de noter qu'il existe plusieurs connections entre les organes OCVs et les noyaux PVH (Johnson and Gross, 1993), supportant ainsi l'hypothèse voulant que les OCVs puissent informer directement l'hypothalamus endocrinien lors d'un stress inflammatoire/immunitaire. Une autre théorie fait plutôt référence à la possibilité que l'IL-6 circulant à l'intérieur des ventricules puisse lier son récepteur localisé dans les noyaux PVH (ceux-ci étant situés à proximité des espaces ventriculaires), et provoquer ainsi l'activation des neurones CRFergiques projetant vers le système infundibulaire. Le fait qu'une injection i.c.v. d'IL-6 provoque une élévation des niveaux plasmatiques d'ACTH soutien cette hypothèse (Lyson and McCann, 1992; Matta *et al.*, 1992; McCann *et al.*, 1993). La forte transcription de l'IL-6 dans les plexus choroïdiens, la sécrétion de la cytokine dans le liquide céphalo-rachidien et l'expression du récepteur de l'IL-6 dans les noyaux PVH durant l'endotoxinémie sont des arguments supplémentaires en faveur de ce mécanisme d'action (LeMay *et al.*, 1990; Vallières and Rivest, 1997). Dans cette perspective, la production centrale d'IL-6, sous l'influence de divers médiateurs inflammatoires du SNC, pourrait également avoir un rôle crucial à jouer. En effet, certains travaux ont révélé que des médiateurs centraux comme l'IL-1 pourraient contribuer aux effets de l'IL-6 sur la sécrétion du CRF neuroendocrinien. Des études *in vitro* ont montré, sur des cellules télencéphaliques en culture, qu'un prétraitement avec un antagoniste du récepteur de l'IL-1 a pour effet d'inhiber la sécrétion d'IL-6 en réponse à une endotoxine bactérienne (Romero *et al.*, 1993). Ainsi, bien qu'une injection i.v. d'IL-1ß soit incapable d'induire l'expression de l'ARNm encodant l'IL-6 et son récepteur dans les cellules parenchymales du SNC (Vallières and Rivest, 1997), il est possible que l'IL-1ß d'origine centrale puisse augmenter la production d'IL-6 à l'intérieur des limites de la barrière hémato-encéphalique. Des études ont d'ailleurs rapporté la présence de concentrations élevées d'IL-6 dans le liquide céphalo-rachidien de rats injectés directement dans le cerveau avec l'IL-1ß (Romero *et al.*, 1996). Finalement, l'AVP et l'OT, deux agents sécrétagogues de l'ACTH, pourraient aussi participer aux effets

de l'IL-6 sur l'axe corticotrope (Mastorakos *et al.*, 1993; Mastorakos *et al.*, 1994; Naitoh *et al.*, 1991; Yasin *et al.*, 1994).

1.1.3 Dérivés de l'acide arachidonique

Au cours des dernières années, il a été possible d'observer un intérêt grandissant dans le domaine de la médecine et de la biologie pour les dérivés de l'acide arachidonique (AA). Aussi connus sous l'appellation d'écosanoïdes, ces acides gras non-saturés sont synthétisés via deux voies métaboliques distinctes: la voie de la cyclooxygénase et celle de la lipooxygénase. Les dérivés de l'AA incluent plusieurs composés dont les prostaglandines (PGs), les thromboxanes, les leukotriènes, les lipoxines et une multitude d'acides gras hydroxylés. Parmi ceux-ci, les PGs ou prostanoïdes sont sans aucun doute les plus étudiées étant donné leurs actions autocrines et paracrines sur une multitude de types cellulaires. Les nombreuses fonctions métaboliques des PGs en tant que second messager assurant la communication entre les milieux extra- et intracellulaires ont également grandement contribué à la popularité des prostanoïdes. Nos connaissances sur les thromboxanes, les leukotriènes et les lipoxines sont, quant à elles, très limitées dû à leur découverte beaucoup plus récente. Malgré de nouvelles évidences démontrant que la plupart de ces composés ont des actions pharmacologiques diverses et puissantes (probablement responsables de nombreuses réponses physiologiques et pathologiques), une attention particulière sera portée aux PGs et à leurs rôles dans l'interaction entre les systèmes immunitaires et neuroendocriniens.

1.1.3.1 Synthèse des prostanoïdes

L'AA est un lipide très abondant incorporé principalement dans la bi-couche phospholipidique constituant la membrane cellulaire (Rosenbaum *et al.*, 1989). C'est sous l'influence d'une grande variété de substances, incluant les molécules inflammatoires et les agents mitogènes, que les écosanoïdes sont synthétisées à partir d'un précurseur commun, l'AA, afin de médier diverses réponses cellulaires, neuronales et physiologiques. Les leukotriène, les lipoxines ainsi que les acides gras hydroxylés sont produits sous l'influence de l'enzyme lipooxygénase. Tel qu'illustré à la figure 3, la formation des PGs et des thromboxanes est initiée par l'action de l'enzyme cyclooxygénase (COX)/prostaglandine endoperoxide G/H synthase, laquelle catalyse deux réactions différentes: l'oxygénation de l'AA en une PG de type G2 (PGG_2) fortement instable et la réduction de cette même PGG_2 en une PG de type H2 beaucoup plus stable. Précisons que la grande majorité des drogues

anti-inflammatoires non-stéroïdiennes (DAINS; ex.: indométhacine, ibuprofène, méclofénamate) agissent en se liant à la place de l'AA, empêchant ainsi la production des PGs et thromboxanes. Quant à l'aspirine, celle-ci inhibe la formation des prostanoïdes en acétylant de façon irréversible les sites actifs de l'enzyme COX. Quoiqu'il en soit, le mécanisme d'action de ces anti-inflammatoires consiste à stopper la production exagérée de ces hormone pouvant causer l'inflammation, des réactions d'hypersensibilité, l'asthme ou même le cancer.

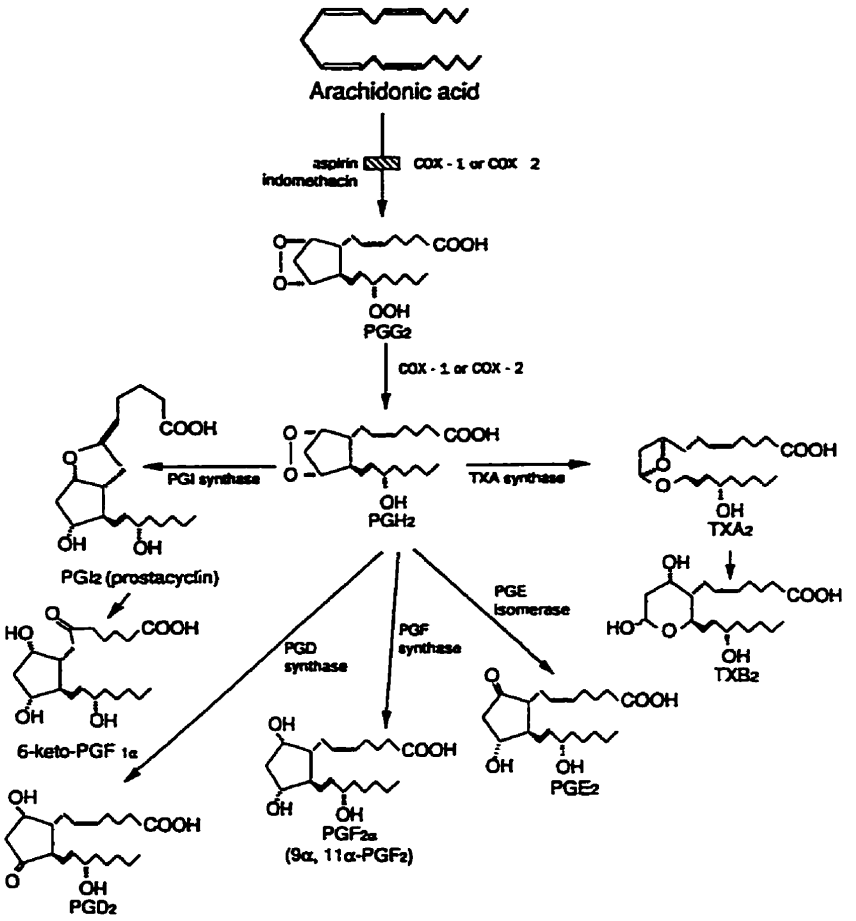


Figure 3. Conversion de l'acide arachidonique via les voies de la cyclooxygénase (figure tirée de: J. R. Vane, Y. S. Bakhle, and R. M. Botting. 1998. Cyclooxygenases 1 and 2. *Annu. Rev. Pharmacol. Toxicol.* 38: 97-120).

Jusqu'à ce jour, deux isoformes différentes de l'enzyme COX ont été clonées, COX-1 et -2 (DeWitt and Smith, 1988; Feng *et al.*, 1993; Kujubu *et al.*, 1991; Merlie *et al.*, 1988; Xie *et al.*, 1991). Bien que plusieurs types de cellules expriment de façon constitutive l'enzyme COX-1, DeWitt et son groupe ont observé que les niveaux d'ARNm et de protéines demeurent inchangés au cours durant la réponse inflammatoire (DeWitt *et al.*, 1990; DeWitt

and Smith, 1988; Goppelt-Struebe, 1995; Robertson, 1995). Ces résultats suggèrent donc que COX-1 puisse jouer un rôle prédominant dans le maintien de l'homéostasie de certains tissus et organes (ex.: reins, estomac, intestins). Cette hypothèse a d'ailleurs été confirmée par les nombreuses observations démontrant que l'inhibiton de la formation des PGs synthétisées par COX-1 pouvait occasionner des dommages gastriques, des ulcères et des hémorragies. En ce qui concerne l'enzyme COX-2, elle semble être indétectable dans la plupart des tissus dans des conditions basales. Par contre, il a été rapporté que l'activité transcriptionnelle du gène COX-2 est fortement induite dans les macrophages et autres types cellulaires traités avec des cytokines pro-inflammatoires ou avec une endotoxine bactérienne, suggérant du même coup une implication de COX-2 dans la pathogénèse inflammatoire/infectieuse (Arias-Negrete *et al.*, 1995; Feng *et al.*, 1993; Goppelt-Struebe, 1995; Hempel *et al.*, 1994; Jones *et al.*, 1993; Lee *et al.*, 1992). Des études réalisées au même moment ont montré que le blocage des voies de la cyclooxygénase peut atténuer les effets d'un stress immunitaire ou des cytokines pro-inflammatoires sur l'activité cellulaire, la transcription des gènes encodant les neuropeptides et plusieurs autres fonctions physiologiques sous le contrôle de populations cellulaires spécifiques du SNC (Rivest and Rivier, 1995; Rivier and Rivest, 1993). Ainsi, ces résultats suggèrent que les PGs produites sous l'action de l'enzyme COX-2 pourraient être responsables de la modulation des fonctions neuronales et endocriniennes au cours de la réponse inflammatoire/immunitaire.

Dans le but précis d'identifier le rôle joué par les PGs dans l'interaction neuroimmunitaire, plusieurs groupes ont utilisé des modèles capables de mimer la réponse immunitaire afin d'étudier la distribution et la régulation de l'enzyme COX-2 dans le SNC. Il est intéressant de constater qu'une augmentation de la production de COX-2 a été observée dans le cerveau, et plus particulièrement la microvasculature cérébrale, de souris et de rats soumis à un stress immunitaire (Breder and Saper, 1996; Cao *et al.*, 1995). En utilisant une technique de double marquage immunohistochimique, Saper et son groupe ont par la suite identifié les cellules non-parenchymales immunoréactives (ir) pour COX-2; c'est-à-dire des cellules microgliales associées aux vaisseaux sanguins et des macrophages dans les méninges et les plexus choroïdiens (Elmquist *et al.*, 1997). Toutefois, ces auteurs ont également observé la présence de cellules COX-2-positives non-colocalisées autour des vaisseaux sanguins, confirmant du même coup que d'autres types cellulaires tels que les cellules endothéliales du cerveau pourraient avoir aussi la capacité de synthétiser des PGs. Des travaux ont prouvé que les cellules endothéliales de la microvasculature cérébrale ont la capacité d'exprimer les récepteurs des différentes cytokines pro-inflammatoires (Bebo and Linthicum, 1995; Cunningham *et al.*, 1992; Ericsson *et al.*, 1995; Vallières and Rivest,

1997; Van Dam *et al.*, 1996). *In vitro*, l'induction de la transcription du gène COX-2, la traduction de son ARNm, ainsi que la biosynthèse des PGs, furent rapportées dans des cellules endothéliales après un traitement avec les cytokines pro-inflammatoires (Jones *et al.*, 1993; Maier *et al.*, 1990) ou avec une endotoxine bactérienne (Renzi and Flynn, 1992). Au meilleur de notre connaissance, Van Dam et ses collaborateurs ont été les premiers à déceler *in vivo* la production de PGs par les cellules endothéliales du cerveau suite à un challenge immunitaire (Van Dam *et al.*, 1993). Toutefois, la question demeure entière quant à savoir si l'expression du gène COX-2 dans le cerveau est un mécanisme général déclenché par la réponse inflammatoire d'origine systémique ou l'endotoxine elle-même. De plus, il est important de vérifier si les cytokines d'origine périphérique sont bel et bien responsables de la production centrale de l'enzyme limitante de la synthèse des PGs au cours de la réponse immunitaire/inflammatoire, et de déterminer quelles sont les cellules impliquées à l'interface des systèmes immunitaire et neuroendocrinien. Ces aspects constitueront d'ailleurs l'essentiel du chapitre 6.

1.1.3.2 Prostaglandines

Les PGs forment une famille complexe de substances aux multiples propriétés biologiques qui varient considérablement d'un type de molécule à un autre. Elles jouent un rôle prépondérant dans l'inflammation et la douleur, mais elles contribuent également, et ce de façon importante, au maintien de plusieurs fonctions physiologiques de l'organisme, notamment au niveau de la muqueuse gastro-intestinale et des reins. Quelques uns des effets biologiques des PGs en périphérie sont d'ailleurs décrits au tableau 1. Même si les diverses actions des PGs sur le SNC sont encore mal comprises, nous savons que les PGs régulent la biosynthèse et la sécrétion de nombreux neuropeptides en réponse aux divers médiateurs inflammatoires. L'inhibition de la production des PGs prévient les effets de l'IL-1 sur différentes fonctions neuroendocriniennes telles que la sécrétion de l'hormone de libération des hormones gonadotropes (GnRH ou LHRH) et LH (Ojeda *et al.*, 1975; Rivest and Rivier, 1993), le relâchement des facteurs hypothalamiques AVP et OT (Yasin *et al.*, 1994). De plus, nous savons également que les PGs jouent un rôle très important dans la régulation centrale de l'osmolarité (Yamaguchi *et al.*, 1997), de la pression artérielle et du rythme cardiaque (Morimoto *et al.*, 1992) à la suite d'une situation de stress. Cependant, la fonction centrale des PGs la plus étudiée jusqu'à présent consiste en la régulation de la température corporelle. Au cours de la réponse immunitaire/inflammatoire, il est en effet bien connu que certains agents pyrogènes, comme les cytokines pro-inflammatoires, communiquent avec le

cerveau afin d'induire une réponse fébrile (Kluger, 1991). Plusieurs études ont confirmé par la suite que la région antéro-ventrale du troisième ventricule est un centre important de contrôle de la fièvre et que les PGs synthétisées localement jouent un rôle crucial dans le divers mécanismes de thermorégulation (Blatteis *et al.*, 1983; Cooper, 1987; Morimoto *et al.*, 1988; Scammell *et al.*, 1996; Stitt, 1985; Stitt, 1986; Stitt, 1991). De plus, l'administration d'indométhacine, un inhibiteur de la synthèse des PGs, directement dans la MPOA atténue considérablement l'élévation de la température corporelle causée par les cytokines circulantes (Stitt, 1986).

Tableau 1

Tableau synthèse des effets biologiques des prostaglandines en périphérie (tableau tiré de: J-P. Falgueyret et J. Y. Gauthier. 1997. Bientôt de nouveaux anti-inflammatoires. Dans *Interface, la revue de la recherche*. Vol. 18, no. 5, p. 36).

Système reproducteur Effets sur le cycle ovulatoire $PGF_{2\alpha}$: réduit le débit sanguin lutéal, inhibe la synthèse de progestérone Effets sur l'utérus PGE_2 / $PGF_{2\alpha}$: rôle dans l'accouchement normal pour déclencher le travail Effets sur le fœtus PGE_2: maintien de la perméabilité du canal artériel	Systèmes cardiovasculaire et rénal Débit sanguin et excrétion du sodium et de l'eau PGA_2 / PGD_2 / PGE_2 / PGG_2 / PGH_2 / PGI_2: augmentent le débit sanguin rénal et le taux de sodium dans l'urine (natriurèse) Libération de la rénine et régulation de la pression artérielle Acide arachidonique / PGA_2 / PGI_2 / PGE_2: stimulent la production de rénine PGA_2: effet antihypertenseur Appareil digestif Sécrétion gastrique et absorption intestinale PGE_2: inhibe la sécrétion gastrique d'HCl stimulée par l'histamine Cytoprotection Les prostaglandines en général protègent la muqueuse intestinale	Appareil respiratoire PGE_2: relâche le muscle lisse des bronchioles et augmente le débit sanguin PGD_2 / $PGF_{2\alpha}$: contractent le muscle lisse des bronchioles Tissu sanguin PGE_2: stimule l'agrégation plaquettaire PGI_2: inhibe l'agrégation plaquettaire et a un effet vasodilatateur TXA_2: provoque l'agrégation plaquettaire et a un effet vasoconstricteur

Au cours des dernières années, les travaux portant sur la régulation de l'axe corticotrope lors d'une activation immunitaire ont permis la mise en évidence d'interactions beaucoup plus complexes qu'on ne l'aurait cru à l'origine. Bien qu'il ait été établi que les cytokines puissent communiquer avec le SNC afin d'induire la synthèse d'immunosuppresseurs endogènes, le rôle d'intermédiaire joué par les métabolites de l'AA dans la médiation des effets des cytokines sur l'axe HPA n'a été étudié que tout récemment. Les premières recherches en ce sens ont démontré que le blocage des voies de la cyclooxygénase inhibe la sécrétion de CRF provenant de fragments hypothalamiques

(Bernardini *et al.*, 1990; Cambronero *et al.*, 1992; Lyson and McCann, 1992; Navarra *et al.*, 1991) ou d'EM isolées (McCoy *et al.*, 1994) suite à un traitement avec l'IL-1, l'IL-6 ou le TNF-α. Presqu'au même moment, des études *in vivo* ont montré que l'inhibition de la synthèse des PGs prévient la libération du CRF dans le système infundibulaire en plus de freiner la sécrétion d'ACTH en réponse aux cytokines pro-inflammatoires (Katsuura *et al.*, 1988; Morimoto *et al.*, 1989; Murakami and Watanabe, 1989; Rivier and Rivest, 1993; Sharp and Matta, 1993; Watanabe *et al.*, 1990). Dernièrement, d'autres groupes ont confirmé la participation des dérivés de l'AA dans l'activation de l'axe HPA survenant après un stress immunitaire ou une inflammation locale stérile (Smith *et al.*, 1994; Turnbull and Rivier, 1996). Le fait que l'injection de drogues anti-inflammatoires non-stéroïdiennes dans les ventricules latéraux ou certaines structures hypothalamiques spécifiques du cerveau supprime la sécrétion du CRF et celle de l'ACTH, confirme l'importance cruciale des PGs d'origine centrale (Katsuura *et al.*, 1990; Katsuura *et al.*, 1988; Thompson and Hedge, 1978). Toutefois, personne n'a pu démontrer si le blocage des voies de la cyclooxygénase prévient l'activation de l'axe corticotrope en supprimant la biosynthèse du CRF et de son récepteur de type 1. Il faut garder en mémoire que ce récepteur semble être impliqué dans les mécanismes responsables du maintien de la transcription du neuropeptide. De plus, le(s) sous-type(s) exact(s) de PGs et le(s) site(s) d'action sollicité(s) dans le SNC afin de médier tous ces effets demeurent à préciser.

Très peu de travaux ont étudié ou comparé les effets des divers types de PGs sur l'activité de l'axe corticotrope et la régulation des fonctions neuronales. Étant donné les nombreuses similarités existant entre les divers types de PGs, il serait de nature très naïf de croire qu'un seul type de PG puisse jouer un rôle déterminant dans la régulation de l'axe HPA. Chacune des PGs est désignée par une lettre de A à F et d'un chiffre de 1 à 3 indiquant la nature et la position de certains groupement chimiques qui leurs sont propres. Les PGs de type E1, E2, F2α et D2 sont sans aucun doute les prostanoïdes ayant reçu le plus d'attention, et par conséquent, les mieux documentées. L'infusion centrale des PGE_1, PGE_2 ou $PGF_{2\alpha}$ stimule la sécrétion d'ACTH (Anthonisen *et al.*, 1997). De plus, il a été démontré qu'un pré-traitement central avec des anticorps dirigés contre les PGE_1, les PGE_2 ou les $PGF_{2\alpha}$ atténue de façon significative l'augmentation des niveaux plasmatiques d'ACTH en réponse à l'injection i.c.v. d'IL-1ß (Watanobe *et al.*, 1995). Ces mêmes auteurs ont également observé que les antisérums spécifiques aux PGE_1 et $PGF_{2\alpha}$ ont réduit davantage les taux élevés d'ACTH observés suite à l'administration centrale d'IL-1ß que l'anticorps des PGE_2. Dans le même ordre d'idée, certains investigateurs avaient rapporté, quelques années auparavant, que les $PGF_{2\alpha}$ pourraient jouer un rôle plus important que les

PGE_2 dans les mécanismes menant à la sécrétion hypothalamique du CRF en réponse à l'IL-1 (Bernardini, 1989; Cambronero *et al.*, 1992). Il y a peu de temps, Nasushita et son groupe ont confirmé l'implication des PGE_1, PGE_2 et $PGF_{2\alpha}$ dans l'augmentation des taux d'ACTH circulante. Bien que l'administration i.v. des PGD_2 s'est avérée sans effet sur la sécrétion d'ACTH, les PGE_1, PGE_2 et $PGF_{2\alpha}$ ont tous provoqué de façon équipotente le relargage de celle-ci dans la circulation sanguine (Nasushita *et al.*, 1997; Watanabe *et al.*, 1991). Des études réalisées par Katsuura et ses collaborateurs en 1990 avaient d'ailleurs laisser planer un premier doute sérieux sur la capacité des PGD_2, contrairement aux PGs de type E1 et 2, à stimuler l'axe HPA. En effet, ces chercheurs ont montré que l'injection des PGE_1 et PGE_2, mais pas des PGD_2, directement dans la région préoptique de l'hypothalamus, entraîne une forte augmentation des concentrations plasmatiques d'ACTH chez le rat (Katsuura *et al.*, 1990). De par ces résultats, il semble donc plus qu'évident que les cytokines inflammatoires stimulent la biosynthèse de plusieurs types d'écosanoïdes qui, à leur tour, pourront agir en tant qu'intermédiaires pour informer le SNC des changements neuroendocriniens à apporter afin d'empêcher une stimulation exagérée de la réaction immune qui pourrait s'avérer néfaste pour l'organisme.

Les effets engendrés au niveau de l'axe HPA, ainsi que les mécanismes et les sites d'action sollicités par ces neuromédiateurs originant de l'AA peuvent différer d'un type de PG à un autre. À titre d'exemple, mentionnons des travaux démontrant l'influence inhibitrice sur la sécrétion d'ACTH, au niveau de l'hypophyse, des PGE_1 et des PGE_2, mais pas des PGD_2 et des $PGF_{2\alpha}$ qui ont été synthétisées localement en réponse au CRF ou à la AVP (Hedge, 1976; Vale *et al.*, 1978; Vlaskovska *et al.*, 1984; Vlaskovska and Knepel, 1984). Par contre, l'infusion des PGE_2 ou des $PGF_{2\alpha}$ dans l'EM favorise la sécrétion d'ACTH (McCoy *et al.*, 1994). De plus, d'autres groupes ont rapporté que les PGD_2, PGE_2 et $PGF_{2\alpha}$ possèdent la capacité de réguler positivement l'axe HPA par l'intermédiaire des facteurs hypothalamiques AVP et OT (Brooks *et al.*, 1986; Hoffman *et al.*, 1982; Inoue *et al.*, 1990; Ishikawa *et al.*, 1981; Poulain and Carette, 1974). Fait intéressant, des sites de liaisons spécifiques aux PGD_2 et $PGF_{2\alpha}$ ont été trouvés dans plusieurs régions du cerveau incluant le système hypothalamo-neurohypophysaire (Shimuzu *et al.*, 1982; Watanabe *et al.*, 1989); les récepteurs EP1 (Batshake *et al.*, 1995) et EP4 (J. Zheng et S. Rivest, papier en préparation) des PGE_2 sont quant à eux très fortement exprimés sur les neurones magnocellulaires des noyaux PVH et SON. En plus d'identifier le(s) type(s) de PGs impliqué(s) dans l'activation de l'axe corticotrope, il est donc très important de vérifier à quel niveau cette action stimulatrice s'effectuera.

1.1.3.3 Prostaglandines de type E2 (PGE_2)

Plusieurs évidences semblent indiquer que les PGE_2 pourraient jouer un rôle clé dans plusieurs des changements observés au cours d'un challenge immunitaire ou d'un traitement avec les cytokines pro-inflammatoires. Les études *in vitro* ont révélé que l'IL-1 stimule la production des PGE_2 à partir de fragments hypothalamiques (Navarra *et al.*, 1992). *In vivo*, l'injection i.v. d'IL-1ß entraîne la production des PGE_2, du CRF et AVP selon un patron temporel très similaire dans les noyaux PVH (Watanobe and Takebe, 1994). Cependant, aucune étude n'a encore vérifié si les neurones impliqués dans le contrôle de l'axe HPA sont activés, et si l'activité transcriptionnelle du CRF est stimulée suite à un traitement avec les PGE_2. Toutefois, puisque l'infusion d'indométhacine directement dans les noyaux PVH ne prévient pas l'activation de l'axe HPA en réponse à l'administration systémique d'IL-1 (Komaki *et al.*, 1992), plusieurs autres structures du SNC pourraient alors participer à la régulation de cette fonction neuroendocrienne essentielle à la survie. Il est donc très intéressant de constater que l'administration i.v. d'IL-1 ou d'une endotoxine bactérienne stimule aussi la sécrétion des PGE_2 dans le liquide céphalo-rachidien et différentes régions hypothalamiques telle que la région médiane préoptique (MPOA)/OVLT (Coceani *et al.*, 1988; Dinarello *et al.*, 1991; Komaki *et al.*, 1992; Sirko *et al.*, 1989). De plus, l'injection des PGE_2 dans l'un des ventricules latéraux (Rassnick *et al.*, 1995) ou directement dans la région préoptique (Katsuura *et al.*, 1990; Morimoto *et al.*, 1989; Murakami and Watanabe, 1989; Watanabe *et al.*, 1990) du rat provoque une augmentation des niveaux plasmatiques d'ACTH et de corticostérone, un effet vraisemblablement médié par les neurones CRFergiques neuroendocriniens (Watanabe *et al.*, 1990). L'importance de la MPOA dans la régulation de l'axe corticotrope a d'ailleurs été confirmée presqu'au même moment suite à une étude rapportant que l'infusion d'un antagoniste des PGs de type E dans la MPOA supprime complètement l'augmentation des niveaux d'ACTH dans le sang à la suite d'une injection d'IL-1ß (Katsuura *et al.*, 1990). La détection des PGE_2 dans le liquide céphalo-rachidien et certains noyaux du cerveau au cours de la réponse immunitaire, et l'existence de nombreux sites de liaisons des PGs à travers l'ensemble du SNC supportent l'hypothèse d'une circuiterie complexe.

1.1.3.4 Récepteurs des PGE_2

Grâce à la technique d'autoradiographie quantitative, Matsumura et son équipe furent les premiers à établir une ébauche complète de la distribution des sites de liaisons des PGE_2

dans le cerveau de rat. Ils ont rapporté une très forte densité de sites de liaison des PGE_2 dans le mur antérieur du troisième ventricule en plus de déceler des niveaux plus modérés dans plusieurs structures du SNC incluant certaines régions de l'hypothalamus (Matsumura *et al.*, 1992; Matsumura *et al.*, 1990). Plus récemment, les différents sous-types des récepteurs des PGE_2 ont été clonés, puis identifiés comme suit: EP1 (Watabe *et al.*, 1993), EP2 (Honda *et al.*, 1993; Sando *et al.*, 1994), EP3α (Sugimoto *et al.*, 1992; Takeuchi *et al.*, 1993), EP3ß (Neuschäfer-Rube *et al.*, 1994; Sugimoto *et al.*, 1993; Takeuchi *et al.*, 1994), EP3γ (Irie *et al.*, 1993) et EP4 (Nishigaki *et al.*, 1995; Regan *et al.*, 1994). Ainsi, l'élaboration d'une cartographie plus détaillée de la répartition de ces récepteurs à travers l'ensemble du cerveau a pu être entreprise. Cette distribution permettra de mieux cerner l'importance relative de chacun des sous-types des récepteurs de la série E au cours de diverses situations, telle que lors de l'activation de l'axe HPA durant un stress immunitaire.

Peu de temps suite au clonage du premier sous-type de récepteur des PGE_2, Batshake et ses collaborateurs ont rapporté la présence du récepteur EP1 dans les noyaux PVH et SON spécifiquement (Batshake *et al.*, 1995). Bien qu'au même moment, des études pharmacologiques aient attribué aux récepteurs EP1 une fonction thermo-régulatrice impliquée dans le développement de la fièvre (Oka and Hori, 1994), la distribution de ce récepteur à l'intérieur du SNC laisse également croire à la possibilité que les signaux de transductions induits à la suite de la liaison des PGE_2 aux récepteurs EP1 pourraient influencer la sécrétion de divers facteurs hypothalamiques.

Des travaux présentement en cours dans les laboratoires du Dr. Rivest ont permis de localiser le récepteur EP2 dans les OCVs et plusieurs régions du SNC reconnues pour leurs connections directes ou indirectes avec les structures neuroendocriniennes. Toutefois, étant donné le clonage récent des récepteurs des prostanoïdes, la littérature ne nous permet pas à l'heure actuelle, de bien cerner la contribution du récepteur EP2 dans la modulation de fonctions neuroendocriniennes au cours de la réponse immunitaire/inflammatoire. Quant au récepteur EP3, deux groupes distincts ont établi sa répartition dans le cerveau. Grâce à l'utilisation d'une sonde radioactive correspondant à une région codante commune aux trois isoformes du récepteur EP3, Sugimoto et ses collègues ont détecté la présence de l'ARNm de EP3 dans plusieurs régions du cerveau de souris incluant la MPOA (Sugimoto *et al.*, 1994). Cependant, contrairement aux travaux d'Ericsson (Ericsson *et al.*, 1995) effectués chez le rat, les travaux réalisés chez la souris ne font aucune allusion à l'expression du récepteur EP3 dans les OCVs. Une chose est néanmoins certaine, aucune de ces structures

neuroendocriniennes ne semble être en mesure de synthétiser le récepteur EP3. De plus, ces deux études n'ont pas rapporté la présence de signal dans les cellules gliales.

De par sa distribution et sa régulation dans le SNC, le récepteur EP4 est sans aucun doute l'un des candidats les plus attrayants afin d'expliquer comment les PGs de type E2 peuvent cibler les neurones responsables de la modulation de l'axe HPA. En effet, bien que le récepteur EP4 ait lui aussi été localisé dans de nombreuses régions du cerveau du rat, l'hybridation *in situ* a permis de déceler la présence de très hauts niveaux d'ARNm de ce récepteur dans la division magnocellulaire des noyaux PVH et les noyaux SON (J. Zhang et S. Rivest, papier en préparation). Quoique très peu de cellules positives pour le récepteur EP4 ont été trouvées dans la division parvocellulaire des noyaux PVH lors de conditions normales, l'induction d'un stress immunitaire a fortement stimulé l'activité transcriptionnelle du récepteur des PGE_2 dans cette subdivision hypothalamique. De plus, les analyses quantitatives de double marquage ont révélé que 60 à 70% des neurones CRFergiques des noyaux PVH expriment le récepteur EP4 lors d'un challenge immunitaire. Ainsi, il est possible de croire que les PGE_2 agissent localement dans les structures neuroendocriniennes, possiblement via les récepteurs EP1 et EP4, dans le but d'activer les facteurs hypothalamiques responsables de la sécrétion ultime des agents immunosuppresseurs.

Compte tenu de la distribution très étendue des différents sous-types des récepteurs de la série E, une circuiterie fonctionnelle beaucoup plus complexe est à envisager dans l'établissement des interactions neuro-immunes. Lors de la réponse immunitaire/inflammatoire, il est donc probable que les cytokines pro-inflammatoires rejoignent leurs récepteurs situés dans les régions localisées à l'extérieur des limites de la barrière hémato-encéphalique, afin d'induire la production de divers neuromédiateurs centraux comme les PGE_2. Par la suite, on peut concevoir que les PGE_2 diffusant dans le liquide extracellulaire peuvent atteindre, puis lier un de leurs récepteurs, afin d'activer certains neurones avoisinants qui, en retour, stimuleront les structures hypothalamiques responsables d'assurer le rétablissement de l'homéostasie. Ne serait-ce que pour compliquer les choses davantage, précisons seulement que les cellules gliales pourraient aussi jouer un rôle déterminant dans la plupart des mécanismes centraux solicités par les cytokines afin d'informer les neurones pouvant moduler l'activité de l'axe HPA. Jusqu'à présent, les résultats démontrant la synthèse des PGs par des cellules neuronales demeurent très controversés et ce, particulièrement au cours d'une situation de stress où il y a libération de médiateurs inflammatoires (Katsuura *et al.*, 1989; Van Dam *et al.*, 1993). Par conséquent, il

ne serait pas étonnant de voir que certains types de cellules gliales, tels les astrocytes, puissent influencer certaines fonctions neuronales. Les astrocytes ont toujours été reconnus comme des cellules de support essentielles à la survie, la croissance et l'activité des neurones. De plus, il a été rapporté que les astrocytes possèdent à leur surface membranaire les récepteurs d'une multitude de cytokines (Ban *et al.*, 1993), en plus d'avoir la capacité de produire des dérivés de l'AA (PGE_2) en réponse à l'IL-1 (Katsuura *et al.*, 1989), aux autres cytokines (Ma *et al.*, 1997) ou à une endotoxine bactérienne (Fontana *et al.*, 1982) et de synthétiser certaines substances capables d'induire l'expression des gènes encodant les récepteurs de la série E sur les neurones avoisinants (Rage *et al.*, 1997).

1.1.4 Sites d'action et circuiterie neuronale

Comme il a été discuté dans les sections précédentes, l'injection systémique des cytokines pro-inflammatoires a pour effet d'influencer plusieurs fonctions neuroendocriniennes, dont l'activité de l'axe HPA. Il a aussi été mentionné qu'un traitement i.v. avec l'IL-1ß, l'IL-6 ou le TNF-α stimule l'expression du gène de réponse précoce c-*fos* (marqueur de l'activité cellulaire) dans de nombreuses régions du cerveau. De plus, l'induction d'une réponse immunitaire systémique provoque la biosynthèse des cytokines pro-inflammatoires, des PGs et des récepteurs de tous ces médiateurs inflammatoires dans des structures spécifiques du SNC. Ainsi, nous avons toutes les raisons de croire qu'une circuiterie très complexe, sollicitant de multiples intervenants et sites d'action, serait impliquée dans la régulation des neurones hypothalamiques de l'axe corticotrope. Toutefois, pour que le système immunitaire puisse communiquer de façon adéquate avec le cerveau, et moduler les fonctions neuroendocriniennes, des sites d'accès pour les cytokines circulantes doivent exister à l'intérieur même du SNC. Dans cette optique, certaines structures comme les OCVs et la microvasculature cérébrale pourraient être des cibles de choix. Quant aux autres régions activées au cours de le réponse immunitaire, celles-ci pourraient plutôt jouer un rôle déterminant dans l'intégration des informations provenant de la périphérie.

Étant donné le faible taux de pinocytose des cellules endothéliales et l'existence de jonctions étanches reliant chacunes de celles-ci autour des vaisseaux sanguins, le passage de diverses substances à l'intérieur du cerveau est très limité. Toutefois, les OCVs sont des structures qui, en plus d'être situées à l'interface entre la périphérie et le cerveau, sont dépourvues de barrière hémato-encéphalique (Oldfield and McKinley, 1995). Il est donc possible que les cytokines circulantes produites par les cellules immunitaires activées

pénètrent les OCVs sensoriels (OVLT, SFO, EM et AP) et activent certaines cellules possédant les récepteurs cytokinergiques appropriés. Fait intéressant, l'administration i.v. des cytokines pro-inflammatoires induit l'expression du gène c-*fos* dans les OCVs (Brady *et al.*, 1994; Ericsson *et al.*, 1994; Rivest and Rivier, 1994; Rivest *et al.*, 1992; Vallières *et al.*, 1997). De plus, les récepteurs des interleukines ont été détectés dans les OCVs dans des conditions basales ou à la suite d'un stress immunitaire (Cunningham *et al.*, 1992; Ericsson *et al.*, 1995; Vallières and Rivest, 1997). Bien entendu, plusieurs groupes ont suggéré que la stimulation de la sécrétion du CRF dans le système infundibulaire, par les cytokines systémiques, pourrait s'effectuer directement au niveau des fibres terminales localisées dans l'EM. Néanmoins, de nombreuses évidences laissent croire que les autres OCVs pourraient eux-aussi avoir un rôle très important à jouer. En effet, des études neuroanatomiques ont rapporté que l'OVLT et le SFO ont des projections efférentes vers les noyaux PVH et SON (voir figure 4) (Johnson and Gross, 1993; Sawchenko and Swanson, 1983; Swanson and Sawchenko, 1983). Grâce à la combinaison de l'immunohistochimie contre la protéine Fos et du transport rétrograde d'un traceur neuroanatomique (toxine du choléra), Elmquist et Saper ont rapporté que certaines régions activées lors d'un challenge immunitaire projettent directement aux noyaux PVH (Elmquist and Saper, 1996). Parmi celles-ci, notons la présence de neurones localisés dans l'OVLT et le SFO. Bien qu'aucune cellule doublement marquée n'ait été observée dans l'AP, des travaux ont démontré l'importance capitale de cette structure dans la régulation de l'hypothalamus neuroendocrinien par l'IL-1ß. En effet, Herkenham et son groupe ont montré que la destruction de l'AP prévient l'activation de l'axe HPA, ainsi que la transcription de c-*fos* dans le NTS et les noyaux PVH spécifiquement, en réponse à l'injection i.v. d'IL-1ß (Lee and Herkenham, 1996). Puisque les études neuroanatomiques n'ont pas rapporté de projections originant de l'AP vers les noyaux PVH, il est fort probable que les influences de l'AP sur l'activité de l'axe corticotrope soient indirectes. Tel qu'illustré à la figure 4, il est donc envisageable que le NTS agisse à titre de centre de relais entre l'AP et les noyaux PVH au cours de la réponse immunitaire; le NTS est un noyau adjacent à l'AP en plus d'être fortement innervé par cet OCV (Cunningham *et al.*, 1994). En réponse à un traitement avec l'IL-1ß ou avec une endotoxine bactérienne, on retrouve dans le NTS plusieurs neurones catécholaminergiques immunoréactifs à la protéine Fos ayant des projections efférentes vers les noyaux PVH (Elmquist and Saper, 1996; Ericsson *et al.*, 1994). Ainsi, l'AP pourrait être une structure cible des molécules immunitaires circulantes et informer l'hypothalamus endocrinien par les neurones catécholaminergiques originant du NTS.

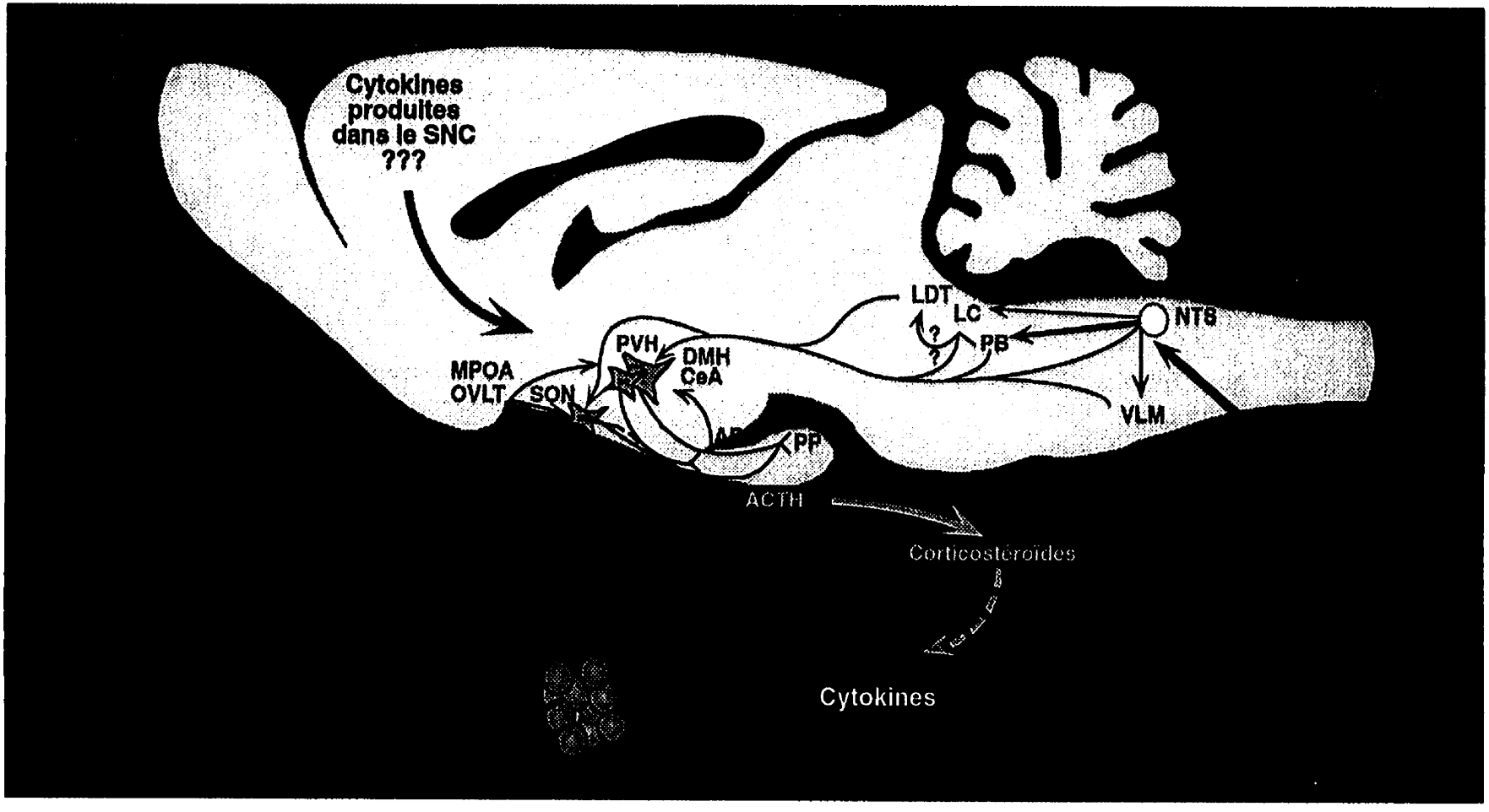


Figure 4. Sites d'action et voies neuronales empruntés à la suite d'un traitement systémique avec l'endotoxine bactérienne LPS. Abréviations: ACTH, hormone adrénocorticotrope; AP, area postrema; ARC, noyau arqué; CeA, noyau central de l'amygdale; DMH, noyau dorsomédial de l'hypothalamus; EM, éminence médiane; LC, locus coeruleus; LDT, noyau latérodorsal tegmental; LPS, lipopolysaccharide; MPOA, aire préoptique médiale; NTS, nucleus tractus solitarius; OVLT, organum vasculosum de la lamina terminalis; PB, noyau parabrachial; PP, hypophyse postérieure; PVH, noyau paraventriculaire de l'hypothalamus (divisions parvocellulaire [pc] et magnocellulaires [mc]); SON, noyau supraoptique; VLM, région ventro-latérale médullaire.

Il semble que les OCVs, en plus de posséder la capacité de répondre aux cytokines d'origine systémique, synthétisent eux-mêmes de l'IL-1, de l'IL-6 et du TNF-α au cours d'une endotoxinémie systémique (Nadeau and Rivest, 1998; Quan *et al.*, 1998; Vallières and Rivest, 1997). Bien que les cytokines pro-inflammatoires produites localement peuvent stimuler des cellules avoisinantes à même les OCVs, plusieurs travaux sont en accord avec le fait que ces cytokines ne peuvent pénétrer à l'intérieur du SNC par diffusion passive entre les cellules épendymales séparant les OCVs du reste du cerveau. Cependant, il est possible que les cytokines localisées dans les OCVs stimulent la production de médiateurs capables de diffuser plus facilement à travers les cellules épendymales. Komaki et ses collaborateurs ont d'ailleurs rapporté que l'injection i.v. d'IL-1ß a pour effet d'augmenter significativement les niveaux de PGE_2 dans l'OVLT (Komaki *et al.*, 1992). Sachant que les PGs sont de petites molécules lipophiles, elles pourraient facilement atteindre les structures avoisinantes des OCVs (Dascombe and Milton, 1979). Dans cette perspective, la MPOA et le NTS sont deux structures particulièrement intéressantes. En plus d'être à proximité de l'OVLT et de l'AP respectivement, la MPOA et le NTS sont les régions possédant les niveaux les plus élevés en récepteurs des PGE_2 (Matsumura *et al.*, 1992; Matsumura *et al.*, 1990). Plusieurs évidences anatomiques ont suggéré l'existence de voies de projections entre la MPOA et les noyaux PVH (voir figure 4) (Conrad and Pfaff, 1975; Sawchenko and Swanson, 1983; Silverman *et al.*, 1981). De plus, la stimulation électrique des neurones localisés dans la MPOA entraîne une augmentation de l'activité électrique dans les noyaux PVH et l'élévation des concentrations plasmatiques en ACTH et corticostérone (Saphier and Feldman, 1986). Finalement, le fait qu'une microinjection d'indométhacine ou d'un antagoniste des PGs de type E dans la MPOA supprime complètement la relâche d'ACTH en réponse à l'injection d'IL-1ß (Katsuura *et al.*, 1990), confirme l'hypothèse que les PGs puissent médier certains des effets activateurs des cytokines sur l'axe corticotrope. Toutefois, il n'existe aucune étude, à notre connaissance, ayant démontré l'existence d'un tel mécanisme dans d'autres structures du SNC. Bien que le NTS ou d'autres structures activées au cours de la réponse immune pourraient constituer des cibles de choix pour les PGs sécrétées sous l'influence des cytokines, des travaux devront être entrepris en ce sens afin d'éclaircir ces questions importantes. À titre d'exemple de structures potentielles, mentionnons la région ventro-latérale médullaire (VLM). Outre la présence des récepteurs EP3 (Ericsson *et al.*, 1995) et EP4 (J. Zhang et S. Rivest, papier en préparation) des PGE_2 dans VLM, des travaux ont rapporté que cette région exprime le gène de réponse précoce c-*fos* durant l'endotoxinémie (Laflamme and Rivest, 1994; Rivest and Laflamme, 1995) et participe à la stimulation des niveaux d'ARNm du CRF en réponse à l'administration i.v. d'IL-1ß (Ericsson *et al.*, 1994).

La microvasculature cérébrale constitue la deuxième porte d'entrée potentielle par laquelle les cytokines circulantes pourraient communiquer avec le cerveau. Jusqu'à présent, plusieurs groupes ont tenté de prouver que les cytokines relarguées en périphérie ont la capacité d'altérer la perméabilité de la barrière hémato-encéphalique, ce qui faciliterait par la même occasion le passage de médiateurs inflammatoires dans le SNC. Cependant, la plupart de ces études n'ont pas été reproduites ou tout simplement contredites par des travaux subséquents (Banks and Kasin, 1992). Une hypothèse beaucoup plus plausible est néanmoins illustrée aux figures 7 et 8 (voir discussion). Celles-ci suggèrent la liaison des cytokines pro-inflammatoires à leurs récepteurs respectifs, qui seraient localisés à la surface membranaire des cellules endothéliales, afin de stimuler la biosynthèse de neuromédiateurs centraux comme les PGs. Bien entendu, plusieurs évidences viennent appuyer cette hypothèse: 1) la présence des récepteurs de l'IL-1, de l'IL-6 et du TNF-α sur les cellules endothéliales de la microvasculature cérébrale dans des conditions basales ou après un stress inflammatoire (Bebo and Linthicum, 1995; Cunningham *et al.*, 1992; Ericsson *et al.*, 1995; Lucas *et al.*, 1997; Vallières and Rivest, 1997; Van Dam *et al.*, 1996); 2) la stimulation de la transcription du gène encodant l'enzyme limitante de la synthèse des PGs (COX-2) dans les vaisseaux sanguins du cerveau suite à l'administration d'IL-1ß ou d'une endotoxine bactérienne (Breder and Saper, 1996; Cao *et al.*, 1995; Cao *et al.*, 1996); 3) la production de PGs par les cellules endothéliales du SNC en réponse à l'injection i.v. d'IL-1ß ou l'induction d'un challenge immunitaire (Van Dam *et al.*, 1993; Van Dam *et al.*, 1996). Ces résultats suggèrent donc que les cytokines pro-inflammatoires d'origine systémique ont la capacité de se lier aux cellules endothéliales de la microvasculature cérébrale afin d'entraîner la synthèse des PGs. Ainsi, les PGs nouvellement formées pourront diffuser librement à travers la barrière hémato-encéphalique et atteindre des sites d'action centraux qui leur sont propres. Bien que l'induction de l'activité transcriptionnelle du gène COX-2 et la biosynthèse des PGs sont des phénomènes qui semblent apparaître de façon non-sélective dans l'ensemble des vaisseaux sanguins du cerveau, il n'en demeure pas moins que les sites activés lors d'un traitement avec les cytokines pro-inflammatoires sont très spécifiques. Il est donc possible que la sélectivité de la réponse centrale soit une conséquence de distribution spécifique des récepteurs des PGs dans le cerveau. Toutefois, nous ne savons pas encore si l'information transférée de l'endothélium vers les éléments du parenchyme, au cours de la réponse immunitaire, renferme des renseignements essentiels à la régulation des diverses fonctions neuronales et neuroendocriniennes ou consiste tout simplement à l'élaboration de la réaction inflammatoire/immunitaire centrale.

La présence de l'ARNm encodant l'IL-1ß et de la protéine elle-même dans les cellules vasculaires et/ou périvasculaires associées suite à un challenge immunitaire (Quan *et al.*, 1998; Van Dam *et al.*, 1992), a compliqué davantage l'hypothèse voulant que les PGs soient les principaux intermédiaires impliqués dans la communication entre les cytokines périphériques et le cerveau. Récemment, différents groupes ont rapporté l'expression des gènes de l'IL-1ß et du TNF-α dans des structures parenchymales autres que les OCVs suite à l'induction d'un stress immunitaire (Nadeau and Rivest, 1998; Quan *et al.*, 1998). Dans les deux cas, les investigateurs ont observé que les cellules positives, vraisemblablement des cellules microgliales, étaient distribuées de façon ubiquitaire à travers l'ensemble du cerveau. Curieusement, ces auteurs ont noté que le signal semblait migrer à partir des structures en contact direct avec la périphérie vers les régions avoisinantes, pour finalement atteindre les cellules parenchymales les moins accessibles du SNC lors d'un challenge immunitaire plus sévère. À la lumière de ces résultats, il est donc possible que les PGs diffusant dans le liquide extracellulaire, ou tout autres médiateurs inflammatoires synthétisés par la microvasculature cérébrale (ex.: IL-1ß), puissent stimuler des cellules localisées à proximité afin d'induire la production et la sécrétion de cytokines centrales. Bien que ceci demeure hypothétique, nous croyons que ces cytokines produites centralement pourraient à leur tour induire la synthèse d'autres neuromédiateurs, et par la même occasion perpétuer la réponse inflammatoire centrale, ou tout simplement influencer directement certains noyaux contrôlant les diverses fonctions neurales sollicitées lors de la réponse immunitaire. Plusieurs études ont démontré jusqu'à présent que les cytokines d'origine centrale ont des effets importants sur les fonctions neuroendocriniennes. Néanmoins, comme le démontrent les travaux cités ci-dessus, les mécanismes, les sites d'action et les voies empruntées par celles-ci peuvent être tout à fait différentes de celles utilisées par les cytokines d'origine systémique.

Comme l'ont suggéré plusieurs études, les voies adrénergiques et noradrénergiques du tronc cérébral semblent elles aussi jouer un rôle important dans la communication entre les cytokines systémiques et les structures neuroendocriniennes (Chuluyan *et al.*, 1992; Ericsson *et al.*, 1994; Li *et al.*, 1996; Weidenfeld *et al.*, 1989). Parmi les régions les plus intéressantes, on retrouve le NTS et la région ventro-latérale médullaire (VLM), deux structures exprimant le gène de réponse précoce c-*fos* en réponse à l'injection i.v. d'IL-1ß (Brady *et al.*, 1994; Ericsson *et al.*, 1994; Rivest and Rivier, 1994; Rivest *et al.*, 1992), en plus de fournir aux noyaux PVH les voies catécholaminergiques (A_1-A_2/C_1-C_2) les plus importantes (Cunningham *et al.*, 1990; Cunningham and Sawchenko, 1988; Sawchenko and Swanson, 1982; Sawchenko and Swanson, 1983). Récemment, Ericsson et son groupe ont démontré qu'une section unilatérale des projections médullaires ascendantes a pour effet

d'atténuer significativement les niveaux d'expression de la protéine Fos et du transcrit encodant le CRF dans le noyau PVH situé du coté ipsilatéral à la lésion, en réponse à l'injection i.v. d'IL-1ß (Ericsson *et al.*, 1994). Ainsi, bien que ces résultats n'excluent pas la possibilité que d'autres types de voies ascendantes (ex.: les voies sérotoninergiques (Laflamme *et al.*, 1998)) pourraient contribuer à l'activation des neurones endocriniens au cours de la réponse immunitaire, les nombreux travaux énumérés ci-dessus semblent suggérer que les voies catécholaminergiques médullaires sont particulièrement importantes.

Malgré le fait que les cytokines produites en périphérie ne peuvent pénétrer la barrière hémato-encéphalique, deux hypothèses distinctes ont été émises afin d'expliquer comment celles-ci peuvent moduler l'activité de certaines structures du tronc cérébral. La première possibilité, bien qu'effleurée dans les paragraphes précédents, implique que les cytokines circulantes atteignent l'AP, qui a son tour pourrait transmettre l'information à de nombreuses autres régions du rhombencéphale incluant: le NTS, le VLM, les noyaux parabrachiaux (PB), le noyau tegmental latérodorsal (LDT) et locus coeruleus (LC); cinq structures activées suite à l'administration systémique d'IL-1ß (Brady *et al.*, 1994; Ericsson *et al.*, 1994; Vallières and Rivest, 1997). Bien que certains médiateurs centraux (tels que les PGs et les cytokines) aient sûrement la capacité de diffuser de l'AP vers le NTS, il est improbable que ces molécules puissent atteindre des régions relativement éloignées comme le VLM, les noyaux PB, le LDT et le LC, compte tenu du pouvoir de diffusion restreint des médiateurs inflammatoires dans le liquide interstitiel (Nicholson, 1980). De plus, les PGs et les cytokines sont reconnues comme des substances possédant une courte durée de vie (Reimers *et al.*, 1991). Toutefois, des études neuroanatomiques ont permis d'établir que ces régions de la médulla et du pons ont des connections qui peuvent être réciproques (voir figure 4). Ainsi, plusieurs groupes ont observé des projections très denses originant de l'AP vers le NTS et le PB (Cunningham *et al.*, 1994; Johnson and Gross, 1993; Shapiro and Miselis, 1985; van der Kooy and Koda, 1983). Bien que peu ou pas de projections axonales aient été décrites de l'AP vers le VLM et le LC, des évidences claires ont montré que le le NTS projette au VLM, au LC et aux noyaux PB (Chan *et al.*, 1995). De plus, des travaux ont montré que toutes ces régions du tronc cérébral, mis à part l'AP, ont des projections efférentes vers les noyaux PVH (Cunningham *et al.*, 1990; Cunningham and Sawchenko, 1988; Sawchenko and Swanson, 1982; Swanson and Sawchenko, 1983). Ainsi, il est possible que ces structures puissent moduler l'action des neurones responsables de stimuler l'ACTH adénohypophysaire.

Bien que plus controversée, la deuxième hypothèse selon laquelle les cytokines périphériques pourraient communiquer avec certaines structures du tronc cérébral fait référence à une route nerveuse plutôt qu'humorale (Dantzer, 1994; Fleshner *et al.*, 1995; Watkins *et al.*, 1995; Watkins *et al.*, 1994). Toujours selon ce modèle, l'augmentation des niveaux tissulaires en cytokines serait amplement suffisante à la régulation des diverses fonctions neuroendocriniennes comme l'axe HPA, l'apparition des cytokines dans la circulation sanguine ne serait pas requise. Ainsi, au cours de la réponse immunitaire, les cytokines pro-inflammatoires auraient la capacité de stimuler directement les nombreuses afférences sensitives du nerf vague en plus des efférences du système nerveux parasympathique regroupées dans le nerf en question. En accord avec cette affirmation, des travaux ont montré qu'une vagotomie subdiaphragmatique atténue l'élévation des concentrations plasmatiques en ACTH et corticostérone en réponse à l'injection intrapéritonéale (i.p.) d'IL-1ß (Fleshner *et al.*, 1995), mais pas suite à l'infusion i.v. d'une forte dose de cette même cytokine (Katsuura *et al.*, 1988). Récemment, Wan et ses collègues ont rapporté qu'une telle chirurgie a pour effet de supprimer l'expression de la protéine Fos dans le cerveau de rats soumis à une administration i.p. de l'endotoxine bactérienne lipopolysaccharide (Wan *et al.*, 1994). Le fait que ce groupe n'ait pu reproduire les mêmes résultats suite à l'injection i.v. de l'endotoxine confirme l'hypothèse voulant que les cytokines tissulaires et systémiques pourraient emprunter des routes différentes pour communiquer avec le SNC. Finalement, il a été démontré que l'IL-1ß et la LPS peuvent être reconnus par les chimiorécepteurs des afférences paraganglionnaires formant des synapses avec le nerf vague au niveau de la région cervicale ainsi que dans les cavités thoraciques et abdominales (Dantzer, 1994; Goehler *et al.*, 1995; Watkins *et al.*, 1995). À la lumière de ces résultats, il semble donc plausible de croire que les cytokines produites localement dans un tissu puissent interagir avec certaines structures du tronc cérébral, via un mécanisme neuronal impliquant le nerf vague afin de stimuler l'axe corticotrope et probablement d'autres fonctions neuronales sollicitées au cours de la réponse immunitaire. Depuis un certain nombre d'années déjà, les neuroanatomistes ont identifié le NTS comme le noyau recevant la grande majorité des terminaisons nerveuses provenant des afférences vagales (Loewy, 1990). Comme mentionné auparavant, le NTS est en contact direct avec plusieurs structures activées lors d'un traitement avec les cytokines pro-inflammatoires, en plus de projeter fortement vers les noyaux PVH. Ainsi, une circuiterie axée sur les voies du tronc cérébral, comme celle décrite dans le paragraphe précédent, pourrait être impliquée dans la communication entre les cytokines tissulaires et l'hypothalamus neuroendocrinien.

1.1.5 Objectifs spécifiques

La présente thèse de doctorat avait pour objectif d'élucider le rôle des PGs dans l'interaction entre les systèmes immunitaire et neuroendocrinien. Plus précisément, nos travaux avaient pour but de clarifier l'importance des PGs dans la médiation des effets des cytokines pro-inflammatoires sur la stimulation des neurones synthétisant le CRF et, par conséquent, l'activation de l'axe HPA. Les mécanismes cellulaires et la circuiterie neuronale par lesquels ces médiateurs inflammatoires influencent les fonctions neuroendocriniennes furent également étudiés. Pour ce, un modèle d'infection à la LPS fut employé afin de mimer la réponse immunitaire de type aigu, et ainsi provoquer la libération des cytokines par les cellules immunitaires activées. Un modèle d'inflammation stérile fut aussi utilisé afin de bien discerner les effets des médiateurs immunitaires et inflammatoires des effets de la LPS en soi. Ces deux modèles ont d'ailleurs été décrits à la section 1.2. Pour mieux orienter le lecteur, les objectifs spécifiques poursuivis lors de cette thèse de doctorat furent énumérés par points, chacun de ceux-ci correspondant à un article et un chapître bien distinct:

- Observer les effets de différentes doses (250, 25 et 2.5 µg/100 g poids corporel) de l'endotoxine bactérienne LPS sur l'expression des gènes de réponse précoce c-*fos* et NGFI-B (ceux-ci sont utilisés comme des index de l'activité cellulaire) dans le cerveau de rats. Étudier le rôle joué par les voies de la cyclooxygénase dans la stimulation de l'activité cellulaire cérébrale et la transcription du CRF neuroendocrinien dans les noyaux PVH de rats soumis à un challenge immunitaire. Vérifier l'hypothèse voulant que les PGs puissent moduler l'activité des groupes de neurones catécholaminergiques A_1/C_1 and A_2/C_2 du tronc cérébral suite à un traitement avec la LPS.

- Investiguer les effets de différentes doses (250, 25 et 2.5 µg/100 g poids corporel) de l'endotoxine bactérienne LPS sur l'expression des gènes encodant les récepteurs du CRF (récepteurs du CRF de type 1, 2α et 2ß) dans le cerveau de rats. Vérifier la possibilité que les voies de la cyclooxygénase puissent médier les influences d'un challenge immunitaire systémique sur la transcription des gènes encodant les différents sous-types des récepteurs du CRF dans des structures spécifiques du SNC.

- Identifier, via l'expression du gène de réponse précoce c-*fos*, les sites activés dans l'ensemble du cerveau de rats traités centralement avec des PGE_2. Déterminer grâce à la combinaison de l'immunohistochimie et de l'hybridation *in situ*, le type cellulaire des

neurones neuroendocriniens (CRF, AVP ou OT) activés suite à l'administration centrale des PGE_2. Évaluer par hybridation *in situ* les effets d'une injection i.c.v. des PGE_2 sur l'activité transcriptionnelle des gènes codant le CRF et son récepteur de type 1 (des sondes introniques et exoniques seront utilisées dans la présente expérience).

• Étudier l'expression des gènes encodant les enzymes limitantes à la production des prostaglandines (COX-1 et 2) au cours de la réponse immunitaire de type aigu et à la suite d'injection systémique des cytokines pro-inflammatoires. Établir si l'induction de la transcription du gène COX-2 dans le cerveau de rats est une conséquence de la réaction inflammatoire systémique ou plutôt un effet direct de l'endotoxine bactérienne LPS (la LPS et la turpentine furent employées à titre de modèles inflammatoires).

• Vérifier l'hypothèse voulant que le SNC possède la capacité d'exprimer le récepteur de la LPS, permettant du même coup à l'endotoxine d'agir directement sur le cerveau afin de stimuler différentes fonctions neuronales. Caractériser la distribution de l'ARNm du CD14 dans le cerveau de rats. Investiguer les effets d'une injection systémique de LPS sur la régulation transcriptionelle de son propre récepteur. Déterminer grâce à la combinaison de l'immunohistochimie et de l'hybridation *in situ*, les populations cellulaires spécifiques exprimant le récepteur CD14 dans des conditions basales ou suite à un challenge immunitaire.

1.2 Modèles infectieux et inflammatoires utilisés afin de mimer la réponse immunitaire de type aigu.

La réponse immunitaire de type aigu est un terme général employé pour décrire la majorité des réponses rapides de l'hôte suite à une invasion par un agent pathogène, un dommage tissulaire, une réaction immunologique ou encore une réaction inflammatoire. Cette réponse survient normalement dans les heures qui succèdent l'infection ou l'inflammation et contraste avec la réponse anticorps primaire et secondaire qui s'échelonne sur une période de temps pouvant même atteindre des mois. La réponse immunitaire de type aigu inclut divers changements des fonctions immunologiques, métaboliques, hématologiques, endocrinologiques et neurologiques. Parmi ceux-ci, on retrouve, entre autres, l'augmentation dramatique de la synthèse des protéines hépatiques, la fièvre, la leukocytose et l'anémie. Cependant, tous sont unanimes pour dire que l'événement le plus fondamental dans l'initiation de la réponse immunitaire de type aigu consiste à la production

des cytokines par les cellules phagocytaires activées (Dinarello, 1989; Rabin *et al.*, 1990). Au cours des dernières années, plusieurs groupes ont tenté de développer des modèles d'activation immunitaire afin de mieux comprendre les mécanismes par lesquels certaines maladies infectieuses/inflammatoires interfèrent avec le système neuroendocrinien. Parmi les nombreux modèles couramment étudiés, deux ont principalement retenu notre attention et feront par conséquent l'objet des expériences présentées dans les chapîtres qui vont suivre.

1.2.1 L'endotoxine bactérienne lipopolysaccharide

L'endotoxine LPS est une composante de la membrane externe des bactéries gram-négatives (Raetz, 1990). Son rôle crucial joué lors d'infections aux bactéries gram-négatives a été démontré par le fait qu'une injection systémique de l'endotoxine, à des animaux ou des humains, entraîne des réponses périphériques et centrales similaires à celles observées suite à une infection bactérienne de type gram-négative (Martich *et al.*, 1993; Redl *et al.*, 1993). Ainsi, la LPS est couramment utilisée afin de mimer les événements endogènes qui apparaissent au cours de l'inflammation et de la septicémie (Ertel *et al.*, 1992; Higgins and Olschowka, 1991; Koenig, 1991; Kushner, 1982). Bien que n'étant pas un vrai modèle d'infection, la LPS représente tout de même un moyen efficace pour augmenter la production de cytokines systémiques et centrales (Bristow *et al.*, 1991; Fontana *et al.*, 1984; Higgins and Olschowka, 1991; Koenig, 1991; Nadeau and Rivest, 1998; Quan *et al.*, 1998; Vallières and Rivest, 1997; Van Dam *et al.*, 1992). L'administration systémique de la LPS induit l'expression des gènes précoces c-*fos* et NGFI-B dans plusieurs structures spécifiques du SNC, ainsi que dans les neurones CRFergiques des noyaux PVH (Laflamme and Rivest, 1994; Rivest and Laflamme, 1995). De plus, l'injection de l'endotoxine bactérienne augmente la transcription du CRF et de son récepteur de type 1 dans cette même structure hypothalamique (Kakucska *et al.*, 1993; Laflamme and Rivest, 1994; Rabin *et al.*, 1988; Rivest and Laflamme, 1995; Rivest *et al.*, 1995) et stimule fortement l'activité de l'axe HPA chez le rat (Rivier and Rivest, 1993).

La sécrétion des cytokines par les monocytes circulants et les macrophages tissulaires suite à un traitement avec la LPS requiert une série d'étapes en cascade. Tout d'abord, l'endotoxine doit rejoindre la circulation sanguine et se lier à une protéine sérique comme la protéine de liaison de la LPS (LBP) ou encore certaines septines (Schumann *et al.*, 1990; Wright *et al.*, 1992). Par la suite, le complexe nouvellement formé devra lier le récepteur CD14 attaché à la surface membranaire des cellules myéloïdes différenciées (principalement

les monocytes et les macrophages mais aussi, à un degré moindre, les neutrophiles) et ainsi stimule la production des cytokines proinflammatoires (Wright *et al.*, 1990). Toutefois, on ne comprend pas encore tous les mécanismes impliqués dans la sécrétion des médiateurs inflammatoires suite à la liaison du complexe LPS-LBP au récepteur membranaire du CD14 (CD14m). *In vitro*, de nombreux groupes ont rapporté que l'immunoneutralisation du CD14 a pour conséquence d'inhiber plusieurs des réponses cellulaires induites en présence de concentrations de LPS pourtant suffisantes au déclenchement d'une septicémie (Kitchens *et al.*, 1992; Lee *et al.*, 1993; Wright *et al.*, 1991; Wright *et al.*, 1990). Récemment, il a été démontré chez des primates injectés avec la LPS, qu'un prétraitement avec un anticorps dirigé contre le CD14 prévient les dommages tissulaires, l'hypotension et l'augmentation des niveaux plasmatiques d'IL-1ß, d'IL-6 et de TNF-α (Leturcq *et al.*, 1996). Ferrero et ses collègues ont observé que des souris transgéniques exprimant le récepteur CD14 humain à la surface de leurs cellules phagocytaires sont hypersensibles à la LPS; comme le démontre leur susceptibilité accrue aux chocs endotoxiques et leur haut taux de mortalité (Ferrero *et al.*, 1993). De plus, la transfection du CD14 humain dans des fibroblastes ovariens d'hamster chinois a eu pour effet de transformer ces cellules considérées comme non-répondantes à la LPS en cellules répondantes (Golenbock *et al.*, 1993).

Plusieurs groupes s'accordent à dire que la sécrétion des cytokines pro-inflammatoires est directement responsable de la grande majorité des effets de l'endotoxine LPS sur le cerveau. Toutefois, certaines évidences semblent aller à l'encontre de cette hypothèse, qui pourtant, n'a presque jamais été remise en question jusqu'à présent. Ainsi, il a été démontré que l'administration systémique de l'antagoniste du récepteur de l'IL-1 est incapable de prévenir l'augmentation des niveaux d'ARNm du CRF dans les noyaux PVH et de corticostérone dans le sang suite à l'injection de LPS (Dunn, 1992; Kakucska *et al.*, 1993). De plus, Dunn et ses collaborateurs ont aussi rapporté que l'injection i.v. d'un anticorps dirigé contre le TNF-α, seule ou combinée à l'infusion de l'antagoniste du récepteur de l'IL-1, n'empêche pas l'activation de l'axe corticotrope en réponse à l'endotoxine bactérienne LPS (Dunn, 1992). La déplétion *in vivo* des macrophages/monocytes ne prévient pas l'augmentation d'ACTH et de corticostérone dans le plasma de rats injectés avec une forte dose de LPS, suggérant du même coup que, lors d'une endotoxinémie, des médiateurs autres que ceux sécrétés par les cellules phagocytaires systémiques sont impliqués dans l'activation de l'axe corticotrope (Derijk *et al.*, 1991). Par ailleurs, il a été prouvé que la montée des taux plasmatiques d'ACTH et de corticostérone, suite à l'administration intra-artérielle de la LPS, précède par au moins 30 minutes l'apparition des cytokines pro-inflammatoires dans la circulation sanguine (Givalois *et al.*,

1994). Il y a peu de temps, des études réalisées chez des souris déficientes pour le gène de l'IL-1ß ou de l'IL-6 ont clairement démontré que la LPS peut activer l'hypothalamus et l'axe HPA malgré l'absence de ces cytokines pyrogéniques endogènes. En effet, contrairement aux souris de souche sauvage injectées avec la LPS, des niveaux plasmatiques normaux de corticostérone ont été mesurés chez les souris IL-1ß -/- et IL-6 -/- traitées avec l'endotoxine bactérienne (Fantuzzi and Dinarello, 1996; Fattori *et al.*, 1994; Zheng *et al.*, 1995). Des travaux entrepris dans le laboratoire du Dr. Serge Rivest ont montré qu'une administration systémique de LPS, mais pas l'induction d'une inflammation locale ou même l'injection i.v. des différentes cytokines pro-inflammatoires, stimule l'activité transcriptionnelle des gènes encodant l'IL-6 et le TNF-α dans les OCVs et les plexus choroïdiens (Nadeau and Rivest, 1998; Vallières and Rivest, 1997). À la lumière de ces résultats, il semble évident que la LPS est capable d'induire par elle-même la biosynthèse des cytokines dans le SNC. Par conséquent, il est aussi envisageable que la LPS puisse réguler directement certaines fonctions neuronales et neuroendocriniennes (axe HPA) au cours d'une endotoxinémie. L'éventualité que la LPS agisse directement sur certaines populations spécifiques du cerveau afin de moduler ces fonctions fera d'ailleurs l'objet du chapître 7. Quant aux cytokines d'origine périphérique, celles-ci pourraient, bien entendu, contribuer de multiples façons aux diverses réponses neuroendocriniennes; certains travaux suggèrent même que les cytokines systémiques pourraient jouer un rôle déterminant dans le prolongement et le maintien des réponses neuroendocriniennes suite à une infection à la LPS (Perlstein *et al.*, 1993; Rivier *et al.*, 1989). Cependant, la possibilité demeure qu'au cours d'une endotoxinémie, la sécrétion des cytokines par les cellules phagocytaires activées en périphérie soit une réponse tout a fait distincte et indépendante.

1.2.2 L'inflammation stérile (turpentine)

Une inflammation aiguë peut-être induite grâce à l'injection d'une grande variété de substances irritantes. Récemment, plusieurs groupes ont commencé à utiliser la turpentine comme modèle d'inflammation stérile (réponse inflammatoire en l'absence de stimuli microbiens). L'injection intramusculaire (i.m.) d'une très faible quantité de ce solvant provoque des dommages tissulaires qui vont ultimement résulter en la production locale d'IL-1ß (Turnbull *et al.*, 1994). Bien qu'il a été démontré que les niveaux plasmatiques d'IL-6 vont par la suite augmenter, aucune élévation des taux d'IL-1α et de TNF-α n'a été rapportée dans la circulation sanguine (Fantuzzi and Dinarello, 1996). Ces mêmes études ont d'ailleurs montré que la sécrétion d'IL-6 dans la systémie était médiée par l'IL-1ß. En effet,

contrairement aux souris sauvages ayant reçu une injection de turpentine, Fantuzzi et ses collaborateurs n'ont pu détecter la présence d'IL-6 dans le sérum de souris déficientes pour le gène IL-1ß. L'injection i.m. de la turpentine induit l'expression du gène précoce c-*fos* dans plusieurs structures sélectives du SNC, ainsi que dans les noyaux PVH (observations personnelles). Dernièrement, Rivier et son groupe ont montré que l'administration de ce solvant augmente les niveaux plasmatiques d'ACTH (Rivier, 1995); un phénomène complètement dépendant du CRF neuroendocrinien (Turnbull and Rivier, 1996). Toutefois, ils ont rapporté que l'activation de l'axe HPA est observée à des temps beaucoup plus tardifs qu'à la suite d'une administration de l'endotoxine LPS. Ainsi, les concentrations d'ACTH dans le plasma ont été significativement augmentées 6 à 12 heures après l'injection de turpentine (Rivier, 1995). Fait intéressant, une élévation encore plus intense des niveaux sanguins d'ACTH a été notée moins d'une heure après un traitement avec la LPS (Givalois *et al.*, 1994). Ces résultats supportent donc l'hypothèse voulant que les cytokines systémiques sécrétées lors d'une endotoxinémie ne soient pas responsables de l'activation précoce des diverse fonctions neuroendocriniennes. Néanmoins, l'implication des cytokines périphériques dans la régulation de l'axe HPA à des temps plus tardifs ne fait aucun doute. L'utilisation d'un deuxième modèle, comme celui de l'inflammation stérile, sera donc d'un précieux secours afin de discriminer si les effets observés suite à un stress immunitaire, sont le résultat de la réponse inflammatoire systémique ou plutôt de diverses actions directes d'une endotoxine par exemple.

1.3 Approche expérimentale utilisée

Afin de bien définir les mécanismes moléculaires et les voies neuronales impliquées lors de la réponse immunitaire de type aigu, plusieurs techniques furent employées sur une base régulière dans le laboratoire du Dr. Serge Rivest. Parmi celles-ci, on retrouve, entre autres; l'hybridation *in situ* histochimique, l'immunohistochimie, le transport neuronal rétrograde ou antérograde et plusieurs techniques de biologie moléculaire incluant le sous-clonage, l'amplification de vecteurs par transformation, les mini- et large-prep, la synthèse de sondes radioactives, etc. De plus, certaines techniques chirurgicales telles que des canulations intracérébroventriculaires, intraveineuses et intrapéritonéales furent nécessaires à la réalisation de ces expériences. Toutefois, parmi ces nombreuses approches expérimentales, l'hybridation *in situ* histochimique (HISH) fut sans aucun doute la technique la plus couramment utilisée lors de la réalisation des divers protocoles inclus dans la présente thèse de doctorat.

L'HISH consiste essentiellement à détecter des brins spécifiques d'ARN grâce à l'utilisation de sondes d'ARN complémentaires marquées avec un isotope radioactif. Bien que plusieurs ont recours à cette technique de haute sensibilité dans le but exclusif d'établir la distribution et la régulation des niveaux d'expression de certains gènes, l'utilisation dont nous en avons fait va beaucoup plus loin. Ainsi, grâce à l'HISH et aux gènes de réponse précoce (IEGs), nous avons été en mesure de détecter la plupart des groupes cellulaires activés dans l'ensemble du cerveau suite à diverses conditions de stress (Lacroix and Rivest, 1997; Lacroix *et al.*, 1996; Nappi *et al.*, 1997; Rivest and Laflamme, 1995; Rivest and Rivier, 1994; Vallières *et al.*, 1997). Parmi les principaux IEGs étudiés dans nos laboratoires, on retrouve, entre autres, le proto-oncogène c-*fos* et NGFI-B, un membre orphelin de la superfamille des récepteurs stéroïdiens aussi connu sous le nom de Nurr 77. Ces gènes ont la caractéristique d'être exprimés à de très faibles niveaux dans les structures impliquées dans la régulation de l'axe HPA lors de conditions basales. Toutefois, une grande variété de stimuli extracellulaires ont été identifiés comme capables d'induire la biosynthèse de ces gènes dans le cerveau. Il est d'autant plus intéressant de constater que la synthèse des IEGs dans les cellules activées varie en fonction du temps et de l'intensité du stimuli. Certains groupes ont d'ailleurs rapporté que l'expression de ces gènes augmente de façon rapide et transitoire dans des régions précises du SNC suite à une stimulation par des neurotransmetteurs, des neuropeptides, des cytokines, des facteurs de croissance, des agents infectieux, des hormones et même des stimuli électriques ou sensoriels (Armstrong and Montminy, 1993; Brady *et al.*, 1994; Bullitt, 1990; Dragunow and Faull, 1989; Ericsson *et al.*, 1994; Jacobson *et al.*, 1990; Morgan and Curran, 1989; Morgan and Curran, 1991; Parkes *et al.*, 1993; Rivest and Laflamme, 1995; Sagar *et al.*, 1988). L'analyse de la distribution des gènes c-*fos* et NGFI-B en fonction du temps représente donc un excellent moyen d'identifier les différentes structures afférentes ou efférentes impliquées dans la régulation des fonctions neurales et neuroendocriniennes, et à quel moment ces structures sont sollicitées au cours de la réponse immunitaire/inflammatoire. Précisons que pour beaucoup de groupes de recherche, l'obtention de ces informations a souvent constitué la première étape afin d'établir une ébauche concrète de la circuiterie responsable de la sécrétion des diverses hormones du système hypothalamo-hypophysaire.

Malgré la grande utilité des IEGs comme indice de l'activité synaptique en réponse à divers stimuli, il est important de prendre en considération que ces marqueurs de l'activité cellulaire ne sont pas parfaits. En effet, des études ont montré que Fos, contrairement à d'autres IEGs, n'était pas exprimé dans certaines cellules spécifiques du cerveau reconnues comme étant stimulées à la suite d'un stress donné (Dragunow and Faull, 1989; Ericsson *et*

al., 1995; Labiner *et al.*, 1993). À titre d'exemple, mentionnons les travaux d'Ericsson et ses collaborateurs qui ont récemment démontré que c-*fos* était un marqueur relativement insensible de l'activité de certains types cellulaires (cellules périvasculaires) du SNC (Ericsson *et al.*, 1995). Une autre limitation majeure de cette technologie réside dans le fait que les IEGs couramment utilisés ne marquent pas les neurones ayant été stimulés par des transmissions synaptiques inhibitrices, une portion considérable des synapses (Chan *et al.*, 1993; Chan and Sawchenko, 1994; Kovacs and Sawchenko, 1993). Enfin, une dernière inquiétude quant à l'utilisation des IEGs afin d'identifier les types cellulaires et sites d'action responsables de l'élaboration de la réponse neurogénique ou neurosécrétoire appropriée, concerne l'expression tardive des facteurs de transcription Fos et NGFI-B à la suite d'un stress (Kovacs and Sawchenko, 1994; Rivest and Laflamme, 1995). Ainsi, il est possible que la biosynthèse de ces IEGs soit une conséquence et non pas la cause de l'activation des cellules responsables du contrôle de l'axe HPA. Bien que ces résultats ne modifient en rien l'usage de Fos et NGFI-B comme marqueur de l'activité synaptique, ils compromettent grandement l'utilité de ces IEGs en tant qu'indice de l'activité transcriptionnelle de certains gènes. L'importance de Fos et NGFI-B dans la régulation de la transcription de certains gènes, comme ceux encodant les facteurs hypothalamiques CRF, AVP et OT, sera d'ailleurs discutée plus en détail au chapître 7.

La technique d'HISH a subi des améliorations constantes au cours des dernières années. Parmi les nombreuses innovations, l'utilisation de sondes introniques a sans aucun doute grandement contribué à augmenter l'efficacité de cette technique. La capacité des sondes introniques à détecter des transcrits primaires (séquences d'ARNhn) dans le cerveau a été observée pour la première fois il y a quelques années déjà (Fremeau *et al.*, 1989; Herman *et al.*, 1992; Herman *et al.*, 1991; Young *et al.*, 1986). L'une des raisons principales pour lesquelles cet outil de travail est maintenant considéré comme essentiel, est la nécessité d'étudier *in vivo* la régulation transcriptionnelle de certains gènes tel que le CRF lors d'une situation de stress. Plusieurs facteurs font en sorte que la mesure des taux d'ARNm, grâce à des sondes exoniques, n'est pas un bon indicateur de l'activité transcriptionnelle. Parmi ceux-ci, on retrouve entre autres l'absence d'informations en ce qui a trait aux taux de dégradation et de traduction des ARNm, de même que la présence de niveaux de base extrêmement élevés des ARNm encodant la plupart des neuropeptides, d'où la difficulté d'observer des modifications de l'activité transcriptionnelle. Pour ces raisons, il semble donc évident que la mesure des concentrations de transcrits primaires soit un reflet plus adéquat de l'activation de la transcription en réponse à un stimulus quelconque. Des études réalisées chez des cellules eucaryotes ont d'ailleurs démontré que suite à un stimulus, les

ARNhn sont rapidement transcrits par les ARN polymérases puis presqu'immédiatement épissés dans le noyau pour donner des ARNm (Darnell, 1983; Lewin, 1980). Le seul problème majeur concernant l'HISH intronique consiste à la formation d'intermédiaires relativement stables en présence de gènes renfermant plusieurs séquences introniques; chacun des différents introns est épissé à une vitesse qui lui est propre d'où la possibilité de voir apparaître un intermédiaire instable. Cependant, puisque le gène qui nous intéresse plus particulièrement est le CRF et que celui-ci ne possède qu'un seul intron (Thompson *et al.*, 1987), la détection de changements dans les taux d'ARNhn du CRF pourra bel et bien être considérée comme une stimulation de la transcription.

En résumé, nous utiliserons la technique d'HISH afin d'étudier l'expression des gènes encodant les IEGs c-*fos* et NGFI-B dans le SNC de rats traités avec la LPS. Ces résultats, de même que diverses analyses effectuées en fonction de la dose de LPS administrée et du temps de sacrifice des animaux suivant l'injection de l'endotoxine, nous permettront d'établir une ébauche complète des sites d'action impliqués dans la médiation des effets d'un stress immunitaire sur les fonctions neuronales et neuroendocriniennes. De plus, suite aux nombreux travaux démontrant que les PGs pouvaient moduler certaines des réponses observées au cours de la réponse immunitaire, nous vérifierons, dans un deuxième temps, si l'inhibition de la synthèse des PGs influence l'activation de certaines structures de même que la transcription du CRF et du R-CRF_1 dans les noyaux PVH et SON. Afin de bien définir l'impact des PG produites centralement sur l'activité cellulaire nerveuse ou gliale ainsi que l'activation de l'axe corticotrope, nous évaluerons ensuite, à l'aide de sondes exoniques et introniques, les niveaux respectifs d'ARNm des IEGs et d'ARNhn du CRF en réponse à l'injection i.c.v. des PGE_2. De plus, grâce à la combinaison de l'HISH à l'immunohistochimie, nous identifierons les types cellulaires activés dans l'hypothalamus endocrinien suite à un tel traitement. Finalement, nos deux dernières études consisteront essentiellement à déterminer les sites de production des PGs de même que les sites d'action de la LPS. Pour ce, nous établirons la distribution complète des régions exprimant les transcrits encodant les enzymes COX et le récepteur CD14m de la LPS dans le cerveau de rats traités avec l'endotoxine bactérienne. À nouveau, l'HISH sera combinée à l'immunohistochimie afin de caractériser les types cellulaires exprimant ces gènes.

CHAPITRE 2. FUNCTIONAL CIRCUITRY IN THE BRAIN OF IMMUNE-CHALLENGED RATS: PARTIAL INVOLVEMENT OF PROSTAGLANDINS.

By

Steve Lacroix and Serge Rivest

Laboratory of Molecular Endocrinology
CHUL Research Center and
Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2

Running Title: Role of PGs in endotoxin-treated rat brain.

Address for Correspondence:
Dr. Serge Rivest
Laboratory of Molecular Endocrinology
CHUL Research Center and
Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2
Tel: (418) 654-2296, Fax: (418) 654-2761
EMail: Serge.Rivest@crchul.ulaval.ca

The Journal of Comparative Neurology, 387 (1997) 307-324

2.1 RÉSUMÉ

L'objectif de cette étude était d'évaluer le rôle des prostaglandines (PGs) dans l'activation neuronale et la transcription du facteur de libération des corticotrophines (CRF) dans le cerveau de rats soumis à un challenge immunitaire. Pour ce, une administration intraveineuse (i.v.) d'indométhacine (0.8 mg/100 g de poids corporel), un inhibiteur de la synthèse des PGs, fut effectuée avant et après l'injection intrapéritonéale (i.p.) de différentes doses (250, 25 and 2.5 µg/100 g de poids corporel) de l'activateur immunitaire lipopolysaccharide (LPS). Nos résultats montrent que l'administration systémique d'une dose forte ou modérée de LPS stimule la transcription des gènes de réponse précoce c-*fos* et NGFI-B dans plusieurs régions du cerveau, tandis que l'injection d'une faible dose d'endotoxine induit l'expression du gène c-*fos* dans les organes circumventriculaires sensoriels exclusivement. Fait intéressant, un prétraitement à l'indométhacine ne prévient pas la transcription de c-*fos* dans le cerveau des rats sacrifiés 3 heures après l'injection d'une dose élevée de LPS. Par contre, l'inhibition de la synthèse des PGs atténue l'activité neuronale chez les animaux traités avec une dose de 25 µg de LPS/100 g de poids corporel; un phénomène dépendant des structures et groupes cellulaires analysés. En effet, le blocage des voies de la cyclooxygénase a altéré significativement l'induction par la LPS des taux d'ARNm de c-*fos* dans: la région médiane préoptique, l'organe vasculaire de la lame terminale, les noyaux périventriculaires, les noyaux paraventriculaires de l'hypothalamus (PVH) et la région ventro-latérale médullaire (VLM). Aucune diminution notable des niveaux de transcrits encodant le gène c-*fos* n'a été décelée dans les autres régions du cerveau incluant: l'organe subfornical, les noyaux centraux de l'amygdale, les noyaux arqués, l'eminence médiane, les noyaux parabrachiaux, les plexus choroïdiens et les noyaux de la tract solitaire (NTS). Dans les noyaux PVH, l'inhibition de la transcription des ARNm de c-*fos* et NGFI-B par l'indométhacine fut associée à une baisse dramatique de l'activité transcriptionnelle du CRF neuroendocrinien; l'expression du transcrit primaire du CRF en réponse à une dose modérée de LPS fut sélective aux noyaux PVH et complètement bloquée par un prétraitement à l'indométhacine. Enfin, une quantité importante des neurones immunoréactifs à la tyrosine hydroxylase (TH) du VLM (A1/C1) et du NTS (A2/C2) ont exprimé l'ARNm de c-*fos* à la suite d'un challenge immunitaire, un effet prévenu dans le VLM, mais pas dans le NTS des rats prétraités à l'indométhacine. Ces résultats indiquent que le rôle joué par les PGs, comme médiateurs des influences stimulatrices de la réponse immunitaire de type aigu, dépend de plusieurs facteurs tels que: la sévérité du stress systémique, les régions du cerveau étudiées, les groupes cellulaires analysés et les marqueurs de l'activité cellulaire utilisés.

2.2 ABSTRACT

This study investigated the role of prostaglandins (PGs) on the neuronal activity and the transcription of corticotropin-releasing factor (CRF) in the brain of conscious immune-challenged rats. Intravenous (i.v.) administration of indomethacin, an inhibitor of PG synthesis, was performed before and after the intraperitoneal (i.p.) injection of different doses (250, 25 and 2.5 μg/100 g of b.w.) of the immune activator lipopolysaccharide (LPS). Systemic administration of the highest and middle doses of LPS caused a robust and widespread induction of both immediate-*early* genes (IEGs), c-*fos* and nerve growth factor-inducible gene B (NGFI-B) mRNAs, whereas injection of the lowest dose selectively triggered c-*fos* expression within the sensorial circumventricular organs. Pretreatment with indomethacin did not prevent c-*fos* transcription in the rat brains challenged with the highest dose of LPS at 3 hours post-injection. Inhibition of PG formation was more effective for interruption of the neuronal activation in animals injected with 25 μg of LPS/100 g b.w., although the influence depended on the structures and the groups of activated cells. Indeed, PG inhibition significantly altered LPS-induced c-*fos* mRNA expression in the medial preoptic area/organum vasculosum of the lamina terminalis, periventricular nucleus, paraventricular nucleus of the hypothalamus (PVN) and the ventrolateral medulla (VLM), but not in many other regions including the subfornical organ, central nucleus of the amygdala, arcuate nucleus/median eminence, parabrachial nucleus, choroid plexus and nucleus of the solitary tract (NTS). In the hypothalamic PVN, inhibition of both c-*fos* and NGFI-B transcripts by indomethacin was also associated to an abolished influence of the endotoxin on the transcription of neuroendocrine CRF; induction of CRF primary transcript by the middle dose of LPS was selective to the PVN and completely blocked by pretreatment with indomethacin. Moreover, a large number of tyrosine hydroxylase (TH)-immunoreactive neurons of the VLM (A1/C1) and NTS (A2/C2) were positive for c-*fos* mRNA in immune-challenged rats, an effect largely prevented by indomethacin in the VLM, but not the NTS. These results indicate that the role of PGs in mediating the stimulatory influence of the acute-phase response depends on the severity of the systemic stressful situation, the brain regions and cell groups as well as the activated target genes.

2.3 INTRODUCTION

Appropriate communication between neuroendocrine and immune systems is essential to maintain homeostasis in the presence of foreign material. Cytokines produced during immune challenge can stimulate the release of endogenous glucocorticoids, which in counterpart could reach the inflammatory site to suppress the immune response (Solomon, 1969). The production of cytokines by activation of macrophages and lymphocytes which occurs rapidly after the contact between organisms and exogenous pyrogen (Dinarello, 1989; Rabin et al., 1990), represents an essential feature of the early events of immune activation characterizing the acute-phase response. Endotoxins are widely used to mimic some of the events that take place during sepsis (Higgins and Olschowka, 1991; Koenig, 1991); lipopolysaccharide (LPS) triggers the synthesis and release of a series of immunoregulatory, cytotoxic, and inflammatory molecules, including tumor necrosis factor (TNF)-α, interleukin-1 (IL-1), and IL-6 (Andersson et al., 1992; Nathan, 1987). Systemic LPS administration causes a strong and prolonged activation of the hypothalamic-pituitary-adrenal (HPA) axis (Rivier and Rivest, 1993), essentially via neuroendocrine corticotropin-releasing factor (CRF)-mediated mechanisms (for review, see Koenig, 1991; Rivest, 1995; Rivest and Rivier, 1995; Rivier and Rivest, 1993). Selective transcriptional activation of CRF was recently reported in the hypothalamic paraventricular nucleus (PVN) of endotoxin-challenged rats (Rivest and Laflamme, 1995), whereas inhibition of CRF abolishes the effects of LPS on the activation of the HPA axis (Rivier and Rivest, 1993).

Systemic administration of the immune activator LPS also causes a profound expression of mRNA encoding the immediate-*early* genes (IEGs) c-*fos* and nerve growth factor-inducible gene B (NGFI-B) as well as their protein products in numerous structures of the brain suggesting that a complex neuronal circuitry is involved in triggering the activity of neuroendocrine neurons controlling the HPA axis (Elmquist and Saper, 1996; Elmquist et al., 1996; Hare et al., 1995; Nappi et al., 1997; Rivest and Laflamme, 1995). The ventrolateral medulla (VLM) and nucleus of the solitary tract (NTS) are among the numerous regions of the brain that are responsive to i.p. injection of the bacterial endotoxin (Rivest and Laflamme, 1995). Catecholamine-synthesizing neurons of these brainstem regions (A1-2/C1-2) are two groups of cells which provide massive projections to hypophysiotrophic cells of the PVN (Cunningham et al., 1990; Cunningham and Sawchenko, 1988; Sawchenko and Swanson, 1982; Swanson et al., 1983), pathways believed to play a role in mediating the effects of immune challenge and systemic cytokines on hypothalamic neuroendocrine

response. It has recently been demonstrated that rats bearing unilateral fiber transection of catecholaminergic inputs had impaired neuronal activation and CRF mRNA expression in the PVN in response to systemic IL-1 challenge (Ericsson et al., 1994), but not following a neurogenic footshock stressor (Li et al., 1996). Influence of catecholaminergic innervation on CRF secretion can nevertheless be located directly at the level of the PVN; depletion of PVN noradrenergic (NA) contents with 6-hydroxydopamine (6-OHDA) has been shown to reduce by 80-82% the increase in plasma corticosterone concentration following i.p. IL-1 injection (Chuluyan et al., 1992).

Most of the pleiotropic effects of IL-1 are known to take place via the activation of prostaglandins (PGs) synthesized by cyclooxygenase pathways (Cominelli et al., 1989; Hughes et al., 1989; Kerr et al., 1989; Kohan, 1989). Blockage of the eicosanoid cyclooxygenase pathways can prevent the stimulation of CRF release by both IL-1 and 6 cytokines from in vitro hypothalamic explants (Lyson and McCann, 1992; Navarra et al., 1991) and isolated median eminence (McCoy et al., 1994), and IL-1-induced adrenocorticotropin (ACTH) release in vivo (Katsuura et al., 1988; Rivier and Rivest, 1993; Watanabe et al., 1990). Inhibition of PG production has been reported to prevent IL-1-induced alteration of other neuroendocrine functions, such as luteinizing hormone-releasing hormone (LHRH) and LH release (Rivest and Rivier, 1993, 1995) as well as hypothalamic vasopressin (AVP) and oxytocin (OT) secretion (Yasin et al., 1994). On the other hand, systemic LPS administration stimulates the release of PGs in the preoptic/hypothalamic area (Ueno et al., 1982), and arachidonic acid metabolism participates in the endotoxin-induced corticosterone secretion in the rat (Smith et al., 1994).

The exact PG subtype(s) and the site(s) of action within the brain involved in these effects still remain unclear. Although various PGs have the potential to mediate the influence of immune-related factors on neuronal activation and neuroendocrine functions, a large body of evidence indicates that PG of the E_2 type might be involved in several changes observed during immune challenge and treatment with cytokines. Intracerebroventricular (i.c.v.) administration of PGE_2 (Rassnick et al., 1995) or direct injection into the medial preoptic area (MPOA)/organum vasculosum of the lamina terminalis (OVLT; Katsuura et al., 1990) elevates plasma levels of ACTH and corticosterone in rats. Moreover, central injection of PGE_2 induces expression of c-*fos* protein (Scammell et al., 1996) and mRNA (Lacroix et al., 1996) in several structures known to be activated during the acute-phase response of an immune challenge. In a similar manner, central treatment with this PG triggers transcription

of CRF and its type 1 receptor essentially in the hypothalamic PVN (Lacroix et al., 1996). Whether the action of PGE_2 takes place directly within the PVN and/or at the level of structures innervating endocrine hypothalamus has yet to be investigated. The fact that systemic injection of IL-1 provokes sharply increased levels of PGE_2 in the PVN (Watanobe and Takebe, 1994) might suggest that local PVN production of the PG participates in the regulation of neuroendocrine functions, such as the HPA axis. On the other hand, strong in situ hybridization histochemical signal for mRNA encoding the EP3 PGE_2 receptor subtypes was observed over neurons of the NTS and VLM (Ericsson et al., 1995). Neuronal populations of the medulla, particularly A1/C1 and A2/C2 cell groups, are likely to be target cells of PGE_2 during the acute-phase response to inform the hypothalamus of the neuroendocrine changes to be accomplished for the restoration of the homeostasis.

The purposes of the present study were to investigate the effects of various doses (250, 25 and 2.5 µg/100 g b.w.) of the bacterial LPS on the expression of the IEGs c-*fos* and NGFI-B (used as an index of cellular activity) throughout the rat brain; to study the role of eicosanoid cyclooxygenase pathways in mediating brain cellular activation and transcription of neuroendocrine CRF in the hypothalamic PVN of immune-challenged rats; to verify the possibility that PGs may modulate LPS-induced activation of A1/C1 and A2/C2 groups of brainstem catecholaminergic neurons.

2.4 MATERIALS AND METHODS

2.4.1 Animals

Adult male Sprague-Dawley rats (~230-260g) were acclimated to standard laboratory conditions (14-hours light, 10-hour dark cycle; lights on at 0600 and off at 2000) with free access to rat chow and water. Each rat was only used once for experimentation, and all protocols were approved by the Laval University Animal Welfare Committee. A total of 120 rats were assigned to three different protocols (each corresponding to a different dose of LPS: 250, 25 or 2.5 µg/100 g b.w.) which were further subdivided into 4 treatments (i.v. vehicle + i.p. vehicle; i.v. vehicle + i.p. LPS; i.v. indomethacin + i.p. vehicle; i.v. indomethacin + i.p. LPS) and two post-injection times (3 and 6 hours following LPS administration).

2.4.2 Surgery

Each rat was anesthetized with an i.p. injection of 0.3 ml of ketamine hydrochloride (91 mg/kg) and xylazine (9.1 mg/kg) mixture. Once anesthetized, rats were implanted with two catheters: one into the jugular vein and the other into the peritoneal cavity which was attached to the abdominal muscle. Catheters were made from a piece of silastic tubing (Silastic medical grade tubing, ID 0.020 inches., OD 0.037 inches.; Dow Corning, Midland, MI) connected to an intramedic polyethylene tubing (PE-50, Caly Adams, Parsippany, N.J.). Outlets of cannulas were placed at the level of the neck, and rats were housed individually in metal cages for a recuperation period of 2 days.

2.4.3 Treatments

On the day of the experiment (~0830 in the morning), the outlet portion of each catheter (i.v. and i.p.) was fixed to a 1.5 cm 27 g needle which was attached to PE-50 tubing. These connectors were then fixed to a 1cc syringe, and rats were placed individually in a quiet room for at least 2 hours before experimentation. This procedure was used to avoid disturbance of the animals during i.v. and i.p. administrations. Intravenous administration of indomethacin (Sigma, Oakville, Ont, Canada; I-7378, lot, 83H0041, 0.8 mg/100 g of b.w.), an inhibitor of prostaglandin synthesis diluted in 300 µl of a sterile saline solution (NaCl 0.9% solution; 5% alcohol), was accomplished 15 minutes before a single i.p. injection of various doses of LPS (250, 25 and 2.5 µg/100 g b.w.; from *Escherichia Coli*, Serotype 055:B5, Sigma, L-2880, lot, 122H4025) diluted in 300 µl of sterile saline (0.9 %). The highest dose of LPS used in this study (250 µg/100 g of b.w.) caused several visible sickness symptoms, such as covering themselves and immobile, diarrhea, shivering, piloerection, but no mortality was observed following this treatment. Twenty-four hours following the i.p. injection of the latter LPS dose, these signs were usually gone in both male and female rats (personal observation). In some animals injected i.p. with 25 µg of LPS/100 g of b.w., few but at least some of the physical symptoms described above (especially covering themselves) were observed, whereas the lowest dose did not cause any apparent signs of sickness. Moreover, the highest dose of LPS elicited a robust activation of both IEGs c-*fos* and NGFI-B in multiple regions of the brain of adult male rats (Rivest and Laflamme, 1995). To insure the complete inhibition of cyclooxygenase pathways, a second and third injection of indomethacin or its vehicle were accomplished 1 and 3 hours after the LPS injection. Similar doses of indomethacin have been shown to reverse the influence of

LPS and IL-1 on many neuroendocrine functions (Lacroix and Rivest, 1996; Rivest and Rivier, 1993; Rivier, 1993; Wan et al., 1994). The rats were conscious and freely moving at all times throughout the experimental procedure. Three or 6 hours after the i.p. treatment with the bacterial endotoxin or the vehicle solution, the animals were deeply anesthetized via an i.v. injection of 0.1 ml of a mixture of ketamine hydrochloride and xylazine and then rapidly perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M borax buffer (pH 9.5 at 4 °C). The time points were chosen on the basis of previous studies, which showed a strong signal for various IEG mRNAs throughout the rat brain and transcriptional activation of the genes encoding CRF and its type 1 receptor in the endocrine hypothalamus 3 and 6 hours after treatment with the bacterial endotoxin (Rivest and Laflamme, 1995; Rivest et al., 1995). Brains were removed from the skulls, postfixed for 2 to 8 days, and then placed in a solution containing 10% sucrose mixed in 4% paraformaldehyde-borax buffer overnight at 4 °C. The frozen brains were mounted on a microtome (Reichert-Jung, Cambridge Instruments Company, Deerfield, IL) and cut into 30-μm coronal sections. The slices were collected in a cold cryoprotectant solution (0.05M sodium phosphate buffer, pH 7.3, 30% ethylene glycol, 20% glycerol) and stored at -20 °C.

2.4.4 *In situ* hybridization histochemistry

Hybridization histochemical localization of each transcript was carried out on every sixth section of the whole rostro-caudal extent of each brain (from the olfactory bulb to the end of the medulla) by using [^{35}S]-labeled cRNA probes. Protocols for riboprobe synthesis, hybridization, and autoradiographic localization of mRNA signal were adapted from Simmons et al. (1989). All solutions were treated with diethylpyrocarbonate (Depc) and sterilized to prevent RNA degradation. Tissue sections mounted onto poly-L-lysine-coated slides were desiccated under vacuum overnight, fixed in 4% paraformaldehyde for 30 minutes, and digested by proteinase K (10 μg/ml in 0.1 M Tris HCl, pH 8.0, and 50 mM EDTA, pH 8.0, at 37 °C for 25 minutes). Thereafter, the brain sections were rinsed in sterile Depc water followed by a solution of 0.1 M triethanolamine (TEA, pH 8.0), acetylated in 0.25% acetic anhydride in 0.1 M TEA, and dehydrated through graded concentrations of alcohol (50, 70, 95, and 100 %). After vacuum drying for a minimum of 2 hours, 90 μl of hybridization mixture (10^7 cpm/ml) was spotted on each slide, sealed under a coverslip, and incubated at 60 °C overnight (~15-20 hours) in a slide warmer. Coverslips were then removed and the slides were rinsed in 4x standard saline citrate (SSC) at room temperature. Sections were digested by RNAse A (20 μg/ml, 37 °C, 30 minutes), rinsed in descending

concentrations of SSC (2x, 1x, 0.5xSSC), washed in 0.1xSSC for 30 minutes at 60 °C (1xSSC: 0.15 M NaCl, 15 mM trisodium citrate buffer, pH 7.0) and dehydrated through graded concentrations of alcohol. After being dried for 2 hours under the vacuum, the sections were exposed at 4 °C to X-ray film (Kodak) for 18-24 hours (depending of the gene), defatted in xylene, and dipped in NTB2 nuclear emulsion (Kodak; diluted 1:1 with distilled water). Slides were exposed for 8-16 days (NGFI-B and c-*fos* mRNA, 8 days; CRF hnRNA, 16 days), developed in D19 developer (Kodak) for 3.5 minutes at 14-15 °C, washed 15 seconds in water, and fixed in rapid fixer (Kodak) for 5 minutes. Thereafter, tissues were rinsed in running distilled water for 1-2 hours, counterstained with thionin (0.25 %), dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX.

2.4.5 cRNA probe synthesis and preparation

c-*fos* and NGFI-B cRNA probes were generated from the *Eco*R 1 fragment of rat c-*fos* cDNA (Dr. I Verma, The Salk Institute) and rat NGFI-B cDNA (Dr. J. Milbrandt; Milbrandt, 1988), subcloned into pBluescript SK-1 (Stratagene, La Jolla, CA) and linearized with *Sma* I and *Bam*H I respectively. pGem3 plasmid containing a pure CRF intronic piece was linearized with *Hin*d III (530 bp) to detect specifically CRF heteronuclear (hn) RNA (Dr. S. Watson, The University of Michigan, Ann Arbor; Herman et al., 1992). Radioactive cRNA copies were synthesized by incubation of 250 ng linearized plasmid in 6 mM $MgCl_2$, 40 mM Tris (pH 7.9), 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 0.2 mM ATP/GTP/CTP, [α-^{35}S]UTP, 40U RNAsin (Promega, Madison, WI) and 20U of either T7 (c-*fos* mRNA and CRF hnRNA antisense probes) or T3 (NGFI-B mRNA antisense probe) RNA polymerase for 60 minutes at 37 °C. Unincorporated nucleotides were removed by using ammonium-acetate method; 100 µl of DNAse solution (1 µl DNAse, 5 µl of 5 mg/ml tRNA, 94 µl of 10 mM Tris/10 mM $MgCl_2$) was added, and 10 minutes later an extraction was accomplished by using a phenol-chloroform solution. The cRNA was precipitated with 80 µl of 5M ammonium acetate and 500 µl of 100% ethanol for 20 minutes on dry ice. The pellet was washed with 500 µl 70% ethanol, dried, and resuspended in 100 µl of 10 mM Tris/1 mM EDTA. A concentration of 10^7 cpm probe was mixed into 1 ml of hybridization solution (500 µl formamide, 60 µl 5 M NaCl, 10 µl 1 M Tris [pH 8.0], 2 µl 0.5 M EDTA [pH 8.0], 50 µl 20x Denhart's solution, 200 µl 50% dextran sulfate, 50 µl 10 mg/ml tRNA, 10 µl 1M dithiothreitol (DTT), [118 µl Depc water - volume of probe used]). This solution was mixed and heated for 5 minutes at 65 °C before being spotted on slides.

2.4.6 Combination of immunocytochemistry with *in situ* hybridization

Immunocytochemistry (tyrosine hydroxylase (TH)-immunoreactive neurons) was combined with the *in situ* hybridization histochemistry protocol (c-*fos* mRNA) to determine the number of TH-activated cells after systemic treatment with LPS and to investigate the role of the PGs in the activation of catecholaminergic neurons during immune challenge, particularly in the NTS and the VLM. Every sixth tissue slice was processed by using the avidin-biotin amplification bridge method with peroxidase as a substrate. Briefly, slices were washed in sterile Depc-treated 50 mM potassium phosphate-buffered saline (KPBS) and incubated at 4 °C with TH antibody mixed in sterile KPBS, 0.4% Triton X-100, 0.25% heparin sodium salt USP (ICN Biomedicals Inc., Aurora, OH) and 1% bovine serum albumin (fraction V, Sigma, St. Louis, MO). Antisera raised in mouse against TH (Incstar Science, Technology and Research, Stillwater, MN, Cat # 22941) was used at a concentration of 1:5,000. Approximately 18 hours after incubation at 4 °C with the primary antibody (TH), the brain slices were rinsed in sterile KPBS and incubated with a mixture of KPBS + Triton X-100 + heparin + biotinylated goat antimouse IgG (1:1,500 dilution; Vector Laboratories, Burlingame, CA) for 90 minutes. Sections were then rinsed with KPBS and incubated at room temperature for 60 minutes with an avidin-biotin-peroxidase complex (Vectastain ABC elite kit, Vector Laboratories). The peroxidase complex was amplified by means of 10-minutes incubation with a 70-nM solution of biotin {sulfosuccinimydyl 6-(biotinamido) hexanoate, Pierce, Rockford, IL; # 21335}-tyramine HCl (4-hydroxyphenethylamine hydrochloride, Sigma T-2879)-H_2O_2 (0.01%), followed by a second incubation of 30 minutes with the ABC elite solution. After several rinses in sterile KPBS, the brain slices were reacted in a mixture containing sterile KPBS, the chromogen 3,3'-diaminobenzidine tetrahydrochloride (DAB, 0.05%), and 0.003 % hydrogen peroxide (H_2O_2).

Thereafter, tissues were rinsed in sterile KPBS, mounted onto poly-L-lysine-coated slides, desiccated under vacuum overnight, fixed in 4% PFA for 30 minutes, and digested by proteinase K (10 µg/ml in 100 mM tris HCl [pH 8.0] and 50 mM EDTA [pH 8.0], at 37 °C for 25 minutes). Prehybridization, hybridization, and posthybridization steps were performed as described above, although dehydration steps (alcohol 50, 70, 95, 100%), were shortened to avoid decoloration of TH cells (brown staining). After being dried for 2 hours under vacuum, sections were exposed at 4 °C to X-ray film (Kodak) overnight, defatted in xylene, and dipped in NTB2 nuclear emulsion (Kodak; diluted 1:1 with distilled water).

Slides were exposed for 8 days, developed in D19 developer (Kodak) for 3.5 minutes at 15 °C, and fixed in rapid fixer (Kodak) for 5 minutes. Thereafter, tissues were rinsed in running distilled water for 1 to 2 hours, rapidly dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX. The presence of c-*fos* transcript was evident as silver grains clearly visible in perikarya, and TH immunoreactivity within the cell cytoplasm which stained in brown.

2.4.7 Quantitative analysis

The c-*fos* mRNA, NGFI-B mRNA and CRF hnRNA signals revealed on dipped, NTB2 nuclear emulsion slides were analyzed and quantified (relative levels) with an Olympus Optical System (BX-50, BMax) coupled to a Macintosh computer (PowerPC 7100/66) and Image software (version 1.59 non-FPU, W. Rasband, NIH). The refraction density (R.D. in arbitrary units) of the hybridization signal was measured under dark-field illumination at a magnification of X10. Sections from the experimental and control animals were matched for rostro-caudal level. Because of the lack of basal expression of c-*fos* mRNA, NGFI-B mRNA and CRF hnRNA in the hypothalamic medial PVN and supraoptic nucleus (SON), the whole nuclei were digitized under brightfield illumination and then subjected to densitometric analysis under darkfield, yielding measurements of integrated R.D. (area of nucleus x average R.D.). The R.D. of each specific region was then corrected for the average background signal, which was determined by sampling cells immediately outside the cell group of interest (McCabe and Pfaff, 1989). The procedure used to quantify the relative levels of refraction density in arbitrary units on nuclear emulsion dipped slides is based on the work published by Ericsson and colleagues (1994).

Measurements of TH-ir cells and double-labeled cells (TH-ir neurons expressing c-*fos* mRNA) was accomplished at a magnification of X100 under brightfield illumination for the whole rostro-caudal NTS (A2/C2) and the VLM (A1/C1). For each rat, an average of 14 to 16 and 36 to 44 bilateral sections were quantified for the NTS and the VLM, respectively. Determination of the double-labeled cells was performed visually for each cell exhibiting clear brown cytoplasm (TH) and a number of silver grains (c-*fos* mRNA) within the cell body that was at least five times higher than background.

2.4.8 Statistical Analysis

Data from Figures 3, 4, 5, 7 and 9 were analyzed by a 2 x 2 analysis of variance (ANOVA), followed by a Bonferroni/Dunn test procedure as post-hoc comparisons for each time post-injection (Statview 4.01). Factors were identified as follows: ***intraperitoneal treatment***, which was composed of two levels (i.p. vehicle or i.p. LPS) and ***intravenous treatment***, which was also divided into two levels (i.v. control or i.v. indomethacin).

2.5 RESULTS

2.5.1 Induction of c-*fos* transcript throughout the brain of immune-challenged rats

Table 1 describes the qualitative analysis of hybridization signal on x-ray films for c-*fos* mRNA in the brain of male rats sacrificed 3 hours after i.p. vehicle or LPS administration for each dose used in this study. Note that c-*fos* was analyzed and presented in detail because it appears to be a more sensitive index of post-synaptic neuronal activation than NGFI-B throughout the brain. In fact, all structures that expressed NGFI-B transcript in response to LPS were also positive for c-*fos*, but not vise-a-versa; compared to the IEG c-*fos* mRNA, positive hybridization signal for NGFI-B transcript appeared limited to fewer LPS-responsive nuclei.

As previously described by several studies, positive hybridization signal for c-*fos* transcript was detected in different regions of the brain under basal conditions. Low to moderate basal signals were generally observed in the suprachiasmatic nucleus, various divisions of the thalamus including the anterodorsal (AD) and anteroventral (AV) nuclei, lateral geniculate complex (LGc), cochlear nucleus (CN), layer 2 of the piriform area (Pir) and multiple layers of the cerebral cortex including layer 2, 3, 4 and 6a. However, vehicle injection did not provoke expression of the IEG in nuclei that are generally stress-responsive, because experiences were accomplished in chronically-implanted animals in absolute quiet conditions. The endotoxin injected i.p. induced expression of c-*fos* in a wide variety of nuclei and areas, an effect dependent on the dose. Representative examples of c-*fos* distribution throughout the brain of animals administered i.p. with a increasing doses of the bacterial endotoxin LPS are depicted by the figure 1.

2.5.1.1 Forebrain

As recently reported (Rivest and Laflamme, 1995) and confirmed by this study, the highest dose of LPS (250 µg/100 g of b.w.), used here as a model of severe induction of the acute-phase response, caused a strong and prolonged (up to 6 hours after the injection) hybridization signal for c-*fos* mRNA in several but selective regions of the diencephalon: SON, PVN, paraventricular nucleus of the thalamus (PVT) and in the arcuate nucleus (ARC). A moderate to low signal was induced in several regions including subependymal zone (SEZ), bed nucleus of the stria terminalis (BnST), periventricular nucleus of the hypothalamus (PV), central nucleus of the amygdala (CeA) and in the dorsomedial nucleus of the hypothalamus (DMH) of rats injected with this high dose. Administration of the middle dose of endotoxin (25 µg/100 g of b.w.) produced a similar pattern in terms of intensity and expressing sites, although the signal for c-*fos* mRNA declined in all these structures 6 hours after injection.

In contrast, injection of the lowest dose (2.5 µg/100 g of b.w.) of LPS caused a modest expression of the IEG c-*fos* in very few regions of the forebrain. Low but significant expression of c-*fos* transcript was indeed detected in the SON and PVT at 3 hours, whereas no positive message was present at 6 hours post-injection.

2.5.1.2 Circumventricular organs (CVOs), meninges and ependymal layer

Injection of the highest dose of the bacterial endotoxin caused a profound expression of c-*fos* mRNA in the MPOA/OVLT and in the leptomeninges. Moreover, numerous other structures including the subfornical organ (SFO), the median eminence (ME) (principally in the internal lamina), the area postrema (AP), the choroid plexus (ChP) and the cuboidal ependymal lining cells of the ventricles exhibited moderate to low signal in rats injected with 250 µg of LPS/100 g b.w.. As mentioned earlier for the forebrain sites, mRNA encoding the IEG remained as long as 6 hours after the treatment.

Injection of a 25 µg of LPS/100 g b.w. activated the same brain regions with similar intensity excluding the SFO and the ME, which exhibited strong hybridization signal. In contrast to the high dose where the signal was still detectable 6 hours after treatment in most

of the above described areas, signal for the IEG largely declined 6 hours after administration of the middle dose except for the sensorial CVOs (OVLT, SFO, ME and AP) where strong hybridization signal was still detected.

Interestingly, the lowest dose of the endotoxin activated transcription of c-*fos* in few regions, including the CVOs the SON and the PVT. Indeed, the MPOA/OVLT, the SFO and the AP of rats injected i.p. with 2.5 µg of LPS/100 g b.w. displayed moderate to low hybridization signal at time 3 hours, whereas the message essentially vanished 6 hours after the administration.

2.5.1.3 Brainstem

Strong hybridization signal for c-*fos* mRNA was observed in the parabrachial nucleus (PB), throughout the entire NTS including the dorsal motor nucleus of the vagus (X), and the caudal ventrolateral medulla (cVLM) at the level of lateral reticular nucleus (LRN)/ambiguus nucleus (AMB) of rats injected with the highest and middle doses of the bacterial endotoxin. The laterodorsal tegmental nucleus (LDT), locus coeruleus (LC) and in some occasions the gracile nucleus (GR) exhibited a moderate to low signals in response to both high and moderate doses of LPS. The only noticeable difference in the effects of both doses (250 and 25 µg/100 g b.w.) of LPS on c-*fos* expression in brainstem regions was detected at 6 hours post-injection; animals treated with the highest dose exhibited persistent expression of the IEG, whereas the message was clearly diminished in rats treated with the dose of 25 µg of LPS/100 g b.w.. Note that no clear induction of both IEGs was detected in any regions of the brainstem of scarcely challenged rats (2.5 µg of LPS/100 g of b.w.) except for the AP as described before.

2.5.2 Effects of indomethacin in the brains of immune-challenged rats

As presented by the table 2, inhibition of PG synthesis had limited influence on LPS-induced c-*fos* expression throughout the rat brain. In fact, 3 hours after injection with the highest dose of endotoxin, the hybridization signal for the IEG was similar (in term of distribution and intensity) in the brain of rats pretreated or not with indomethacin. However, at time 6 hours post LPS injection, some structures exhibited a reduced c-*fos* mRNA signal following blockage of cyclooxygenase pathways. Indeed, a lower expression of the gene

encoding c-*fos* was observed in the PVN, SON and LRN/ABMd of severely immune-challenged rats receiving indomethacin in comparison to animals i.v. injected with the vehicle solution. Inhibition of PG was more efficient in attenuating c-*fos* expression in different brain nuclei of rats administered with the middle dose of LPS; the PV, PVN, LRN/AMBd and the leptomeninges displayed a notable attenuated hybridization signal for c-*fos* transcript 3 hours after systemic injections with both indomethacin and LPS. Representative examples of such phenomenon are depicted by the figure 2 which illustrates darkfield photomicrographs of right PVN hybridized with c-*fos* riboprobe and dipped in NTB2 nuclear emulsion to reveal the silver grains. Although significantly reduced, transcription of c-*fos* was not completely abolished by indomethacin 3 hours after injection with the dose of 25 μg of endotoxin/100 g b.w. (Figs. 2 and 3, middle panel) indicating a partial influence of PGs in mediating the effects of LPS on brain neuronal activation. Image analysis was performed on the SON and PVN, because both hypothalamic nuclei are extremely well defined structures avoiding therefore drawing inaccuracies between brains. Moreover, different neuropeptidergic cell groups located in these nuclei were found to be activated by systemic i.p. LPS (Rivest and Laflamme, 1995).

Indomethacin had a modest influence to prevent c-*fos* expression in few regions of the brain, such as the SON, even in animals treated with the dose of 25 μg LPS/100 g of b.w.. The c-*fos* signal intensity in the SON was similar in rats sacrificed 3 hours after injection of high and middle doses of LPS, whereas pretreatment with indomethacin did not significantly modulate such effect (Fig. 4, top and middle panels). On the other hand, inhibition of cyclooxygenase pathways largely abolished the increase in the average refraction density (R.D.) for c-*fos* mRNA 6 hours after treatment with the high dose of endotoxin (Fig. 4, top panel). Pretreatment with indomethacin did not modify the hybridization signal for c-*fos* mRNA in several responsive structures, including the SFO, PVT, CeA, ARC/ME, PB, LC, NTS, AP, and the choroid plexus of animals injected with a moderate dose of LPS (Table 2).

Figure 5 depicts the average O.D. of the hybridization signal for the IEG NGFI-B in the PVN and SON after i.p. administration of the middle dose of the bacterial endotoxin LPS (25 μg/100 g b.w.). As previously described for c-*fos* mRNA, the signal intensity was very strong 3 hours after a single i.p. administration with such a dose of LPS and largely vanished at 6 hours post-injection time. Inhibition of cyclooxygenase pathways significantly ($P < 0.05$) prevented LPS-induced expression of NGFI-B mRNA in these hypothalamic regions

at 3 hours post-injection time; a 1.7- and 0.8-fold increase was detected in rats receiving the endotoxin alone compared to those submitted to a treatment combining both i.v. indomethacin and i.p. LPS treatments, in the PVN (top panel) and SON (bottom panel) respectively.

2.5.3 Effects of indomethacin on LPS-induced CRF gene transcription in the endocrine hypothalamus

Because indomethacin is capable of attenuating PVN neuronal activation in response to systemic LPS (middle dose), it was of interest to investigate the effect of both treatments on neuroendocrine CRF gene transcription using intronic probe as a tool to detect the CRF primary transcript (CRF hnRNA). We have previously reported that i.p. LPS administration triggered CRF transcription selectively within the parvocellular division of the rat PVN (Rivest and Laflamme, 1995). Although not expressed under basal conditions, i.p. injection of 25 μg of LPS/100 g of b.w. caused a selective expression of CRF primary transcript within the parvocellular division of the PVN (Fig. 6), without inducing detectable signals in other areas (results not shown). Positive hybridization signal for CRF hnRNA was detected in the PVN at both times post LPS administration but was more intense at 3 than 6 hours following systemic injection with the endotoxin. Inhibition of cyclooxygenase pathways interrupted transcription of CRF hnRNA in animals injected with the middle dose of LPS, indicating that PGs can influence the expression of the gene encoding the neuropeptide directly responsible for the control of the HPA axis during the acute-phase response.

The average R.D. of the hybridization signal for CRF primary transcript in the PVN after i.p. administration of highest (top panel), intermediary (middle panel) or lowest (bottom panel) dose of the bacterial endotoxin is presented by the figure 7. In contrast to the results obtained with the middle dose of endotoxin, inhibition of PG synthesis did not interfere with the signal intensity of CRF hnRNA in the PVN of rats injected with 250 μg of LPS/100 g b.w. and sacrificed 3 hours after. However, as for c-*fos* mRNA, pretreatment with indomethacin tended to inhibit, at least in part, the expression of PVN CRF hnRNA at 6 hours post-injection. A low signal was detected in the endocrine hypothalamus 3 and 6 hours after treatment with the lowest dose of the bacterial endotoxin (Fig. 7, bottom panel).

2.5.4 Effect of indomethacin on the activation of catecholaminergic neurons during immune challenge

As previously described, systemic LPS injection provoked a robust induction of the IEG c-*fos* in the NTS and the VLM, suggesting that noradrenergic (A1, A2) and adrenergic (C1, C2) pathways arising from these two brainstem nuclei could be activated during acute-phase response. To investigate such possibility, the percentage of TH-immunoreactive (ir) cells displaying c-*fos* mRNA in the NTS and VLM was determined in combining immunocytochemistry to *in situ* hybridization histochemistry on the same brain sections. Measurements of TH-ir cells and double-labeled cells (TH-ir neurons expressing c-*fos* mRNA) was accomplished at a magnification of X100 under brightfield illumination on the entire NTS (A2/C2) and the full rostro-caudal extent of the VLM (A1/C1). Figure 8 depicts high magnification photomicrographs of TH-ir neurons expressing c-*fos* transcript in the NTS (top panels) and the VLM (bottom panels) of rats sacrificed 3 hours after the injection of the middle dose of LPS. Darkfield photomicrographs illustrate the exact location from which high magnification brightfield photomicrographs of the NTS (top panels) and the VLM (bottom panels) were taken. In the NTS, the number of TH-ir cells displaying c-*fos* mRNA (filled arrowheads) was almost similar in animals treated or not with indomethacin before receiving the bacterial endotoxin at a dose of 25 µg/100 g b.w.. In the VLM of immune-challenged rats, agglomerations of silver grains delineating c-*fos* mRNA positive cells were largely co-localized within TH-ir neurons (filled arrowheads), whereas inhibition of the cyclooxygenase pathways largely prevented expression of the IEG c-*fos* within TH-ir cells located in this brainstem region.

23.89% ± 4.67 and 5.25% ± 1.80 of the catecholaminergic neurons of the NTS were positive for c-*fos* mRNA 3 and 6 hours after injection of the intermediary dose of LPS, respectively (Fig. 9, top panel). Interestingly, pretreatment with indomethacin did not significantly interfere with the number of double-labeled cells of the NTS 3 hours after LPS administration. However, Bonferroni/Dunn post-hoc test revealed significant differences between vehicle- and LPS-injected rats sacrificed 6 hours after immunogenic challenge, but not between both indomethacin-treated groups. In the VLM, the number of TH-ir cells expressing c-*fos* mRNA peaked 3 hours after a single i.p. administration of 25 µg of LPS/100 g b.w. (22.58% ± 6.73), whereas this value dropped to 3.42% ± 0.74, 6 hours following the i.p. injection. In contrast to the results obtained previously for the NTS, inhibition of cyclooxygenase pathways significantly ($P < 0.05$) attenuated the number of

activated TH cells in the VLM (Fig. 9, bottom panel); the number of double-labeled cells fell to 6.47% ± 3.20 at 3 hours post-injection and was almost undetectable in this brainstem region (0.25% ± 0.10) 6 hours after LPS and indomethacin combined treatments.

2.6 DISCUSSION

The present study demonstrates that the three doses of the bacterial endotoxin LPS have different effects on the brain cellular activity as illustrated by the selective and transient expression of c-*fos* transcript. Systemic administration of the highest dose of LPS leads to a robust, widespread and prolonged induction of both c-*fos* and NGFI-B mRNAs throughout the brain. The middle dose of endotoxin causes similar effects at 3 hours post-injection, but hybridization signal for c-*fos* mRNA vanished rapidly 3 hours after except for the sensorial CVOs where strong signal was still detectable. On the other hand, injection of the lowest dose selectively triggered c-*fos* expression within the CVOs at 3 hours, whereas the message returned to basal expression at time 6 hours. Pretreatment with indomethacin did not significantly influence c-*fos* transcription in rats challenged with the highest dose of LPS at 3 hours post-injection. Inhibition of PG formation was more effective in interrupting the neuronal activation in animals injected with the moderate dose of LPS, although the influence depended on the structures and the groups of activated cells. Indeed, PG inhibition significantly altered LPS-induced c-*fos* mRNA expression in the PV, PVN, cVLM (LRN/AMBd) and the leptomeninges. In the hypothalamic PVN, inhibition of both c-*fos* and NGFI-B transcripts by indomethacin was also associated to a diminished expression of CRF hnRNA. In fact, induction of CRF primary transcript by 25 µg of LPS/100 g b.w. was selective to the PVN and completely blocked by pretreatment with indomethacin. Moreover, a large number of TH-ir neurons of the VLM (A1/C1) and NTS (A2/C2) were positive for c-*fos* mRNA in immune-challenged rats, an effect essentially abolished by indomethacin in the VLM, but not the NTS. The role of PGs as mediators of the stimulatory influence of the acute-phase response seems therefore to depend on the severity of the systemic stressful situation, the brain regions and cell groups as well as the activated target genes.

As presented by table 1 and figure 1, the effects of systemic injection of LPS on c-*fos* mRNA expression in the brain of male rats depended on the dose used during the experimentation. Injection of the low dose triggered transcription of c-*fos* in very selective sites, including the sensorial CVOs at 3 hours post-injection. The exact mechanisms involved in the influence of low dose of the bacterial endotoxin on neuronal activity are

unknown and whether production of specific cytokines by LPS is responsible for induction of c-*fos* mRNA within the CVOs remains to be established. Berkenbosch et al. (1991) have reported that similar low dose of LPS caused an increase in plasma IL-6 levels without affecting those of circulating IL-1. Another group has also reported that circulating IL-1ß was hardly noticeable until 8 hours after the administration of a low dose of the endotoxin, whereas a significant increase in the concentrations of IL-6 was detected (Givalois et al., 1994). Moreover, we have recently reported that systemic i.v. injection of IL-6 induced a robust expression of c-*fos* in most of the sensorial CVOs, including OVLT, SFO, ME and AP (Vallières et al., 1997). It is therefore tempting to speculate that IL-6 may mediate the influence of a low dose of LPS on the activity of CVOs during a modest activation of the acute-phase response.

Systemic i.p. administration of the highest and middle doses of the bacterial endotoxin LPS induced a widespread neuronal activity throughout the rat brain. In fact, similar patterns of intensity for c-*fos* mRNA signal were observed at 3 hours post-injection in various nuclei of the forebrain, CVOs and brainstem regions of animals treated with both doses of LPS. Circulating IL-1 can be rapidly detected at similar doses of LPS (Berkenbosch et al., 1991) and i.v. injected IL-1 mimics quite well the endotoxin-induced distribution of c-*fos* mRNA throughout the brain, except for most of the CVOs (Ericsson et al., 1994; Rivest and Laflamme, 1995). Whether circulating IL-1 is responsible to trigger brain cellular activity in animals treated with such doses of the bacterial endotoxin has yet to be confirmed, but is a likely mechanism of action. It is also possible that IL-1 and IL-6 act in parallel and at different levels of the brain to stimulate neuronal activity during acute-phase response.

Animals injected with 250 µg of LPS/100 g b.w. and sacrificed 6 hours after still exhibited strong hybridization signal for the IEGs throughout the brain, whereas at that time the message returned to basal expression in rats treated with 25 µg/100 g b.w. except for the sensorial CVOs. This phenomenon could be explained in part by the dose/time-dependent influence of LPS on circulating cytokines (Berkenbosch et al., 1991). Moreover, *in vivo* macrophage depletion is able to prevent the increase in plasma ACTH and corticosterone levels in animals injected with a low dose of LPS, but not following high dose of the endotoxin (Derijk et al., 1991). These results are in agreement with the hypothesis that macrophage-independent mechanisms are involved in the activation of HPA axis during severe immune-challenge (Derijk et al., 1991). It is possible that many other pathways

related to changes in blood pressure and osmolarity, pain, oxygen consumption, fever and energy metabolism participate to restore the homeostasis in response to profound emergency situations, such as those provoked by the high dose of endotoxin. It is worth reminding here that rats receiving the high dose of LPS displayed signs of sickness (covering themselves and immobility, shivering, piloerection, while some had diarrhea), whereas barely any visible sickness symptoms were observed in animals administered with the middle dose. However, c-*fos* was still highly expressed throughout the brain of this latter group, which also exhibited activation of the sensorial CVOs up to 6 hours post LPS injection. These results would once again support the hypothesis of a dose- and time-dependent influence of the endotoxin on the production of selective cytokines having the ability to produce these events.

Pretreatment with indomethacin did not prevent transcription of c-*fos* in the brains of rats challenged with the highest dose of LPS at 3 hours post-injection. The possibility that the dose of indomethacin used in the present study did not completely block the PG formation in LPS-treated rats has to be considered, although this dose has been shown to be effective in preventing the effect of LPS and IL-1 on various neuroendocrine functions. Indeed, the HPA axis response to LPS and IL-1 has been reported to be inhibited by systemic pretreatment with similar or lower doses of indomethacin (Katsuura et al., 1988; McCoy et al., 1994; Rivier and Vale, 1991; Watanabe et al., 1990). Blockage of PG production with the same dose of the cyclooxygenase inhibitor also prevented the IL-1-induced alteration of neuroendocrine LHRH system and plasma LH levels in female rats (Rivest and Rivier, 1993). Therefore, our results may suggest that the role of PGs in mediating the stimulatory influence of immune challenge on the transcription of the IEGs throughout the brain depends on the severity of this systemic stressful situation.

On the other hand, indomethacin significantly abolished the expression of both IEG c-*fos* and NGFI-B transcripts in the neuroendocrine hypothalamic PVN 3 hours after injection of 25 μg of LPS/100 g b.w.. These results are in agreement with the known influence of eicosanoid cyclooxygenase pathways in mediating several neuroendocrine responses to immune challenge and acute exposure to cytokines. Indeed, the effect of cytokines on the release of CRF, vasopressin (AVP), oxytocin (OT) and the activity of the HPA axis can be antagonized by drugs blocking PG synthesis (Bernardini et al., 1990; Navarra et al., 1991; Yasin et al., 1994). In the hypothalamic PVN, the influence of the endotoxin (middle dose) on the transcription of neuroendocrine CRF (this study) and its type 1 receptor (Lacroix and Rivest, 1996) is inhibited by the administration of indomethacin. Whether the action of PGs

take place directly within the PVN or at the level of the structures innervating the endocrine hypothalamus remains an open question. It has been reported that intravenous administration of IL-1ß provokes sharp increase in the levels of PGE_2 within the PVN (Watanobe and Takebe, 1994), which provides evidence that local PVN production of the PG may participate in the regulation of neuroendocrine functions, such as the HPA axis. However, this might be a simplistic view of this complicated issue because PGE_2 has also be found to be increased in many other structures and perfusion of indomethacin within the PVN failed to prevent IL-1-induced activation of the HPA axis (Komaki et al., 1992). Systemic LPS and IL-1 administrations have recently been shown to stimulate the transcription of the gene encoding prostaglandin G/H synthase 2 (COX-2, the limiting enzyme for the central PG production) throughout the entire brain microvasculatures (Cao et al., 1995; 1996). Moreover, central i.c.v. injection of PGE_2 caused expression of c-*fos* mRNA in numerous structures throughout the brain including the PVN (Lacroix et al., 1996), although neurons of this hypothalamic region do not seem to express the genes that encode PGE_2 receptors (Ericsson et al., 1995).

Synthesis of PGs within the MPOA/OVLT could be a determinant mechanism through which immune-related factors trigger the transcription of c-*fos* in the hypothalamic PVN and other hypothalamic nuclei. High density of PGE_2 binding sites (Matsumura et al., 1992; 1990) and positive hybridization signal for the mRNA encoding EP3 PGE_2 receptor (Ericsson et al., 1995) have been found in the OVLT/MPOA and this region has direct neuronal connection with the endocrine PVN (Sawchenko and Swanson, 1983). Stimulation of the rat POA increases the firing rates of neurons located in the PVN and raises plasma corticosterone levels (Saphier and Feldman, 1986), whereas microinjection of PGE_2 in the POA induces Fos in parvocellular division of the hypothalamic PVN (Scammell et al., 1996). Systemic injection with bacterial LPS causes PG production in the OVLT/preoptic area (Ueno et al., 1982) and intravenous administration of IL-1ß raises PGE_2 levels in these structures (Komaki et al., 1992). In addition, microinjection of PGE_2 within the OVLT/MPOA increases plasma ACTH release, while infusion of indomethacin or a PGE antagonist into this area is able to significantly prevent the stimulatory influence of i.v. IL-1ß administration on the HPA axis (Katsuura et al., 1990). Induction of c-*fos* mRNA in the MPOA/OVLT, transcriptional activation of neuroendocrine CRF (Lacroix et al., 1996) and release of ACTH and corticosterone (Rassnick et al., 1995) following i.c.v. PGE_2 injection provide additional evidences leading to believe that OVLT/MPOA PGs may play an important role in mediating the information received from circulating cytokines to the endocrine hypothalamus.

Expression of c-*fos* mRNA was detected in the VLM after systemic injections of IL-1 (Ericsson et al., 1994), LPS (Rivest and Laflamme, 1995) and central treatment with PGE_2 (Lacroix et al., 1996). Here we demonstrate that a high concentration of these cells are TH-ir neurons and that indomethacin essentially abolished A1/C1 activation in animals administered with the middle dose of LPS used in this study. These results suggest that PGs participate in the activation of A1/C1 catecholaminergic neurons in response to immunogenic challenges. Since rat brain endothelial cells have been shown to express the type 1 IL-1 receptor (IL-1R1) (Ericsson et al., 1995; Van Dam et al., 1996), it is possible that such circulating cytokine acts on its receptor at the level of medullary microvasculatures to stimulate the synthesis PGs in response to LPS. As mentioned, injection of LPS (Cao et al., 1995) and IL-1ß (Cao et al., 1996) induces COX-2 mRNA within the brain microvasculatures (most likely in endothelial and/or perivascular microglial-associated cells), a phenomenon particularly robust in the VLM (Lacroix and Rivest, unpublished data). The fact that the mRNA encoding the EP3 PGE_2 receptor subtype is expressed over neurons of the VLM (Ericsson et al., 1995), and that A1/C1 neurons that are responsive to i.v. IL-1ß project to the PVN (Ericsson et al., 1994) indicate an additional circuitry through which PGs could modulate neuroendocrine functions during immunogenic challenges.

VLM and NTS are two interconnected structures, whereas this latter (A2/C2) is known to provide the largest NA input to the PVN (Cunningham et al., 1990; Cunningham and Sawchenko, 1988; Sawchenko and Swanson, 1982; Swanson et al., 1983). Interestingly, disruption of afferent medullary catecholaminergic pathways has been shown to significantly prevent the increase in CRF mRNA levels in the PVN of IL-1ß-injected rats (Ericsson et al., 1994). It has also been demonstrated that NTS displayed the highest density of PGE_2 binding sites (Matsumura et al., 1992) and PG facilitates excitatory synaptic transmission in voltage-clamped neurons in rat NTS slices (Seriyama et al., 1995). The role of PGs in the activation of A2/C2 group of cells in response to LPS remains however uncertain; in contrast to the VLM, indomethacin did not significantly alter the number of TH-ir cells positive for c-*fos* mRNA in the NTS 3 hours after the i.p. treatment with a moderate dose of the endotoxin, suggesting a PG-independent mechanism. Interestingly, the involvement of the vagus nerve in mediating the effect of systemic (i.p.) LPS on Fos-ir in the NTS and the PVN has been demonstrated (Wan et al., 1994). On the other hand, neurons of the NTS receive efferent projections from the AP (Cunningham et al., 1994), a circumventricular organ devoid of the blood-brain-barrier. Circulating cytokines (such as IL-1) produced during the acute-phase response could therefore reach their receptors (IL-1R1)

associated with either neurons and/or perivascular elements of the AP (Ericsson et al., 1995) and indirectly activate, without the implication of PGs, catecholaminergic neurons of the NTS. In accordance with this hypothesis is the elegant study performed by Lee and Herkenham (1996) demonstrating the essential role played by the AP in the effects of i.v. IL-1ß on the endocrine hypothalamus. More precisely, AP destruction was able to prevent the IL-1-induced activation of the HPA axis and transcription of c-*fos* in the NTS and PVN, but not in any other regions (Lee and Herkenham, 1996).

In conclusion, systemic i.p. injection of a low dose of endotoxin (2.5 µg/100 g b.w.) induced expression of the IEGs quite selectively within the sensorial CVOs, whereas 25 and 250 µg of the bacterial LPS/100 g b.w. caused strong transcription of c-*fos* mRNA in numerous structures throughout the rat brain. Administration of the eicosanoid cyclooxygenase inhibitor indomethacin attenuated IEG induction in the PV, PVN, cVLM (LRN/AMBd) and the leptomeninges of rats treated with 25 µg/100 g b.w., but not in animals receiving the highest dose of LPS i.p. In a similar manner, pretreatment with indomethacin was significantly effective to prevent the transcription of neuroendocrine CRF only in rats treated with the middle dose of LPS (25 µg/100 g b.w.). Finally, PG production in response to such a dose of endotoxin seems largely responsible for the activation of A1/C1, but not A2/C2 groups of brainstem catecholaminergic neurons. These results indicate that the role played by PGs as mediators of systemic immune response, is partial and dependent of various factors, such as the severity of the stressful situation, the brain regions and cell groups as well as the activated target genes.

2.7 ACKNOWLEDGMENTS

This research was supported by the Medical Research Council (MRC) of Canada. Serge Rivest is a Medical Research Council Scholar. Steve Lacroix holds a Studentship from the National Sciences and Engineering Research Council of Canada (NSERC). We thank Dr. I. Verma (The Salk Institute, La Jolla, CA) for the generous gift of the rat c-*fos* cDNA, Dr. J. Milbrandt (Washington University, St. Louis, Missouri) for the rat NGFI-B cDNA and Dr. S. Watson (The University of Michigan, Ann Arbor, MI) for the pGem3 plasmid containing a pure CRF intronic piece. The authors also thank Ms. Nathalie Laflamme for invaluable technical assistance.

2.8 REFERENCES

Andersson, J., S. Nagy, L. Björk, J. Abrams, S. Holm, and U. Andersson (1992) Bacterial toxin-induced cytokine production studied at the single-cell level. Immunological Rev. *127*:69-96.

Berkenbosch, F., D.A.W. Wolvers, and R. DeRijk (1991) Neuroendocrine and immunological mechanisms in stress-induced immunomodulation. J. Steroid Biochem. Biol. *40*:639-647.

Bernardini, R., A.E. Calogero, G. Mauceri, and G. Chrousos (1990) Rat hypothalamic corticotropin-releasing hormone secretion in vitro is stimulated by interleukin-1 in an eicosanoid-dependent manner. Life Sci. *47*:1601-1607.

Cao, C., K. Matsumura, K. Yamagata, and Y. Watanabe (1995) Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in the rat brain; its possible role in the febrile response. Brain Res. *697*:187-196.

Cao, C.Y., K. Matsumura, K. Yamagata, and Y. Watanabe (1996) Endothelial-cells of the rat-brain vasculature express cyclooxygenase-2 messenger-RNA in response to systemic interleukin-1: A possible site of prostaglandin synthesis responsible for fever. Brain Res. *733*:263-272.

Chuluyan, H.E., D. Saphier, W.M. Rohn, and A.J. Dunn (1992) Noradrenergic innervation of the hypothalamus participates in adrenocortical responses to interleukin-1. Neuroendocrinology *56*:106-111.

Cominelli, F., C.C. Nast, C.A. Dinarello, P. Gentilini, and R.D. Zipser (1989) Regulation of eicosanoid production in rabbit colon by interleukin-1. Gastroenterology *97*:1400-1405.

Cunningham, E.T., M.C. Bohn, and P.E. Sawchenko (1990) Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J. Comp. Neurol. *292*:651-667.

Cunningham, E.T., R.R. Miselis, and P.E. Sawchenko (1994) The relationship of efferent projections from the area postrema to vagal motor and brain stem catecholamine-containing cell groups: An axonal transport and immunohistochemical study in the rat. Neuroscience *58*:635-648.

Cunningham, E.T., and P.E. Sawchenko (1988) Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. J. Comp. Neurol. *274*:60-76.

Derijk, R., N. Van Rooijen, F.J.H. Tilders, H.O. Besedovsky, A. Del Rey, and F. Berkenbosch (1991) Selective depletion of macrophages prevent pituitary-adrenal activation in response to subpyrogenic, but not to pyrogenic, doses of the bacterial endotoxin in rats. Endocrinology *129*:330-338.

Dinarello, C.A. (1989) Interleukin-1 and its biologically related cytokines. Adv. Immunol. *44*:153-161.

Elmquist, J.K., and C.B. Saper (1996) Activation of neurons projecting to the paraventricular nucleus by intravenous lipopolysaccharide. J. Comp. Neurol. *374*:315-331.

Elmquist, J.K., T.E. Scammell, C.D. Jacobson, and C.B. Saper (1996) Distribution of Fos-like immunoreactivity in the rat-brain following intravenous lipopolysaccharide administration. J. Comp. Neurol. *371*:85-103.

Ericsson, A., M. Ek, and N. Lindefors (1995) Distribution of prostaglandins E2 receptor (EP3 subtype) mRNA containing cells in the rat central nervous system. Soc. Neurosci. Abstr. *21*:98.

Ericsson, A., K.J. Kovacs, and P.E. Sawchenko (1994) A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. J. Neurosci. *14*:897-913.

Ericsson, A., C. Liu, R.P. Hart, and P.E. Sawchenko (1995) Type 1 interleukin-1 receptor in the rat brain: distribution, regulation, and relationship to sites of IL-1-induced cellular activation. J. Comp. Neurol. *361*:681-698.

Givalois, L., J. Dornand, M. Mekaouche, M.D. Solier, A.F. Bristow, G. Ixart, P. Siaud, I. Assenmacher, and G. Barbanel (1994) The temporal cascade of plasma level surges in ACTH, corticosterone and cytokines in endotoxin-challenged rats. Am. J. Physiol. *266*:R164-R170.

Hare, A.S., G. Clarke, and S. Tolchard (1995) Bacterial lipopolysaccharide-induced changes in Fos protein expression in the rat brain: Correlation with thermoregulatory changes and plasma corticosterone. J. Neuroendocrinol. *7*:791-799.

Herman, J.P., M. Schafer, R.C. Thompson, and S.J. Watson (1992) Rapid regulation of corticotropin-releasing hormone gene transcription *in vivo*. Mol. Endocrinol. *6*:1061-1069.

Higgins, G.A., and J.A. Olschowka (1991) Induction of interleukin-1ß mRNA in adult rat brain. Mol. Brain Res. *9*:143-148.

Hughes, J.H., R.A. Easom, B.A. Wolf, J. Turk, and M.L. McDaniel (1989) Interleukin-1-induced prostaglandin E2 accumulation by isolated pancreatic islets. Diabetes *38*:1251-1257.

Katsuura, G., A. Arimura, K. Koves, and P.E. Gottschall (1990) Involvement of organum vasculosum of the lamina terminalis and preoptic area in interleukin-1ß-induced ACTH release. Am. J. Physiol. *258*:E163-E171.

Katsuura, G., P.E. Gottschall, R.R. Dahl, and A. Arimura (1988) Adrenocorticotropin release induced by intracerebroventricular injection of recombinant human interleukin-1 in rats: Possible involvement of prostaglandin. Endocrinology *122*:1773-1779.

Kerr, J.S., T.M. Stevens, G.L. Davis, J.A. Mclaughlin, and R.R. Harris (1989) Effects of recombinant interleukin-1 beta on phospholipase A2 mRNA levels, and eicosanoid formation in rabbit chondrocytes. Biochem. Biophys. Res. Com. *165*:1079-1084.

Koenig, J.I. (1991) Presence of cytokines in the hypothalamic-pituitary axis. Prog. NeuroEndocrImmunol. *4*:143-153.

Kohan, D.E. (1989) Interleukin-1 regulation of prostaglandin E2 synthesis by the papillary collecting duct. J. Lab. Clin. Med. *114*:717-723.

Komaki, G., A. Arimura, and K. Koves (1992) Effect of intravenous injection of IL-1ß on PGE2 levels in several brain areas as determined by microdialysis. Am. J. Physiol. *262*:E246-E251.

Lacroix, S., and S. Rivest (1996) Role of cyclo-oxygenase pathways in the stimulatory influence of immune challenge in the transcription of a specific CRF receptor subtype in the rat brain. J. Chem. Neuro. *10*:53-71.

Lacroix, S., L. Vallières, and S. Rivest (1996) C-*fos* mRNA pattern and corticotropin-releasing factor neuronal activity throughout the brain of rats injected centrally with a prostaglandin of E2 type. J. Neuroimmunol. *70*:163-179.

Lee, H.Y., and M. Herkenham (1996) Area postrema removal abolishes stimulatory effects of intravenous interleukin-1ß on HPA axis activity and c-fos mRNA in the hypothalamic paraventricular nucleus. Soc. Neurosci. Abstr. *22*:87.

Li, H.-Y., A. Ericsson, and P. Sawchenko (1996) Distinct mechanisms underlie activation of hypothalamic neurosecretory neurons and their medullary catecholaminergic afferents in categorically different stress paradigms. Proc. Natl. Acad. Sci. USA *93*:2359-2364.

Lyson, K., and S.M. McCann (1992) Involvement of arachidonic acid cascade pathways in interleukin-6-stimulated corticotropin-releasing factor release in vitro. Neuroendocrinology *55*:708-713.

Matsumura, K., Y. Watanabe, K. Imai-Matsumura, M. Connolly, Y. Koyama, H. Onoe, and Y. Watanabe (1992) Mapping of prostaglandin E2 binding sites in rat brain using quantitative autoradiography. Brain Res. *581*:292-298.

Matsumura, K., Y. Watanabe, H. Onoe, Y. Watanabe, and O. Hayaishi (1990) High density of prostaglandin E2 binding sites in the anterior wall of the 3rd ventricle: a possible site of its hyperthermic action. Brain Res. *533*:147-151.

McCabe, J.T., and D.W. Pfaff (1989) *In situ* hybridization: a methodological guide. Methods Neurosci. *1*:98-117.

McCoy, J.G., S.G. Matta, and B.M. Sharp (1994) Prostaglandins mediate the ACTH response to interleukin-1-beta instilled into the hypothalamic median eminence. Neuroendocrinology *60*:426-435.

Milbrandt, J. (1988) Nerve growth factor induces a gene homologous to the glucocorticoid receptor gene. Neuron *1*:183-188.

Nappi, R.E., M.J. Bonneau, and S. Rivest (1997) C-*fos* and CRF gene transcription in the brains of endotoxin-challenged cycling female rats: A possible relevance for neuroendocrine-immunological sexual dimorphism. Neuroendocrinology *65*:29-46.

Nathan, C.F. (1987) Secretory products of macrophages. J. Clin. Invest. *79*:319-326.

Navarra, P., S. Tsagarakis, M.S. Faria, L.H. Rees, G.M. Besser, and A.B. Grossman (1991) Interleukin-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. Endocrinology *128*:37-44.

Rabin, B.S., J.E. Cunnick, and D.T. Lysle (1990) Stress-induced alteration of immune function. Prog. NeuroEndocrinImmunol. *3*:116-125.

Rassnick, S., D.H. Zhou, and B.S. Rabin (1995) Central administration of prostaglandin E (2) suppresses in vitro cellular immune responses. Am. J. Physiol. *R92-R97*:

Rivest, S. (1995) Molecular mechanisms and neural pathways mediating the influence of interleukin-1 on the activity of neuroendocrine CRF motoneurons in the rat. Int. J. Devl. Neurosci. *13*:135-146.

Rivest, S., and N. Laflamme (1995) Neuronal activity and neuropeptide gene transcription in the brain of immune-challenged rats. J. Neuroendocrinol. *7*:501-525.

Rivest, S., N. Laflamme, and R.E. Nappi (1995) Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. J. Neurosci. *15*:2680-2695.

Rivest, S., and C. Rivier (1993) Centrally injected interleukin-1ß inhibits the hypothalamic LHRH secretion and circulating LH levels via prostaglandins in rats. J. Neuroendocrinol. *5*:445-450.

Rivest, S., and C. Rivier (1995) The role of CRF and interleukin-1 in the regulation of neurons controlling reproductive functions. Endocrine Rev. *16*:177-199.

Rivier, C. (1993) Neuroendocrine effects of cytokines in the rat. Rev. Neurosci. *4*:223-237.

Rivier, C., and S. Rivest (1993) Mechanisms mediating the effects of cytokines in neuroendocrine functions in the rat. In: Corticotropin-releasing factor, Ciba Foundation Symposium 172 (Chadwick DJ, Marsh J and Ackrill K, ed.) pp 204-225. Chichester: John Wiley & Sons Ltd.

Rivier, C., and W. Vale (1991) Stimulatory effect of interleukin-1 on ACTH secretion in the rat: is it modulated by prostaglandins? Endocrinology *129*:384-388.

Saphier, D., and S. Feldman (1986) Effects of stimulation of the preoptic area on hypothalamic paraventricular nucleus unit activity and corticosterone secretion in freely moving rats. Neuroendocrinology *42*:167-173.

Sawchenko, P.E., and L.W. Swanson (1982) The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. Brain Res. Rev. *4*:275-325.

Sawchenko, P.E., and L.W. Swanson (1983) The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. J. Comp. Neurol. *218*:121-144.

Scammell, T.E., J.K. Elmquist, J.D. Griffin, and C.B. Saper (1996) Ventromedial preoptic prostaglandin E2 activates fever-producing autonomic pathways. J. Neurosci. *16*:6246-6254.

Seriyama, N., S. Mizuta, A. Hori, and S. Kobayashi (1995) Prostaglandin E(2) facilitates excitatory synaptic transmission in the nucleus-tractus-solitarii of rats. Neurosc. Let. *188*:101-104.

Simmons, D.M., J.L. Arriza, and L.W. Swanson (1989) A complete protocol for *in situ* hybridization of messenger RNAs in brain and other tissus with radio-labeled single-stranded RNA probes. J. Histotechnol. *12*:169-181.

Smith, T., A.K. Hewson, L. Quarrie, J.P. Leonard, and M.L. Cuzner (1994) Hypothalamic PGE2 and cAMP production and adrenocortical activation following intraperitoneal endotoxin injection: *in vivo* microdialysis studies in Lewis and Fischer rats. Neuroendocrinology *59*:396-405.

Solomon, G.F. (1969) Stress and antibody response in rats. Int. Arch. Allergy *35*:97-108.

Swanson, L.W., P.E. Sawchenko, J. Rivier, and W.W. Vale (1983) Organization of ovine corticotropin releasing factor (CRF)-immunoactive cells and fibers in the rat brain: An immunohistochemical study. Neuroendocrinology *36*:165-186.

Ueno, R., S. Narumiya, T. Ogorochi, T. Nakayama, Y. Ishikawa, and O. Hayaishi (1982) Role of prostaglandin D2 in the hypothermia of rats caused by bacterial lipopolysaccharide. Proc. Natl. Acad. Sci. U.S.A. *79*:6093-6097.

Vallières, L., S. Lacroix, and S. Rivest (1997) Influence of interleukin-6 on neural activity and transcription of the gene encoding corticotropin-releasing factor in the rat brain: an effect depending upon the route of administration. Eur. J. Neurosci. *9*:1461-1472

Van Dam, A.M., H.E. DeVries, J. Kuiper, F.J. Zijlstra, A.G. DeBoer, F.J.H. Tilders, and F. Berkenbosch (1996) Interleukin-1 receptors on rat brain endothelial cells: a role in neuroimmune interaction? FASEB J. *10*:351-356.

Wan, W., L. Wetmore, C.M. Sorensen, A.H. Greenberg, and D.M. Nance (1994) Neural and biochemical mediators of endotoxin and stress-induced c-*fos* expression in the rat brain. Brain Res. Bull. *34*:7-14.

Watanabe, T., A. Morimoto, Y. Sakata, and N. Murakami (1990) ACTH response induced by interleukine-1 is mediated by CRF secretion stimulated by hypothalamic PGE. Experientia *46*:481-484.

Watanobe, H., and K. Takebe (1994) Effects of intravenous administration of interleukin-1-beta on the release of prostaglandin E2, corticotropin-releasing factor, and arginine vasopressin in several hypothalamic areas of freely moving rats: estimation by push-pull perfusion. Neuroendocrinology *60*:8-15.

Yasin, S.A., A. Costa, M.L. Forsling, and A. Grossman (1994) Interleukin-1ß and interleukin-6 stimulate neurohypophysial hormone release in vitro. J. Neuroendocrinol. *6*:179-184.

2.9 ABBREVIATIONS

A1-2/C1-2	catecholamine-synthesizing groups of neurons
ACTH	adrenocorticotropin
AMB	ambiguus nucleus
AP	area postrema
ARC	arcuate nucleus
AVP	vasopressin
BnST	bed nucleus of the stria terminalis
b.w.	body weight
CeA	central nucleus of the amygdala
ChP	choroid plexus
CN	cochlear nucleus
COX-2	prostaglandin G/H synthase 2
CRF	corticotropin-releasing factor
cVLM	caudal ventrolateral medulla
CVOs	circumventricular organs
DMH	dorsomedial nucleus of the hypothalamus
EP3	PGE_2 receptor subtypes
Epd	ependymal lining cells of lateral ventricle
GR	gracile nucleus
hnRNA	heteronuclear RNA (primary transcript)
HPA	hypothalamic-pituitary-adrenal axis
i.c.v.	intracerebroventricular
IEGs	immediate-*early* genes
IL-1	interleukin-1
IL-6	interleukin-6

IL-1R1	type 1 IL-1 receptor
IND	indomethacin
i.p.	intraperitoneal
ir	immunoreactive
i.v.	intravenous
LC	locus coeruleus
LDT	laterodorsal tegmental nucleus
LGc	lateral geniculate complex
LHRH	luteinizing hormone-releasing hormone
LPS	lipopolysaccharide
LRN	lateral reticular nucleus
ME	median eminence
Men	meninges (lepto)
MPOA	medial preoptic area
NTS	nucleus of the solitary tract
NTSc	nucleus of the solitary tract, caudal part
O.D.	optical density
OT	oxytocin
OVLT	organum vasculosum of the lamina terminalis
PB	parabrachial nucleus
PGE_2	prostaglandins of E_2 type
PGs	prostaglandins
Pir	piriform area
PV	periventricular nucleus of the hypothalamus
PVN	paraventricular nucleus of the hypothalamus
PVT	paraventricular nucleus of the thalamus
SEZ	subependymal zone

SFO	subfornical organ
SON	supraoptic nucleus of the hypothalamus
TH	tyrosine hydroxylase
TNF-α	tumor necrosis factor-alpha
VLM	ventrolateral medulla

Table 1:

Qualitative analysis of hybridization signal for C-*fos* mRNA in the brain of immune-challenged rats (3 hours after i.p. lipopolysaccharide administration).

Region \ Dose of LPS	250µg/ 100 g b.w.	25 µg/ 100 g b.w.	2,5 µg/ 100 g b.w.	VEH
Claustrum	0/+	0/+	0/+	0/+
Subependymal zone	+/++	+/++	0/+	—
Lateral septal nucleus	0/+	0/+	0/+	0/+
Bed nucleus of the stria terminalis	++	+/++	0/+	0/+
Suprachiasmatic nucleus	+/++	+/++	+/++	+/++
Medial preoptic area/OVLT	+++/++++	+++/++++	+/++	0/+
Periventricular nucleus of the hypothalamus	+	+/++	—	—
Supraoptic nucleus	+++/++++	+++/++++	+	0/+
Anterior hypothalamic nucleus	0/+	0/+	0/+	0/+
Subfornical organ	++	++/+++	+/++	0/+
Hypothalamic paraventricular nucleus	+++/++++	+++/++++	0/+	0/+
Paraventricular nucleus of the thalamus	++/+++	++/+++	+/++	0/+
Thalamus (anterodorsal and anteroventral)	+	+	+	+
Central nucleus of the amygdala	+/++	+/++	0/+	0/+
Arcuate nucleus	++/+++	++/+++	0/+	0/+
Median eminence	+/++	++/+++	0/+	0/+
Dorsomedial nucleus of the hypothalamus	+/++	+/++	0/+	0/+
Subthalamic nucleus	0/+	0/+	—	—
Lateral geniculate complex	+	+	+	+
Laterodorsal tegmental nucleus	+/++	+/++	0/+	0/+
Parabrachial nucleus (external lateral part)	++/+++	++/+++	0/+	0/+
Locus coeruleus	++	++	0/+	0/+
Cochlear nucleus	+	+	+/++	+/++
Spinal nucleus of the trigeminal	0/+	0/+	0/+	0/+
Nucleus of the solitary tract	+++/++++	+++/++++	0/+	0/+
Lateral reticular nucleus/ambiguus nucleus	++/+++	++/+++	0/+	0/+
Area postrema	++	+/++	+	—
Cortex (general)	+	+	+	+
Choroid plexus	++	++/+++	0/+	0/+
Ependymal cells of ventricles	+/++	+/++	0/+	—
Meninges (lepto)	++/+++	++/+++	0/+	—

In situ hybridization histochemistry was accomplished by using a [^{35}S]-labeled cRNA probe encoding the immediate-early gene c-*fos*. The cDNA-encoding the rat c-*fos* was generously provided by Dr. I. Verma (The Salk Institute, La Jolla, CA). ++++, very strong signal; +++, strong signal; ++, moderate signal; +, low but positive signal; —, undetectable signal; b.w., body weight; IND, indomethacin pretreatment; LPS, lipopolysaccharide; OVLT, organum vasculosum of the lamina terminalis; PGs, prostaglandins; VEH, vehicle treatment.

Table 2:

Hybridization signal for C-*fos* mRNA in the brain of immune-challenged rats (3 hours after i.p. lipopolysaccharide administration): Role of prostaglandins.

Dose of LPS	250μg/100 g b.w.		25 μg/100 g b.w.		2.5 μg/100 g b.w.			
Region	LPS	LPS-IND	LPS	LPS-IND	LPS	LPS-IND	IND	VEH
Claustrum	0/+	0/+	0/+	0/+	0/+	0/+	0/+	0/+
Subependymal zone	+/++	+/++	+/++	+/++	0/+	0/+	—	—
Lateral septal nucleus	0/+	0/+	0/+	0/+	0/+	0/+	0/+	0/+
Bed nucleus of the stria terminalis	++	++	+/++	+	0/+	0/+	0/+	0/+
Suprachiasmatic nucleus	+/++	+/++	+/++	+/++	+/++	+/++	+/++	+/++
Medial preoptic area/OVLT	+++/++++	+++/++++	+++/++++	+++	+/++	0/+	0/+	0/+
Periventricular nucleus of the hypothal.	+	0/+	+/++	0/+	—	—	—	—
Supraoptic nucleus	+++/++++	+++/++++	+++/++++	+++	+	0/+	0/+	0/+
Anterior hypothalamic nucleus	0/+	0/+	0/+	0/+	0/+	0/+	0/+	0/+
Subfornical organ	++	++	++/+++	++/+++	+/++	+/++	0/+	0/+
Hypothalamic paraventricular nucleus	+++/++++	+++	+++/++++	++	0/+	0/+	0/+	0/+
Paraventricular nucleus of the thalamus	++/+++	++/+++	++/+++	++/+++	+/++	+	0/+	0/+
Thalamus (anterodorsal and ventral)	+	+	+	+	+	+	+	+
Central nucleus of the amygdala	+/++	+/++	+/++	+/++	0/+	0/+	0/+	0/+
Arcuate nucleus	++/+++	++/+++	++/+++	+++	0/+	0/+	0/+	0/+
Median eminence	+/++	+/++	++/+++	++/+++	0/+	0/+	0/+	0/+
Dorsomedial nucleus of the hypothal.	+/++	+/++	+/++	+	0/+	0/+	0/+	0/+
Subthalamic nucleus	0/+	0/+	0/+	+	—	—	—	—
Lateral geniculate complex	+	+	+	+	+	+	+	+
Laterodorsal tegmental nucleus	+/++	+/++	+/++	+	0/+	0/+	0/+	0/+
Parabrachial nucleus (ext. lateral part)	++/+++	++/+++	++/+++	++/+++	0/+	0/+	0/+	0/+
Locus coeruleus	++	+/++	++	+/++	0/+	0/+	0/+	0/+
Cochlear nucleus	+	+	+	+	+/++	+	+	+/++
Spinal nucleus of the trigeminal	0/+	0/+	0/+	0/+	0/+	0/+	0/+	0/+
Nucleus of the solitary tract	+++/++++	+++/++++	+++/++++	+++/++++	0/+	0/+	0/+	0/+
Lateral reticular and ambiguus nuclei	++/+++	+/++	++/+++	+	0/+	0/+	0/+	0/+
Area postrema	++	+/++	+/++	+/++	+	+	—	—
Cortex (general)	+	+/++	+	+/++	+	+/++	+	+
Choroid plexus	++	++	++/+++	++/+++	0/+	0/+	0/+	0/+
Ependymal cells of ventricles	+/++	+/++	+/++	+	0/+	0/+	—	—
Meninges (lepto)	++/+++	++/+++	++/+++	+/++	0/+	0/+	—	—

Figure 1:

Representative example of the distribution of the mRNA encoding the immediate-*early* gene c-*fos* in the rat brain after intraperitoneal (i.p.) administration of three different doses of the endotoxin lipopolysaccharide (LPS). Animals were deeply anesthetized and rapidly perfused with 4% paraformaldehyde 3 hours after treatment with LPS (2.5 μg/100 g b.w.; lowest dose, 25 μg/100 g b.w.; middle dose, 250 μg/100 g b.w.; highest dose). These rostro-caudal coronal sections (30 μm) of LPS-treated rat exhibited a positive signal on x-ray film (Biomax, Rochester, NY) for c-*fos* transcript in various structures throughout the brain, a phenomenon dependent on the dose administered. AP, area postrema; ARC, arcuate nucleus; BnST, bed nucleus of the lamina terminalis; CeA, central nucleus of the amygdala; Epd, ependymal lining cells of lateral ventricle; LC, locus coeruleus; ME, median eminence; Men, meninges (lepto); MPOA, medial preoptic area; NTS, nucleus of the solitary tract; NTSc, nucleus of the solitary tract, caudal part; PVN, paraventricular nucleus of the hypothalamus; SFO, subfornical organ; SON, supraoptic nucleus of the hypothalamus; VLM, ventrolateral medulla.

LPS I.P.
low dose
(2.5 µg/100g b.w.)

LPS I.P.
moderate dose
(25 µg/100g b.w.)

LPS I.P.
high dose
(250 µg/100g b.w.)

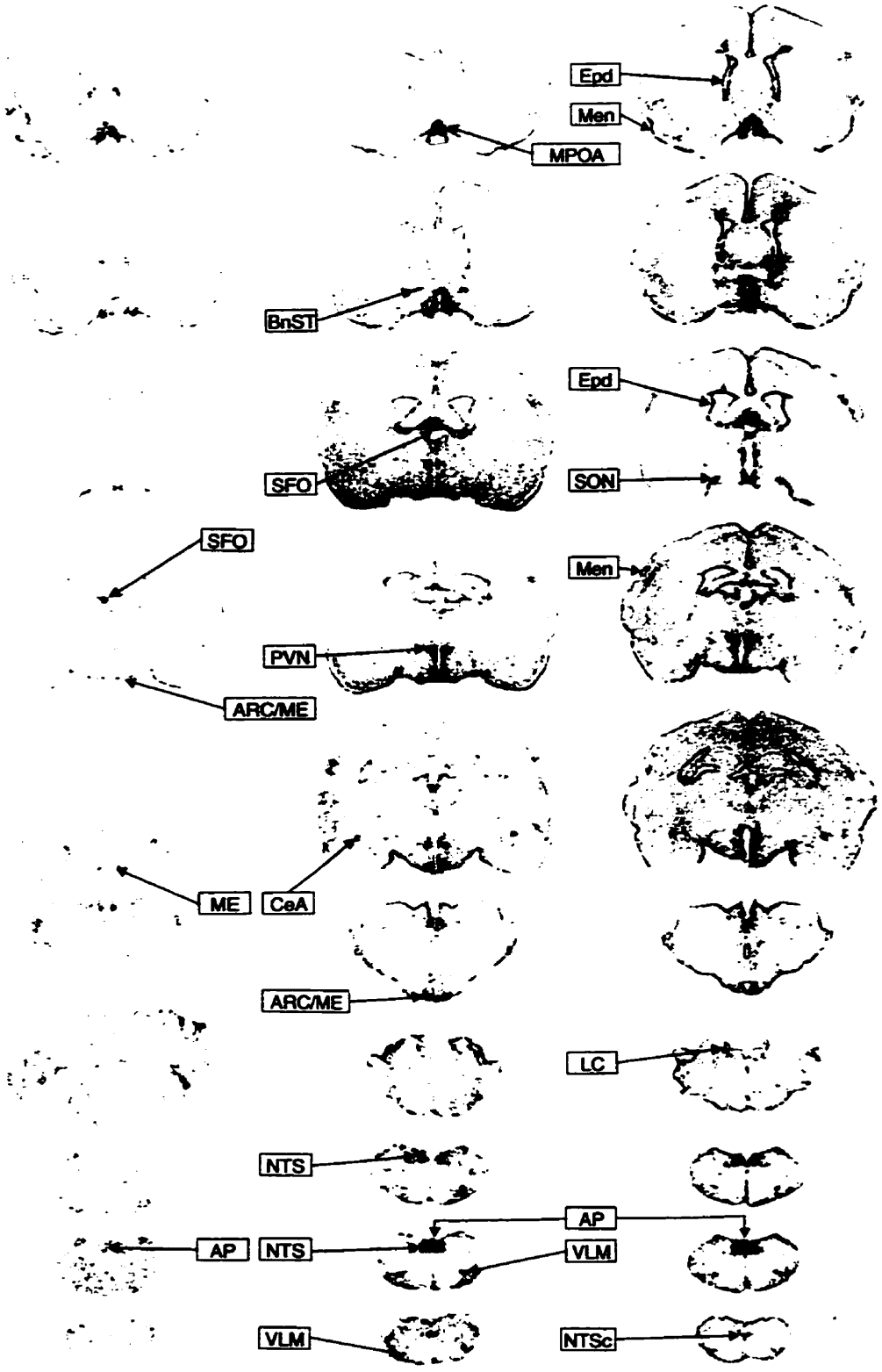


Figure 2:

Effect of indomethacin (IND) intravenous (i.v.) injection on the expression of c-*fos* mRNA in the hypothalamic paraventricular nucleus (PVN) 3 and 6 hours after intraperitoneal (i.p.) administration of the middle dose of the bacterial endotoxin lipopolysaccharide (LPS, 25 μg/100 g b.w.). These dark-field photomicrographs show in situ hybridization signals for the mRNA encoding the immediate-*early* gene throughout similar areas of the right PVN. Note that inhibition of cyclooxygenase pathways attenuated c-*fos* transcription in animals injected i.p. with such dose of LPS at both post-injection times (3 and 6 hours). In addition, a stronger expression of the mRNA encoding Fos was selectively detected in the parvocellular PVN. Veh, Vehicle. Magnification X25, Scale bar = 100 μm.

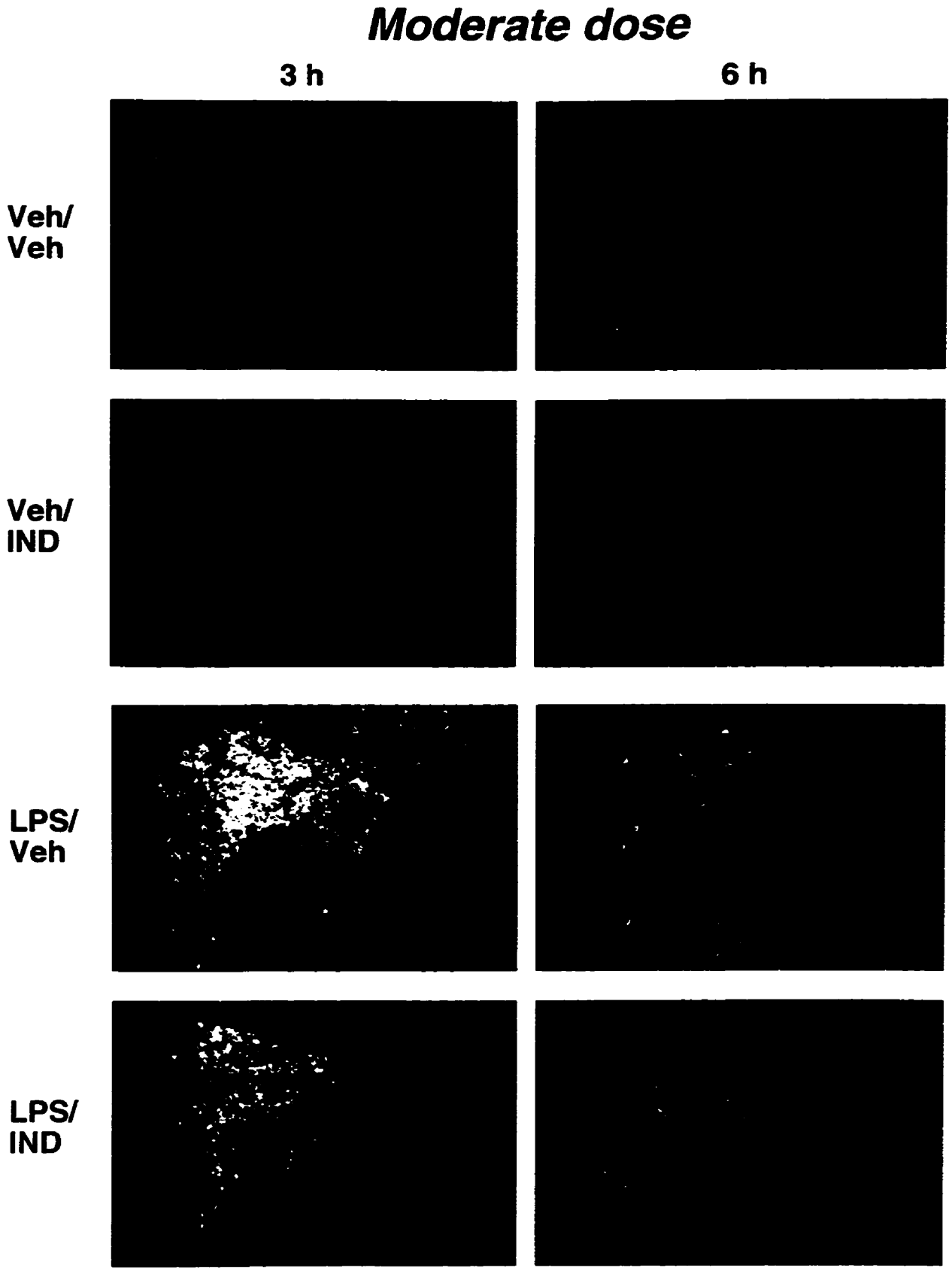

Moderate dose
3 h
6 h
Veh/
Veh
Veh/
IND
LPS/
Veh
LPS/
IND

Figure 3:

Average refraction density (R.D. in arbitrary units) of the hybridization signal for the relative levels of c-*fos* transcript in the hypothalamic paraventricular nucleus (PVN) after i.p. administration of the highest (top panel), intermediary (middle panel) or lowest (bottom panel) dose of the bacterial endotoxin lipopolysaccharide (LPS). Results represent means ± SEM of four rats; an average of two medial PVNs were digitized for each rat. Statistical analysis was performed using a 2 x 2 analysis of variance (ANOVA) followed by a Bonferroni/Dunn post hoc test for each post-treatment time (Statview 4.01). The asterisk between the lines indicates a main effect of the LPS treatment without significant interaction between i.p. LPS and i.v. indomethacin (IND) treatments. Significant interaction between i.p. and i.v. treatments; the asterisk alone indicates a significant difference ($P < 0.05$) from their appropriate control groups. A double asterisk indicates a significant difference ($P < 0.05$) from all the other groups. For more information on image analysis, see MATERIALS AND METHODS.

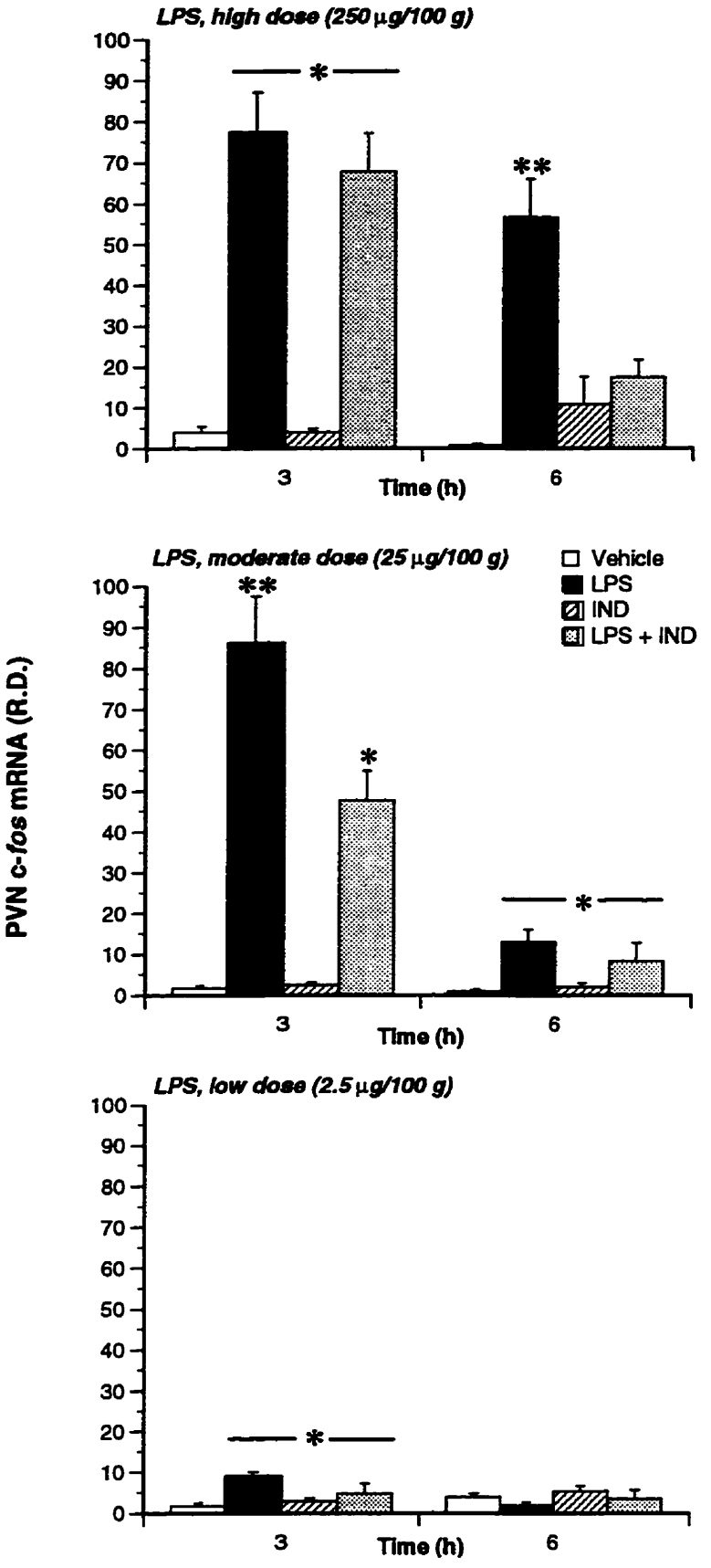

LPS, high dose (250 µg/100 g)
LPS, moderate dose (25 µg/100 g)
LPS, low dose (2.5 µg/100 g)
Vehicle
LPS
IND
LPS + IND
PVN c-fos mRNA (R.D.)
Time (h)
3
6
*
**

Figure 4:

Effect of intraperitoneal (i.p.) injection of the endotoxin lipopolysaccharide (LPS) on the average refraction density (R.D. in arbitrary units) of the hybridization signal for c-*fos* transcript in the SON of rats pretreated or not with the cyclooxygenase inhibitor indomethacin (IND). Results represent means ± SEM of four rats; an average of four medial SONs were digitized for each rat. Statistical analysis was performed using a 2 x 2 analysis of variance (ANOVA) followed by a Bonferroni/Dunn post hoc test for each post-injection time (Statview 4.01). The asterisk between the lines indicates a main effect of the LPS treatment without significant interaction between i.p. LPS and intravenous (i.v.) IND treatments. Significant interaction between i.p. and i.v. treatments; Single asterisk, significantly different ($P < 0.05$) from their appropriate control groups. Double asterisk, significantly different ($P < 0.05$) from all the other groups. For more information on image analysis, see MATERIALS AND METHODS.

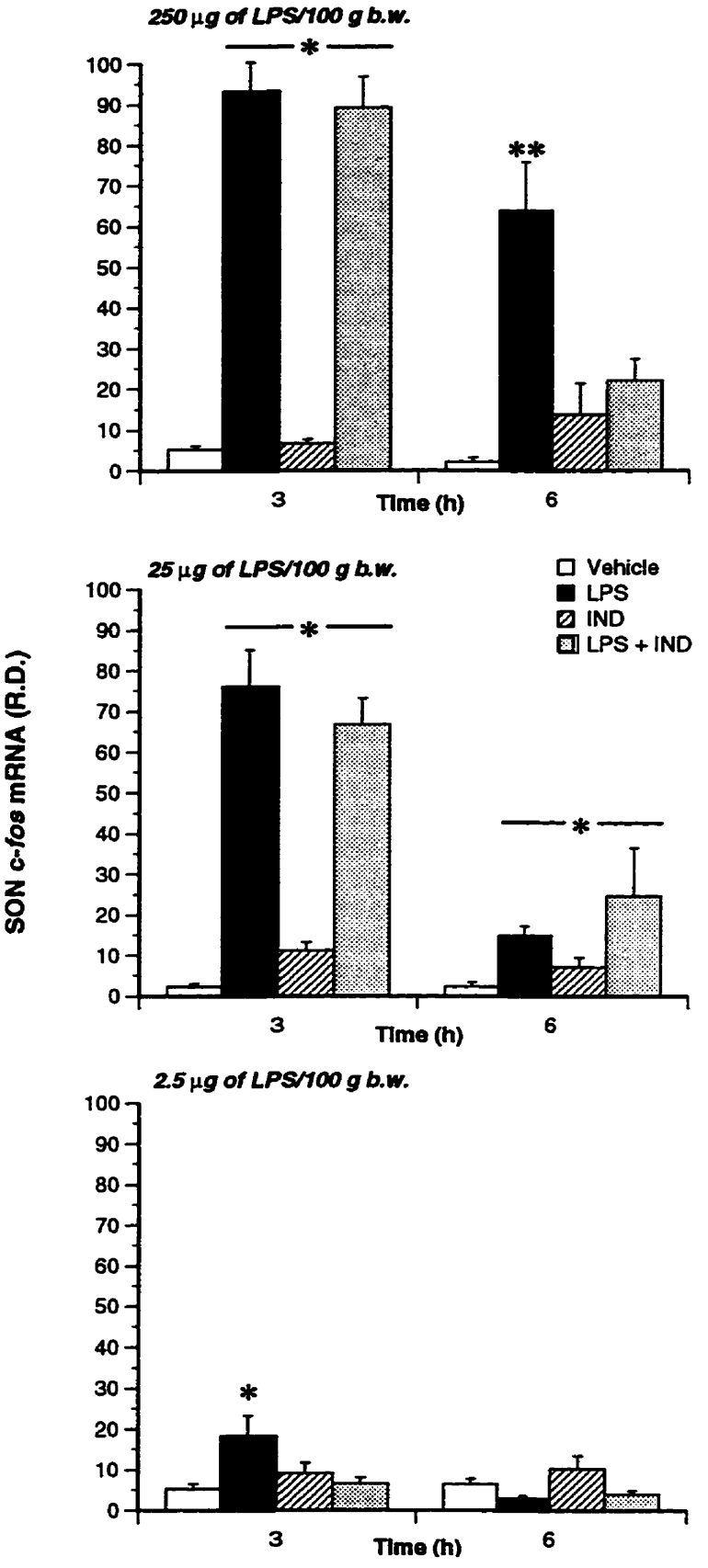
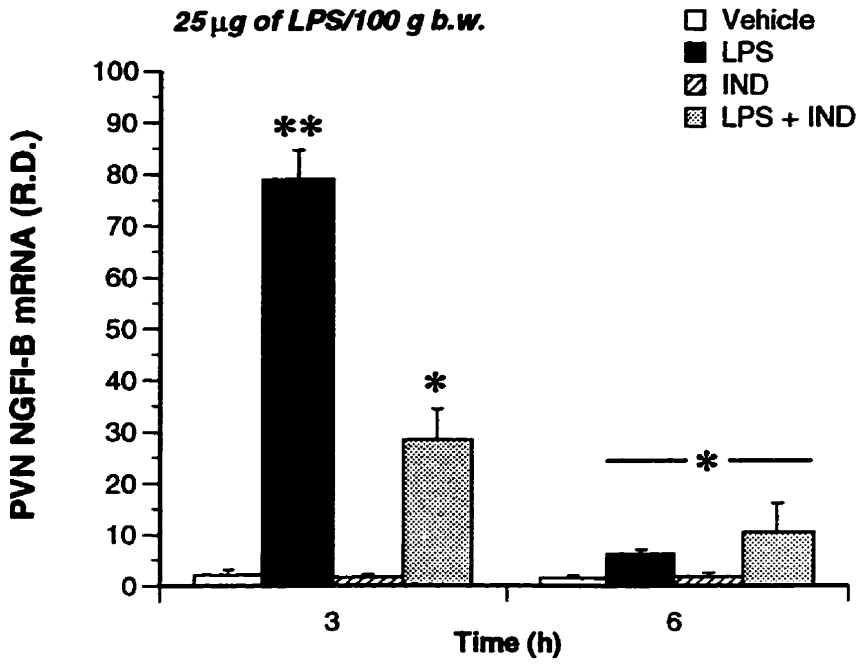

250 µg of LPS/100 g b.w.
25 µg of LPS/100 g b.w.
2.5 µg of LPS/100 g b.w.
SON c-fos mRNA (R.D.)
Time (h)
3
6
Vehicle
LPS
IND
LPS + IND
*
**

Figure 5:

Average refraction density (R.D. in arbitrary units) of the hybridization signal for the immediate-*early* gene nerve growth factor-inducible gene B (NGFI-B) in the PVN and SON after intraperitoneal (i.p.) administration of the middle dose of the bacterial endotoxin lipopolysaccharide (LPS; 25 μg/100 g b.w.). Results represent means ± SEM of four rats; an average of two medial PVNs and four medial SONs were digitized for each rat. Statistical analysis was performed by using a 2 x 2 analysis of variance (ANOVA) followed by a Bonferroni/Dunn post hoc test for each post-injection time (Statview 4.01). Asterisk between the lines indicates a main effect of the LPS treatment without significant interaction between i.p. LPS and i.v. indomethacin (IND) treatments. Significant interaction between i.p. and i.v. treatments; Single asterisk, significantly different ($P < 0.05$) from their appropriate control groups. Double asterisk, significantly different ($P < 0.05$) from all the other groups. For more information on image analysis, see MATERIALS AND METHODS. Abbreviations as in Fig. 1.


25 µg of LPS/100 g b.w.
Vehicle
LPS
IND
LPS + IND
PVN NGFI-B mRNA (R.D.)
100
90
80
70
60
50
40
30
20
10
0
**
*
*
3
6
Time (h)

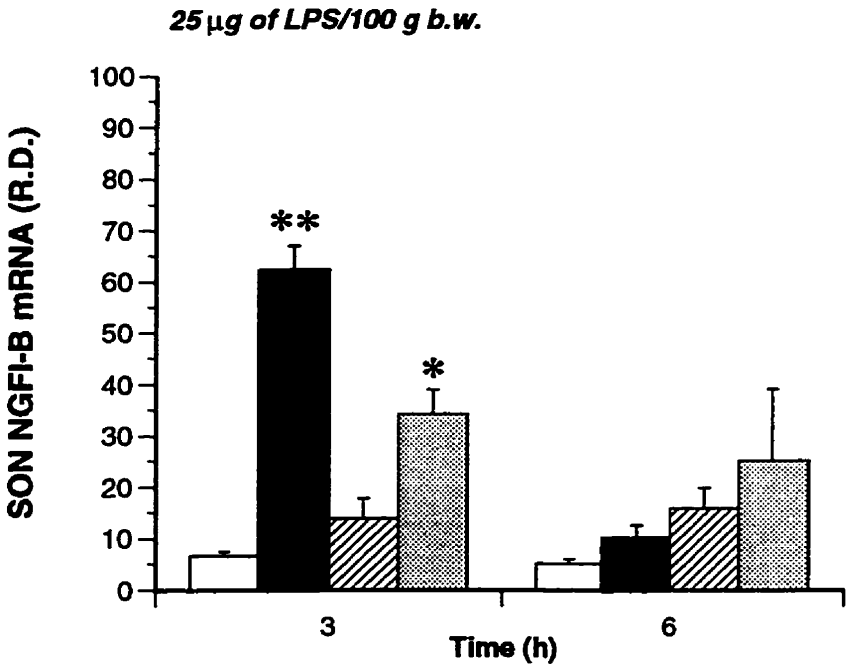

25 µg of LPS/100 g b.w.
SON NGFI-B mRNA (R.D.)
100
90
80
70
60
50
40
30
20
10
0
**
*
3
6
Time (h)

Figure 6:

Influence of cyclooxygenase pathways on the expression of corticotropin-releasing factor (CRF) primary transcript in the hypothalamic paraventricular nucleus (PVN) of rats treated i.p. with the middle dose of the bacterial lipopolysaccharide (LPS, 25 μg/100 g b.w.). These darkfield photomicrographs show *in situ* hybridization signals for CRF heteronuclear (hn) RNA through similar right medial PVN. Note that inhibition of prostaglandin production by intravenous indomethacin administration decreased expression of CRF hnRNA (index of transcriptional activity) in the PVN of animals injected i.p. with such a dose of LPS at both post-injection times (3 and 6 hours). Magnification X25, Scale bar = 100 μm.


Veh/
Veh
Veh/
IND
LPS/
Veh
LPS/
IND

Figure 7:

Average refraction density (R.D. in arbitrary units) of the hybridization signal for the relative levels CRF primary transcript in the PVN after intraperitoneal (i.p.) administration of 250 μg (top panel), 25 μg (middle panel) or 2.5 μg (bottom panel) of the bacterial endotoxin lipopolysaccharide (LPS)/100 g b.w. Results represent means ± SEM of four rats; an average of two medial PVNs were digitized for each rat. Statistical analysis was performed by using a 2 x 2 analysis of variance (ANOVA) followed by a Bonferroni/Dunn post hoc test for each time post-administration (Statview 4.01). Asterisk between lines, main effect of the LPS treatment without significant interaction between i.p. LPS and intravenous (i.v.) indomethacin (IND) treatments. Significant interaction between i.p. and i.v. treatments; Double asterisk, significantly different ($P < 0.05$) from all the other groups. For more information on image analysis, see MATERIALS AND METHODS.

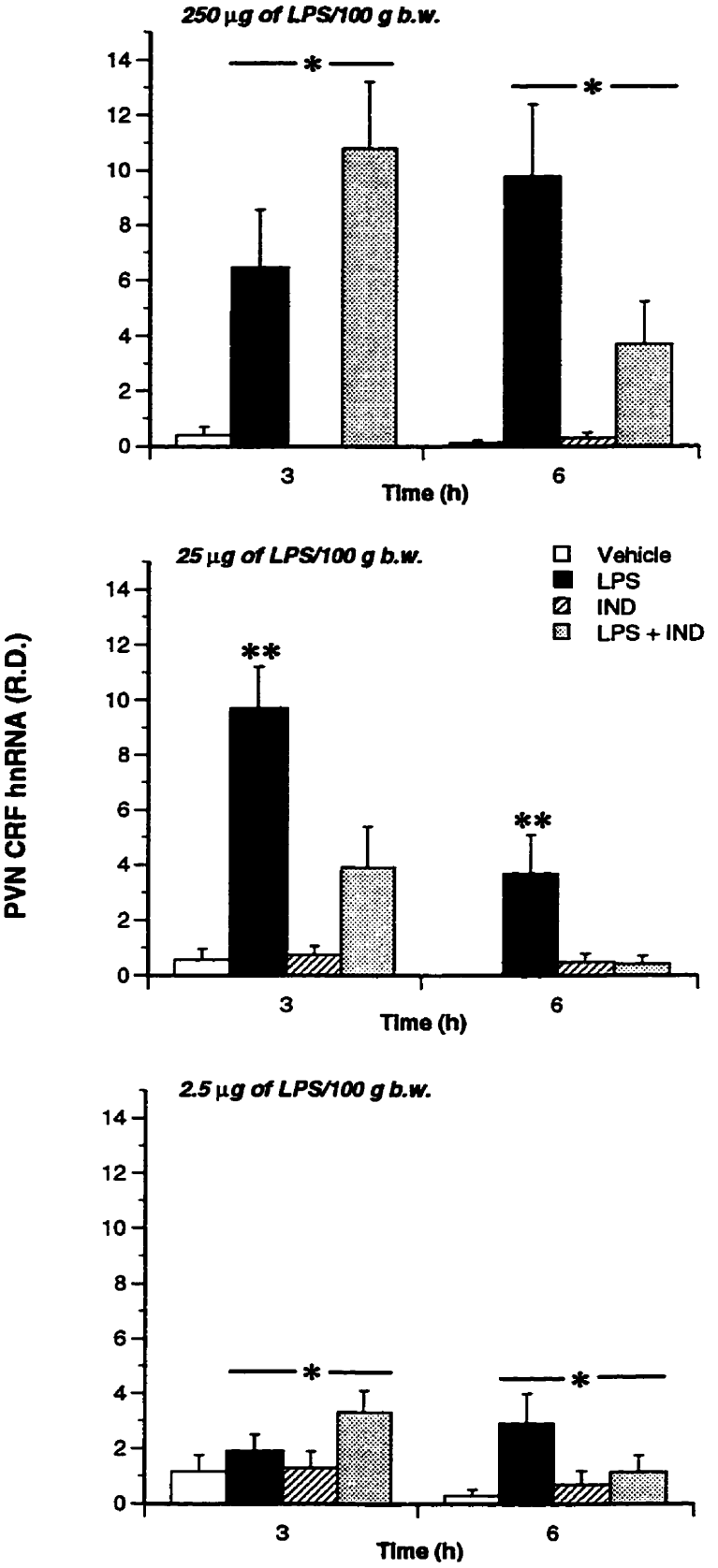

250 µg of LPS/100 g b.w.
25 µg of LPS/100 g b.w.
2.5 µg of LPS/100 g b.w.
Vehicle
LPS
IND
LPS + IND
PVN CRF hnRNA (R.D.)
Time (h)
3
6
*
**

Figure 8:

High power bright-field photomicrographs representing tyrosine hydroxylase (TH)-immunoreactive (ir) neurons expressing c-*fos* transcript in the nucleus of the solitary tract (NTS, top panels) and the ventrolateral medulla (VLM, bottom panels) of rats killed humanely 3 hours after the injection of the middle dose of lipopolysaccharide (LPS, 25 μg/100 g b.w.). Immunocytochemistry (TH protein, stained cytoplasms and fibers) was performed on the same brain sections (30 μm) before in situ hybridization histochemistry (c-*fos* mRNA, silver grains). Dark-field photomicrographs (left panels) of the NTS (top) and VLM (bottom) of a representative LPS-treated rat illustrate the exact level of the nucleus from which the high magnification brightfield photomicrographs were taken. LPS/Veh: animal receiving 25 μg of LPS i.p./100 g b.w. and vehicle solution i.v.; LPS/IND: animal i.p. injected with the same dose of LPS and pretreated i.v. with indomethacin (IND). Filled arrows, TH-ir neurons expressing the mRNA encoding c-*fos*; open arrows, c-*fos* positive neurons alone; curved arrows, TH-ir neurons alone. Magnification of the dark-field panels X10, Scale bar = 250 μm. Magnification of the bright-field panels X250, Scale bar = 10 μm.

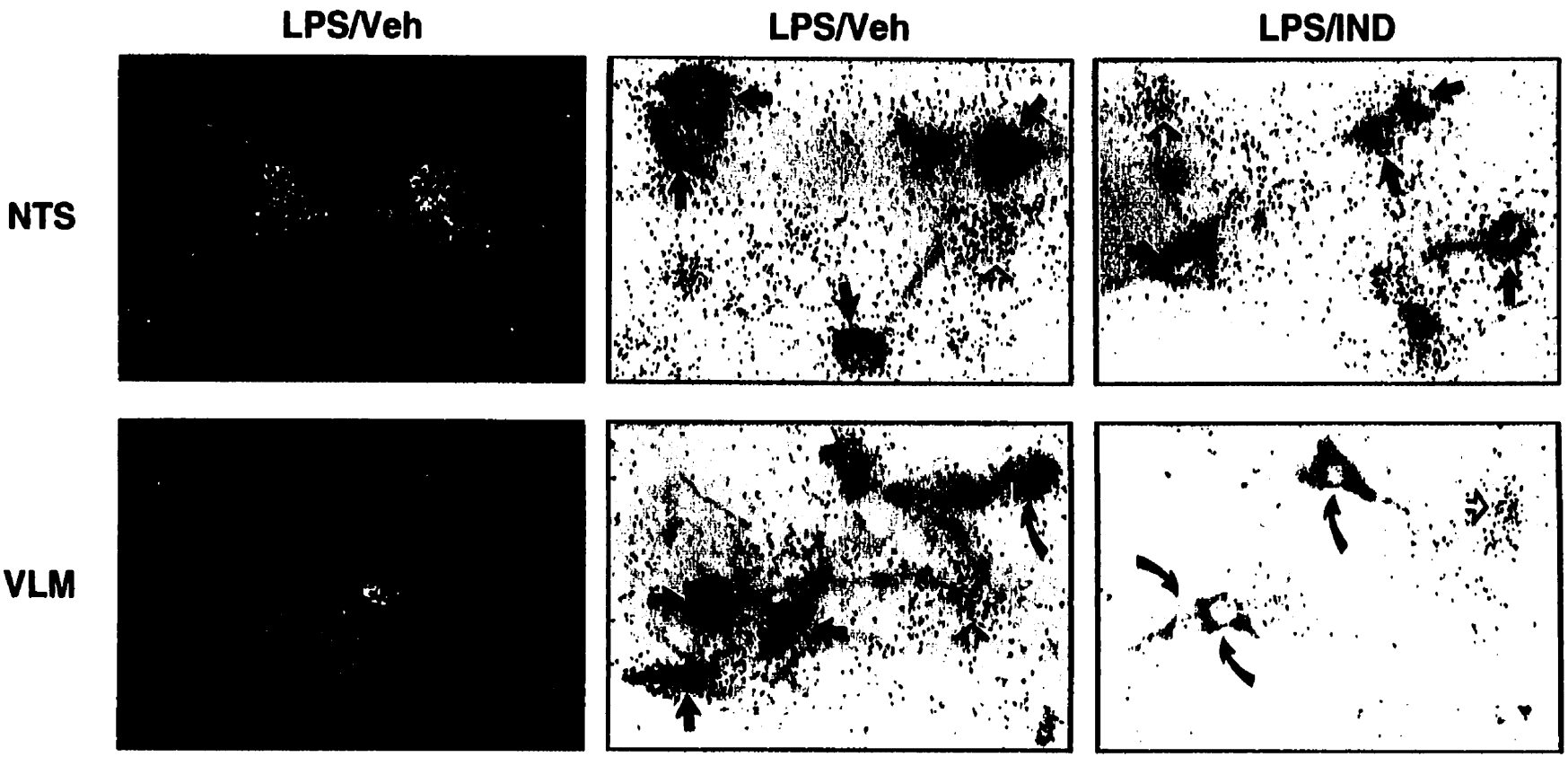
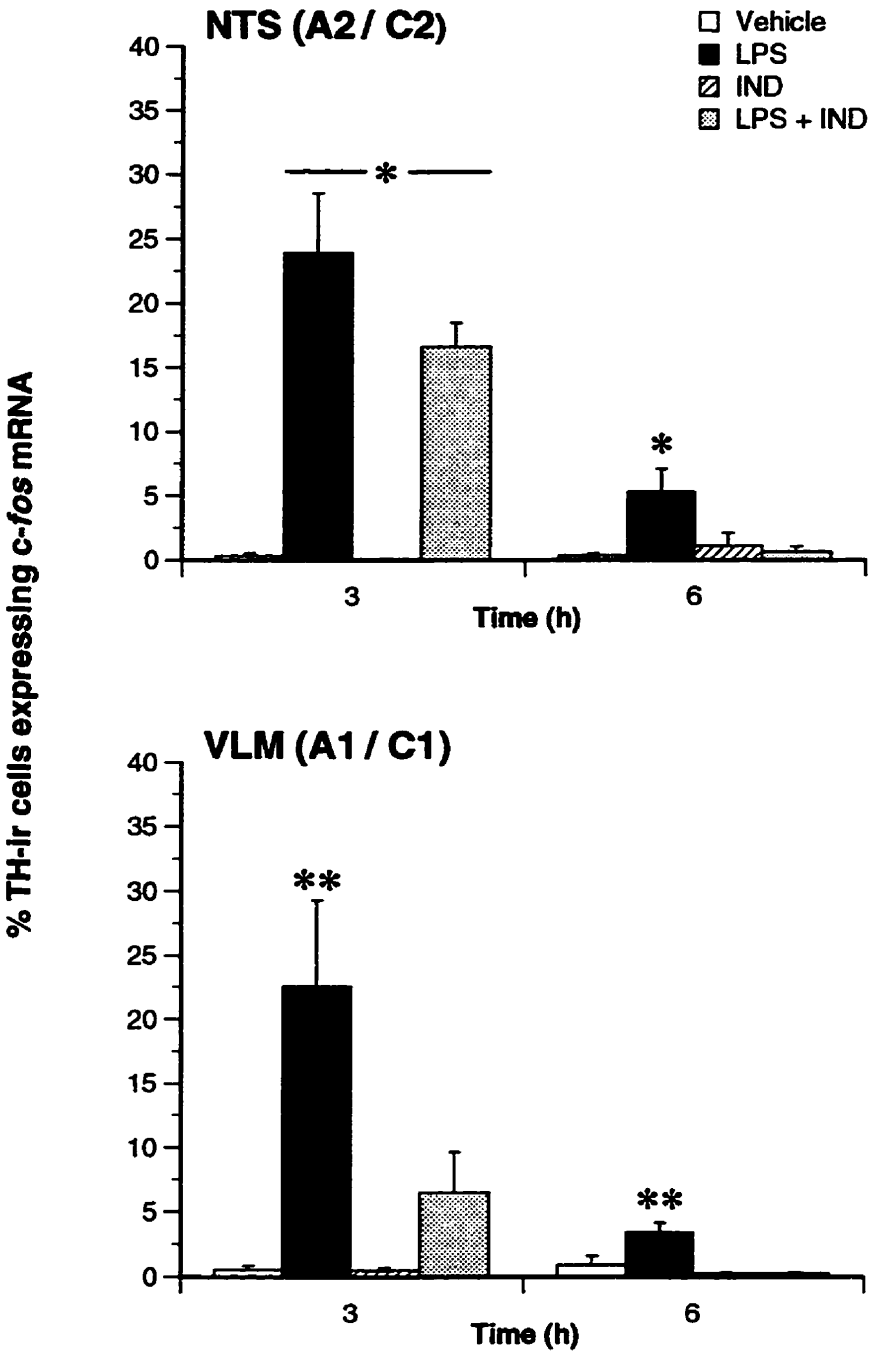

LPS/Veh
LPS/Veh
LPS/IND
NTS
VLM

Figure 9:

Percentage (%) of tyrosine hydroxylase (TH)-immunoreactive (ir) cells exhibiting positive signal for c-*fos* mRNA in the nucleus of the solitary tract (NTS) and the ventrolateral medulla (VLM) of endotoxin-challenged rats (LPS i.p., 25 μg/100 g b.w.) receiving either i.v. vehicle or indomethacin (IND) injections. Immunocytochemistry was performed before in situ hybridization histochemistry on the same brain sections, and measurements of TH-ir cells and double-labeled cells (TH-ir neurons expressing c-*fos* mRNA) were performed at a magnification of X100 under bright-field illumination for the entire rostro-caudal structures delineating the NTS (A2/C2) and VLM (A1/C1). Results represent means ± SEM of four rats for an average of 14 to 16 and 36 to 44 bilateral sections corresponding to the NTS and the VLM, respectively, for each rat. Statistical analysis was performed by using a 2 x 2 analysis of variance (ANOVA) followed by a Bonferroni/Dunn post hoc test for each post-injection time (Statview 4.01). Asterisk between lines, main effect of the LPS treatment without significant interaction between i.p. LPS and i.v. indomethacin (IND) treatments. Significant interaction between i.p. and i.v. treatments; Single asterisk, significantly different ($P < 0.05$) from their appropriate control groups. Double asterisk, significantly different ($P < 0.05$) from all the other groups.


NTS (A2 / C2)
Vehicle
LPS
IND
LPS + IND
% TH-ir cells expressing c-fos mRNA
40
35
30
25
20
15
10
5
0
*
*
3
6
Time (h)
VLM (A1 / C1)
**
**
3
6
Time (h)

CHAPITRE 3. ROLE OF CYCLO-OXYGENASE PATHWAYS IN THE STIMULATORY INFLUENCE OF IMMUNE CHALLENGE ON THE TRANSCRIPTION OF A SPECIFIC CRF RECEPTOR SUBTYPE IN THE RAT BRAIN.

By

Steve Lacroix and Serge Rivest

Laboratory of Molecular Endocrinology
CHUL Research Center and
Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2

Running Title: CRF receptor gene expression in immune-challenged rat brains: Role of PGs.

Address for Correspondence:
Dr. Serge Rivest
Laboratory of Molecular Endocrinology
CHUL Research Center and
Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2
Tel: (418) 654-2296, Fax: (418) 654-2761
EMail: Serge.Rivest@crchul.ulaval.ca

The Journal of Chemical Neuroanatomy, 10 (1996) 53-71

3.1 RÉSUMÉ

Le but de cette étude était d'investiguer le rôle des prostaglandines (PGs) dans l'expression des différents types de récepteurs du facteur de libération des corticotrophines (CRF) dans le cerveau de rats soumis à un challenge immunitaire. Pour ce, une administration intraveineuse (i.v.) d'indométhacine (0.8 mg/100 g de poids corporel), un inhibiteur de la synthèse des PGs, fut effectuée 15 min précédent l'injection intrapéritonéale (i.p.) d'une dose forte (250 µg/100 g de poids corporel), modérée (25 µg/100 g de poids corporel), ou faible (2.5 µg/100 g de poids corporel) de l'endotoxine bactérienne lipopolysaccharide (LPS). Les rats mâles Sprague-Dawley (230-260g) furent sacrifiés trois et six heures suivant le traitement i.p. avec l'endotoxine LPS, et leur cerveau coupé en tranches coronales de 30-µm à partir du bulbe olfactif jusqu'à la médulla. L'analyse de l'expression des transcrits encodant les récepteurs du CRF (R-CRF; type 1, 2α, et 2β) a été accomplie par hybridation *in situ* à l'aide de sondes marquées au S^{35}. Dans des conditions basales, des taux élevés d'ARNm du R-CRF_1 ont été détectés dans plusieurs régions du cerveau. Nos résultats révèlent également que le transcrit codant le R-$CRF_{2\alpha}$ est fortement exprimé dans quelques structures du système limbique, tandis que seul les plexus choroïdiens ont montré un signal positif pour l'ARNm du R-CRF_{28}. Contrairement aux récepteurs de type 2, la transcription du gène encodant le R-CRF_1 a été nettement stimulée dans des régions spécifiques de l'hypothalamus en réponse à la LPS. Ainsi, une forte induction des niveaux d'ARNm du R-CRF_1 a été observée dans les divisions parvo- et magnocellulaires des noyaux paraventriculaires de l'hypothalamus (PVH) et les noyaux supraoptiques (SON) de rats injectés avec une dose élevée de LPS. Il est intéressant de noter qu'une dose modérée ou faible de l'endotoxine a stimulé l'activité transcriptionnelle du R-CRF_1 dans la division parvocellulaire des noyaux PVH exclusivement. Nos analyses montrent également qu'un prétraitement à l'indométhacine ne prévient pas l'induction de la transcription du R-CRF_1 dans les noyaux PVH de rats injectés avec la dose forte de LPS. Toutefois, le blocage des voies de la cyclooxygénase a inhibé significativement l'expression du R-CRF_1 dans les noyaux PVH et SON de rats sacrifiés 6 heures après l'administration d'une dose modérée ou faible de LPS; les niveaux d'ARNm du R-CRF_1 étaient approximativement trois (dose modérée) et deux (dose faible) fois plus élevés chez les rats injectés avec l'endotoxine seulement que chez les animaux soumis à un double traitement combinant l'infusion i.v. d'indométhacine et l'administration i.p. de LPS. Ces résultats suggèrent donc que l'ARNm encodant le R-CRF_1, mais pas ceux des récepteurs de type 2 du CRF, est spécifiquement régulé dans l'hypothalamus endocrinien du rat en réponse à un challenge immunitaire. Le rôle joué par les PGs comme médiateurs des influences

stimulatrices de la réponse immunitaire sur la transcription du R-CRF_1 dans les noyaux PVH et SON, semble quant à lui dépendre de la sévérité du stress systémique.

3.2 ABSTRACT

The aim of this study was to investigate the role of prostaglandins (PGs) on the expression of corticotropin-releasing factor (CRF) receptors in the brains of immune-challenged rats. Intravenous (i.v.) administration of indomethacin (0.8 mg/100 g of b.w.), an inhibitor of PG synthesis, was performed 15 min before the intraperitoneal (i.p.) injection of a high (250 µg/100 g of b.w.), moderate (25 µg/100 g of b.w.), or low (2.5 µg/100 g of b.w.) dose of the immune activator lipopolysaccharide (LPS). Three and 6 hours after the i.p. treatment with the endotoxin LPS, male Sprague-Dawley rats (230-260g) were sacrificed. Frozen brains were mounted on a microtome and cut from the olfactory bulb to the medulla in 30-µm coronal sections. mRNAs encoding CRF receptors (type 1, 2α, and 2β) were assayed by *in situ* hybridization using ^{35}S-labeled riboprobes. Strong basal levels of CRF_1 receptor transcript were detected in multiple regions of the brain, whereas $CRF_{2\alpha}$ receptor message was highly localized in few structures of the limbic system and positive signal for $CRF_{2\beta}$ receptor mRNA was observed only in the choroid plexus. The transcription of the gene encoding the CRF type 1 (but not types 2) receptor was highly stimulated by LPS administration in selective hypothalamic nuclei. Indeed, a high dose of LPS caused strong expression of CRF_1 receptor mRNA in both parvo- and magnocellular paraventricular nucleus (PVN) and in the supraoptic nucleus (SON), although low and moderate doses of endotoxin induced a more specific expression of this transcript in the parvocellular division of the PVN. Pretreatment with indomethacin did not prevent the induction of CRF_1 receptor transcription in the PVN of rats injected with a high dose of LPS. In contrast, inhibition of cyclo-oxygenase pathways significantly inhibited the expression of CRF_1 receptor in the PVN and SON of rats sacrificed 6 h after being injected with a moderate or a low dose of LPS; the CRF_1 receptor mRNA levels were approximately three (moderate dose) and two (low dose) times higher in rats receiving the endotoxin alone than those submitted to a treatment combining both i.v. indomethacin and i.p. LPS. These results indicate that the mRNA encoding the type 1 but not the type 2 CRF receptor is specifically regulated in endocrine hypothalamus of immune-challenged rats, whereas the role of PGs in mediating the stimulatory influence of immune challenge on the transcription of CRF_1 receptor in the PVN and SON seems to depend on the severity of this systemic stressful situation.

3.3 INTRODUCTION

In the presence of foreign material, the appropriate interaction between immune, endocrine, and neural systems is considered essential to the survival of mammalian organisms. Although communication between immune and endocrine systems has long been recognized because of the immunosuppressive actions of glucocorticoids, this concept has been largely expanded by the observation that immune-derived proteins are also potent modulators of multiple neuroendocrine functions. The increased production of cytokines, proteins released by activated macrophages and lymphocytes upon presentation of an antigen (Dinarello, 1989; Rabin et al., 1990), represents one element of the early events of immune activation and is called the acute-phase response. Endotoxins are widely used to mimic some of the events that occur during sepsis (Higgins and Olschowka, 1991; Koenig, 1991); lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria (Raetz, 1990) that triggers the synthesis and release of a series of immunoregulatory, cytotoxic, and inflammatory molecules, including tumor necrosis factor (TNF)-α, interleukin-1 (IL-1), and IL-6 (Nathan, 1987). Systemic LPS administration stimulates transcription of the gene encoding corticotropin-releasing factor (CRF) selectively in the rat PVN (Rivest and Laflamme, 1995) and causes a strong activation of the hypothalamic-pituitary-adrenal (HPA) axis (Rivier and Rivest, 1993). Although it has been suggested that lymphocyte-derived ACTH can play a role in triggering corticosterone release in immune-challenged animals (Blalock and Smith, 1985), this hypothesis remains highly controversial, and solid evidence supports the concept of neuroendocrine CRF-mediated mechanisms (for review, see Koenig, 1991; Rivest, 1995; Rivest and Rivier, 1995; Rivier and Rivest, 1993).

Although the wide distribution of CRF and its type 1 receptor (CRF_1 receptor) throughout the rat brain (Potter et al., 1994; Rivest et al., 1995; Sawchenko et al., 1993; Sawchenko and Swanson, 1990) support the evidence for the fundamental role of this neuropeptide in integrating the endocrine, behavioral, and autonomic responses to stressful stimuli, the gene encoding the CRF_1 receptor appears to be selectively upregulated under stress conditions. Indeed, immobilization stress induced a highly selective expression of CRF_1 receptor mRNA within the hypothalamic PVN without affecting the basal expression of the gene encoding this receptor in other regions of the brain in both male (Rivest et al., 1995) and female (Nappi and Rivest, 1995) rats. Intraperitoneal (i.p.) administration of the immune activator LPS also caused a profound expression of mRNA encoding the CRF_1 receptor in selective hypothalamic nuclei, including the parvocellular and magnocellular

divisions of the PVN and the supraoptic nucleus (SON) (Rivest et al., 1995), an effect also observed in salt-loading stressed rats (Luo et al., 1994). Interestingly, CRF-immunoreactive perikarya of the parvocellular PVN displayed positive signal for CRF_1 receptor transcript in both immobilized and severely immune-challenged rats (Rivest et al., 1995) suggesting that CRF might play a direct role in controlling the activity of neuroendocrine CRF motoneurons.

The mechanisms through which immune-challenge can trigger transcription of the CRF_1 receptor within selective neuroendocrine nuclei remain to be fully investigated. Most of the pleomorphic effects of interleukin-1 on its target cells are known to take place via the activation of prostaglandins (PGs) synthesized by cyclo-oxygenase pathways (Cominelli et al., 1989; Hughes et al., 1989; Kerr et al., 1989; Kohan, 1989). Furthermore, pituitary secretion of various hormones, including luteinizing hormone (Ojeda et al., 1975), prolactin (Harms et al., 1973), growth hormone (Stachura and Tyler, 1986), and ACTH (Weidenfeld et al., 1983) are associated with eicosanoids. The importance of arachidonic acid pathways in mediating cytokine-induced alteration of hypothalamic neurons was ascertained by the fact that IL-1 and IL-6 stimulated release of CRF can be blocked by cyclo-oxygenase (but not lipo-oxygenase) inhibitors (Bernardini et al., 1990; Navarra et al., 1991), whereas IL-1-induced inhibition of LHRH neuronal activity and plasma LH release can be prevented by indomethacin (Rivest and Rivier, 1993). On the other hand, systemic LPS administration induces release of PGs in the preoptic/hypothalamic area, a phenomenon involved in the hypothermic effects of the endotoxin (Ueno et al., 1982). Arachidonic acid metabolism also participates in the hypothalamic response to endotoxin-mediated adrenocortical activation in the rats (Smith et al., 1994) but whether cyclo-oxygenase pathways are involved in the influence of immune challenge on the expression of the gene encoding CRF_1 receptor remains to be investigated.

Other CRF receptor subtypes ($CRF_{2\alpha}$ and $_{2\beta}$ receptor) have recently been cloned by several groups (Kishimoto et al., 1995; Lovenberg et al., 1995b; Perrin et al., 1995; Stenzel et al., 1995). In contrast to the type 1 CRF receptor which is widely distributed throughout the rat brain (Nappi and Rivest, 1995; Potter et al., 1994; Rivest et al., 1995), the mRNA encoding the type 2α CRF receptor is expressed in very few brain structures (Lovenberg et al., 1995b), but whether expression of this CRF receptor subtype is altered in the brains of immune-challenged rats has yet to be investigated. The purposes of the present study were therefore to investigate the effects of different doses (low, moderate, and high) of the bacterial LPS on the expression of CRF receptors throughout the rat brains and to study the

possibility that eicosanoid cyclo-oxygenase pathways can mediate the influence of systemic immune challenge on transcription of the gene encoding specific CRF receptor subtypes in selective nuclei of the rat brains.

3.4 MATERIALS AND METHODS

3.4.1 Animals

Adult male Sprague-Dawley (~230-260g) were acclimated to standard laboratory conditions (14-h light, 10-h dark cycle; lights on at 0600 and off at 2000) with free access to rat chow and water. Each rat was used for experimentation only once, and all protocols were approved by the Laval University Animals Welfare Committee. A total of 96 rats were assigned to three different protocols (each corresponding to a different dose of LPS: high, moderate, and low) which were further subdivided into 4 treatments (i.v. vehicle + i.p. vehicle; i.v. vehicle + i.p. LPS; i.v. indomethacin + i.p. vehicle; i.v. indomethacin + i.p. LPS) and two times post-injection (3 and 6 h following LPS administration).

3.4.2 Surgery

Each rat was anesthetized with an i.p. injection 0.3 ml of a mixture of ketamine hydrochloride (91 mg/kg) and xylazine (9.1 mg/kg). Once anesthetized, rats were implanted with two catheters: one into the jugular vein and the other into the peritoneal cavity which was attached to the abdominal muscle. Catheters were made from a piece of silastic tubing (Silastic medical grade tubing, ID 0.020 in., OD 0.037 in.; Dow Corning, Midland, MI) connected to an intramedic polyethylene tubing (PE-50, Caly Adams, Parsippany, N.J.). Outlet of cannulas was placed at the level of the neck and rats were housed individually in metal cages for a period of recuperation of two days.

3.4.3 Treatments

On the day of the experient (~0830 in the morning), the outlet portion of each catheter (i.v. and i.p.) was fixed to a tronqued 27 g needle which was attached to a PE-50 tubing. These connectors were then fixed to a 1cc syringe and rats were placed individually in a quiet room for at least 2 hours before experimentation. This procedure was used to avoid

disturbing the animals during i.v. and i.p. administrations. Intravenous administration of indomethacin (Sigma, I-7378, lot, 83H0041, 0.8 mg/100 g of b.w.), an inhibitor of prostaglandin synthesis diluted in 300 µl of a sterile saline solution (NaCl 0.9% solution; 5% alcohol), was accomplished 15 min before a single i.p. injection of high (250 µg/100 g of b.w.), moderate (25 µg/100 g b.w.), or low (2.5 µg/100 g b.w.) doses of LPS (Sigma, L-2880, lot, 122H4025) diluted in 300 µl of sterile saline (0.9 %). These doses were selected to compare the expression of the genes encoding specific CRF receptor subtypes in the brain of *severely*, *moderately*, and *scarcely* immune-challenged rats. The criteria used to define this terminology were based on the physical appearance of animals following the i.p. endotoxin treatment. High dose of LPS (250 µg/100 g of b.w.) caused a "*severe*" immune-challenge because the animals looked very sick (cover on themselves and immobile, diarrhea, shivering, irritated hairs, ...) but no mortality was observed following this treatment. Twenty-four h after i.p. injection with this dose of LPS, the symptoms of sickness are usually gone in both male and female rats (personal observation). The terminology "*moderate*" was used for the dose 25 µg/100 g of b.w. in respect to the fact that very few but some of the physical symptoms described above were observed, whereas the lowest dose did not cause any apparent symptoms of sickness and explain the term "*scarcely*". Moreover, high dose of LPS elicited a robust activation of both immediate *early* genes (IEGs) c-*fos* and NGFI-B in multiple regions of the brain (Rivest and Laflamme, 1995) and CRF_1 receptor gene expression in the PVN and SON (Rivest et al., 1995) of adult male rats. To insure the inhibition of cyclo-oxygenase pathways, a second and third injection of indomethacin or its vehicle were accomplished 1 and 3 h after the LPS injection. Similar doses of indomethacin have been shown to reverse the influence of LPS and IL-1 on many neuroendocrine functions (Rivest and Rivier, 1993; Rivier, 1993; Wan et al., 1994). The rats were conscious and freely moving at all times throughout the experimental procedure. Three and 6 h after the i.p. treatment with the bacterial endotoxin or the vehicle solution, the animals were deeply anesthetized via an i.v. injection of 0.1 ml of a mixture of ketamine hydrochloride and xylazine and then rapidly perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M borax buffer (pH 9.5 at 4 °C). The time points were chosen on the basis of previous studies, which showed a strong signal for various IEG mRNAs at 3 h post-injection (Rivest and Laflamme, 1995) and profound transcription of the gene encoding the CRF_1 receptor in selective hypothalamic nuclei 6 h after treatment with the bacterial endotoxin (Rivest et al., 1995). Brains were removed from the skulls, postfixed for 2 to 8 days, and then placed in 10% sucrose in the solution of 4% paraformaldehyde-borax buffer overnight at 4 °C. The frozen brains were mounted on a microtome (Reichert-Jung, Cambridge Instruments Company, Deerfield, IL) and cut into 30-µm coronal sections. The

slices were collected in a cold cryoprotectant solution (0.05M sodium phosphate buffer, pH 7.3, 30% ethylene glycol, 20% glycerol) and stored at -20 °C.

3.4.4 *In situ* hybridization histochemistry

Hybridization histochemical localization of each transcript (CRF_1, $CRF_{2\alpha}$, and $CRF_{2\beta}$ receptor mRNAs) was carried out in 1 in 6 series (every sixth section) of brain slices throughout the brain (from the olfactory bulb to the end of the medulla) using ^{35}S-labeled cRNA probes. Protocols for riboprobe synthesis, hybridization, and autoradiographic localization of mRNA signal were adapted from Simmons et al. (1989). All solutions were treated with diethylpyrocarbonate (Depc) and sterilized to prevent RNA degradation. Tissue sections mounted onto poly-L-lysine-coated slides were desiccated under vacuum overnight, fixed in 4% paraformaldehyde for 30 min, and digested by proteinase K (10 µg/ml in 0.1 M tris HCl, pH 8.0, and 50 mM EDTA, pH 8.0, at 37 °C for 25 min). Thereafter, the brain sections were rinsed in sterile Depc water followed by a solution of 0.1 M triethanolamine (TEA, pH 8.0), acetylated in 0.25% acetic anhydride in 0.1 M TEA, and dehydrated through graded concentrations of alcohol (50, 70, 95, and 100 %). After vacuum drying for a minimum of 2 h, 90 µl of hybridization mixture (10^7 cpm/ml) was spotted on each slide, sealed under a coverslip, and incubated at 60 °C overnight (~15-20 h) in a slide warmer. Coverslips were then removed and the slides were rinsed in 4xSSC at room temperature. Sections were digested by RNAase A (20 µg/ml, 37 °C, 30 min), rinsed in descending concentrations of SSC (2x, 1x, 0.5xSSC), washed in 0.1xSSC for 30 min at 60 °C (1xSSC: 0.15 M NaCl, 15 mM trisodium citrate buffer, pH 7.0) and dehydrated through graded concentrations of alcohol. After being dried for 2 h under the vacuum, the sections were exposed at 4 °C to X-ray film (Kodak) for 18-48 h, defatted in xylene, and dipped in NTB2 nuclear emulsion (Kodak; diluted 1:1 with distilled water). Slides were exposed for 8-16 days, developed in D19 developer (Kodak) for 3.5 min at 14-15 °C, washed 15 sec. in water, and fixed in rapid fixer (Kodak) for 5 min. Thereafter, tissues were rinsed in running distilled water for 1-2 h, counterstained with thionin (0.25 %), dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX.

3.4.5 cRNA probe synthesis and preparation

Specific rat CRF_1 receptor probe (1.3 kb) was generated from the *PstI-PstI* fragment of the rat prCRF PP1.3-BS cDNA {Dr. W. Vale, Peptide Biology Laboratory, The Salk

Institute, (Perrin et al., 1993)}, subcloned into pBluescript II SK (Stratagene, La Jolla, CA), and linearized with *Bam*H I and *Hin*dIII (Pharmacia) for antisense and sense probes, respectively (Chen et al., 1993; Perrin et al., 1993; Rivest et al., 1995). The pBluescript (SK+) plasmids containing either a 275 bp insert of the rat $CRF_{2\alpha}$ receptor cDNA or a 200 bp insert of a rat $CRF_{2\beta}$ receptor cDNA {Dr. T. Lovenberg, Neurocrine Biosciences Inc. San Diego, CA (Lovenberg et al., 1995b)} were linearized with *Hin*dIII and *Bam*HI to generate antisense and sense probes, respectively. These two probes ($CRF_{2\alpha}$ and $CRF_{2\beta}$) have no overlap with one another and have no similarity to the CRF_1 receptor probe (T. Lovenberg, personal communication). Radioactive cRNA copies were synthesized by incubation of 250 ng linearized plasmid in 6 mM $MgCl_2$, 40 mM Tris (pH 7.9), 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 0.2 mM ATP/GTP/CTP, [α-^{35}S]UTP, 40U RNAsin (Promega, Madison, WI) and 20U T7 (CRF_1 receptor antisense; $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptor sense probes) and T3 (CRF_1 receptor sense; $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptor antisense probes) RNA polymerase for 60 min at 37 °C. Unincorporated nucleotides were removed using ammonium-acetate method; 100 µl of DNAse solution (1 µl DNAse, 5 µl of 5 mg/ml tRNA, 94 µl of 10 mM tris/10 mM $MgCl_2$) was added and 10 min later an extraction was accomplished using a phenol-chloroform solution. The cRNA was precipitated with 80 µl of 5M ammonium acetate and 500 µl of 100% ethanol for 20 min on dry ice. The pellet was washed with 500 µl 70% ethanol, dried, and resuspended in 100 µl of 10 mM Tris/1 mM EDTA. A concentration of 10^7 cpm probe was mixed into 1 ml of hybridization solution (500 µl formamide, 60 µl 5 M NaCl, 10 µl 1 M Tris [pH 8.0], 2 µl 0.5 M EDTA [pH 8.0], 20 µl 50x Denhart's solution, 200 µl 50% dextran sulfate, 50 µl 10 mg/ml tRNA, 10 µl 1M DTT, [118 µl Depc water - volume of probe used]). This solution was mixed and heated for 5 min at 65 °C before being spotted on slides.

3.4.6 Quantitative analysis

Semiquantitative analysis of hybridization signals for CRF_1 receptor mRNA was carried out in nuclear emulsion-dipped slides over the confines of cells within defined structures expressing the receptor using a Olympus Optical System (BX-50, BMax) coupled to a Macintosh computer (PowerPC 7100/66) and Image software (version 1.55 non-FPU, W. Rasband, NIH). The optical density (O.D.) of the hybridization signal was measured under darkfield illumination at a magnification of X10. Sections from the experimental and control animals were matched for rostro-caudal level. The regions (PVN, SON) were digitized and subjected to densitometric analysis, yielding measurements of integrated O.D.

(area of nucleus x average optical density). The O.D. of each specific region was then corrected for the average background signal, which was determined by sampling cells immediately outside the cell group of interest (McCabe and Pfaff, 1989).

3.4.7 Statistical Analysis

Data from Figures 7 and 9 are expressed as O.D. for CRF_1 receptor mRNA in the hypothalamic PVN and SON of control and immune-challenged rats. Results were analyzed by a 2 x 2 analysis of variance (ANOVA), followed by a Bonferroni/Dunn test procedure as post-hoc comparisons for each time post-injection (Statview 4.01). Factors were identified as follows: *intraperitoneal treatment*, which was composed of two levels (i.p. vehicle or i.p. LPS) and *intravenous treatment*, which was also divided into two levels (i.v. control or i.v. indomethacin).

3.5 RESULTS

3.5.1 Distribution of CRF_1 receptor mRNA throughout the rat brains

Figure 1 illustrates a representative example of the distribution of the CRF_1 receptor gene expression in the brains of immune-challenged (first three columns) and control (right column) male rats. Hybridized tissues with the sense probe did not exhibit detectable signal in any of the regions that showed positive signal with the antisense probe (results not shown). As recently reported (Potter et al., 1994; Rivest et al., 1995) and confirmed by this study (see table 1 for a detailed qualitative analysis of hybridization signal on x-ray film), strong basal levels of CRF_1 receptor transcript were observed in several regions of the brain, such as the piriform cortex, medial nucleus of the amygdala, basolateral nucleus of the amygdala, subthalamic nucleus, red nucleus, laterodorsal tegmental nucleus, pontine gray, Purkinje and granule cell layers of the cerebellum, nucleus incertus, spinal nucleus of the trigeminal nerve (oral part), principal sensory nucleus of the trigeminal nerve, external cuneate nucleus, and various layers of the cerebral cortex. A low to moderate signal was also detected in multiple sites, including the medial septal nucleus, nucleus of the diagonal band, bed nucleus of the stria terminalis, supraoptic nucleus (SON), central nucleus of the amygdala, dorsomedial nucleus of the hypothalamus, caudal division of the zona incerta, pretectal area, lateral geniculate complex, substantia nigra, interpeduncular nucleus (central

subnucleus), parabrachial nucleus, medial vestibular nucleus, nucleus prepositus, spinal nucleus of the trigeminal nerve (interpolar part), lateral reticular nucleus, and gracile nucleus.

3.5.2 CRF of type 2 receptors

In contrast to CRF_1 receptor which is widely distributed throughout the rat brain, the mRNA encoding the $CRF_{2\alpha}$ receptor is highly localized in very few regions of the brain (Figure 2). Indeed, positive hybridization signal was detected in the lateral septal nucleus, principal nucleus of the bed nucleus of the stria terminalis, ventromedial hypothalamic nucleus, cortico-amygdaloid nucleus, entorhinal cortex, and interpeduncular nucleus. LPS administration did not alter the endogenous expression of $CRF_{2\alpha}$ receptor transcript in these spontaneously expressing structures (Figure 2, left column). As opposed to Lovenberg et al. (1995a), we have not seen any convincing evidence of positive signal for the mRNA encoding the CRF type 2α receptor within the hypothalamic PVN and the SON on brain sections either exposed on x-ray film or dipped in NTB-2 nuclear emulsion.

On the other hand, positive signal was clearly hybridized with $CRF_{2\beta}$ antisense probe only in the choroid plexus and not in any other regions of control, immune-challenged, and immobilized rat brains (data not shown). These results demonstrate a very distinct and selective distribution of each CRF receptor subtypes in the rat brains.

3.5.3 Site-specific induction of CRF_1 receptor transcription in the brain of immune-challenged animals

Vehicle-treated rats hardly displayed detectable signal for CRF_1 receptor mRNA in the paraventricular nucleus (PVN), but the transcription of that gene was highly stimulated in this hypothalamic region following administration of various doses of LPS (Figure 1). High dose of endotoxin (250 µg/100 g of b.w.) caused a robust expression of CRF_1 receptor transcript in both parvocellular and magnocellular subdivisions of the PVN 6 h after treatments. A more selective induction of the mRNA encoding this receptor was detected in the parvocellular PVN in animals administered with a moderate (25 µg/100 g of b.w.) or a low (2.5 µg/100 g of b.w.) dose of LPS. Indeed, while a strong level of transcripts was observed in the parvocellular PVN, the magnocellular division of this hypothalamic nucleus exhibited a low signal in moderately immune-challenged rats. Interestingly, the low

expression of the type 1 receptor in the magnocellular division of the PVN of animals treated with the moderate dose of LPS seems to correspond to the pattern of oxytocin containing cells (external ring), whereas high dose invoked a stronger and more uniform expression of this gene within the magnocellular PVN.

Although a small basal expression of CRF_1 receptor mRNA was detected in the SON of several vehicle-treated rats, a notable increase in the levels of transcript was obtained 3 and 6 h following systemic treatment with a high and moderate doses of bacterial endotoxin. However, the low dose of LPS did not markedly raise the expression of the gene encoding the CRF_1 receptor in this hypothalamic structure.

As presented in the table 1, i.p. endotoxin administration did not appear to modulate significantly the expression of CRF_1 receptor mRNA in other regions, which provides evidence that immune challenge triggers transcription of the gene encoding the type 1 receptor in very selective hypothalamic nuclei directly involved in the regulation of neuroendocrine functions.

3.5.4 Effects of indomethacin in the brains of immune-challenged rats

Figures 3, 4, and 5 show the effect of indomethacin i.v. injection on the expression of CRF_1 receptor transcript in the PVN of rats treated with high (250 µg/100 g of b.w.), moderate (25 µg/100 g of b.w.), and low dose (2.5 µg/100 g of b.w.) of the bacterial endotoxin, respectively. Inhibition of cyclo-oxygenase pathways did not prevent stimulation of CRF_1 receptor transcription in the PVN of severely immune-challenged rats (Figure 3, bottom panels). Indeed, high dose of LPS induced expression of CRF_1 receptor in parvocellular and magnocellular divisions of the PVN in both indomethacin- and vehicle-pretreated animals. In contrast, inhibition of PGs interrupted biosynthesis of CRF_1 receptor in animals injected i.p. with a moderate (Figure 4, bottom panels) and a low (Figure 5, bottom panels) dose of LPS at both times post-injection.

Figure 6 depicts a high magnification brightfield photomicrographs of the influence of PG inhibition on the intensity of silver grains in CRF_1 receptor positive neurons of the PVN. Agglomeration of silver grains delineating positive cells in the parvocellular PVN was similar in animals treated or not with indomethacin i.v. before being administered with high dose of

the endotoxin i.p. In contrast, inhibition of cyclo-oxygenase pathways largely prevented induction of CRF_1 receptor positive neurons in moderately immune-challenged animals (Figure 6, middle panels).

The average optical density (O.D.) of the hybridization signal for CRF_1 receptor transcript in the PVN after i.p. administration of high (top panels), moderate (middle panels) or low (bottom panels) dose of the bacterial endotoxin is presented by Figure 7. The O.D. was quantified under darkfield illumination to reveal the silver grains on hybridized 30-μm sections dipped in NTB-2 nuclear emulsion. The average O.D. for the CRF_1 receptor hybridized signal in the PVN peaked 6 h after a single i.p. administration of high and moderate dose of LPS, whereas a modest increase was detected 3 and 6 h after treatment with the low dose (Figure 7, bottom panel). Pretreatment with indomethacin did not interfere with the signal intensity of CRF_1 receptor transcript in the PVN of rats injected with a high dose of LPS. In contrast, inhibition of cyclo-oxygenase pathways significantly ($P < 0.05$) abolished expression of CRF_1 receptor mRNA in this hypothalamic region in rats injected with a moderate or a low dose of LPS; the CRF_1 receptor mRNA levels were three (moderate dose) and two (low dose) times higher in rats receiving the endotoxin alone than those submitted to a treatment combining both i.v. indomethacin and i.p. LPS treatments.

Figure 8 exhibits representative examples of CRF_1 receptor mRNA levels in the SON of rats administered with a moderate dose of the bacterial LPS. Inhibition of PG synthesis prevented the endotoxin-induced expression of the mRNA encoding the CRF_1 receptor in the SON in moderately immune-challenged rats (Figure 8, bottom panels). On the other hand, the intensity of CRF_1 receptor signal was similar in rats sacrificed 3 h after injection of high dose of LPS and pretreated or not with indomethacin i.v. (Figure 9, top panel). Inhibition of cyclo-oxygenase pathways significantly ($P < 0.05$) attenuated the increase in the average O.D. for CRF_1 receptor mRNA 6 h after treatment with high dose of endotoxin. However, the SON still displayed high levels of transcript in this group of rats (Figure 9, top panel), whereas pretreatment with indomethacin totally abolished the LPS-induced stimulation of CRF_1 receptor expression in the SON of moderately immune-challenged rats (Figure 9, middle panel).

3.6 DISCUSSION

Although the mRNA encoding the type 1 receptor for the stress-related neuropeptide CRF is widely distributed throughout the brain, as previously reported (Rivest et al., 1995) and further confirmed in the present study, immune challenge does not seem to alter the hybridization signal for this receptor in most of these spontaneously expressing regions (see table I). Similarly, the bacterial endotoxin did not interfere with the endogenous expression of the highly localized CRF type 2 receptors; $CRF_{2\alpha}$ receptor mRNA was detected in few nuclei of the limbic system while the choroid plexus was the only structure exhibiting a positive hybridization signal for the mRNA encoding the 2β form in the brains of control and challenged rats. Interestingly, administration of a low dose of LPS (2.5 μg/100g b.w.) induced a specific expression of CRF_1 receptor mRNA in the parvocellular PVN, whereas high dose of the endotoxin (250 μg/100 g of b.w.) produced a strong activation of CRF_1 receptor transcription in both parvo- and magnocellular divisions of the hypothalamic PVN as well as in the SON. Indomethacin did not significantly modulate the signal intensity of CRF_1 receptor mRNA in the PVN of severely immune-challenged rats, whereas inhibition of PG synthesis completely prevented transcription of the receptor in this hypothalamic nucleus following i.p. injection with moderate and low doses of the bacterial endotoxin. Taken together, the present study provides evidence that systemic LPS administration induces expression of the gene encoding specifically the CRF receptor of type 1 in the PVN and the SON of male rats, an event probably related to the control of the HPA axis activity. The role of PGs as mediators of immune system to trigger CRF_1 receptor gene expression appears to depend on the severity of the immune challenge; stimulation of cyclo-oxygenase pathways is in fact a mechanism involved in the induction of CRF_1 receptor mRNA in neuroendocrine nuclei following systemic administration with a moderate and low dose of the endotoxin while production of PGs in severely immune-challenged rats does not seem to be a decisive mechanism involved in this phenomenon.

The role of eicosanoid cyclo-oxygenase pathways in mediating several neuroendocrine responses to immune challenge and to acute exposure to cytokines is well known. Several studies have shown that the effect of cytokines on the CRF release and the activity of the HPA axis can be antagonized by drugs which block PG synthesis; the stimulatory effect of IL-1 and IL-6 on CRF, vasopressin (AVP), and oxytocin (OT) release from rat hypothalami *in vitro* is inhibited by indomethacin (Bernardini et al., 1990; Navarra et al., 1991; Yasin et al., 1994). Blockage of PG production also prevents the IL-1-induced

alteration of neuroendocrine LHRH system and plasma LH levels in female rats (Rivest and Rivier, 1993). Smith et al (1994) recently reported that PGE_2 participates in the hypothalamic response to endotoxin-mediated adrenocortical activation in female rats. Systemic injection with bacterial LPS causes production of PGD_2 in the OVLT/preoptic area (Ueno et al., 1982) whereas IL-1ß and IL-6 specifically stimulate PGE_2 release from hypothalamic explants *in vitro* in a dose dependent manner (Navarra et al., 1992). Moreover, the production of PGE_2 in the hypothalamus is increased selectively during LPS-induced fever (Sirko et al., 1989). Our results demonstrate that the PGs have an essential role in the activation of CRF_1 receptor gene transcription in the brains of moderately and scarcely immune-challenged rats. Indeed, inhibition of cyclo-oxygenase pathways significantly inhibited the expression of CRF_1 receptor in specific neuroendocrine nuclei of rats sacrificed after being injected with a moderate or a low dose of LPS.

The exact circuitry through which PGs of various types can interfere with mechanisms involved in the regulation of CRF_1 receptor within the hypothalamic PVN and SON of immune-challenged rats has yet to be clarified. Systemic administration of the bacterial LPS increases circulating concentrations of cytokines, including IL-1 and IL-6 (Berkenbosch et al., 1991). It is thus possible that these cytokines of systemic origin penetrate the fenestrated ependymal cells forming the ventricular surfaces of the circumventricular organs, such as the OVLT which contains a rich vascular plexus with specialized arrangements of the blood vessels (for review, see Oldfield and Mckinley, 1995). The tight junctions normally present between the endothelial cells are shifted in part to the ventricular surface and partly to the boundary between the OVLT and the adjacent structures explaining that large molecules can diffuse into perivascular region (Oldfield and Mckinley, 1995), in particular during fever. Interestingly, i.p. injection with the bacterial LPS induces strong expression of the immediate *early* gene c-*fos* mRNA in the OVLT/MPOA along with numerous other structures of the rat brain (Rivest and Laflamme, 1995). Whether endothelial cells, astrocytes, and/or neurons located in the OVLT/MPOA display positive signal for c-*fos* transcript in immune-challenged rats is presently under investigation, but all these cell types could be involved in the interaction between systemic immune system and neuroendocrine functions. Cultured murine astrocytes bind with IL-1α and ß (Ban et al., 1993) and recombinant human IL-1ß increases PGE_2 in a dose-dependent manner in rat astrocyte cultures (Katsuura et al., 1989). Moreover, microinjection of indomethacin directly into the OVLT/MPOA region suppresses the ACTH response to i.v. IL-1ß (Katsuura et al., 1990) suggesting that production of PGE_2 by astrocytes of the OVLT/MPOA could participate in neuroendocrine responses to IL-1 and LPS challenges. Intrapreoptic microinjection of PGE_2

activates the activity of the HPA axis possibly via a CRF-dependent pathway (Katsuura et al., 1990), although Bernardini et al. (1989) failed to observe any direct stimulatory influence of this PG on hypothalamic CRF secretion from explanted rat hypothalami in culture.

Local production of PGs within the OVLT/MPOA could be a determinant mechanism through which immune challenge triggers the transcription of the gene encoding CRF_1 receptor in the hypothalamic PVN and SON; high density of PGE_2 binding sites is found in the OVLT/MPOA (Matsumura et al., 1990) and this region has direct neuronal connection with the PVN (Sawchenko and Swanson, 1983). Stimulation of the rat POA increases the firing rates of neurons located in the PVN and raises plasma corticosterone levels (Saphier and Feldman, 1986). Neurons of the OVLT have also been shown to project to the SON (Sawchenko and Swanson, 1983) and may therefore represent critical sensors of circulating material. Elegant study by Kovacs and Sawchenko reported that OVLT can serve both to monitor the osmotic composition of the blood and transmit this information to the PVN and the SON (Kovacs and Sawchenko, 1993). Indeed, discrete transsections of descending projections from structures associated with the lamina terminalis, as well as excitotoxin lesions centered in one lamina terminalis-associated structures, the organum vasculosum, abolish the effect of salt loading stress on the CRF gene expression in both magno- and parvocellular neurosecretory systems (Kovacs and Sawchenko, 1993). It is thus possible that a production of circulating cytokines act through a similar circuitry during a moderate immune challenge to activate the transcriptional machinery of CRF_1 receptor in selective neuroendocrine nuclei of the rat hypothalamus.

Pretreatment with indomethacin did not prevent transcription of CRF type 1 receptor in the PVN and partly prevented expression of the transcript in the SON of rats injected with a high dose of the bacterial LPS. It is however important to keep in mind the possibility that the dose of indomethacin used in the present study did not completely inhibit the PG formation in LPS-treated rats, although as mentioned before, this dose has been shown to be quite efficient to prevent the effect of LPS and IL-1 on various neuroendocrine functions. Therefore, our results, with all reserves, indicate that the role of PGs in mediating the stimulatory influence of immune challenge on the transcription of CRF_1 receptor in the PVN and SON might depend on the severity of this systemic stressful situation. In fact, the production of PGs seems to be a key mechanism involved in triggering the biosynthetic machinery of the gene encoding this receptor in the PVN during a weak and moderate immune challenge but not during a severe activation of the acute-phase response. Although

the involvement of eicosanoid cyclo-oxygenase remains possible in this situation, several other factors can be considered as potential mediators through which immune system can trigger transcription of the gene encoding CRF_1 receptor in selective hypothalamic nuclei of severely immune-challenged animals. As mentioned, systemic administration with LPS activates expression of the immediate *early* genes c-*fos* and NGFI-B in the OVLT/MPOA but also in numerous other structures including the subfornical organ, the arcuate nucleus/median eminence, the central nucleus of the amygdala, laterodorsal tegmental nucleus (LDT), locus coeruleus (LC), parabrachial nucleus (PB), area postrema, nucleus of the solitary tract (NTS), and A1 cell group (Rivest and Laflamme, 1995). Immune system might therefore use several pathways and sites of entry to communicate with the brain and to activate the CRF neurons within selective endocrine nuclei. The noradrenergic (A_1, A_2, A_6) and adrenergic (C_1-C_3) pathways from the brainstem could mediate the influence of different cytokines of systemic origin produced during the acute-phase response to stimulate the parvo- and magnocellular neurons of the hypothalamic PVN as well as in the SON. Indeed, Ericsson et al. (1994) recently suggested that C_1, C_2, and A_2 aminergic cell groups are involved in the hypothalamic response to IL-1ß. This group also raised the possibility that circulating IL-1 may be transduced peripherally by a sensory component of the cranial nerves or centrally at the level of the aminergic neurons themselves to interact with CRF neuronal activity in the PVN. Interestingly, the involvement of the vagus nerve in mediating the effect of systemic (i.p.) LPS on Fos-ir in the NTS and the PVN was recently demonstrated (Wan et al., 1994). However, in the brainstem of LPS-treated rats, c-*fos* mRNA is expressed not only in the NTS and in the A_1-C_1 regions, but also in the LC, PB, and the LDT. These nuclei could either relay the information from the NTS to the neuroendocrine nuclei or modulate themselves some of the effects of cytokines on neuroendocrine functions. A complex neuronal circuitry is thus probably involved to trigger the activity of neuroendocrine CRF and the expression of its type 1 receptor in the PVN and SON of severely immune-challenged animals.

The physiological relevance of the CRF_1 receptor induction in endocrine nuclei of immune-challenged rats still remains highly hypothetical. CRF might play a direct role in controlling the activity of neuroendocrine CRF motoneurons during stress; CRF-immunoreactive perikarya of the parvocellular PVN express the gene encoding the CRF_1 receptor following immobilization stress (Nappi and Rivest, 1995) and i.p. LPS administration (Rivest et al., 1995). Because of the late expression of the mRNA encoding the type 1 CRF receptor following systemic treatment with the bacterial LPS (3 and 6 h post-injection), its involvement as mechanism participating to stimulate the release of CRF in the

median eminence is unlikely. We recently reported that intracerebroventricular administration of CRF causes expression of c-*fos* and NGFI-B and increases relative levels of CRF mRNA in the parvocellular neurons of the PVN (Parkes et al., 1993), suggesting that CRF may be a potential modulator of its own biosynthesis in the PVN of stressed rats. However, the presence of CRF_1 receptor mRNA does not precede the transcription of CRF in the parvocellular PVN of LPS-treated rats. One hour after systemic injection with the endotoxin, a strong signal for CRF primary transcript {CRF heteronuclear (hn)RNA} is detected in the parvocellular PVN (Rivest and Laflamme, 1995), whereas at that time, the same structure hardly exhibits detectable signal for the gene encoding the CRF receptor (Rivest et al., 1995). Consequently, the temporal changes in the expression of both CRF_1 receptor mRNA and CRF hnRNA do not provide evidence that induction of the receptor is a mechanism involved in activating neuroendocrine CRF transcription. The fact that CRF itself can participate in post-transcriptional events to restore the depletion of neuroendocrine CRF secreted in the infundibulum during immune challenge should, however, be considered.

The presence of CRF_1 receptor transcript in the magnocellular division of the hypothalamic PVN and SON is quite intriguing. These data provide evidence that CRF play a role in modulating magnocellular neurons of immune-challenged animals, although whether the receptor is expressed in oxytocin (OT) and/or vasopressin (AVP) neurons as yet to be determined. Immune challenge can activate the release of AVP into the hypophyseal-portal circulation (Harbuz et al., 1992) and AVP is well known for its supportive role for CRF in stimulating the release of ACTH in the rat (Rivier and Plotsky, 1986). It is also possible that AVP neurons stimulate directly neuroendocrine CRF neurons through magnocellular neurons of the SON and PVN innervating CRF perikarya located in the parvocellular division of the PVN (for review, see Swanson, 1991). The indirect action of CRF on these magnocellular neurons could be a mechanism involved in maintaining and restoring the peptide depletion because of the strong need of neuroendocrine CRF during a long lasting stress such as severe immune response. On the other hand, the presence of CRF in the magnocellular PVN and SON might be directly related to osmotic and blood pressure changes caused by systemic LPS, in particular with high dose of the bacterial endotoxin. The levels of CRF mRNA and peptide in the magnocellular PVN and SON is hardly detectable under normal conditions, but the increased plasma osmolarity associated with salt loading causes a considerable expression of the gene encoding CRF (Kovacs and Sawchenko, 1993; Young, 1986) and its type 1 receptor (Luo et al., 1994) in these hypothalamic structures. It is possible that CRF is involved in the control of AVP and/or OT neurons of neurohypophyseal system and therefore

represents an adaptive response to a severe systemic immune challenge to maintain blood homeostasis.

In conclusion, while the gene encoding CRF_1 receptor is widely distributed in the rat brain, positive hybridization signal for CRF of type 2 receptors is highly localized. Immune challenge activated the transcription of the CRF_1 receptor subtype in very selective endocrine nuclei; systemic injection of a low dose of endotoxin induced a specific expression of CRF_1 receptor mRNA in the parvocellular PVN, whereas high dose of the bacterial LPS caused strong transcription of the gene encoding this particular receptor in both parvo- and magnocellular divisions of the PVN and the SON. Administration of the eicosanoid cyclo-oxygenase inhibitor indomethacin largely abolished transcription of CRF_1 receptor in the PVN and SON of moderately and scarcely immune-challenged rats but not in animals receiving high dose of LPS i.p. These results indicate that PGs play a limited role as mediators of systemic immune response on the hypothalamic expression of CRF_1 receptor transcript.

3.7 ACKNOWLEDGMENTS

This research was supported by the Medical Research Council (MRC) of Canada. Serge Rivest is a Medical Research Council Scholar. We thank Dr. Wylie W. Vale, PBL, The Salk Institute, La Jolla, CA, for the generous gift of rat CRF_1 receptor cDNA and Dr. Timothy W. Lovenberg, Department of Molecular Biology, Neurocrine Biosciences, Inc. San Diego, CA for the gift of plasmids containing rat $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptor cDNAs. The authors would also like to thank Miss Nathalie Laflamme for the invaluable technical assistance and Dr Rossella E. Nappi for precious advice and discussion.

3.8 REFERENCES

Ban EM, Sarlième LL and Haour FG (1993) Interleukin-1 binding sites on astrocytes. Neuroscience 52: 725-733.

Berkenbosch F, Wolvers DAW and DeRijk R (1991) Neuroendocrine and immunological mechanisms in stress-induced immunomodulation. J. Steroid Biochem. Biol. 40: 639-647.

Bernardini R (1989) Arachidonic acid metabolites modulate rat hypothalamic cortocotropin-releasing hormone secretion *in vitro*. Neuroendocrinology 50: 708-715.

Bernardini R, Calogero AE, Mauceri G and Chrousos G (1990) Rat hypothalamic corticotropin-releasing hormone secretion in vitro is stimulated by interleukin-1 in an eicosanoid-dependent manner. Life Sciences 47: 1601-1607.

Blalock J and Smith EM (1985) A complete loop between the immune system and neuroendocrine systems. Fed. Proc. 44: 108-111.

Chen R, Lewis KA, Perrin MH and Vale WW (1993) Expression cloning of a human corticotropin-releasing factor receptor. Proc. Natl. Acad. Sci. (USA) 90: 8967-8971.

Cominelli F, Nast CC, Dinarello CA, Gentilini P and Zipser RD (1989) Regulation of eicosanoid production in rabbit colon by interleukin-1. Gastroenterology 97: 1400-1405.

Dinarello CA (1989) Interleukin-1 and its biologically related cytokines. Adv. Immunol. 44: 153-161.

Ericsson A, Kovacs KJ and Sawchenko PE (1994) A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. J. Neurosci. 14: 897-913.

Harbuz MS, Rees RG, Eckland D, Jessop DS, Brewerton D and Lightman SL (1992) Paradoxical responses of hypothalamic corticotropin-releasing factor (CRF) messenger ribonucleic acid (mRNA) and CRF-41 peptide and adenohypophysial pro-opiomelanocortin mRNA during chronic inflammatory stress. Endocrinology 130: 1394-1400.

Harms PG, Ojeda SR and McCann SM (1973) Prostaglandin involvement in the hypothalamic control of gonadotropin and prolactin release. Science 181: 700-700.

Higgins GA and Olschowka JA (1991) Induction of interleukin-1ß mRNA in adult rat brain. Mol. Brain Res. 9: 143-148.

Hughes JH, Easom RA, Wolf BA, Turk J and McDaniel ML (1989) Interleukin-1-induced prostaglandin E2 accumulation by isolated pancreatic islets. Diabetes 38: 1251-1257.

Katsuura G, Arimura A, Koves K and Gottschall PE (1990) Involvement of organum vasculosum of the lamina terminalis and preoptic area in interleukin-1ß-induced ACTH release. Am. J. Physiol. 258: E163-E171.

Katsuura G, Gottschall PE, Dahl RR and Arimura A (1989) Interleukin-1ß increases prostaglandin E2 in rat astrocyte cultures: modulating effect of neuropeptides. Endocrinology 124: 3125-3127.

Kerr JS, Stevens TM, Davis GL, Mclaughlin JA and Harris RR (1989) Effects of recombinant interleukin-1 beta on phospholipase A2 mRNA levels, and eicosanoid formation in rabbit chondrocytes. Biochem. Biophys. Res. Com. 165: 1079-1084.

Kishimoto T, II RVP, Lin CR and Rosenfeld MG (1995) A sauvagine/corticotropin-releasing factor receptor expressed in the heart and skeletal muscle. Proc. Natl. Acad. Sci. USA 92: 1108-1112.

Koenig JI (1991) Presence of cytokines in the hypothalamic-pituitary axis. Prog. NeuroEndocrImmunol. 4: 143-153.

Kohan DE (1989) Interleukin-1 regulation of prostaglandin E2 synthesis by the papillary collecting duct. J. Lab. Clin. Med. 114: 717-723.

Kovacs KJ and Sawchenko PE (1993) Mediation of osmoregulatory influences on neuroendocrine corticotropin-releasing factor expression by the ventral lamina terminalis. Proc. Natl. Acad. Sci. USA 90: 7681-7685.

Lovenberg TW, Chalmers DT, Liu C and Souza EBD (1995a) CRF2α and CRF2β receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. Endocrinology 136: 4139-4142.

Lovenberg TW, Liam CW, Grigoriadis DE, Clevenger W, Chalmers DT, DeSouza EB and Oltersdorf T (1995b) Cloning and characterization of a functional distinct corticotropin-releasing factor receptor subtype from the rat brain. Proc. Natl. Acad. Sci. USA 92: 836-840.

Luo X, Kiss A, Makara G, Lolait SJ and Aguilera G (1994) Stress-specific regulation of corticotropin-releasing hormone receptor expression in the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J. Neuroendocrinol. 6: 689-696.

Matsumura K, Watanabe Y, Onoe H, Watanabe Y and Hayaishi O (1990) High density of prostaglandin E2 binding sites in the anterior wall of the 3rd ventricle: a possible site of its hyperthermic action. Brain Res. 533: 147-151.

McCabe JT and Pfaff DW (1989) *In situ* hybridization: a methodological guide. Methods Neurosci. 1: 98-117.

Nappi RE and Rivest S (1995) Ovulatory cycle influences the stimulatory effect of stress on the expression of corticotropin-releasing factor receptor messenger ribonucleic acid in the paraventricular nucleus of the female rat hypothalamus. Endocrinology 136: 4073-4083.

Nathan CF (1987) Secretory products of macrophages. J. Clin. Invest. 79: 319-326.

Navarra P, Pozzoli G, Brunetti L, Ragazzoni E, Besser M and Grossman A (1992) Interleukin-1ß and interleukin-6 specifically increase the release of prostaglandin E2 from rat hypothalamic explants *in vitro*. Neuroendocrinology 56: 61-68.

Navarra P, Tsagarakis S, Faria MS, Rees LH, Besser GM and Grossman AB (1991) Interleukin-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. Endocrinology 128: 37-44.

Ojeda SR, Harms PG and McCann SM (1975) Effect of inhibitors of prostaglandin synthesis on gonadotropin release in the rat. Endocrinology 97: 843-854.

Oldfield BJ and Mckinley MJ (1995) Circumventricular organs. In: The rat nervous system (Paxinos G, ed.) pp 391-403. San Diego: Academic Press.

Parkes D, Rivest S, Lee S, Rivier C and Vale W (1993) Corticotropin-releasing factor activates c-*fos*, NGFI-B and CRF gene expression within the paraventricular nucleus of the rat hypothalamus. Mol. Endocrinol. 7: 1357-1367.

Perrin M, Donaldson C, Chen R, Blount A, Berggren T, Bilezikjian L, Sawchenko P and Vale W (1995) Identification of a second corticotropin-releasing factor receptor gene and caracterization of a cDNA expressed in heart. Proc. Natl. Acad. Sci. USA 92: 2969-2973.

Perrin MH, Donaldson CJ, Chen R, Lewis KA and Vale WW (1993) Cloning and functional expression of a rat brain corticotropin-releasing factor (CRF) receptor. Endocrinology 133: 3058-3061.

Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE and Vale WW (1994) The distribution of CRF receptor mRNA expression in the rat brain and pituitary. Proc. Natl. Acad. Sci., USA 91: 8777-8781.

Rabin BS, Cunnick JE and Lysle DT (1990) Stress-induced alteration of immune function. Prog. NeuroEndocrinImmunol. 3: 116-125.

Raetz CR (1990) Biochemistry of endotoxins. Annu. Rev. Biochem. 59: 129-170.

Rivest S (1995) Molecular mechanisms and neural pathways mediating the influence of interleukin-1 on the activity of neuroendocrine CRF motoneurons in the rat. Int. J. Devl. Neuroscience 13: 135-146.

Rivest S and Laflamme N (1995) Neuronal activity and neuropeptide gene transcription in the brain of immune-challenged rats. J. Neuroendocrinol. 7: 501-525.

Rivest S, Laflamme N and Nappi RE (1995) Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. J. Neurosci. 15: 2680-2695.

Rivest S and Rivier C (1993) Centrally injected interleukin-1ß inhibits the hypothalamic LHRH secretion and circulating LH levels via prostaglandins in rats. J. Neuroendocrinol. 5: 445-450.

Rivest S and Rivier C (1995) The role of CRF and interleukin-1 in the regulation of neurons controlling reproductive functions. Endocrine Rev. 16: 177-199.

Rivier C (1993) Neuroendocrine effects of cytokines in the rat. Rev. Neurosci. 4: 223-237.

Rivier C and Rivest S (1993) Mechanisms mediating the effects of cytokines in neuroendocrine functions in the rat. In: Corticotropin-releasing factor, Ciba Foundation Symposium 172 (Chadwick DJ, Marsh J and Ackrill K, ed.) pp 204-225. Chichester: John Wiley & Sons Ltd.

Rivier CL and Plotsky PM (1986) Mediation by corticotropin-releasing factor (CRF) of adenohypophysial hormone secretion. Ann. Rev. Physiol. 48: 475-494.

Saphier D and Feldman S (1986) Effects of stimulation of the preoptic area on hypothalamic paraventricular nucleus unit activity and corticosterone secretion in freely moving rats. Neuroendocrinology 42: 167-173.

Sawchenko PE, Imaki T, Potter E, Kovács K and Vale W (1993) The functional neuroanatomy of corticotropin-releasing factor. In: Corticotropin-releasing factor, Ciba Foundation Symposium 172 (Chadwick DJ, Marsh J and Ackrill K, ed.) pp 5-29. Chichester: John Wiley & Sons Ltd.

Sawchenko PE and Swanson LW (1983) The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. J. Comp. Neurol. 218: 121-144.

Sawchenko PE and Swanson LW (1990) Organization of CRF immunoreactive cells and fibers in the rat brain: Immunohistochemical studies. In: Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide. (DeSouza EB and Nemeroff CB, ed.) pp 29-51. Boca Raton, Florida: CRC Press, Inc.

Simmons DM, Arriza JL and Swanson LW (1989) A complete protocol for *in situ* hybridization of messenger RNAs in brain and other tissus with radio-labeled single-stranded RNA probes. J. Histotechnol. 12: 169-181.

Sirko S, Bishai I and Coceani F (1989) Prostaglandin formation in the hypothalamus in vivo: effect of pyrogens. Am. J. Physiol. 256: R616-R624.

Smith T, Hewson AK, Quarrie L, Leonard JP and Cuzner ML (1994) Hypothalamic PGE2 and cAMP production and adrenocortical activation following intraperitoneal endotoxin injection: *in vivo* microdialysis studies in Lewis and Fischer rats. Neuroendocrinology 59: 396-405.

Stachura ME and Tyler JM (1986) Functional substructure of the rat somatotroph immediate release pool: Definition by response to N6, 2'-O-dibutyryl cyclic adenosine 3, 5' monophosphate, potassium ion or prostaglandin E1. Endocrinology 119: 2168-2176.

Stenzel P, Kesterson R, Yeung W, Cone RD, Rittenberg MB and Stenzel-Poore MP (1995) Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. Mol. Endocrinol. 9: 637-645.

Swanson LW (1991) Biochemical switching in hypothalamic circuits mediating responses to stress. In: Progress in Brain Research (Holstege G, ed.) pp 181-200. Amsterdam: Elsevier Science Publishers B.V. (Biochemical Division).

Ueno R, Narumiya S, Ogorochi T, Nakayama T, Ishikawa Y and Hayaishi O (1982) Role of prostaglandin D2 in the hypothermia of rats caused by bacterial lipopolysaccharide. Proc. Natl. Acad. Sci. U.S.A. 79: 6093-6097.

Wan W, Wetmore L, Sorensen CM, Greenberg AH and Nance DM (1994) Neural and biochemical mediators of endotoxin and stress-induced c-*fos* expression in the rat brain. Brain Res. Bull. 34: 7-14.

Weidenfeld J, Siegel RA, Conforti N and Chowers I (1983) ACTH and corticosterone secretion following indomethacin, in intact, adrenalectomized and dexamethasone-pretreated male rats. Neuroendocrinology 36: 49-52.

Yasin SA, Costa A, Forsling ML and Grossman A (1994) Interleukin-1ß and interleukin-6 stimulate neurohypophysial hormone release in vitro. J. Neuroendocrinol. 6: 179-184.

Young WS (1986) Corticotropin-releasing factor mRNA in the hypothalamus is affected differently by drinking saline and by dehydratation. FEBS Lett. 208: 158-162.

Table 1:

Qualitative analysis of hybridization signal for CRF receptor mRNAs in the rat brain under basal conditions.

Region	CRF_1-R	$CRF_{2\alpha}$-R	$CRF_{2\beta}$-R
Piriform cortex	++/+++	—	—
Medial septal nucleus	+/++	—	—
Lateral septal nucleus	—	++	—
Nucleus of the diagonal band	+	—	—
Bed nucleus of the stria terminalis	+	+	—
Supraoptic nucleus	0/+	—	—
Hypothalamic paraventricular nucleus	0/+	—	—
Basolateral nucleus of the amygdala	++/+++	—	—
Central nucleus of the amygdala	0/+	—	—
Medial nucleus of the amygdala	++/+++	—	—
Ventromedial nucleus of the hypothalamus	—	++/+++	—
Dorsomedial nucleus of the hypothalamus	+	—	—
Cortico-amygdaloid nucleus	—	0/+	—
Lateral geniculate complex	+/++	—	—
Pretectal area	+	—	—
Zona incerta, caudal division	0/+	—	—
Subthalamic nucleus	++/+++	—	—
Substantia nigra	+/++	—	—
Red nucleus	+++	—	—
Interpeduncular nucleus	++	0/+	—
Pontine gray	+++/++++	—	—
Dorsal raphe nucleus	—	0/+	—
Laterodorsal tegmental nucleus	+++	—	—
Parabrachial nucleus	+++	—	—
Cerebellum	+++/++++	—	—
Nucleus incertus	++/+++	—	—
Principal sensory nucleus of the trigeminal nerve	++/+++	—	—
Spinal nucleus of the trigeminal, oral part	++/+++	—	—
Nucleus prepositus	+/++	—	—
External cuneate nucleus	++/+++	—	—
Spinal nucleus of the trigeminal, interpolar part	+/++	—	—
Entorhinal cortex	+++	0/+	0/+
Cortex (general)	+++	—	—
Choroid plexus	—	—	++
Blood vessels	—	—	+

In situ hybridization histochemistry was accomplished via ^{35}S-labeled cRNA probes encoding specifically each CRF receptor subtype. The cDNA encoding the rat CRF_1 receptor was generously provided by Dr. Wylie Vale (The Salk Institute, La Jolla, CA) whereas the cDNAs encoding the CRF receptor of 2 subtypes were obtained from Dr. Tim Lovenberg (Neurocrine Biosciences, Inc., San Diego, CA). ++++, very strong signal; +++, strong signal; ++, moderate signal; +, low but positive signal; —, undetectable signal.

Table 2:

Qualitative analysis of hybridization signal for CRF_1 receptor transcript in the brain of immune-challenged rats (6 h after i.p. LPS administration) treated or non-treated with indomethacin.

Dose of LPS	250 μg/100 g b.w.		25 μg/100 g b.w.		2.5 μg/100 g b.w.			
Region	LPS	LPS-IND	LPS	LPS-IND	LPS	LPS-IND	IND	VEH
Piriform cortex	++/+++	+++	++/+++	++/+++	++/+++	+++	+++	++/+++
Medial septal nucleus	+/++	++	+/++	+/++	+/++	+/++	++	+/++
Nucleus of the diagonal band	+	+/++	+	+	+	+/++	+/++	+
Bed nucleus of the stria terminalis	+/++	+	+	+	+/++	+	+/++	+
Supraoptic nucleus	+++/++++	++/+++	+++/++++	+/++	+/++	0/+	0/+	0/+
Hypothalamic paraventricular nucleus	++/+++	++/+++	+++/++++	+	+	0/+	0/+	0/+
Basolateral nucleus of the amygdala	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++
Central nucleus of the amygdala	0/+	0/+	0/+	+	0/+	0/+	0/+	0/+
Medial nucleus of the amygdala	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++
Dorsomedial nucleus of the hypothalamus	+/++	+	+	+	+	+	+	+
Lateral geniculate complex	++	+/++	++	+/++	+/++	++	+/++	+/++
Pretectal area	0/+	0/+	0/+	+	+	+	+	+
Zona incerta, caudal division	0/+	0/+	0/+	0/+	0/+	0/+	0/+	0/+
Subthalamic nucleus	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++
Substantia nigra	+/++	+/++	+/++	+/++	+/++	+/++	+/++	+/++
Red nucleus	+++	++	++/+++	+++	++/+++	+++	++/+++	+++
Interpeduncular nucleus	+/++	+/++	+/++	+/++	+/++	++/+++	+/++	++
Pontine gray	+++/++++	+++/++++	+++/++++	+++/++++	+++/++++	++++	+++/++++	+++/++++
Laterodorsal tegmental nucleus	+++	++/+++	++/+++	++/+++	++/+++	+++	+++	+++
Cerebellum	+++/++++	+++/++++	+++/++++	+++/++++	+++/++++	+++/++++	+++/++++	+++/++++
Nucleus incertus	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++
Principal sensory nucl. of the trigeminal	++	++	++	+/++	++/+++	+/++	++	++/+++
Spinal nucl. of the trigeminal, oral part	++/+++	++/+++	++	+/++	++/+++	+/++	++	++/+++
Nucleus prepositus	+/++	+/++	+/++	+/++	+/++	+/++	+/++	+/++
External cuneate nucleus	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++
Spinal nucl. of the trigeminal, interpolar	+/++	+	+/++	+	+/++	+/++	+/++	+/++
Cortex (general)	+++	+++	+++/++++	+++/++++	+++/++++	+++/++++	+++/++++	+++

These values represent the average of 4 to 12 animals per group. ++++, very strong signal; +++, strong signal; ++, moderate signal;+, low but positive signal. b.w., body weight; IND, indomethacin; LPS, lipopolysaccharide; VEH, vehicle.

Figure 1:

Representative example of the distribution of the mRNA encoding the corticotropin-releasing factor (CRF) type 1 receptor in the rat brain after intraperitoneal (i.p.) administration of different doses (high, 250 μg/100 g b.w.; moderate, 25 μg/100 g b.w.; low, 2.5 μg/100 g b.w.) of the endotoxin lipopolysaccharide (LPS) or vehicle. Animals were deeply anesthetized and rapidly perfused with 4% paraformaldehyde 6 h after treatment with LPS or the vehicle solution. These rostrocaudal coronal sections (30 μm) of LPS- and vehicle-treated rat brains exhibit positive signal on X-ray film (kodak XAR 5) for the CRF_1 receptor transcript in the basolateral nucleus of the amygdala (BLA), central nucleus of the amygdala (CeA), external cuneate nucleus (ECU), interpeduncular nucleus (IPN), nucleus of the diagonal band (NDB), piriform cortex (Pir), nucleus prepositus (PRP), red nucleus (RN), substantia nigra, compact part (SNc), spinal nucleus of the trigeminal nerve, interpolar part (SPVi), and in various layers of the cerebral cortex. Note the selective induction of the CRF_1 receptor mRNA in the hypothalamic paraventricular nucleus (PVN) of immune-challenged male rats (first three columns). As illustrated, a high dose of endotoxin caused a robust expression of CRF_1 receptor transcript in both parvocellular and magnocellular subdivisions of the PVN. On the other hand, a more selective induction of the mRNA encoding this receptor was detected in the parvocellular PVN in animals administered with a moderate or low dose of LPS.

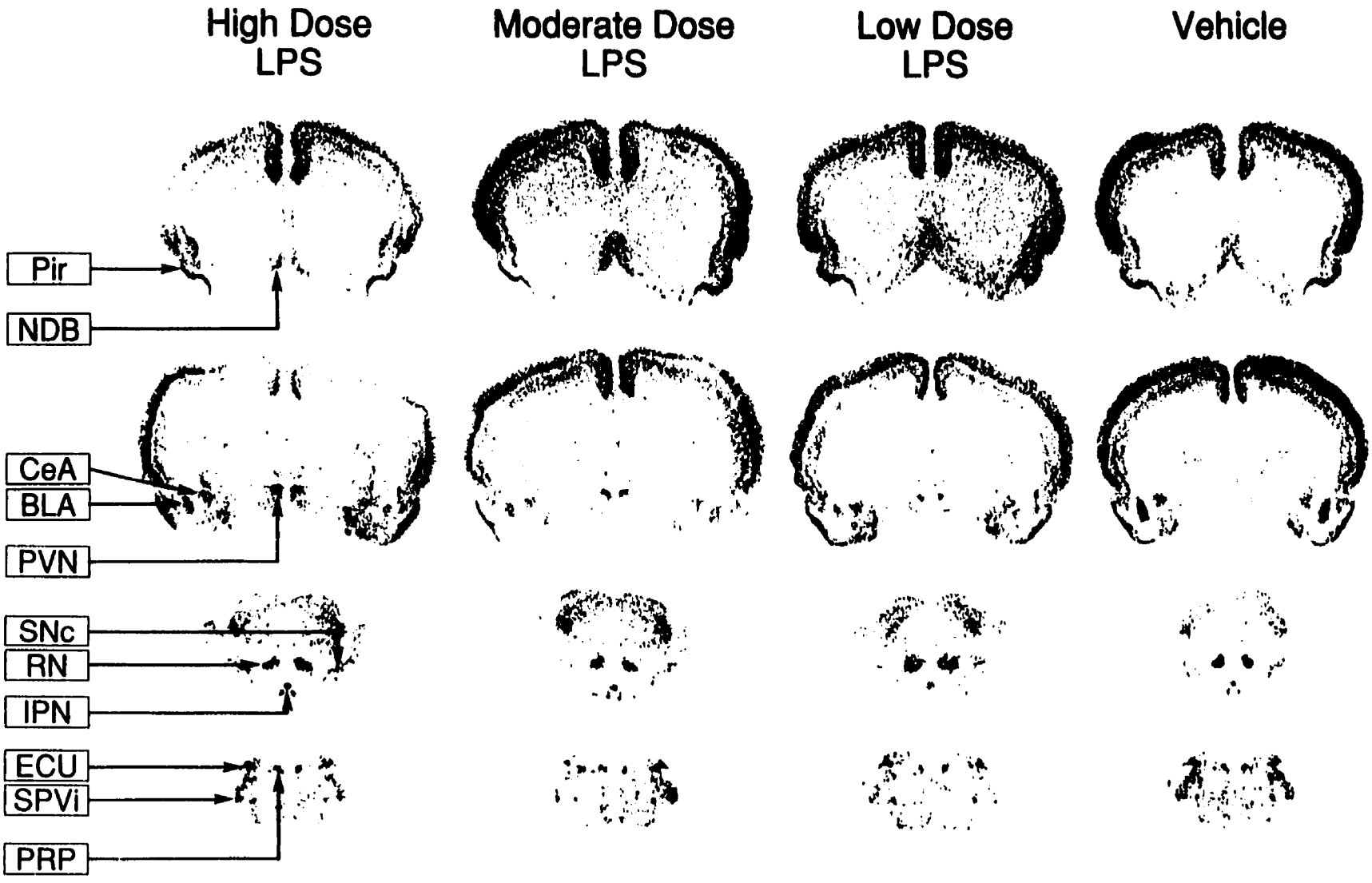

High Dose LPS
Moderate Dose LPS
Low Dose LPS
Vehicle
Pir
NDB
CeA
BLA
PVN
SNc
RN
IPN
ECU
SPVi
PRP

Figure 2:

Distribution of the mRNA encoding the $CRF_{2\alpha}$ receptor in the brains of immune-challenged and vehicle-treated rats. Animals were sacrificed 6 h after intraperitoneal treatment with the immune activator lipopolysaccharide (LPS, 25 μg/100 g b.w.) or the vehicle solution. These rostrocaudal coronal sections (30 μm) of LPS- and vehicle-treated rat brains exhibit a highly localized positive signal on X-ray film for the $CRF_{2\alpha}$ receptor transcript. BnST, bed nucleus of the stria terminalis, principal nucleus; IPN, interpeduncular nucleus; LS, lateral septal nucleus; VMH, ventromedial hypothalamic nucleus.

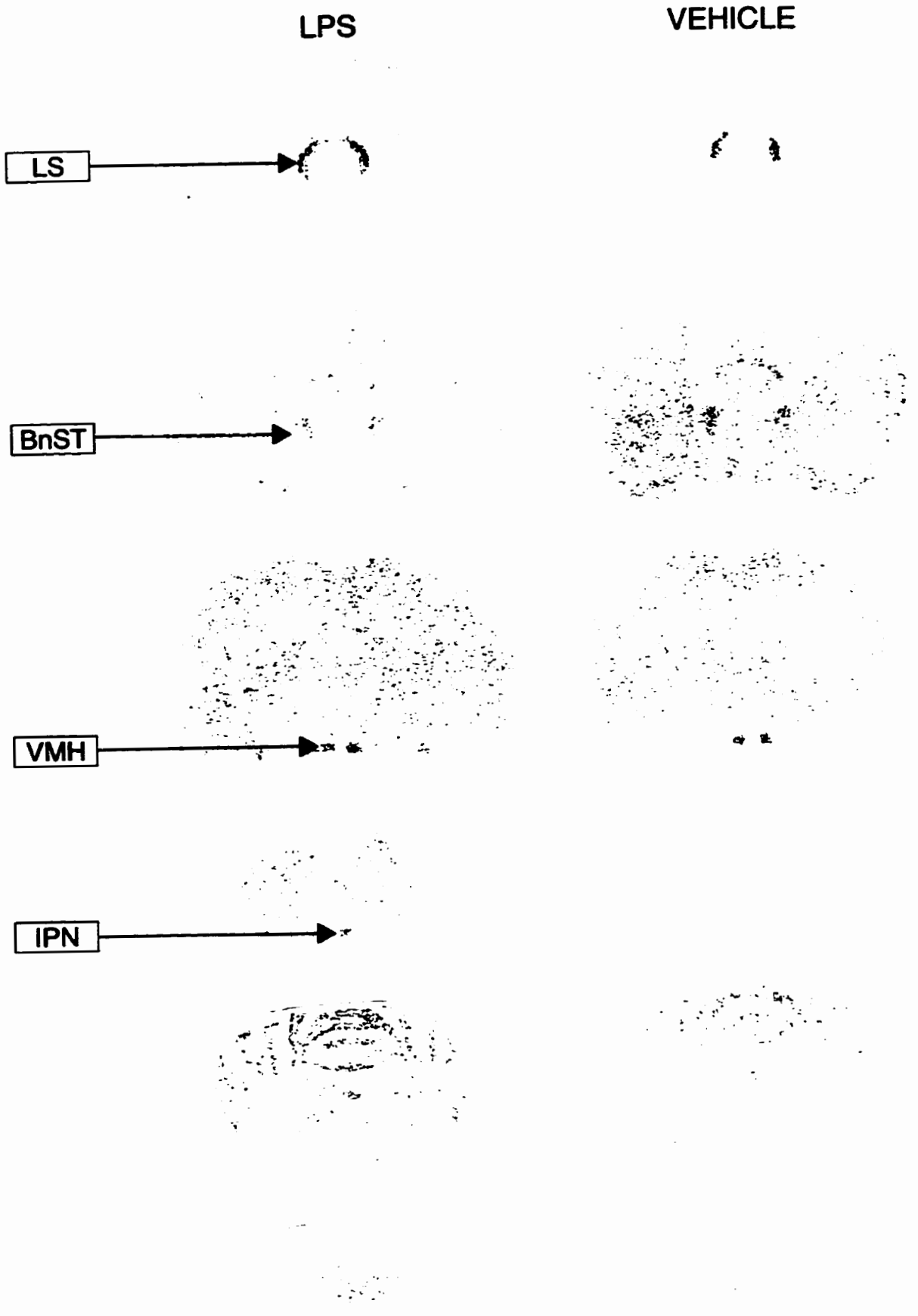

LPS
VEHICLE
LS
BnST
VMH
IPN

Figure 3:

Effect of indomethacin (IND) i.v. injection on the expression of CRF_1 receptor mRNA in the paraventricular nucleus (PVN) of the hypothalamus of rats treated with a high dose of the endotoxin lipopolysaccharide (LPS, 250 μg/100 g b.w.). These photos depict darkfield photomicrographs of dipped autoradiographs of hybridized 30-μm sections with CRF_1 receptor riboprobe through identical areas of the right PVN. Animals were deeply anesthetized and rapidly perfused with 4% paraformaldehyde 3 and 6 h after the treatments. Note that the inhibition of cyclo-oxygenase pathways did not prevent stimulation of CRF_1 receptor transcription in the PVN of severely immune-challenged rats. Moreover, it is possible to observe a strong hybridization signal in both parvo- and magnocellular divisions of the PVN following high dose of LPS, particularly at 6 h post-injection, in both indomethacin- and vehicle (Veh)-pretreated animals, whereas the PVN of control rats displayed hardly detectable CRF_1 receptor transcript. Magnification X25.

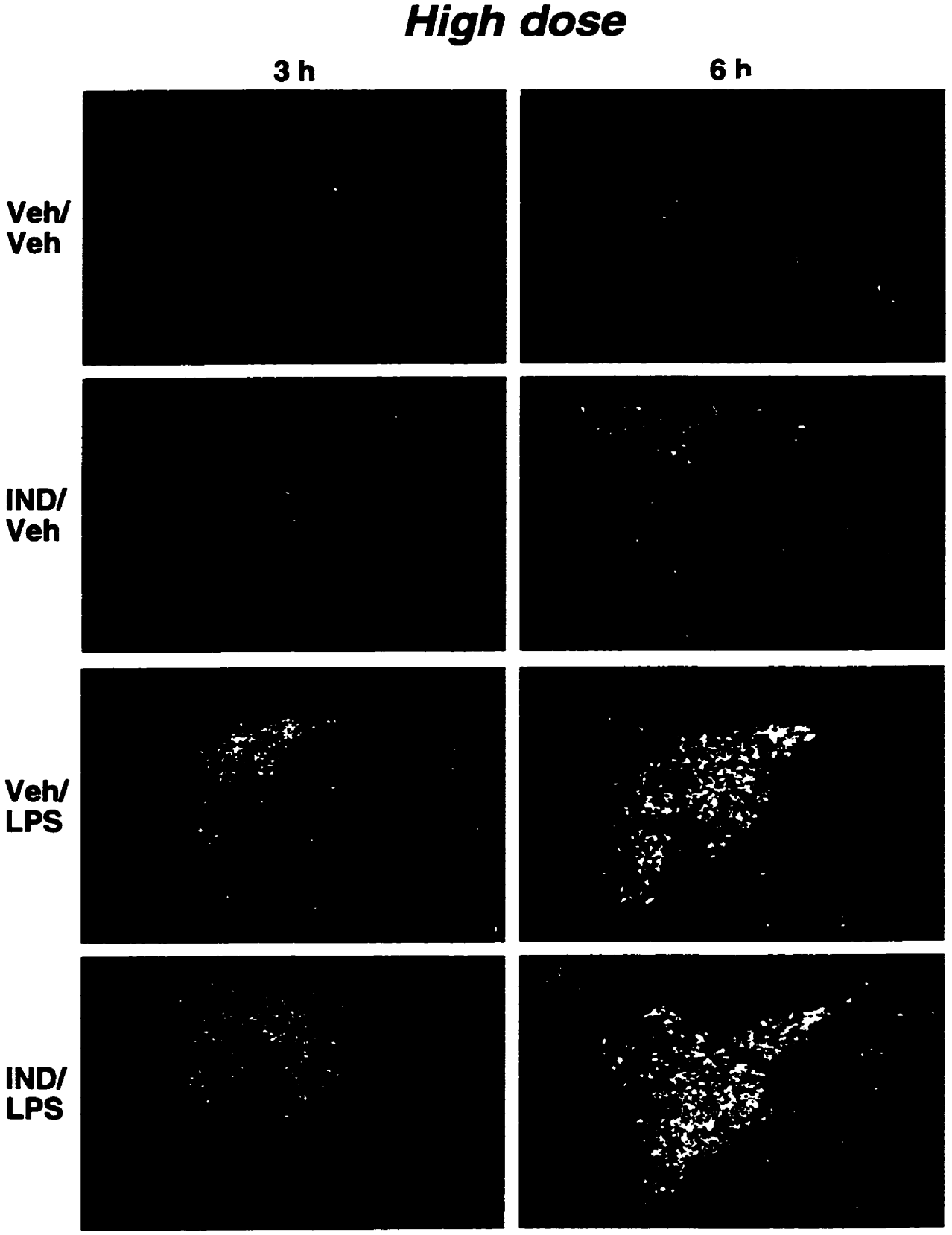

High dose
3 h
6 h
Veh/
Veh
IND/
Veh
Veh/
LPS
IND/
LPS

Figure 4:

Influence of cyclo-oxygenase pathways on the expression of CRF_1 receptor mRNA in the hypothalamic paraventricular nucleus (PVN) of rats treated i.p. with a moderate dose of the bacterial lipopolysaccharide (LPS, 25 μg/100 g b.w.). These photos depict darkfield photomicrographs of dipped autoradiographs of hybridized 30-μm sections with CRF_1 receptor riboprobe through identical areas of the right PVN. Note that inhibition of cyclo-oxygenase pathways interrupted transcription of CRF_1 receptor in animals injected i.p. with a moderate dose of LPS at both times post-injection (3 and 6 h). In addition, a more selective induction of the mRNA encoding this receptor was detected in the parvocellular PVN (the magnocellular division of this hypothalamic nucleus exhibited a low signal). Magnification X25.

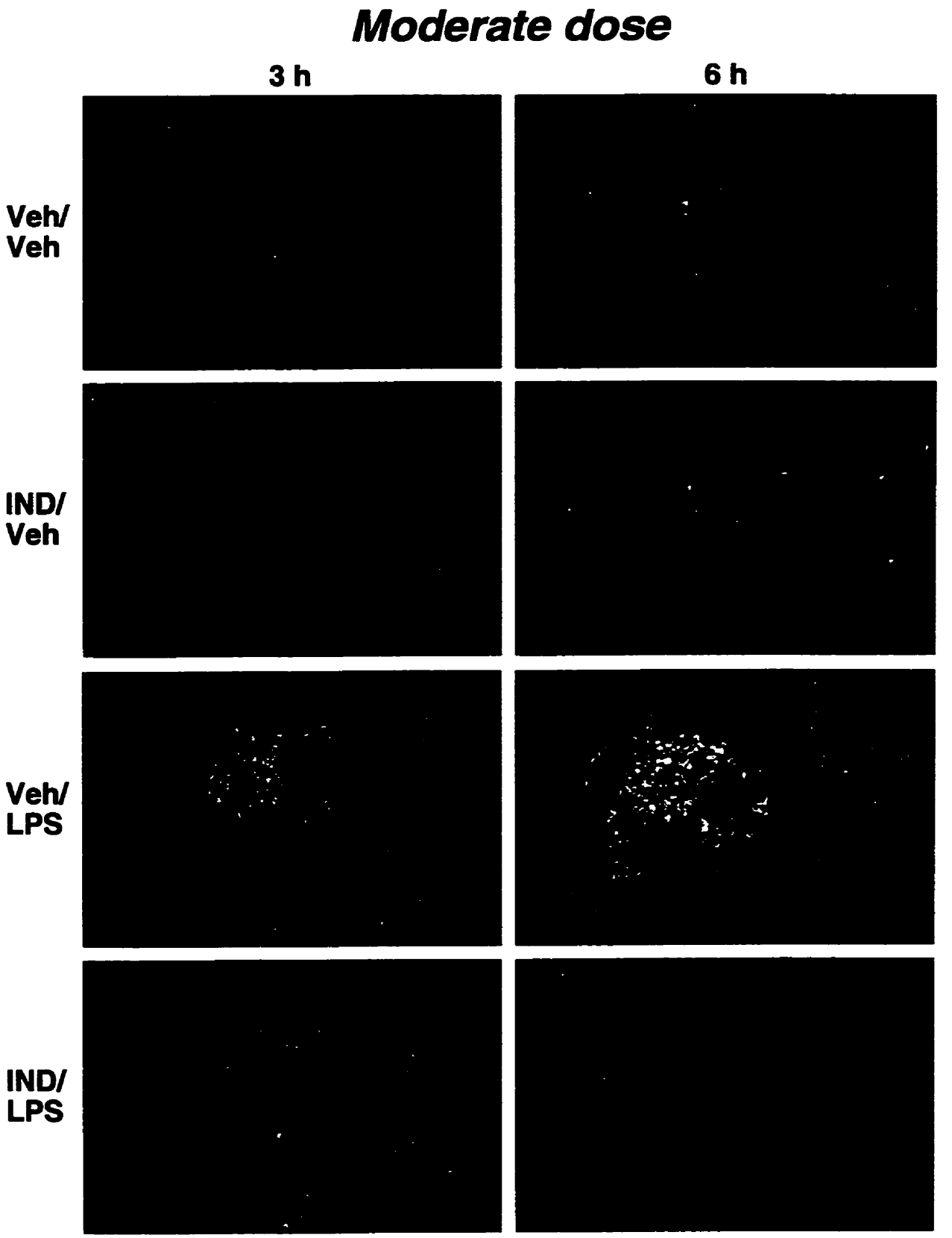

Moderate dose
3 h
6 h
Veh/
Veh
IND/
Veh
Veh/
LPS
IND/
LPS

Figure 5:

Expression of CRF_1 receptor mRNA in the rat paraventricular nucleus (PVN) following i.v. pre-treatment with indomethacin (IND) and i.p. administration of a low dose of lipopolysaccharide (LPS, 2.5 μg/100 g b.w.). These photos depict darkfield photomicrographs of dipped autoradiographs of hybridized 30-μm sections with CRF_1 receptor riboprobe through identical areas of the right PVN. Note that inhibition of cyclo-oxygenase pathways prevented the low expression of the mRNA encoding the CRF_1 receptor in the parvocellular PVN of rats treated with a low dose of LPS. Magnification X25.

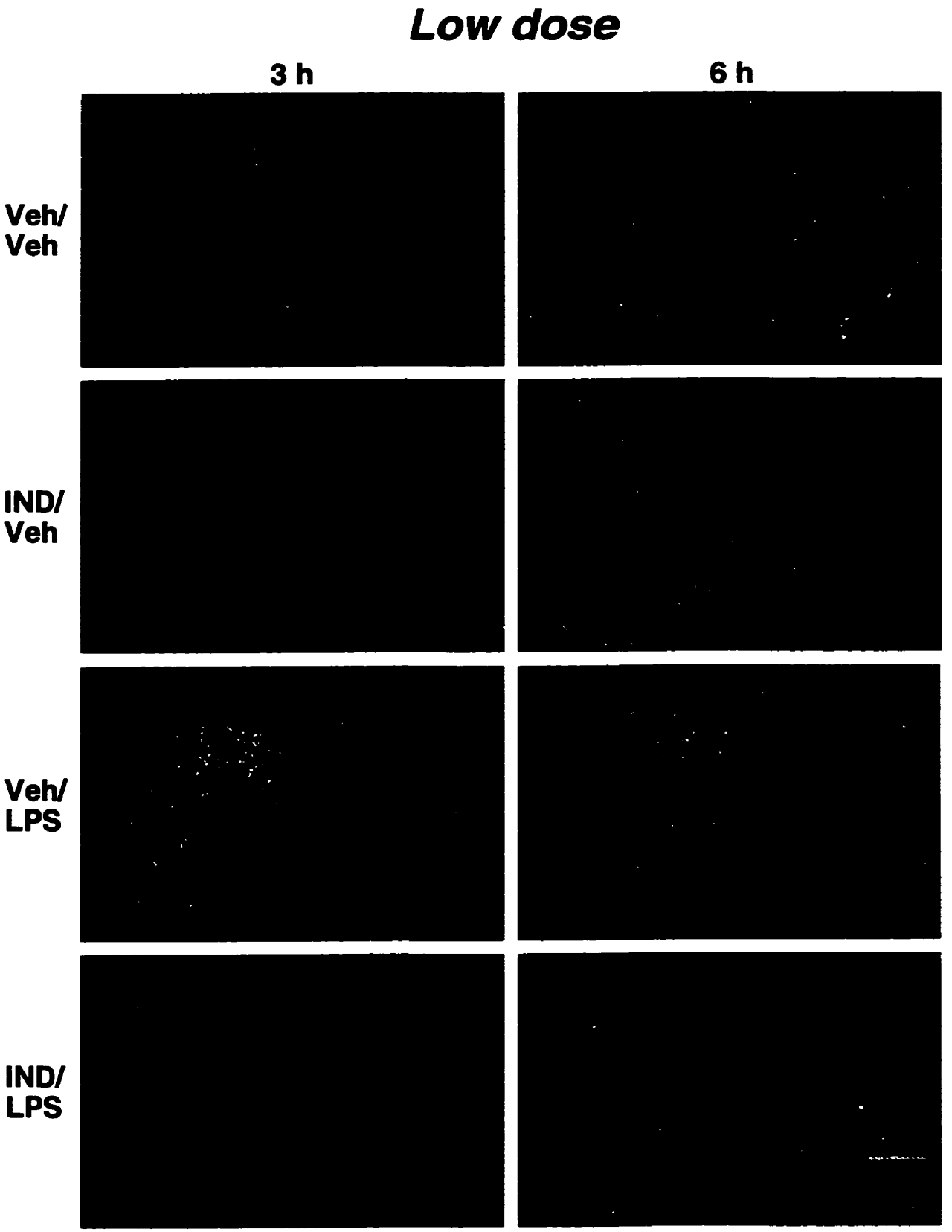

Low dose
3 h
6 h
Veh/
Veh
IND/
Veh
Veh/
LPS
IND/
LPS

Figure 6:

High power brightfield photomicrographs of dipped autoradiographs of hybridized 30-µm sections with CRF_1 receptor riboprobe through similar areas of the parvocellular division of the paraventricular nucleus (PVN) of rats treated or not with indomethacin (IND) and the bacterial endotoxin. Animals were deeply anesthetized and rapidly perfused with 4% paraformaldehyde 6 h after the i.p. administration of the various doses of lipopolysaccharide (LPS). High dose of LPS, 250 µg/100 g b.w. (top panels); moderate dose of LPS, 25 µg/100 g b.w. (middle panels); low dose of LPS, 2.5 µg/100 g b.w. (bottom panels). Note the amount of silver grains delineating several neurons expressing the gene encoding the CRF_1 receptor following the i.p. administration of a high dose of LPS, a phenomenon not prevented by indomethacin i.v. (top panels). In contrast, inhibition of cyclo-oxygenase pathways largely prevented induction of CRF_1 receptor positive neurons in moderately immune-challenged animals (middle panels). Magnification X250.

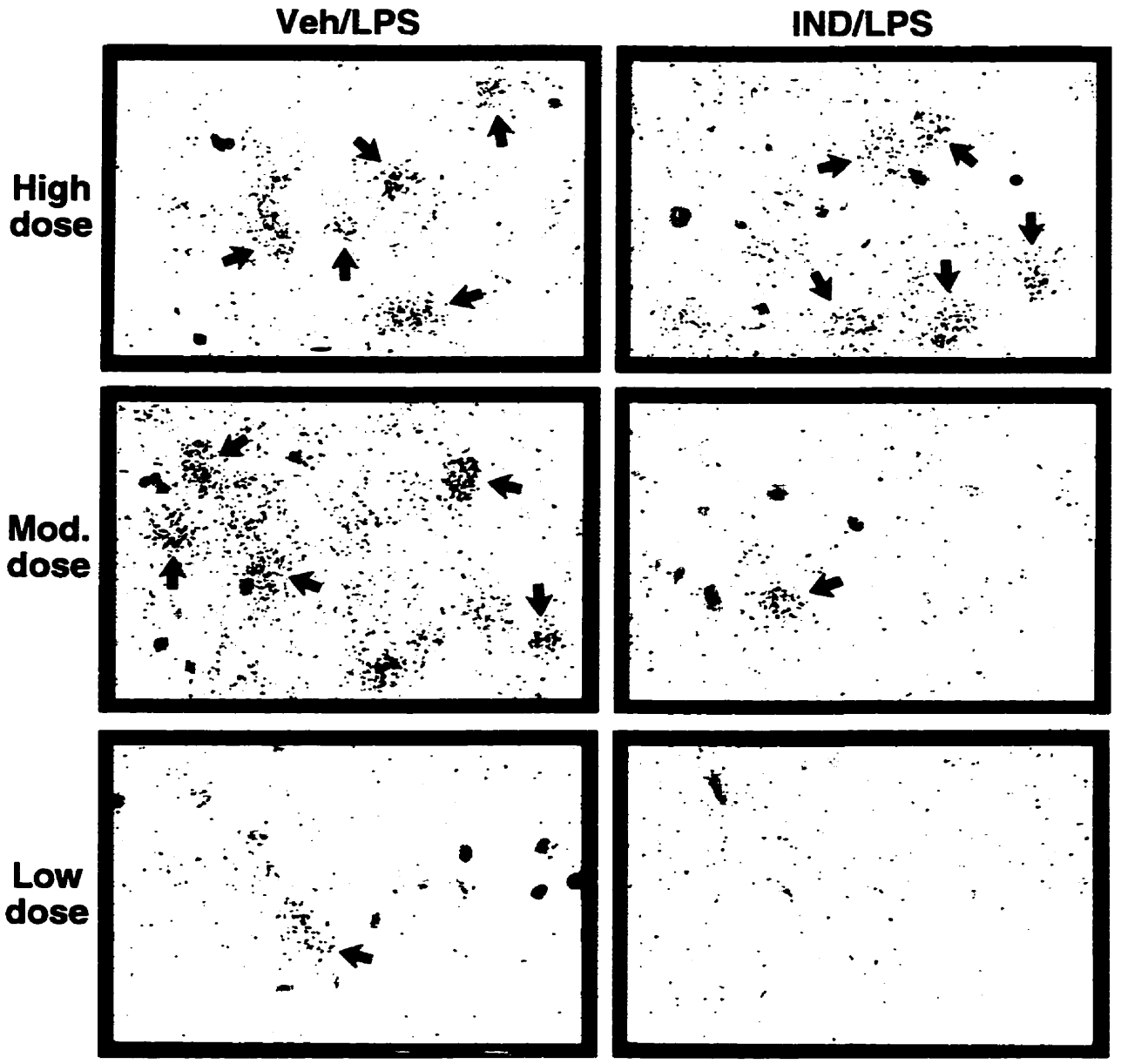

Veh/LPS
IND/LPS
High dose
Mod. dose
Low dose

Figure 7:

Average optical density (O.D.) of the hybridization signal for CRF_1 receptor transcript in the PVN after i.p. administration of high (top panel), moderate (middle panel) or low (bottom panel) dose of the bacterial endotoxin lipopolysaccharide (LPS). The O.D. was quantified on darkfield photomicrographs of dipped autoradiographs of hybridized 30-μm sections using a Olympus Optical System (BX-50, BMax) coupled to a Macintosh computer (PowerPC 7100/66) and Image software (version 1.55 non-FPU, W Rasband, NIH). Results represent means ± SEM of 4 rats; an average of at least 2 medial PVNs were digitized for each rat. Statistical analysis was performed using a 2 x 2 analysis of variance (ANOVA) followed by a Bonferroni/Dunn post hoc test for each time post-treatment (Staview 4.01). *, Significantly different ($P < 0.05$) from their appropriate control groups of rats of the same time after LPS injection. **, Significantly different ($P < 0.05$) from all the other groups of rats of the same time after LPS administration. For more information on image analysis, see Materials and Methods. IND, Indomethacin.

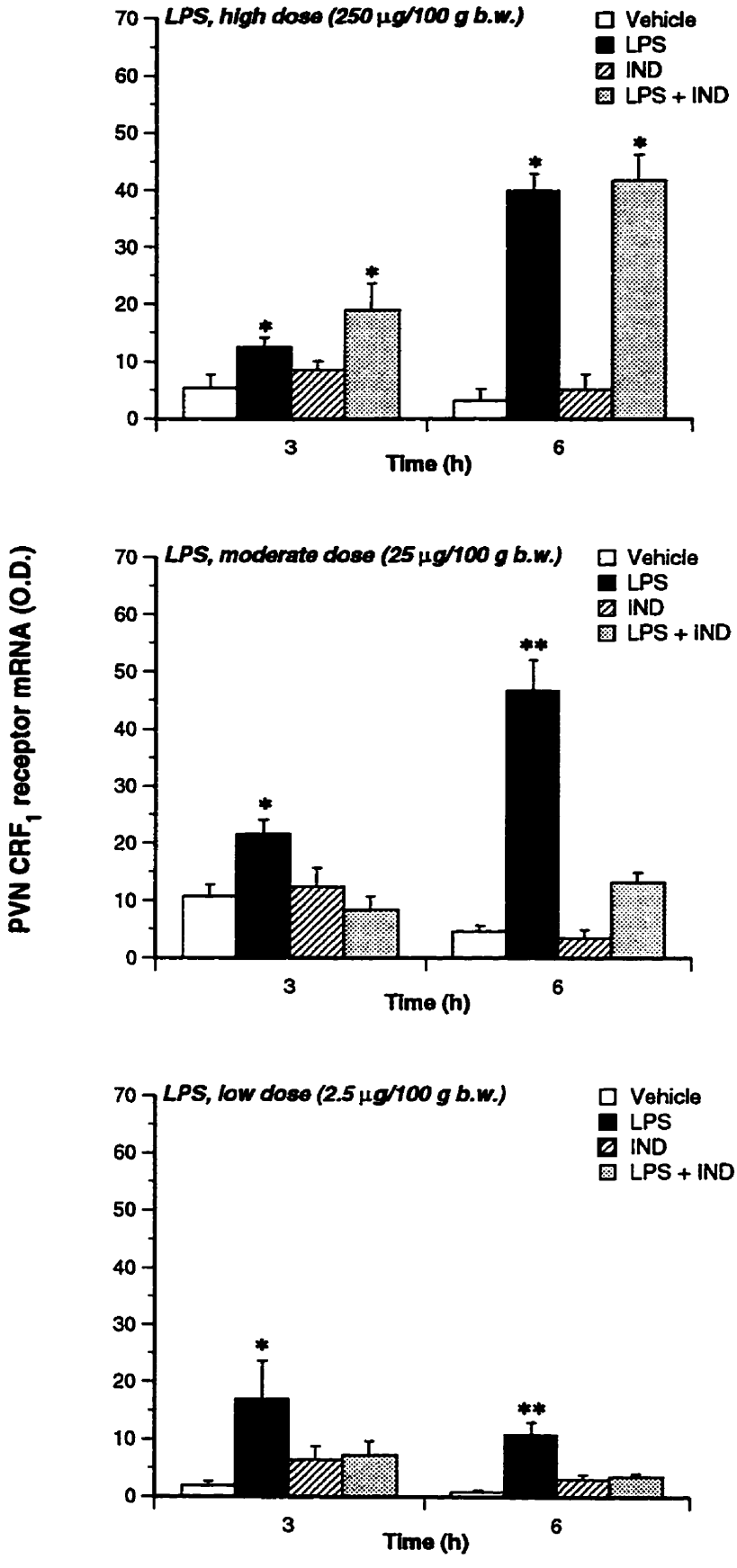

LPS, high dose (250 µg/100 g b.w.)
LPS, moderate dose (25 µg/100 g b.w.)
LPS, low dose (2.5 µg/100 g b.w.)
Vehicle
LPS
IND
LPS + IND
PVN CRF1 receptor mRNA (O.D.)
Time (h)
0
10
20
30
40
50
60
70
3
6
*
**

Figure 8:

Effect of indomethacin (IND) i.v. injection on the expression of CRF_1 receptor mRNA in the supraoptic nucleus (SON) of rats treated with a moderate dose (25 μg/100 g b.w.) of the endotoxin lipopolysaccharide (LPS). These photos depict darkfield photomicrographs of dipped autoradiographs of hybridized 30-μm sections with CRF_1 receptor riboprobe through identical areas of the right SON. Animals were deeply anesthetized and rapidly perfused with 4% paraformaldehyde 3 and 6 h after the treatment with LPS or the vehicle solution (Veh). Note that inhibition of prostaglandin production prevented transcription of CRF_1 receptor in the SON of moderately immune-challenged rats (bottom panels). Magnification X25.

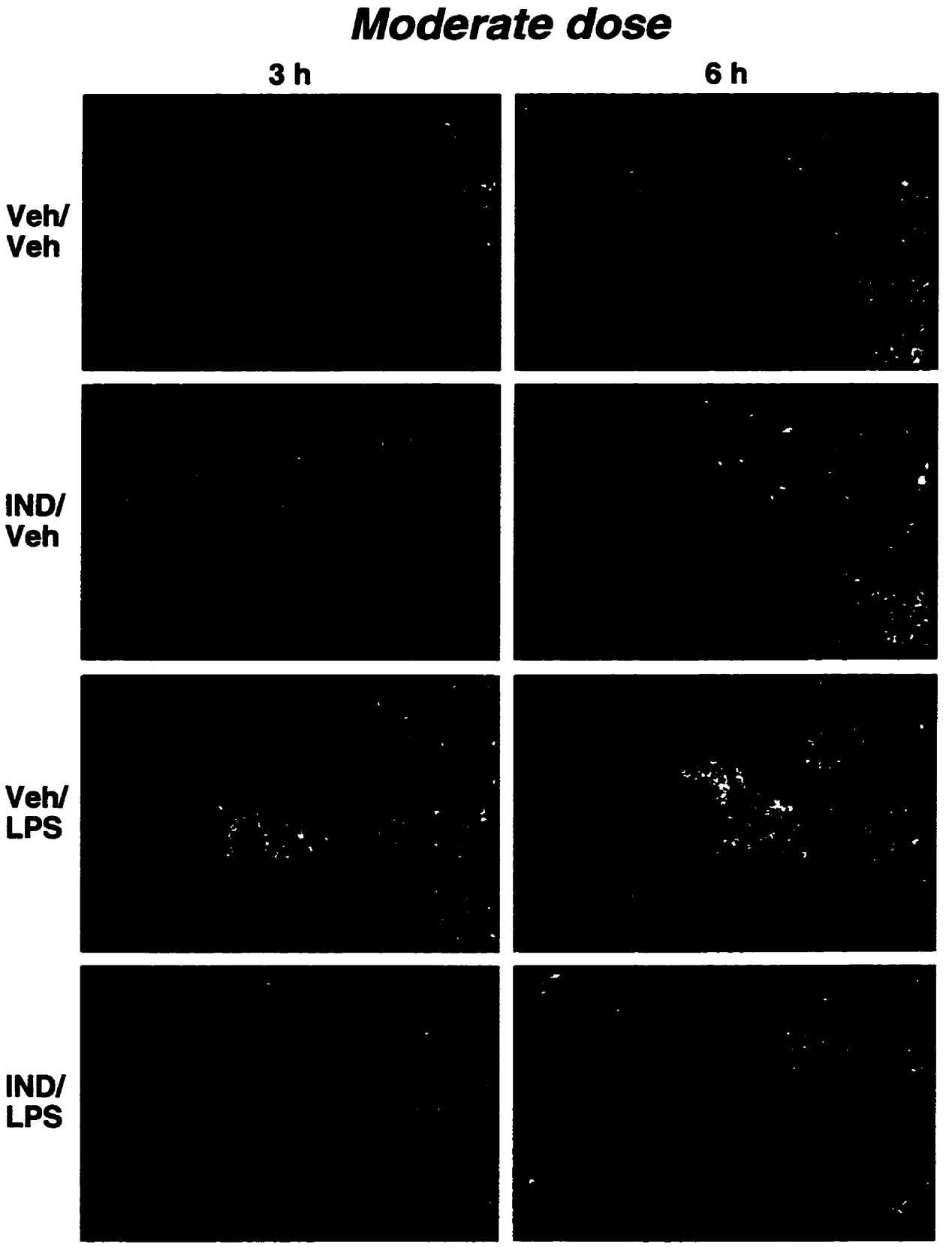
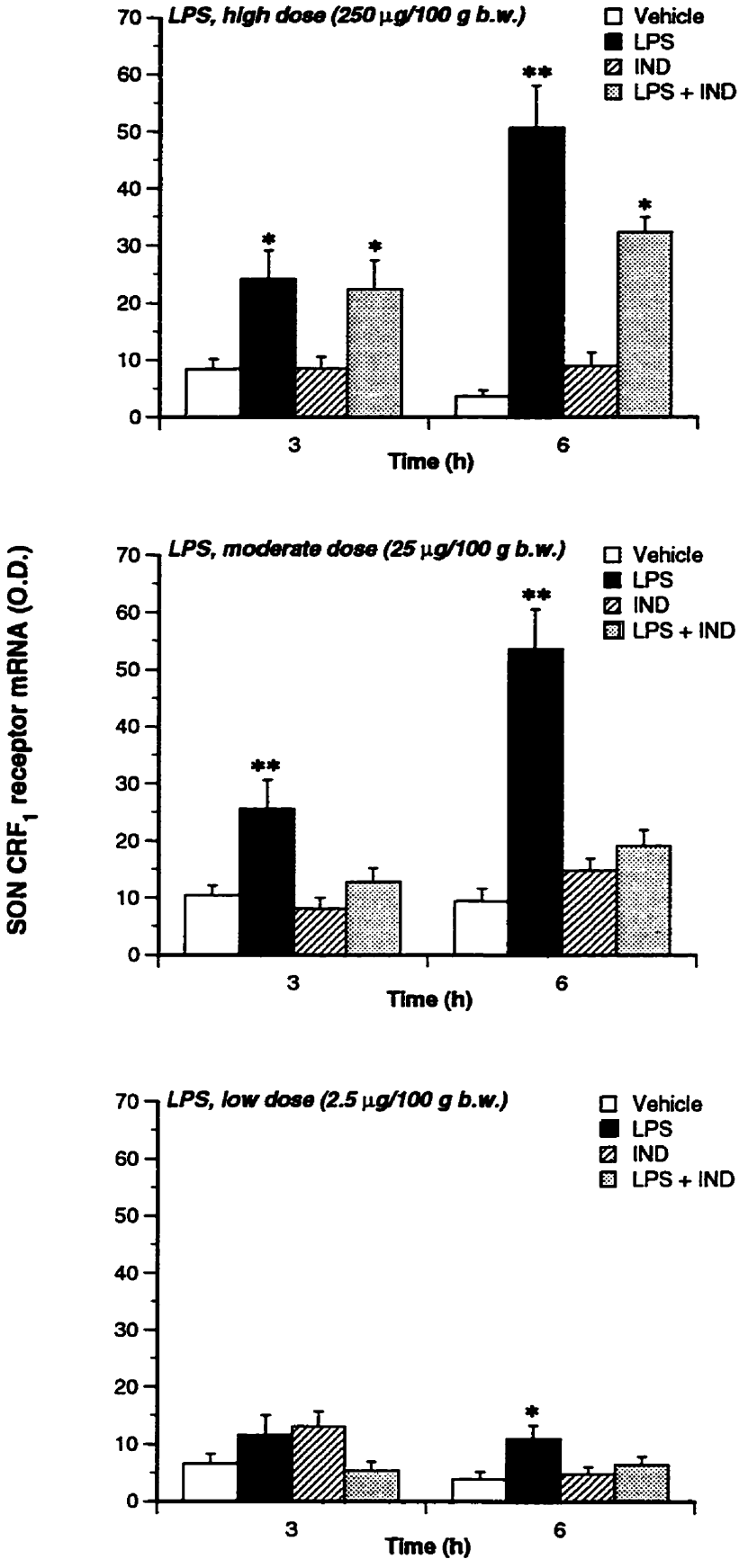

Moderate dose
3 h
6 h
Veh/
Veh
IND/
Veh
Veh/
LPS
IND/
LPS

Figure 9:

Average optical density (O.D.) of the hybridization signal for CRF_1 receptor transcript in the SON after i.p. administration of high (top panel), moderate (middle panel), or low (bottom panel) dose of the bacterial endotoxin lipopolysaccharide (LPS). The O.D. was quantified on darkfield photomicrographs of dipped autoradiographs of hybridized 30-μm sections using a Olympus Optical System (BX-50, BMax) coupled to a Macintosh computer (PowerPC 7100/66) and Image software (version 1.55 non-FPU, W Rasband, NIH). Results represent means ± SEM of 4 rats; an average of at least 4 medial (full) SONs were digitized for each rat. Statistical analysis was performed using a 2 x 2 analysis of variance (ANOVA) followed by a Bonferroni/Dunn post hoc test for each time post-treatment (Staview 4.01). *, Significantly different ($P < 0.05$) from their appropriate control groups of rats of the same time after LPS injection. **, Significantly different ($P < 0.05$) from all the other groups of rats of the same time after LPS administration. For more information on image analysis, see Materials and Methods. IND, Indomethacin.


LPS, high dose (250 µg/100 g b.w.)
LPS, moderate dose (25 µg/100 g b.w.)
LPS, low dose (2.5 µg/100 g b.w.)
Vehicle
LPS
IND
LPS + IND
SON CRF1 receptor mRNA (O.D.)
Time (h)
0
10
20
30
40
50
60
70
3
6
*
**

CHAPITRE 4. C-*FOS* mRNA PATTERN AND CRF NEURONAL ACTIVITY THROUGHOUT THE BRAIN OF RATS INJECTED CENTRALLY WITH A PROSTAGLANDIN OF E2 TYPE.

By

Steve Lacroix, Luc Vallières and Serge Rivest

Laboratory of Molecular Endocrinology
CHUL Research Center and
Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2

Running Title: Neuronal circuitry involved in PGE_2-treated rats.

Address for Correspondence: Dr. Serge Rivest
Laboratory of Molecular Endocrinology
CHUL Research Center and
Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2
Tel: (418) 654-2296, Fax: (418) 654-2761
EMail: Serge.Rivest@crchul.ulaval.ca

The Journal of Neuroimmunology, 70 (1996) 163-179

4.1 RÉSUMÉ

L'objectif de cette étude était d'évaluer les effets d'une administration centrale des prostaglandines de type E_2 (PGE_2) sur la distribution de l'ARNm encodant le gène de réponse précoce c-*fos* et l'activité transcriptionnelle des gènes codant le facteur de libération des corticotrophines (CRF) et son récepteur de type 1. Pour ce faire, des rats mâles adultes ont été sacrifiés 30 minutes et 2 heures suivant l'injection des PGE_2 (2 µg/10 µl) dans le ventricule latéral droit et les cerveaux coupés en tranches de 30 µm. L'analyse de l'expression du gène de réponse précoce c-*fos* et du récepteur du CRF de type 1 a été accomplie par hybridation *in situ* à l'aide de sondes exoniques marquées au S^{35}. Étant donné les taux élevés d'ARNm du CRF dans des conditions basales, une sonde intronique (indice de l'activité transcriptionnelle) fut utilisée afin de détecter le transcrit primaire (ARN hétéronucléaire [hn]) du neuropeptide CRF. La colocalisation de l'ARNm de c-*fos* dans le neurones CRF, vasopressine (AVP) et oxytocine (OX) fut déterminée grâce à la combinaison des techniques d'immunohistochimie et d'hybridation *in situ* sur les mêmes tranches de cerveau. Ainsi, 30 minutes après l'injection intracérébroventriculaire (i.c.v.) des PGE_2, une induction forte à modérée des niveaux d'ARNm du gène c-*fos* a été observée dans plusieurs régions du cerveau incluant la région médiane préoptique, l'organe vasculaire de la lame terminale, les noyaux supraoptiques (SON), les divisions parvo- et magnocellulaires des noyaux paraventriculaires de l'hypothalamus (PVH), le noyau central de l'amygdale, le noyau du tractus solitaire, le complexe dorso-vagal, l'area postrema, la division dorsale du noyau ambigu, les plexus choroïdiens et les leptoméninges. Un message plus faible, mais tout de même significatif, a aussi été décelé dans plusieurs autres structures dont: l'organe subfornicale, le noyau du lit de la strie terminale, les noyaux arqués et les noyaux périventriculaires de l'hypothalamus. Deux heures après le traitement aux PGE_2, le signal a complètement disparu dans la plupart de ces régions. Dans la division parvocellulaire des noyaux PVH, c-*fos* fut exprimé en majorité dans les neurones CRFergiques et oxytocinergiques, tandis que dans les neurones magnocellulaires des noyaux PVH et SON, le transcrit a été détecté essentiellement des neurones immunoréactifs à la protéine OT. Nos résultats montrent que l'activation des neurones CRFergiques neuroendocriniens a été associée à une augmentation sélective de la transcription du CRF dans le noyaux PVH; aucune autre structure du cerveau n'a montré la présence d'ARNhn du CRF suite à l'injection centrale des PGE_2. De plus, le traitement avec les PGE_2 a stimulé la transcription du gène encodant le récepteur de type 1 du CRF dans la division parvocellulaire des noyaux PVH. Ces résultats nous procurent des évidences anatomiques claires, à savoir que l'injection i.c.v. des PGE_2 entraîne l'expression sélective et spécifique du gène c-*fos* dans plusieurs structures

reconnues comme étant activées dans le cerveau d'animaux traités avec l'endotoxine bactérienne lipopolysaccharide. Il est donc plus que probable que les PGE_2 jouent un rôle crucial dans le système nerveux central afin de moduler les diverses fonctions neuroendocrinienne, principalement l'axe hypothalamo-hypophyso-corticosurrénalienne, au cours de la réponse immunitaire.

4.2 ABSTRACT

The present study investigated the effect of central administration of the prostaglandin of E_2 type (PGE_2) on the distribution of the immediate *early* gene (IEG) c-*fos* mRNA and the transcriptional activity of corticotropin-releasing factor (CRF) and its type 1 receptor in the brain of conscious rats. Adult male rats were sacrificed 30 min and 2 h after a single infusion of PGE_2 into the right lateral ventricle (2 μg/10 μl) and their brains cut from the olfactory bulb to the end of the medulla in 30-μm coronal sections. mRNAs encoding the IEG c-*fos* and CRF_1 receptor were assayed by *in situ* hybridization histochemistry using ^{35}S-labeled exonic riboprobes whereas the primary transcript {heteronuclear (hn)RNA} for CRF was detected using intronic probe technology as an index of CRF transcriptional activity. Colocalization of c-*fos* mRNA within CRF, vasopressin (AVP), and oxytocin (OT) neurons was determined by means of a combination of immunocytochemistry and *in situ* hybridization techniques on the same brain sections. Thirty min after PGE_2 injection, a moderate to strong positive signal for c-*fos* mRNA was detected in multiple structures of the brain such as the medial preoptic area/organum vasculosum of the lamina terminalis, supraoptic nucleus (SON), parvocellular and magnocellular divisions of the paraventricular nucleus (PVN) of the hypothalamus, central nucleus of the amygdala, nucleus of the solitary tract, dorsal motor nucleus of the vagus, area postrema, dorsal division of the ambiguus nucleus, and throughout the choroid plexus and leptomeninges. A smaller but significant c-*fos* expression was observed in various structures including the subfornical organ, bed nucleus of the stria terminalis, arcuate nucleus, and periventricular nucleus of the hypothalamus. Two h after treatment with the PG, the signal for c-*fos* mRNA in most of these brain nuclei vanished. In the parvocellular nucleus of the PVN, c-*fos* was expressed in CRF-immunoreactive (ir) and OT-ir neurons, whereas in the magnocellular part of that nucleus and in the SON, this transcript was essentially colocalized in OT-ir neurons. Activation of CRF neuroendocrine cells was also associated with an increase in CRF transcription as revealed by the selective presence of CRF primary transcript (hnRNA), which was stimulated only in the PVN but not in any other nuclei in the brains of PGE_2-treated rats. Central administration of PGE_2 also induced expression of the CRF type 1 receptor in the parvocellular PVN. Taken together, these results provide clear anatomical evidence that central PGE_2 injection causes specific and selective expression of c-*fos* in several brain structures recognized to be activated in the brains of endotoxin-challenged rats. It is therefore possible that PG of E_2 type plays a crucial role within the CNS in the interface between the immune and nervous systems to modulate neuroendocrine responses, such as the hypothalamic-pituitary-adrenal axis.

4.3 INTRODUCTION

An appropriate regulation of the mechanisms involved in the interaction between immune and neuroendocrine systems seems crucial for the defense of the organism in presence of foreign material. These mechanisms are poorly understood and depend on several variables including the type and the severity of immune challenge, the gender, age and species of animals, and the frequent presence of other stressful circumstances at the time of infection. The activation of the hypothalamic-pituitary-adrenal (HPA) axis is, however, a phenomenon regularly observed during immune challenge, at least during the acute phase response (for reviews, see Berkenbosch et al., 1991; Koenig, 1991; Rivest, 1995; Rivier, 1993; Rivier and Rivest, 1993). This neuroendocrine response was in fact a turning point because of the elegant concept that production of cytokines (such as interleukin-1) during immune challenge may stimulate the release of glucocorticoids which are potent endogenous immunosuppressors (Solomon, 1969). A large body of evidence suggests that this bi-directional communication involves a complex neuronal circuitry to trigger the activity of neuroendocrine neurons controlling the HPA axis (Ericsson et al., 1994; Rivest, 1995; Rivest and Laflamme, 1995; Rivest and Rivier, 1995; Rivier, 1993; Rothwell, 1991). Although widely distributed throughout the brain, corticotropin-releasing factor (CRF) neurons that project to the externa lamina of the median eminence and therefore release the peptide into the infundibular system originate from the parvocellular division of the paraventricular nucleus (PVN) of the hypothalamus (Plotsky, 1990; Plotsky and Sawchenko, 1987; Sawchenko et al., 1993; Sawchenko and Swanson, 1990). Induction of the acute-phase response with the bacterial endotoxin lipopolysaccharide (LPS) as well as the cytokine IL-1 have been shown to cause a robust activation of CRF neurons in the hypothalamic PVN (Barbanel et al., 1990; Ericsson et al., 1994; Rivest and Laflamme, 1995; Rivest and Rivier, 1994; Saphier and Ovadian, 1990), a phenomenon directly related to the IL-1 induced plasma ACTH release (Kovacs and Elenkov, 1995; Rivest and Rivier, 1991).

Among the numerous intra- and extracellular mechanisms involved in influence of immune-related factors on the HPA axis, several reports have shown that prostaglandins (PGs) are potent modulators of various cytokines on the activity of neuroendocrine CRF neurons. Indeed, blockage of the eicosanoid cyclooxygenase pathways can prevent the stimulation of CRF release by both IL-1 and 6 from *in vitro* hypothalamic explants (Lyson and McCann, 1992; Navarra et al., 1991) and median eminence (McCoy et al., 1994), and IL-1-induced ACTH release *in vivo* (Katsuura et al., 1988; Rivier, 1993). Inhibition of PG

production has been reported to prevent other IL-1-induced alteration of neuroendocrine functions, such as LHRH and LH release (Rivest and Rivier, 1993) as well as hypothalamic vasopressin (AVP) and oxytocin (OT) release (Yasin et al., 1994). The exact PG subtype(s) and the site(s) of action within the brain involved in these effects still remain unclear, but a large body of evidence indicates that PG of E_2 type might be involved in several changes observed during immune challenge and treatment with cytokines. IL-1 increases the release of PGE_2 from rat hypothalamic explants *in vitro* (Navarra et al., 1992), medial preoptic area (MPOA)/organum vasculosum lamina terminalis (OVLT), PVN, dorsal hippocampus, lateral ventricle *in vivo* (Komaki et al., 1992), rat astrocyte cultures (Katsuura et al., 1989), isolated pancreatic islets (Hughes et al., 1989), and papillary collecting duct (Kohan, 1989). Mapping of PGE_2 binding sites in the rat brain using quantitative autoradiography revealed very high density of binding sites in the nucleus of the solitary tract and the anterior wall of the third ventricle and moderate density within several brain structures including the hypothalamus (Matsumura et al., 1992, 1990). Moreover, intracerebroventricular (i.c.v.) administration of PGE_2 (Rassnick et al., 1995) or directly into the MPOA/OVLT (Katsuura et al., 1990) elevated plasma ACTH and corticosterone in rats, an effect most likely mediated through neuroendocrine CRF neurons. PG of E_2 type could therefore be a determinant mediator within the brain to stimulate the HPA axis during immune challenge, but whether this PG can trigger the transcription of CRF and its receptor within the brain has yet to be investigated.

Central treatment with PGE_2 is not only associated with an increase in the HPA axis but is also known to decrease blood lymphocyte proliferative responses to the T-cell mitogens, phytohemagglutinin and concanavalin-A, and decline spleen lymphocyte proliferative responses to the bacterial endotoxin lipopolysaccharide (LPS), a potent immune activator of type B (Rassnick et al., 1995). This treatment has been reported to produce many other physiological responses such as the alteration of the cardiovascular and sympathetic nervous system functions (Ando et al., 1995; Feuerstein et al., 1982), and hyperthermia in rats (Oka and Hori, 1994). It is thus permit to believe that PGE_2 may play a role as intermediate within the CNS for the peripheral immune system to modulate the appropriate physiological responses to maintain the homeostasis in response to immunogenic challenges. However, little is known regarding exact neuronal circuitry and molecular mechanisms mediating the action of PG of E_2 type in the brain. The purposes of the present study were therefore to outline the sites of activation throughout the brains of rats treated centrally with PGE_2 via the expression of the immediate *early* gene c-*fos* mRNA; to determine whether neurons directly involved in the control of the HPA axis (CRF,

vasopressin, and oxytocin) were activated by combining both *in situ* hybridization and immunocytochemistry techniques on the same brain sections; and to investigate the transcriptional activity of CRF and its receptor of type 1 by means of *in situ* hybridization histochemistry using intronic and exonic probe technology throughout the rat brain.

4.4 MATERIALS AND METHODS

4.4.1 Animals

Adult male Sprague-Dawley rats (~4 month old) were acclimated to standard laboratory conditions (14-h light, 10-h dark cycle; lights on at 0600 and off at 2000) and given free access to rat chow and water. Seven to eight rats were used per group and time post-injection and all protocols were approved by the Laval University's Animal Welfare Committee.

4.4.2 Central infusion of PGE_2

Rats were anesthetized with a i.p. injection of 100 µl/100 g of a mixture of ketamine hydrochloride (91 mg/ml) and xylazine (9 mg/ml). The right lateral ventricle was reached stereotaxically (David Kopf instruments, Tujunga, CA) using the Paxinos and Watson atlas (1986). With the incisor bar placed at 3.3 mm below the interaural line (horizontal zero), the coordinates from bregma for the guide cannula were as follows: anteroposterior (A-P): -0.6, lateral (L): -1.4, dorsoventral (D-V): -2.8 mm. A 22-gauge stainless steel guide cannula was implanted close to the right lateral ventricle and was secured with screws and cranioplastic cement (cranioplastic powder, Plastic One Inc., Roanoke, VA; Dentsply repair material, Dentsply International Inc., York, PA). Eight to ten days after the i.c.v. surgery, infusions were made via an internal cannula (28-gauge, 1 mm projection beyond the tip of the guide cannula) connected to a 100-µl Hamilton glass syringe using a microinjection pump (Razel, model A-99, Razel Scientific Instruments, Inc., Stanford, Conn). Two µg of PGE_2 {([5^z, 11α, t3E, 15S]-11, 15-dihydroxy-9 oxoprosta 5, 13-diehoic acid), lot 71H3795, Sigma}, diluted in 10 µl of sterile saline solution was infused in the right lateral ventricle over a period of 3 min. This dose was selected on the basis of previous studies showing alteration of cellular immune responses, activation of the HPA axis, and elevation of plasma IL-6 levels (Rassnick et al., 1995). Sham-treated rats were infused intracerebroventricularly (i.c.v.) with 10 µl of the vehicle solution. Rats were conscious and freely moving at all times

throughout the experimental procedure. Thirty min and 2 hours after the central administration with the PG, animals were deeply anesthetized via an intraperitoneal injection of 0.5 ml ketamine-xylazine mixture, then rapidly perfused transcardially with saline followed by 4 % paraformaldehyde (PF) in 100 mM borax buffer (pH 9.5 at 4 °C). Brains were removed from the skull, postfixed for 5 to 8 days and then placed in 10 % sucrose in the solution of 4 % PF-borax buffer overnight at 4 °C. Frozen brains were mounted on a microtome (Reichert-Jung, Cambridge Instruments Company, Deerfield, IL) and were cut in 30 µm coronal sections from the olfactory bulb to the end of the medulla. The slices were collected in a cold cryoprotectant solution (50 mM sodium phosphate buffer, 30% ethylene glycol, 20% glycerol) and then stored at -20 °C. The placement of i.c.v. cannula was verified visually during brain sectioning, and animals with misplaced cannulae were excluded from the analysis.

4.4.3 Endotoxin treatment

We and other have previously reported the pattern of c-*fos* mRNA distribution throughout the brain of rats injected systemically with the bacterial endotoxin lipopolysaccharide (LPS). In order to compare this distribution with the one observed in animals injected i.c.v. with PGE_2, 8 rats (4 vehicle, 4 LPS) received either an intraperitoneal (i.p.) injection of LPS (25 µg/100 g of body weight) or vehicle solution (sterile saline) and were sacrificed 3 h after as described previously. This time was selected on the basis of previous studies showing a maximal signal for c-*fos* mRNA in the brain of i.p. LPS-treated animals (Rivest and Laflamme, 1995). It is worth reminding that LPS is a component of Gram-negative bacteria capable of inducing fever, shock and the acute-phase response in several animal species, including human being (for review, see Andersson et al., 1992). Bacterial toxins constitute a group of many virulence factors by which bacteria cause disease and their interaction with the host's immune system, in particular by inducing the release of cytokines, has been the object of a large number of studies. In the present series of experiments, we used LPS to mimic some of the events occurring during the acute-phase response to show the distribution of c-*fos* mRNA in the brain of immune-challenged rats in comparison to the brain cellular activity caused by central PGE_2 administration.

4.4.4 *In situ* hybridization histochemistry

Hybridization histochemical localization of each transcript was carried out in 1 in 6 series (every sixth section) of slices through the brain (from the olfactory bulb to the end of the medulla) using ^{35}S-labeled cRNA probes. Protocols for riboprobe synthesis, hybridization, and autoradiographic localization of mRNA signal were adapted from Simmons et al. (1989). All solutions were treated with diethylpyrocarbonate (Depc) and sterilized to prevent RNA degradation. Tissue sections mounted onto poly-L-lysine-coated slides were desiccated under vacuum overnight, fixed in 4% PF for 30 min, and digested by proteinase K (10 µg/ml in 100 mM tris HCl, pH 8.0, and 50 mM EDTA, at 37 °C for 25 min). Thereafter, the brain sections were rinsed in sterile Depc water followed by a solution of 100 mM triethanolamine (TEA, pH 8.0), acetylated in 0.25% acetic anhydride in 100 mM TEA, and dehydrated through graded concentrations of alcohol (50, 70, 95, and 100%). After vacuum drying for a minimum of 2 h, 90 µl of hybridization mixture (10^7 cpm/ml) was spotted on each slide, sealed under a coverslip, and incubated at 60 °C overnight (~15-20 h) in a slide warmer. Coverslips were then removed and the slides were rinsed in 4x standard saline citrate (SSC) at room temperature. Sections were digested by RNSase A (20 µg/ml, 37 °C, 30 min), rinsed in descending concentrations of SSC (2x, 1x, 0.5x SSC), washed in 0.1xSSC for 30 min at 60 °C (1x SSC: 150 mM NaCl, 15 mM trisodium citrate buffer, pH 7.0), and dehydrated through graded concentrations of alcohol. After being dried for 2 h under vacuum, the sections were exposed at 4 °C to x-ray film (Kodak) for 15 to 30 h (depending on the probe), defatted in xylene, and dipped in NTB2 nuclear emulsion (Kodak; diluted 1:1 with distilled water). Slides were exposed for approximately 7 to 12 days, developed in D19 developer (Kodak) for 3.5 min at 14-15 °C, and fixed in rapid fixer (Kodak) for 5 min. Thereafter, tissues were rinsed in running distilled water for 1 to 2 h, counterstained with thionin (0.25 %), dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX.

4.4.5 cRNA probe synthesis and preparation

c-*fos* probe was generated from the *Eco* R 1 fragment of rat c-*fos* cDNA (Dr. I Verma, The Salk Institute), subcloned into pBluescript SK-1 (Stratagene, La Jolla, CA), and linearized with *Sma* I. Specific rat CRF_1 receptor probe (1.3 kb) was produced from the *Pst* I-*Pst* I fragment of the rat prCRF PP1.3-BS cDNA {Dr. W. Vale, Peptide Biology Laboratory, The Salk Institute, (Perrin et al., 1993)}, subcloned into pBluescript II SK

(Stratagene, La Jolla, CA), and linearized with *Bam* HI and *Hin*d III (Pharmacia) for antisense and sense probes, respectively (Perrin et al., 1993; Rivest et al., 1995). pGem3 plasmid containing a pure CRF intronic piece was linearized with *Hin*d III (530 bp) to detect specifically CRF heteronuclear (hn) RNA {Dr. S. Watson, The University of Michigan, Ann Arbor (Herman et al., 1992)}.

Radioactive antisense cRNA copies were synthesized by incubation of 250 ng of linearized plasmid in 6 mM $MgCl_2$, 30-40 mM Tris (pH 7.9), 2 mM spermidine, 10 mM Nacl, 10 mM dithiothreitol, 0.2 mM ATP/GTP/CTP, [α-^{35}S]UTP, 40U RNAsin (Promega, Madison, WI), and 20U of T7 RNA polymerase for 60 min at 37°C. Unincorporated nucleotides were removed using the ammonium-acetate method; 100 µl of DNAse solution (1 µl DNAse, 5 µl of 5 mg/ml tRNA, 94 µl of 10 mM Tris/10 mM $MgCl_2$) was added, and 10 min later an extraction was accomplished using a phenol-chloroform solution. The cRNA was precipitated with 80 µl of 5M ammonium acetate and 500 µl of 100% ethanol for 20 min on dry ice. The pellet was washed with 500 µl ethanol, dried, and resuspended in 100 µl of 10 mM Tris/1 mM EDTA (pH 8.0). A concentration of 10^7 cpm probe was mixed into 1 ml of hybridization solution (500 µl formamide, 60 µl 5 M NaCl, 10 µl 1 M Tris [pH 8.0], 2 µl 0.5 M EDTA [pH 8.0], 20 µl 50x Denhart's solution, 200 µl 50% dextran sulfate, 50 µl 10 mg/ml tRNA, 10 µl 1M DTT, [118 µl Depc water - volume of probe used]). This solution was mixed and heated for 5 min at 65 °C before being spotted on slides. Radioactive sense (control) cRNA copies were also prepared to verify the specificity of each probe. Hybridization with these probes did not reveal any positive signal in the brain of vehicle- and PGE_2-treated rats.

4.4.6 Combination of immunocytochemistry with *in situ* hybridization

Immunocytochemistry (CRF-, AVP-, or OT-immunoreactive neurons) was combined with the *in situ* hybridization histochemistry protocol (c-*fos* mRNA) to determine the types of cells, particularly in the PVN and the SON, that express the IEG c-*fos* after central treatment with PGE_2. Every sixth tissue slice for each antibody (CRF, AVP, or OT) was processed by using the avidin-biotin amplification bridge method with peroxidase as a substrate. Briefly, slices were washed in sterile Depc-treated 50 mM potassium phosphate-buffered saline (KPBS) and incubated at 4 °C with either CRF, AVP, or OT antibody mixed in sterile KPBS, 0.4% Triton X-100, 0.25% heparin sodium salt USP (ICN Biomedicals Inc., Aurora, OH) and 1% bovine serum albumin (fraction V, Sigma, St. Louis, MO). Rabbit

antihuman/rat CRF serum (code PBL rc 70, 8/9/83 bleed), a generous gift from Dr. Wylie Vale (Peptide Biology Laboratory, The Salk Institute, La Jolla, CA), was used at a concentration of 1:10000. On the other hand, antisera raised in rabbit against AVP (Incstar Science Technology and Research, Stillwater, MN, Cat # 20069) and OT (Incstar Science Technology and Research, Stillwater, MN, Cat # 20068) were used at a concentration of 1:10000 and 1:5000, respectively. AVP and OT antibodies were very specific to each neuropeptide because the staining of AVP neurons, for example, was completely eliminated by pretreatment of 1 ml of the diluted antibody with 200 μg of AVP, whereas pretreatment with OT did not diminish AVP neuronal staining in the rat hypothalamus (Incstar). On the other hand, pretreatment with OT but not AVP prevented OT-immunoreaction with the serum raised in rabbit against OT (Incstar). Approximately 18 h after incubation at 4 °C with the primary antibody (CRF, AVP, or OT), the brain slices were rinsed in sterile KPBS and incubated with a mixture of KPBS + triton-X + heparin + biotinylated goat antirabbit IgG (1:1500 dilution; Vector Laboratories, CA) for 90 min. Sections were then rinsed with KPBS and incubated at room temperature for 60 min with an avidin-biotin-peroxidase complex (Vectastain ABC elite kit, Vector Laboratories, CA). The peroxidase complex was amplified by means of 10-min incubation with a 70-nM solution of biotin {sulfosuccinimydyl 6-(biotinamido) hexanoate, Pierce # 21335}-tyramine HCl (4-hydroxyphenethylamine hydrochloride, Sigma T-2879)-H_2O_2 (0.01%), followed by a second incubation of 30 min with the ABC elite solution. After several rinses in sterile KPBS, the brain slices were reacted in a mixture containing sterile KPBS, the chromagen 3,3'-diaminobenzidine tetrahydrochloride (DAB, 0.05%), and 0.003 % hydrogen peroxide (H_2O_2).

Thereafter, tissues were rinsed in sterile KPBS, mounted onto poly-L-lysine-coated slides, desiccated under vacuum overnight, fixed in 4% PF for 30 min, and digested by proteinase K (10 μg/ml in 100 mM tris HCl [pH 8.0] and 50 mM EDTA [pH 8.0], at 37 °C for 25 min). Prehybridization, hybridization, and posthybridization steps were performed as described above with the difference of dehydration (alcohol 50, 70, 95, 100%), which was shortened to avoid decoloration of CRF, AVP, or OT cells (brown staining). After being dried for 2 h under the vacuum, sections were exposed at 4 °C to x-ray film (Kodak) overnight, defatted in xylene, and dipped in NTB2 nuclear emulsion (Kodak; diluted 1:1 with distilled water). Slides were exposed for 6 to 8 days, developed in D19 developer (Kodak) for 3.5 min at 15 °C, and fixed in rapid fixer (Kodak) for 5 min. Thereafter, tissues were rinsed in running distilled water for 1 to 2 h, rapidly dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX. The presence of c-

fos transcript was evident as silver grains in perikarya, and CRF, AVP, or OT immunoreactivity within the cell cytoplasm was stained in brown.

4.4.7 Qualitative analysis

Qualitative evaluation of signal intensity for the mRNA encoding the IEG Fos throughout the entire rostro-caudal brains of PGE_2 and LPS-injected rats was performed on X-ray films. Meticulous analysis of visual intensity (very strong, ++++; strong, +++; moderate, ++; low, +) for each structure displaying positive signal was accomplished for every rat providing therefore an average number in arbitrary units.

4.4.8 Quantitative analysis

The c-*fos* mRNA, CRF hnRNA, and CRF_1 receptor mRNA signals revealed on dip NTB2 nuclear emulsion slides were analyzed and quantified using an Olympus Optical System (BX-50, ß-MAX) coupled to a Macintosh computer (Power PC 7100/66) and Image software (version 1.55 non-FPU, W. Rasband, NIH). The optical density (O.D.) of the hybridization signal was measured under darkfield illumination at a magnification of X10. Sections for the experimental and control animals were matched for rostrocaudal level. Because of the lack of basal expression of c-*fos* mRNA, CRF hnRNA, and CRF_1 receptor mRNA in the hypothalamic PVN, the whole medial PVN was digitized under brightfield illumination and then subjected to densitometric analysis under darkfield, yielding measurements of integrated O.D. (area of PVN x average O.D.). The O.D. of each specific transcript was then corrected for the average background signal as determined by sampling cells immediately outside the cell group of interest (McCabe and Pfaff, 1989).

4.4.9 Statistical Analysis

Data from figures 2 and 8 are expressed as O.D. (arbitrary units) and analyzed by a 2 x 2 analysis of variance (ANOVA). Factors were identified as follows: *central treatment*, which was composed of two levels (i.c.v. vehicle or i.c.v. PGE_2) and *time post-injection*, which combined 2 levels (30 min and 2 h post-injection). Bonferroni/Dunn test procedure (Statview 4.01) was used as post hoc comparisons when significant interaction between factors was obtained.

4.5 RESULTS

4.5.1 Basal expression of c-*fos* transcript throughout the rat brain

Vehicle-treated animals displayed a strong to moderate signal for the mRNA encoding c-*fos* in various structures of the brain, although the intensity varied from an animal to another (Table 1). Strong basal expression of c-*fos* mRNA was generally detected in multiple layers of the cerebral cortex including the layer 2, 3, 4, and 6a, in the layer 2 of the piriform area (Pir), the claustrum (CLA), the cuboidal ependymal cells lining of the right lateral ventricle (site of infusion), the laterodorsal tegmental nucleus (LDT), and the cerebellum. A moderate to low signal was detected in the lateral septal nucleus, the caudate putamen (CPu), the suprachiasmatic nucleus (SCh), several thalamic nuclei including the paraventricular nucleus (PVT), the arcuate nucleus (ARC), the dorsomedial nucleus of the hypothalamus (DMH), the parabrachial nucleus (PB), the locus coeruleus (LC), the cochlear nucleus (CN), the spinal nucleus of the trigeminal (SPV), the choroid plexus (Chp) and a low but positive signal in the leptomeninges of few vehicle-treated animals. The basal expression of c-*fos* transcript in these structures was detected in both groups of vehicle-treated animals sacrificed at either 30 or 120 min post-injection.

4.5.2 Induction of the IEG c-*fos* mRNA in selective regions of PGE_2-administered rats

Figure 1 illustrates representative examples of various areas of the brain exhibiting a moderate to strong hybridization signal for c-*fos* transcript 30 min after the injection of the PG of E_2 type into the right lateral ventricle (left column), whereas treatment with the vehicle solution (right column) caused either low or undetectable expression of the IEG in each of these brain areas. The organum vasculosum of the lamina terminalis (OVLT)/medial preoptic area (MPOA), the supraoptic nucleus (SON), the hypothalamic paraventricular nucleus (PVN), the central nucleus of the amygdala (CeA), and the dorsovagal complex/area postrema (AP) are among the regions profoundly activated by the PG. At the level of the OVLT/MPOA, several cells were positive for c-*fos* mRNA within the rostral vascular plexus as well as in the parachymal zone and uniformly distributed in the rostral MPOA, the neuronal compartment surrounding the OVLT. In the PVN, both parvocellular and magnocellular divisions displayed strong positive signal, although the intensity was generally more pronounced in the parvocellular than in the magnocellular PVN. At the level of the

dorsovagal complex, a moderate to strong hybridization signal was found in the AP, the medial and caudal commissural part of the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus (X). The Chp, leptomeninges and the dorsal division of the ambiguus nucleus (AMBd) were also clearly activated by central administration with the PG.

Injection of PGE_2 into the lateral ventricle induced a lower but significant expression of c-*fos* transcript in several other regions including subependymal zone (SEZ), bed nucleus of the stria terminalis (BnST), subfornical organ (SFO), periventricular of the hypothalamus (Pv), ARC, and nucleus raphé pallidus. To compare the brain distribution of c-*fos* mRNA following a systemic immunogenic challenge with the one of centrally PGE_2-treated animals, table 1 also presents the qualitative analysis of hybridization signal for the IEG in endotoxin-injected rats (left column). Animals were sacrificed 3 h after receiving a single i.p. injection (25 µg/100 g of body weight) of the immune activator of B type (B-lymphocyte mitogen), the bacterial endotoxin lipopolysaccharide (LPS). Interestingly, the large majority of brain regions expressing c-*fos* in systemically LPS-treated animals was also positive for the IEG after i.c.v. injection with PGE_2. Among these structures, most of the circumventricular organs, MPOA, PVN, SON, CeA, NTS/X and the AMBd were quite similar in term of distribution and intensity in the brains of i.p. endotoxin- and i.c.v. PGE_2-challenged rats.

4.5.3 Type of neurons expressing c-*fos* mRNA in the PVN and the SON of PGE_2-challenged rats

The influence of central PGE_2 treatment on c-*fos* induction was rapid and of short duration in most of the brain structures described above. In the PVN for example, strong hybridization signal for c-*fos* mRNA was detected 30 min following i.c.v. injection with the PGE_2 whereas at 2 h, the optical density for this transcript was comparable in both vehicle and PG-treated groups of rats (Fig. 2). To determine the types of neuropeptidergic neurons in the PVN and the SON that express the gene encoding c-*fos* following central injection with the PG of E_2 type, immunocytochemistry was performed before *in situ* hybridization histochemistry on the same brain sections. The top panel of Fig. 3 shows examples of CRF-immunoreactive (ir) neurons (brown staining) expressing c-*fos* mRNA (filled arrowheads) in the parvocellular PVN of PGE_2-treated rats. A large concentration of c-*fos* positive neurons were colocalized within CRF-ir perikarya in the parvocellular division of the PVN suggesting activation of these neurons by the central treatment with PGE_2. However, not all c-*fos* positive cells of the parvocellular PVN were co-localized within CRF-ir neurons (open

arrowheads) and some CRF cell bodies alone were also detected in this hypothalamic division 30 min after the i.c.v. PGE_2 injection (curved arrowheads). Double-labeled OT-ir neurons were also noted in the ventral part of the medial parvocellular division as well as within the lateral-caudal parvocellular section of the PVN (result not shown).

C-*fos* mRNA positive cells in the magnocellular division of the PVN rats were largely co-localized within OT-ir neurons 30 min after PGE_2 administration (Fig. 3, bottom panel). Indeed, several OT-ir neurons displayed positive signal for c-*fos* mRNA in the magnocellular PVN (filled arrowheads) and very few OT perikarya alone were found in the magnocellular PVN of PGE_2-injected animals. Interestingly, some OT-ir cells positive for c-*fos* mRNA were also found in the anterior hypothalamic area and lateral hypothalamic area (results not shown). In contrast, very few AVP-ir cell bodies expressed the IEG c-*fos* (Fig. 3, middle panel); at both times post-injection, most of the AVP-ir neurons of the magnocellular PVN were devoid of positive signal for the mRNA encoding the IEG in PGE_2-treated rats. Neither OT-ir nor AVP-ir perikarya expressed c-*fos* transcript in the PVN of vehicle-administered rats (results not shown).

Figure 4 shows representative examples of immunocytochemistry (AVP-ir, top panel; OT-ir, bottom panel) combined with *in situ* hybridization histochemistry (c-*fos* mRNA, silver grains) in the SON 30 min after central PGE_2 injection. In the SON, several, if not all, OT-ir cell bodies were positive for c-*fos* transcript (bottom panel, filled arrowheads) whereas few, but some, AVP-ir perikarya expressed the IEG in the SON of PGE_2-treated animals.

4.5.4 PGE_2 and CRF gene transcription

To investigate the possibility that central injection with the PG of E_2 type triggers transcription of CRF within selective regions of the brain, we used the intronic probe technology as a tool to detect the CRF primary transcript {heteronuclear (hn)RNA} throughout the rat brain. Interestingly, apart from the inferior olivary complex which displayed a low but positive signal for CRF hnRNA in both vehicle and PGE_2-injected animals, basal expression of this transcript was not detected in other regions of the brain (Fig. 5, right column). On the other hand, PGE_2 caused a selective and rapid expression of CRF primary transcript within the parvocellular division of the PVN without inducing detectable signal in other areas (Fig. 5, left column). The hybridization signal for CRF hnRNA was positive and significant in the PVN 30 min after i.c.v. PGE_2 administration and

very low to undetectable in vehicle-treated rats and in animals sacrificed 2 h after injection with the PG (Fig. 6). This indicates a rapid but transient stimulatory influence of central PGE_2 on CRF transcription in the neuroendocrine nucleus directly responsible for the control of the HPA axis.

4.5.5 CRF receptor gene expression in the brains of PGE_2-treated rats

Rivest et al., 1995), whereas the type 2 (the α splice variant) is expressed in more define structures of the limbic system (Lacroix and Rivest, 1995; Lovenberg et al., 1995). Strong basal levels of CRF_1 receptor transcript are observed in several regions of the brain, such as the Pir, medial nucleus of the amygdala, basolateral nucleus of the amygdala, subthalamic nucleus, red nucleus, laterodorsal tegmental nucleus, pontine gray, Purkinje and granule cell layers of the cerebellum, nucleus incertus, spinal nucleus of the trigeminal nerve (oral part), principal sensory nucleus of the trigeminal nerve, external cuneate nucleus, and various layers of the cerebral cortex. A low to moderate signal is also detected in multiple sites, including the medial septal nucleus, nucleus of the diagonal band, BnST, SON, CeA, DMH, caudal division of the zona incerta, pretectal area, lateral geniculate complex, substantia nigra, interpeduncular nucleus (central subnucleus), PB, medial vestibular nucleus, nucleus prepositus, spinal nucleus of the trigeminal nerve (interpolar part), lateral reticular nucleus, and gracile nucleus. While the PVN of vehicle-treated animals displayed low to barely detectable hybridization signal for CRF_1 receptor mRNA, a significant induction of that transcript was detected in this endocrine nucleus 2 h after i.c.v. administration of PG (Figs. 7 and 8). A notable increase in the relative levels of CRF_1 receptor mRNA was also observed in the SON of PG-administered rats (Fig. 7 and 8). After a meticulous qualitative analysis of the brains of vehicle- and PGE_2-treated animals, it was not possible to detect significant changes in other structures of the brain following central injection with the PG of E_2 type.

4.6 DISCUSSION

The present study provides clear evidence that central treatment with PGE_2 causes a selective cellular activation as indicated by the rapid and transient expression of the IEG c-*fos* mRNA within multiple regions of the brain. Among these regions, the OVLT, MPOA, SON, PVN, CeA, NTS/X, AP, and the AMBd displayed a moderate to strong hybridization signal for the IEG 30 min following central treatment with the PG and the signal for this

transcript vanished 90 min after. In the parvocellular PVN, CRF-ir and OT-ir neurons expressed c-*fos* mRNA, whereas in the magnocellular PVN and the SON, the IEG was essentially located within OT-ir perikarya. Activation of CRF neurons was also associated with an increase in the transcription of the neuropeptide; PGE_2 caused expression of CRF hnRNA selectively in this neuroendocrine nucleus and not in any other regions of the brain. Moreover, the CRF_1 receptor mRNA was significantly increased in the PVN of PGE_2-treated-animals suggesting that CRF can play a role within this neuroendocrine nucleus under central PGE_2 influence.

As presented by the table 1, systemic treatment with a moderate dose of LPS, an activator of B type acute phase immune response, induces a c-*fos* mRNA pattern quite similar to the one observed in the brains of centrally-administered PGE_2 rats. It is thus permit to believe that production of PGE_2 in selective regions of the brain of systemically immune-challenged animals is an important step in the integration by the brain of the information received from the periphery to restore the homeostasis when the organism is challenged by foreign material. PGE_2 synthesis and release have been shown to be stimulated in various regions of the brain by cytokines. In a microdialysis study, Komaki et al. (1992) have reported that intravenous administration of IL-1ß induced a significant increase in PGE_2 levels in the OVLT and the medial part of the MPOA, a structure profoundly activated by systemic treatment with IL-1ß (Brady et al., 1994; Ericsson et al., 1994), IL-6 (Vallières et al., paper in preparation) and the bacterial endotoxin LPS (Hare et al., 1995; Rivest and Laflamme, 1995). This later region also exhibits high density of PGE_2 binding sites in the rat brain (Matsumura et al., 1990) and is activated by central treatment with the PG as revealed by expression of c-*fos* mRNA in the present study. Interestingly, microinjection of PGE_2 within the OVLT/MPOA increases plasma ACTH release, whereas indomethacin or a PGE antagonist infused into this area of the brain significantly prevented the stimulatory influence of i.v. IL-1ß administration in rats (Katsuura et al., 1990). The fact that the PVN receives afferent innervation from the OVLT/MPOA (Sawchenko and Swanson, 1983) and i.c.v. treatment with PGE_2 causes transcriptional activation of neuroendocrine CRF (Figs. 5 and 6) and ACTH and corticosterone release (Rassnick et al., 1995) are evidences leading to speculate that PG of E_2 type are the interface between circulating cytokines (in particular IL-1) and cells of the OVLT/MPOA involved in providing the information to the PVN to trigger the HPA axis.

Although this hypothesis is quite attractive, it might be too simplistic and as presented here, the OVLT is not the only sensorial CVO's activated by PGE_2; the SFO and the AP also displayed positive signal for c-*fos* mRNA. Because of the structural heterogeneity of the CVO's, it is difficult to describe the cell types expressing the IEG following central PGE_2 injection and most likely involved several distinct classes of cells including endothelial, ependymal, glial, as well as neuronal. Interestingly, we (Rivest and Laflamme, 1995) and others (Hare et al., 1995) have reported that immune activation of B-type by systemic administration of the bacterial endotoxin LPS caused expression of c-*fos* mRNA and protein within the OVLT, SFO and AP suggesting therefore possible link between local production of PGE_2 within these structures during immune challenge. Whether all the CVO's are involved in the control of neuroendocrine CRF and the HPA remains to be resolved, but neuronal projections from SFO to PVN have been found. Indeed, as previously described for the OVLT, a moderate number of retrogradely-labeled neurons can be found in the SFO after injection of a transporter within the hypothalamic PVN (Johnson and Gross, 1993; Larsen and Mikkelsen, 1995; Liposits, 1993; Sawchenko and Swanson, 1983). Most of the projections of the AP to the PVN are, on the other hand, likely to be indirect; this CVO provides dense input to the commissural and medial parts of the NTS as well as the external lateral part of the parabrachial nucleus (Cunningham et al., 1994), two structures which massively innervate endocrine hypothalamus (Swanson et al., 1987). The targets of the AP are therefore in very good position to mediate the information received from systemic circulation as those observed during immune challenge and that PG of E_2 type can be an important step in this cascade of events.

As previously reported in animals injected systemically with IL-1 (Ericsson et al., 1994) and LPS (Rivest and Laflamme, 1995), central treatment with the PG causes expression of c-*fos* mRNA within the MPOA, BnST, CeA, SON, PVN, NTS/X, and AMB/ventrolateral medulla (Fig. 1). These brain structures are believed to be involved in many stress responses including cardiovascular, neuroendocrine and behavioral changes. The NTS/X is a region of particular interest here because this region, along with the MPOA, displays the highest density of PGE_2 binding site (Matsumura et al., 1992) whereas the PG facilitates excitatory synaptic transmission in voltage-clamped neurons in rat NTS slices (Seriyama et al., 1995). The NTS is therefore another good candidate to mediate some of the effects of PGE_2 during immune challenge on neuroendocrine functions. In fact, this nucleus provides the largest noradrenergic (NA) input to the PVN (Cunningham and Sawchenko, 1988) and several of these neurons are activated by systemic IL-1ß injection (Ericsson et al., 1994), whereas unilateral brain stem transections significantly attenuated the activation of

PVN CRF neurons by the cytokine (Ericsson et al., 1994). Chuluyan and colleagues have also recently reported that noradrenergic innervation of the hypothalamus participates in adrenocortical responses to IL-1 (Chuluyan et al., 1992). The interaction between ascending NA fibers and PGE_2 in mediating the activation of neuroendocrine CRF neurons could however be located directly within the PVN; injection of 6-OHDA into the PVN depleted its NA content by 85 % and reduced by 80-82 % the increase in plasma corticosterone levels following i.p. injection of IL-1 (Chuluyan et al., 1992) and i.v. treatment with IL-1ß stimulates production of PGE_2 within the PVN measured by both push-pull (Watanobe and Takebe, 1994) and microdialysis (Komaki et al., 1992) techniques.

Central injection with the PGE_2 provokes a profound expression of c-*fos* within the PVN, in particular in the parvocellular division, where several CRF-ir neurons were positive for the IEG. This activation of CRF neurons within this endocrine nucleus was associated with an increase in CRF gene transcription as revealed by the rapid but transient presence of CRF primary transcript (hnRNA) in the rat PVN. Interestingly, only the CRF neurons of the PVN seemed to respond to central PGE_2 treatment; the colocalization between c-*fos* mRNA and CRF-ir was not detected in other CRF groups of cells and although basal expression of CRF hnRNA was observed in the inferior olivary complex, the PVN was the unique structure exhibiting increased hybridization signal for the primary transcript. Selective activation of CRF transcription within the rat PVN has also been recently reported in the brains of immune-challenged rats (Rivest and Laflamme, 1995), a phenomenon significantly prevented by pre-treatment with indomethacin (Lacroix and Rivest, unpublished data). These results are in agreement with the stimulatory influence of PGE_2 on the activity of HPA axis and provide anatomical evidences that such phenomenon involved activation of neuroendocrine CRF cells. Injection of PGE_2 into the ventricular space has also been reported to suppress *in vitro* cellular immune responses (Rassnick et al., 1995) and decreased mitogenic responses of spleen and blood lymphocytes is mediated by stimulation of HPA axis (Cunninck et al., 1990). Taken together, these results suggest that local production of PGE_2 within selective structures of the brain may be an interface to activate stress neuronal circuitry involved to restore and maintain the homeostasis during infection and tissue trauma. In this concept, neuroendocrine CRF neurons appear to play a crucial role in orchestrating the appropriate concentration of glucocorticoids to restrain and prevent exaggerated immune responses.

Animals treated centrally with PGE_2 showed expression of CRF_1 receptor gene in the parvocellular division of the PVN while this transcript was barely detectable in this endocrine nucleus in vehicle-treated animals (Fig. 7). In the past two years, two distinct CRF receptors have been isolated; the type 1 is widely distributed throughout the brain (Potter et al., 1994; Rivest et al., 1995), whereas the type 2 has two different splice variants, a shorter (also called α) and a longer form (also called β) (Lovenberg et al., 1995; Perrin et al., 1995). $CRF_{2\alpha}$ receptor mRNA is located in very few define structures of the limbic system such as the lateral septum, ventromedial hypothalamus and the medial amygdaloid nuclei while the type 2β is expressed in several peripheral tissue but not in the brain (Perrin et al., 1995). We have recently reported that systemic treatment with the bacterial LPS induces transcription of CRF_1 receptor in the rat PVN (Rivest et al., 1995) but similar treatment did not alter expression of the type 2 in the rat brain (Lacroix and Rivest, 1995). The role played by the CRF_1 receptor in the PVN of immune- and PGE_2-challenged animals is far from been clear but because this receptor is expressed in a large concentration of neuroendocrine CRF neurons in challenged animals (Nappi and Rivest, 1995; Rivest et al., 1995), we believe that CNS CRF can modulate in a positive manner the activity of a selective group of cells within the hypothalamic PVN, a phenomenon most likely related to the HPA axis during the acute-phase response.

In the magnocellular division of the PVN and the SON, most of the c-*fos* expressing cells were colocalized in OT-ir neurons, a phenomenon recently observed in rats treated systemically with IL-1ß (Ericsson et al., 1994) and LPS (Rivest and Laflamme, 1995). Several OT-ir neurons located in the ventral part of the medial parvocellular division as well as within the lateral-caudal parvocellular section of the PVN also displayed positive hybridization signal for c-*fos* mRNA. The role played by OT neurons during immune challenge has yet to be investigated but it is possible that some of these cells, in particular those localized in the parvocellular parts of the nucleus which project to dorsal vagal complex and the intermedio-lateral column of the spinal cord, participate to trigger directly the sympathetic nervous system (SNS) activity. Central injection of PGE_2 increases splenic sympathetic nerve activity in rats (Ando et al., 1995) and involvement of this system in the immunosuppression has been suggested (Sundar et al., 1990), whereas the stimulatory influence of IL-1ß on SNS activity can be prevented by cyclooxygenase inhibitors (Ichijo et al., 1994). PGE_2 of central origin could therefore be involved in the activation OT neurons projecting to the spinal cord to stimulate the SNS in immune-challenged animals, although this hypothesis still remains to be fully investigated. We have also detected OT-ir neurons in the lateral magnocellular component of the PVN that were positive for the IEG c-*fos* mRNA

and these neurons, as for those of the SON, send their projections to the posterior pituitary. The physiological relevance of these results remains uncertain but OT belongs to a family of neuropeptides able to potentiate the action of neuroendocrine CRF on the secretion of ACTH from corticotroph cells of the adenohypophysis during various types of challenges. Secretion of OT into the infundibular process and in the circulation would therefore be another candidate to help stimulating the HPA axis during immune challenge. As mentioned before, in the magnocellular PVN, very few AVP neurons expressed c-*fos* transcript after central treatment with PGE_2 whereas in the SON, some double-labeled neurons (AVP-ir + c-*fos* mRNA) were detected. Although involvement of vasopressin system in response to cytokines and immune challenge has been suggested (Berkenbosch et al., 1987; Harbuz et al., 1992; Watanobe and Takebe, 1994), the number of AVP neurons activated by central PGE_2 administration and systemic LPS treatment is by no doubt much lower than OT cells.

In conclusion, i.c.v. injection of PGE_2 induces c-*fos* expression in several structures recognized to be activated during the acute phase response of an immune challenge and in a similar manner, central treatment with this PG, triggers transcription of CRF and its type 1 receptor essentially in the hypothalamic PVN. Local production of PGE_2 might therefore be a crucial step within the CNS to mediate the effects of cytokines and other immune-related systemic factors on the neuronal circuitry involved in the activation of the HPA axis and the sympathetic nervous system. Reinforcing this concept is the fact that indomethacin can significantly prevent LPS-induced neuronal activity in the rat brains, although this seems to depend upon the dose and the activated structures by the endotoxin (Lacroix and Rivest, paper in preparation).

4.7 ACKNOWLEDGMENTS

This research was supported by the Medical Research Council (MRC) of Canada. Serge Rivest is a Medical Research Council Scholar. We thank Dr. Wylie W. Vale (PBL, The Salk Institute, La Jolla, CA) for the generous gift of the rabbit antihuman/rat CRF serum and the rat CRF_1 receptor cDNA, Dr. I. Verma (The Salk Institute, La Jolla, CA) for the rat c-*fos* cDNA, and Dr. S. Watson (The University of Michigan, Ann Arbor, MI) for the pGem3 plasmid containing a pure CRF intronic piece. The authors also thank Ms. Nathalie Laflamme for invaluable technical assistance.

4.8 REFERENCES

Andersson, J., Nagy, S., Björk, L., Abrams, J., Holm, S. and Andersson, U. (1992) Bacterial toxin-induced cytokine production studied at the single-cell level. Immunological Rev. 127, 69-96.

Ando, T., Ichijo, T., Katafuchi, T. and Hori, T. (1995) Intracerebroventricular injection of prostaglandin E2 increases splenic sympathetic nerve activity in rats. Am. J. Physiol. 269, R662-R668.

Barbanel, G., Ixart, G., Szafarczyk, A., Malaval, F. and Assenmacher, I. (1990) Intrahypothalamic infusion of interleukin-1ß increases the release of corticotropin-releasing hormone (CRH 41) and adrenocorticotropic hormone (ACTH) in free-moving rats bearing a push-pull cannula in the median eminence. Brain Res. 516, 31-56.

Berkenbosch, F., Oers, J.V., Rey, A.D., Tilders, F. and Besedovsky, H. (1987) Corticotropin-releasing factor producing neurons in the rat activated by interleukin-1. Science 238, 524-526.

Berkenbosch, F., Wolvers, D.A.W. and DeRijk, R. (1991) Neuroendocrine and immunological mechanisms in stress-induced immunomodulation. J. Steroid Biochem. Biol. 40, 639-647.

Brady, L., Lynn, A.B., Herkenham, M. and Gottesfeld, Z. (1994) Systemic interleukin-1 induces early and late patterns of c-*fos* mRNA expression in brain. J. Neurosci. 14, 4951-4964.

Chuluyan, H.E., Saphier, D., Rohn, W.M. and Dunn, A.J. (1992) Noradrenergic innervation of the hypothalamus participates in adrenocortical responses to interleukin-1. Neuroendocrinology 56, 106-111.

Cunninck, J.E., Lysle, D.T., Kucinski, B.J. and Rabin, B.S. (1990) Evidence that shock-induced immune supression is mediated by adrenal hormones and peripheral B-adrenergic receptors. Pharmacol. Biochem. Behav. 36, 645-651.

Cunningham, E.T., Miselis, R.R. and Sawchenko, P.E. (1994) The relationship of efferent projections from the area postrema to vagal motor and brain stem catecholamine-containing cell groups: An axonal transport and immunohistochemical study in the rat. Neuroscience 58, 635-648.

Cunningham, E.T. and Sawchenko, P.E. (1988) Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. J. Comp. Neurol. 274, 60-76.

Ericsson, A., Kovacs, K.J. and Sawchenko, P.E. (1994) A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. J. Neurosci. 14, 897-913.

Feuerstein, G., Adelberg, S.A., Kopin, I.J. and Jacubowitz, D.M. (1982) Hypothalamic sites for cardiovascular and sympathetic modulation by prostaglandin E2. Brain Res. 231, 335-342.

Harbuz, M.S., Rees, R.G., Eckland, D., Jessop, D.S., Brewerton, D. and Lightman, S.L. (1992) Paradoxical responses of hypothalamic corticotropin-releasing factor (CRF) messenger ribonucleic acid (mRNA) and CRF-41 peptide and adenohypophysial pro-opiomelanocortin mRNA during chronic inflammatory stress. Endocrinology 130, 1394-1400.

Hare, A.S., Clarke, G. and Tolchard, S. (1995) Bacterial lipopolysaccharide-induced changes in Fos protein expression in the rat brain: Correlation with thermoregulatory changes and plasma corticosterone. J. Neuroendocrinol. 7, 791-799.

Herman, J.P., Schafer, M., Thompson, R.C. and Watson, S.J. (1992) Rapid regulation of corticotropin-releasing hormone gene transcription *in vivo*. Mol. Endocrinol. 6, 1061-1069.

Hughes, J.H., Easom, R.A., Wolf, B.A., Turk, J. and McDaniel, M.L. (1989) Interleukin-1-induced prostaglandin E2 accumulation by isolated pancreatic islets. Diabetes 38, 1251-1257.

Ichijo, T., Katafuchi, T. and Hori, T. (1994) Central interleukin-1ß enhances splenic sympathetic nerve activity in rats. Brain Res. Bull. 34, 547-553.

Johnson, A.K. and Gross, P.M. (1993) Sensory circumventricular organs and brain homeostatic pathways. Faseb 7, 678-686.

Katsuura, G., Arimura, A., Koves, K. and Gottschall, P.E. (1990) Involvement of organum vasculosum of the lamina terminalis and preoptic area in interleukin-1ß-induced ACTH release. Am. J. Physiol. 258, E163-E171.

Katsuura, G., Gottschall, P.E., Dahl, R.R. and Arimura, A. (1988) Adrenocorticotropin release induced by intracerebroventricular injection of recombinant human interleukin-1 in rats: Possible involvement of prostaglandin. Endocrinology 122, 1773-1779.

Katsuura, G., Gottschall, P.E., Dahl, R.R. and Arimura, A. (1989) Interleukin-1ß increases prostaglandin E2 in rat astrocyte cultures: modulating effect of neuropeptides. Endocrinology 124, 3125-3127.

Koenig, J.I. (1991) Presence of cytokines in the hypothalamic-pituitary axis. Prog. NeuroEndocrImmunol. 4, 143-153.

Kohan, D.E. (1989) Interleukin-1 regulation of prostaglandin E2 synthesis by the papillary collecting duct. J. Lab. Clin. Med. 114, 717-723.

Komaki, G., Arimura, A. and Koves, K. (1992) Effect of intravenous injection of IL-1ß on PGE2 levels in several brain areas as determined by microdialysis. American Journal of Physiology 262 (Endocrinol. Metab. 25), E246-E251.

Kovacs, K.J. and Elenkov, I.J. (1995) Diffencial dependence of ACTH secretion induced by various cytokines on the integrity of the paraventricular nucleus. J. Neuroendocrinol. 7, 15-23.

Lacroix, S. and Rivest, S. (1996) Role of cyclo-oxygenase pathways in the stimulatory influence of immune challenge in the transcription of a specific CRF receptor subtype in the rat brain. J. Chem. Neuro. 10, 53-71.

Larsen, P.J. and Mikkelsen, J.D. (1995) The functional identification of central afferent projections conveying information of acute "stress" to the hypothalamic paraventricular nucleus. J. Neurosc. 15, 2609-2627.

Liposits, Z. (1993) Ultrastructure of hypothalamic paraventricular neurons. Crit. Rev. Neurobiol. 7, 89-162.

Lovenberg, T.W., Liam, C.W., Grigoriadis, D.E., Clevenger, W., Chalmers, D.T., DeSouza, E.B. and Oltersdorf, T. (1995) Cloning and characterization of a functional distinct corticotropin-releasing factor receptor subtype from the rat brain. Proc. Natl. Acad. Sci. USA 92, 836-840.

Lyson, K. and McCann, S.M. (1992) Involvement of arachidonic acid cascade pathways in interleukin-6-stimulated corticotropin-releasing factor release in vitro. Neuroendocrinology 55, 708-713.

Matsumura, K., Watanabe, Y., Imai-Matsumura, K., Connolly, M., Koyama, Y., Onoe, H. and Watanabe, Y. (1992) Mapping of prostaglandin E2 binding sites in rat brain using quantitative autoradiography. Brain Res. 581, 292-298.

Matsumura, K., Watanabe, Y., Onoe, H., Watanabe, Y. and Hayaishi, O. (1990) High density of prostaglandin E2 binding sites in the anterior wall of the 3rd ventricle: a possible site of its hyperthermic action. Brain Res. 533, 147-151.

McCabe, J.T. and Pfaff, D.W. (1989) *In situ* hybridization: a methodological guide. Methods Neurosci. 1, 98-117.

McCoy, J.G., Matta, S.G. and Sharp, B.M. (1994) Prostaglandins mediate the ACTH response to interleukin-1-beta instilled into the hypothalamic median eminence. Neuroendocrinology 60, 426-435.

Nappi, R.E. and Rivest, S. (1995) Ovulatory cycle influences the stimulatory effect of stress on the expression of corticotropin-releasing factor receptor messenger ribonucleic acid in the paraventricular nucleus of the female rat hypothalamus. Endocrinology 136, 4073-4083.

Navarra, P., Pozzoli, G., Brunetti, L., Ragazzoni, E., Besser, M. and Grossman, A. (1992) Interleukin-1ß and interleukin-6 specifically increase the release of prostaglandin E2 from rat hypothalamic explants *in vitro*. Neuroendocrinology 56, 61-68.

Navarra, P., Tsagarakis, S., Faria, M.S., Rees, L.H., Besser, G.M. and Grossman, A.B. (1991) Interleukin-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. Endocrinology 128, 37-44.

Oka, T. and Hori, T. (1994) EP1-receptor mediation of prostaglandin E2-induced hyperthermia in rats. Am. J. Physiol. 267, R289-R294.

Paxinos, G. and Watson, C. (1986) The Rat Brain in Stereotaxic Coordinates. San Diego: Academic Press.

Perrin, M., Donaldson, C., Chen, R., Blount, A., Berggren, T., Bilezikjian, L., Sawchenko, P. and Vale, W. (1995) Identification of a second corticotropin-releasing factor receptor gene and caracterization of a cDNA expressed in heart. Proc. Natl. Acad. Sci. USA 92, 2969-2973.

Perrin, M.H., Donaldson, C.J., Chen, R., Lewis, K.A. and Vale, W.W. (1993) Cloning and functional expression of a rat brain corticotropin-releasing factor (CRF) receptor. Endocrinology 133, 3058-3061.

Plotsky, P.M. (1990) Pathways to the secretion of adrenocorticotropin: A view from the portal. J. Neuroendocrinol. 3, 1-9.

Plotsky, P.M. and Sawchenko, P.E. (1987) Hypophysial-portal plasma levels, median eminence content and immunohistochemical staining of corticotropin releasing factor, arginine vasopressin and oxytocin following pharmacological adrenalectomy. Endocrinology 120, 1361-1369.

Potter, E., Sutton, S., Donaldson, C., Chen, R., Perrin, M., Lewis, K., Sawchenko, P.E. and Vale, W.W. (1994) The distribution of CRF receptor mRNA expression in the rat brain and pituitary. Proc. Natl. Acad. Sci., USA 91, 8777-8781.

Rassnick, S., Zhou, D.H. and Rabin, B.S. (1995) Central administration of prostaglandin E (2) suppresses in vitro cellular immune responses. Am. J. Physiol. R92-R97.

Rivest, S. (1995) Molecular mechanisms and neural pathways mediating the influence of interleukin-1 on the activity of neuroendocrine CRF motoneurons in the rat. Int. J. Devl. Neuroscience 13, 135-146.

Rivest, S. and Laflamme, N. (1995) Neuronal activity and neuropeptide gene transcription in the brain of immune-challenged rats. J. Neuroendocrinol. 7, 501-525.

Rivest, S., Laflamme, N. and Nappi, R.E. (1995) Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. J. Neurosci. 15, 2680-2695.

Rivest, S. and Rivier, C. (1991) Influence of the paraventricular nucleus of the hypothalamus in the alteration of neuroendocrine functions induced by intermittent footshock or interleukin. Endocrinology 129, 2049-2057.

Rivest, S. and Rivier, C. (1993) Centrally injected interleukin-1ß inhibits the hypothalamic LHRH secretion and circulating LH levels via prostaglandins in rats. J. Neuroendocrinol. 5, 445-450.

Rivest, S. and Rivier, C. (1994) Stress and interleukin-1ß-induced activation of c-*fos*, NGFI-B and CRF gene expression in the hypothalamic PVN: Comparison between Sprague-Dawley, Fisher-344 and Lewis rats. J. Neuroendocrinol. 6, 101-117.

Rivest, S. and Rivier, C. (1995) The role of CRF and interleukin-1 in the regulation of neurons controlling reproductive functions. Endocrine Rev. 16, 177-199.

Rivier, C. (1993) Neuroendocrine effects of cytokines in the rat. Rev. Neurosci. 4, 223-237.

Rivier, C. and Rivest, S. (1993) Mechanisms mediating the effects of cytokines in neuroendocrine functions in the rat. In: Corticotropin-releasing factor, Ciba Foundation Symposium 172 (Chadwick DJ, Marsh J and Ackrill K, ed.) pp 204-225. Chichester: John Wiley & Sons Ltd.

Rothwell, N.J. (1991) Functions and mechanisms of interleukin-1 in the brain. Trends Pharmacol. Sci. 12, 430-436.

Saphier, D. and Ovadian, H. (1990) Selective facilitation of putative corticotropin-releasing factor-secreting neurons by interleukin-1. Neurosci. Lett. 114, 283-288.

Sawchenko, P.E., Imaki, T., Potter, E., Kovács, K. and Vale, W. (1993) The functional neuroanatomy of corticotropin-releasing factor. In: Corticotropin-releasing factor, Ciba Foundation Symposium 172 (Chadwick DJ, Marsh J and Ackrill K, ed.) pp 5-29. Chichester: John Wiley & Sons Ltd.

Sawchenko, P.E. and Swanson, L.W. (1983) The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. J. Comp. Neurol. 218, 121-144.

Sawchenko, P.E. and Swanson, L.W. (1990) Organization of CRF immunoreactive cells and fibers in the rat brain: Immunohistochemical studies. In: Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide. (DeSouza EB and Nemeroff CB, ed.) pp 29-51. Boca Raton, Florida: CRC Press, Inc.

Seriyama, N., Mizuta, S., Hori, A. and kobayashi, S. (1995) Prostaglandin E(2) facilitates excitatory synaptic transmission in the nucleus-tractus-solitarii of rats. Neurosc. Let. 188, 101-104.

Simmons, D.M., Arriza, J.L. and Swanson, L.W. (1989) A complete protocol for *in situ* hybridization of messenger RNAs in brain and other tissus with radio-labeled single-stranded RNA probes. J. Histotechnol. 12, 169-181.

Solomon, G.F. (1969) Stress and antibody response in rats. Int. Arch. Allergy 35, 97-108.

Sundar, S.K., Cierpial, M.A., Kilts, C., Ritchie, J.C. and Weiss, J.M. (1990) Brain IL-1-induced immunosuppression occurs through activation of both pituitary-adrenal axis and sympathetic nervous system by corticotropin-releasing factor. J. Neurosci. 10, 3701-3706.

Swanson, L.W., Sawchenko, P.E., Lind, R.W. and Rho, J.H. (1987) The CRH Motoneuron: Differential peptide regulation in neurons with possible synaptic, paracrine, and endocrine outputs. Ann. N.Y. Acad. Sci. 512, 12-23.

Watanobe, H. and Takebe, K. (1994) Effects of intravenous administration of interleukin-1-beta on the release of prostaglandin E2, corticotropin-releasing factor, and arginine vasopressin in several hypothalamic areas of freely moving rats: estimation by push-pull perfusion. Neuroendocrinology 60, 8-15.

Yasin, S.A., Costa, A., Forsling, M.L. and Grossman, A. (1994) Interleukin-1ß and interleukin-6 stimulate neurohypophysial hormone release in vitro. J. Neuroendocrinol. 6, 179-184.

Table 1:

Qualitative analysis of hybridization signal for c-*fos* mRNA in the brain of rats treated centrally with PGE_2: A comparison with the distribution of the immediate *early* gene throughout the brains of immune-challenged rats with the bacterial endotoxin lipopolysaccharide (LPS).

Region	LPS i.p. 25 μg/ 100 g b.w.	PGE_2 i.c.v. 2 μg/ 10μl	Vehicle
Claustrum	0/+	++/+++	++/+++
Subependymal zone	+/++	+/++	—
Lateral septal nucleus	0/+	++/+++	++
Bed nucleus of the stria terminalis	+/++	+/++	—
Suprachiasmatic nucleus	+/++	+/++	+/++
Medial preoptic area (MPOA/OVLT)	+++/++++	++/+++	—
Periventricular nucleus of the hypothalamus	+/++	+/++	0/+
Supraoptic nucleus	+++/++++	+++/++++	+
Anterior hypothalamic nucleus	0/+	0/+	0/+
Subfornical organ	++/+++	+	—
Hypothalamic paraventricular nucleus	+++/++++	+++/++++	+
Paraventricular nucleus of the thalamus	++/+++	++	++
Thalamus (general)	0/+	+/++	+/++
Central nucleus of the amygdala	+/++	++/+++	—
Arcuate nucleus	++/+++	++/+++	+/++
Median eminence	++/+++	0/+	—
Dorsomedial nucleus of the hypothalamus	+/++	+/++	+/++
Subthalamic nucleus	0/+	0/+	0/+
Lateral geniculate complex	+	+	+
Laterodorsal tegmental nucleus	+/++	++/+++	++/+++
Parabrachial nucleus (external lateral part)	++/+++	+	+
Locus coeruleus	++	+/++	+/++
Cochlear nucleus	+	++	+/++
Spinal nucleus of the trigeminal	0/+	++	+/++
Nucleus raphé pallidus	—	+/++	0/+
Nucleus of the solitary tract	+++/++++	+++/++++	0/+
Ambiguus nucleus (dorsal division)	++/+++	++/+++	+
Lateral reticular nucleus	+/++	+/++	+/++
Area postrema	+/++	++/+++	—
Cortex (general)	+	+++	+++
Choroid plexus	++/+++	++/+++	+/++
Ependymal cells of ventricles	+/++	+++	++/+++
Meninges (lepto)	++/+++	++	0/+

In situ hybridization histochemistry was accomplished via ^{35}S-labeled cRNA probe encoding the gene c-*fos*. Animals were sacrificed 30 min after an intracerebroventricular (i.c.v.) injection of the prostaglandin of E_2 type (PGE_2, middle column) or the vehicle solution (right column). For comparison, distribution of c-*fos* throughout the brains of animals sacrificed 3h after systemic (i.p.) treatment with the immune activator of B-type lipopolysaccharide (LPS) is included (left column). Legends: ++++, very strong signal; +++, strong signal; ++, moderate signal; +, low but positive signal; —, undetectable signal.

Figure 1:

Representative examples of the distribution of the mRNA encoding the immediate *early* gene c-*fos* in the rat brain after intracerebroventricular (i.c.v.) administration of prostaglandin E_2 (PGE_2) or vehicle. Animals were deeply anesthetized and rapidly perfused with 4% paraformaldehyde 30 min after treatment with PGE_2 (2 μg/10 μl) or the vehicle solution. These photos depict darkfield photomicrographs of dipped autoradiographs of hybridized 30-μm sections with c-*fos* riboprobe through identical areas of the rat brain. A) medial preoptic area; B) supraoptic nucleus of the hypothalamus; C) paraventricular nucleus of the hypothalamus; D) central nucleus of the amygdala; E) area postrema/nucleus of the solitary tract/dorsal motor nucleus of the vagus. X10 (A, C, D, E), X 25 (B).

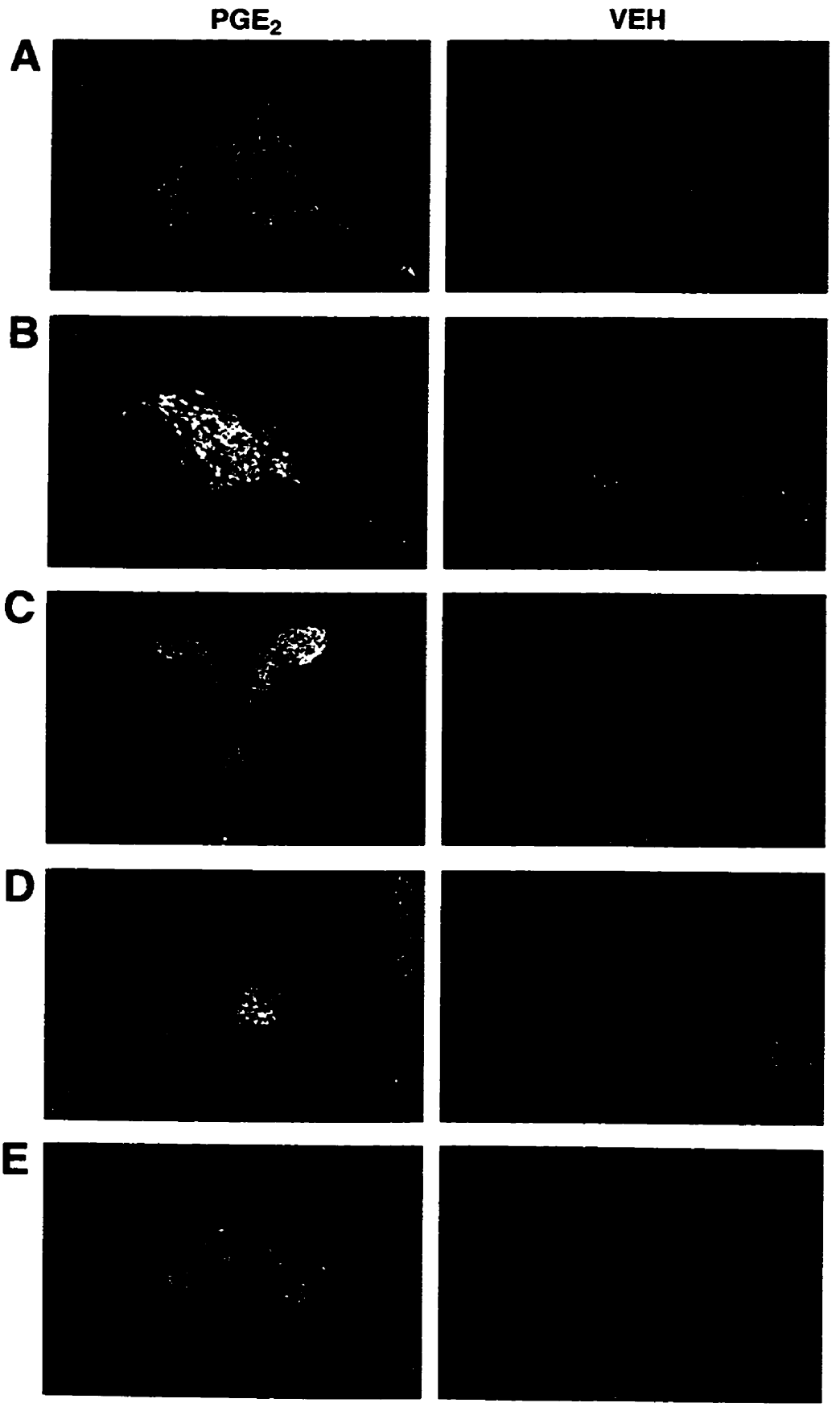

PGE2
VEH
A
B
C
D
E

Figure 2:

Influence of intracerebroventricular (i.c.v.) administration of PGE_2 or vehicle on the average optical density (O.D., arbitrary units) for c-*fos* mRNA hybridization signal in the paraventricular nucleus (PVN) of the rat hypothalamus. The O.D. was quantified in both sides of the medial PVN using an Olympus Optical System (BX-50, BMax) coupled to a Macintosh computer (PowerPC 7100/66) and Image software (version 1.55 non-FPU, W Rasband, NIH). Results represent means ± SEM and statistical analysis was performed using a two-way analysis of variance (2 x 2) followed by a Bonferroni/Dunn post hoc test (Staview 4.01). *, Significantly different ($P < 0.05$) from all the other groups of rats. For more information on image analysis, see Materials and Methods.

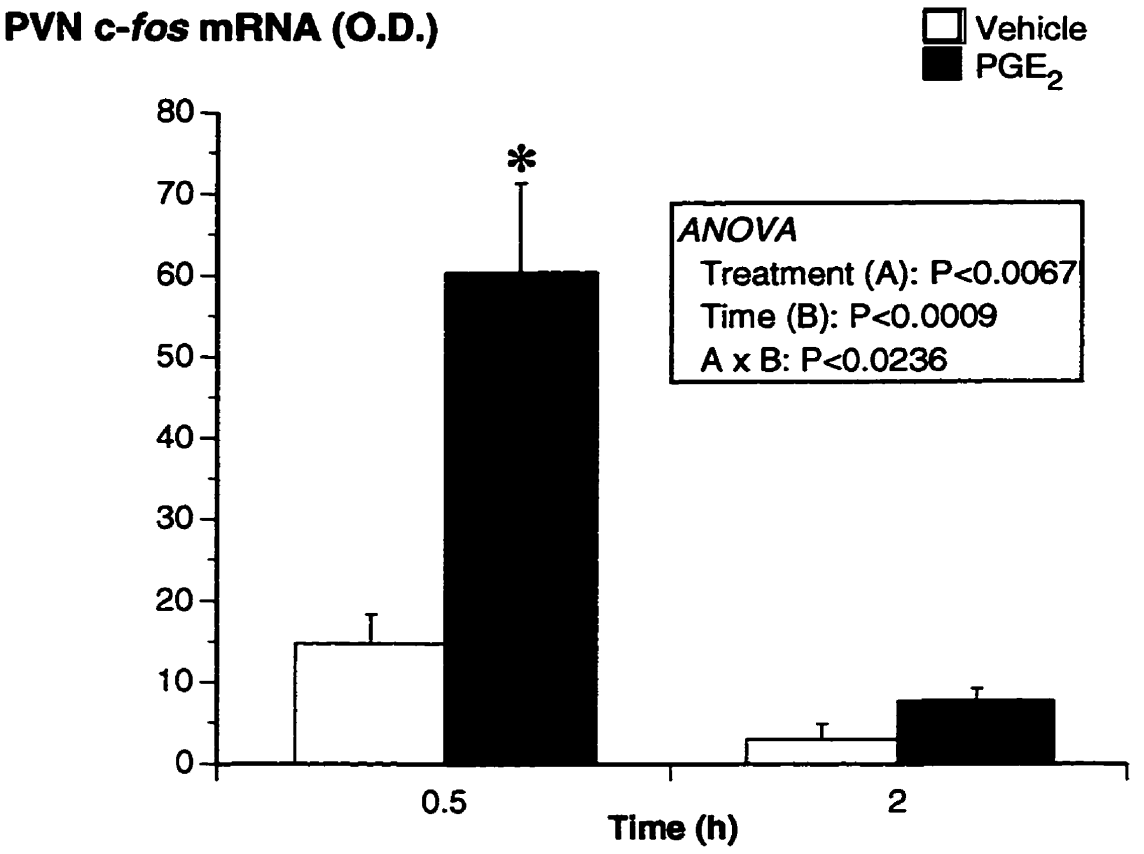
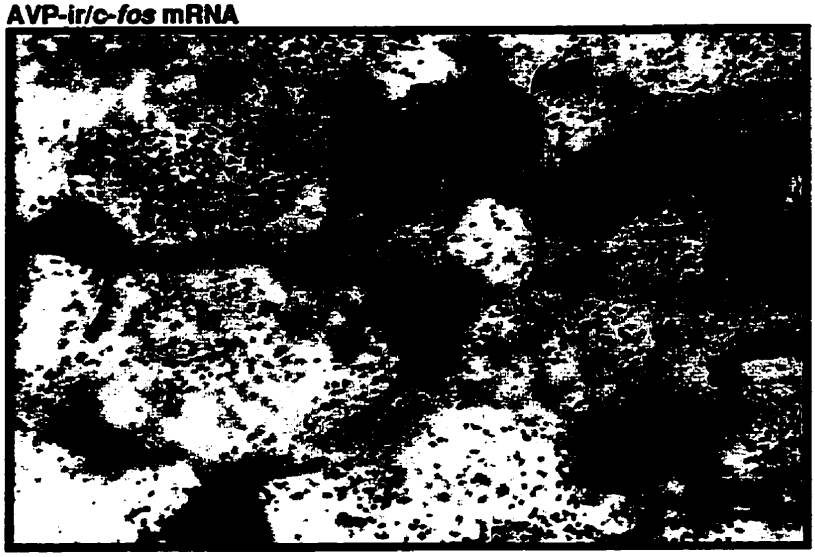

PVN c-*fos* mRNA (O.D.)
Vehicle
PGE_2
80
70
60
50
40
30
20
10
0
*
ANOVA
Treatment (A): P<0.0067
Time (B): P<0.0009
A x B: P<0.0236
0.5
2
Time (h)

Figure 3:

Expression of the mRNA encoding the immediate *early* gene c-*fos* in selective neuropeptidergic perikarya located in the hypothalamic paraventricular nucleus (PVN) of PGE_2-treated rats. Animals were killed 30 min after an intracerebroventricular (i.c.v.) administration of PGE_2 (2 μg/10 μl) and colocalization of corticotropin-releasing factor (CRF), vasopressin (AVP), or oxytocin (OT) perikarya with c-*fos* expressing neurons was performed. Immunocytochemistry (CRF, AVP, or OT protein, brown neurons) was accomplished on the same brain sections (30 μm) before *in situ* hybridization histochemistry (c-*fos* mRNA, silver grains). For more details on the combination of both immunocytochemistry and *in situ* hybridization techniques, see Materials and Methods. Filled arrowheads, CRF (top picture), AVP (middle picture), or OT (bottom picture) neurons expressing the mRNA encoding c-*fos*; Open arrowheads, c-*fos* positive neurons alone; Curved arrowheads, neuropeptidergic neuron alone. CRF-ir, corticotropin-releasing factor-immunoreactive neurons; AVP-ir, vasopressin-immunoreactive neurons; OT-ir, oxytocin-immunoreactive neurons. X250.

CRF-ir/c-*fos* mRNA

AVP-ir/c-*fos* mRNA



OT-ir/c-*fos* mRNA

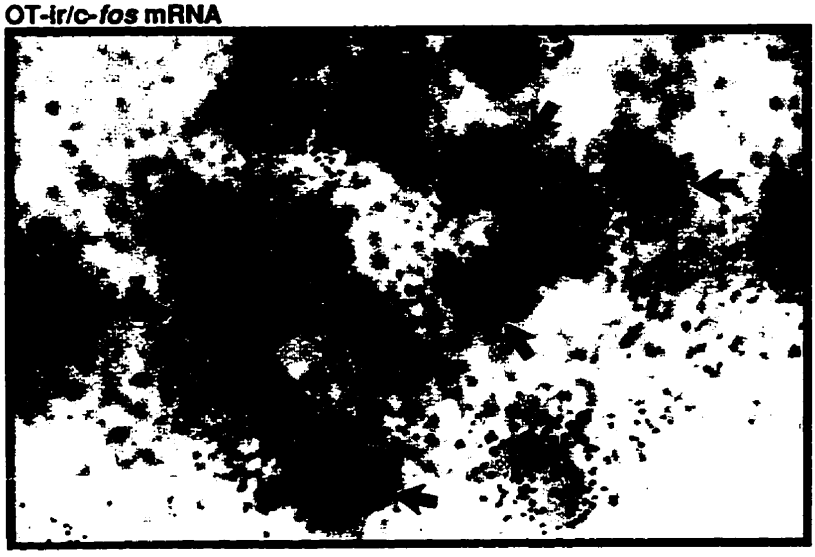
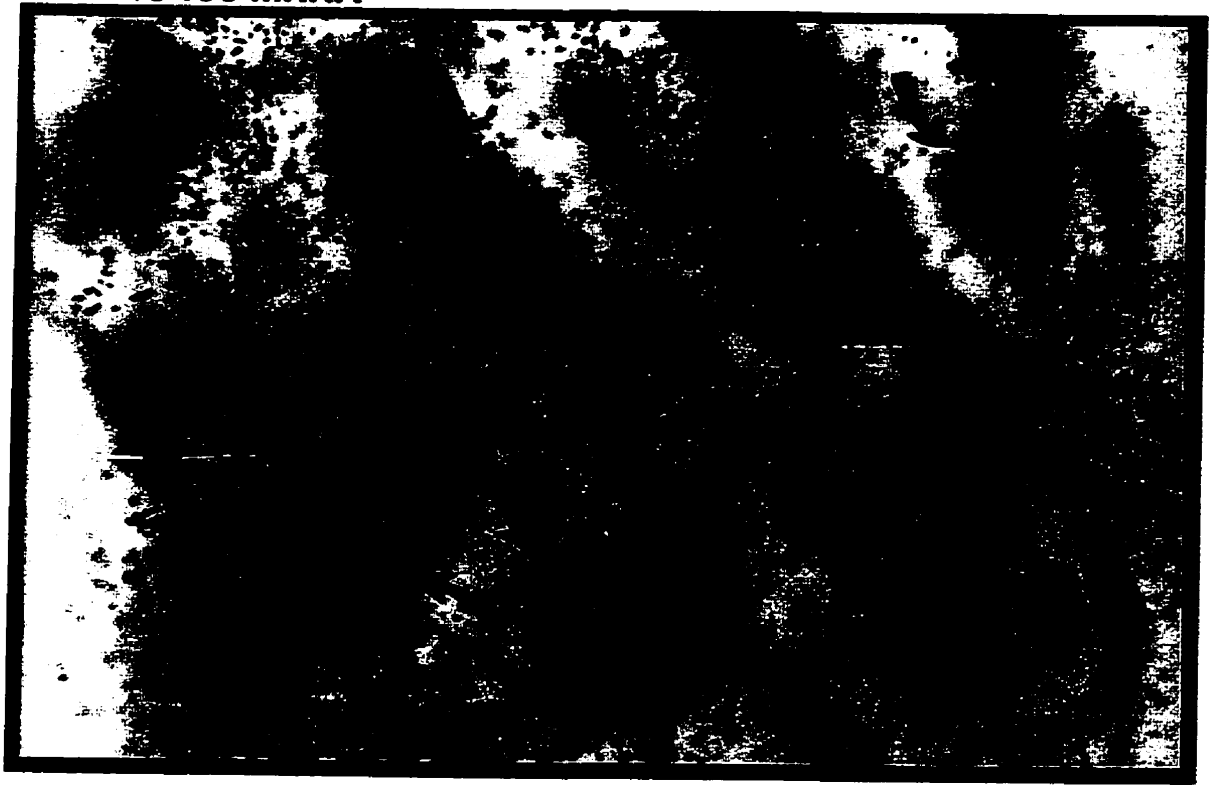


Figure 4:

Colocalization of vasopressin (AVP) and oxytocin (OT)-immunoreactive (ir) neurons with c-*fos* expressing cells in the hypothalamic supraoptic nucleus (SON) of animals treated centrally with the prostaglandin of E_2 type (PGE_2). Animals were killed 30 min after the treatment. Immunocytochemistry (AVP or OT protein, brown neurons) was accomplished on the same brain sections (30 μm) before *in situ* hybridization histochemistry (c-*fos* mRNA, silver grains). Filled arrowheads, AVP (top picture) or OT (bottom picture) neurons expressing the mRNA encoding c-*fos*; Open arrowheads, c-*fos* positive neurons alone; Curved arrowheads, neuropeptidergic neuron alone. X250.

AVP-ir/c-*fos* mRNA



OT-ir/c-*fos* mRNA

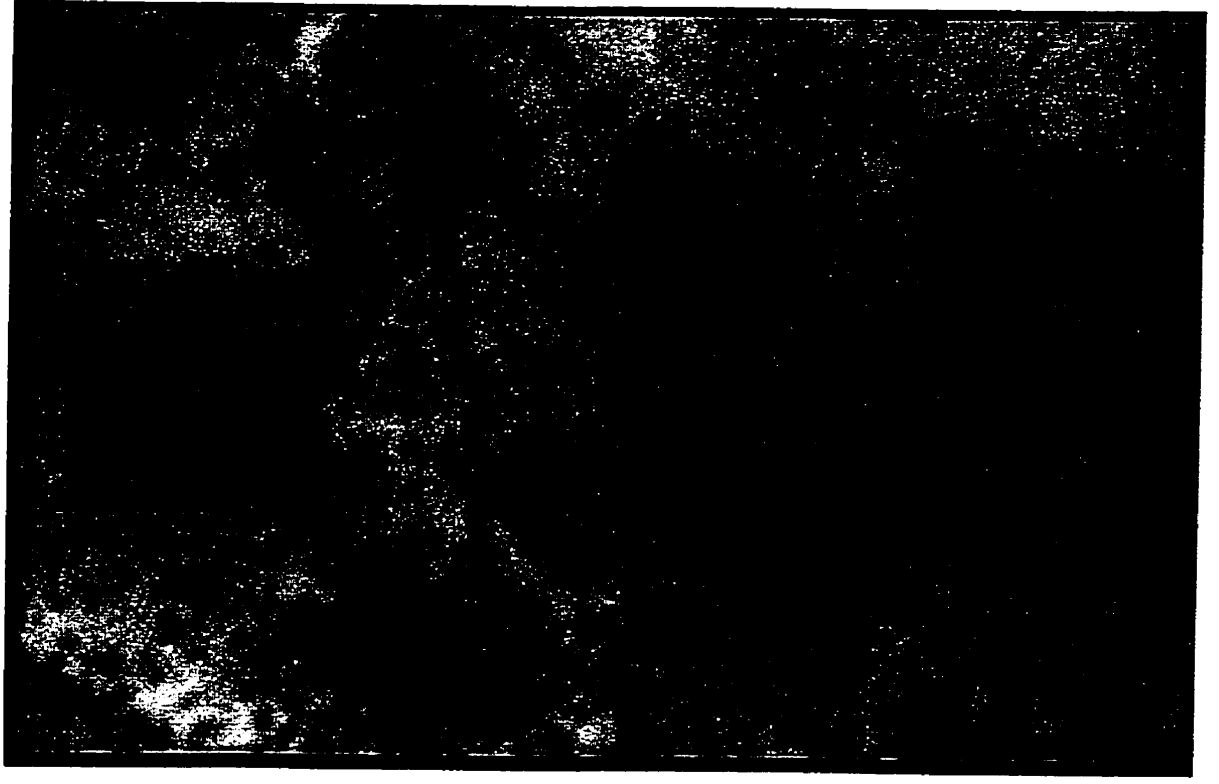


Figure 5:

Distribution of CRF primary transcript in the brain of animals treated intracerebroventricularly (i.c.v.) with prostaglandin E_2 (PGE_2). Animals were deeply anesthetized and rapidly perfused with 4% paraformaldehyde 30 min after the i.c.v. treatment with PGE_2 (2 μg/10 μl, left column) or the vehicle solution (right column). These rostro-caudal coronal sections (30 μm) were hybridized with a selective probe that was complementary to the rat CRF intron, providing an indication of early transcription of this stress-related neuropeptide. Note the very selective induction of CRF heteronuclear (CRF hnRNA) transcript in the hypothalamic paraventricular nucleus (PVN) by i.c.v. PGE_2 (left column). Cer, cerebellum; CP, caudate putamen; IOC, inferior olivary complex; LV, lateral ventricle; ME, median eminence; MGC, medial geniculate complex; MM, mammillary body; SPVI, spinal trigeminal nucleus; V3, third ventricle; V4, fourth ventricle.

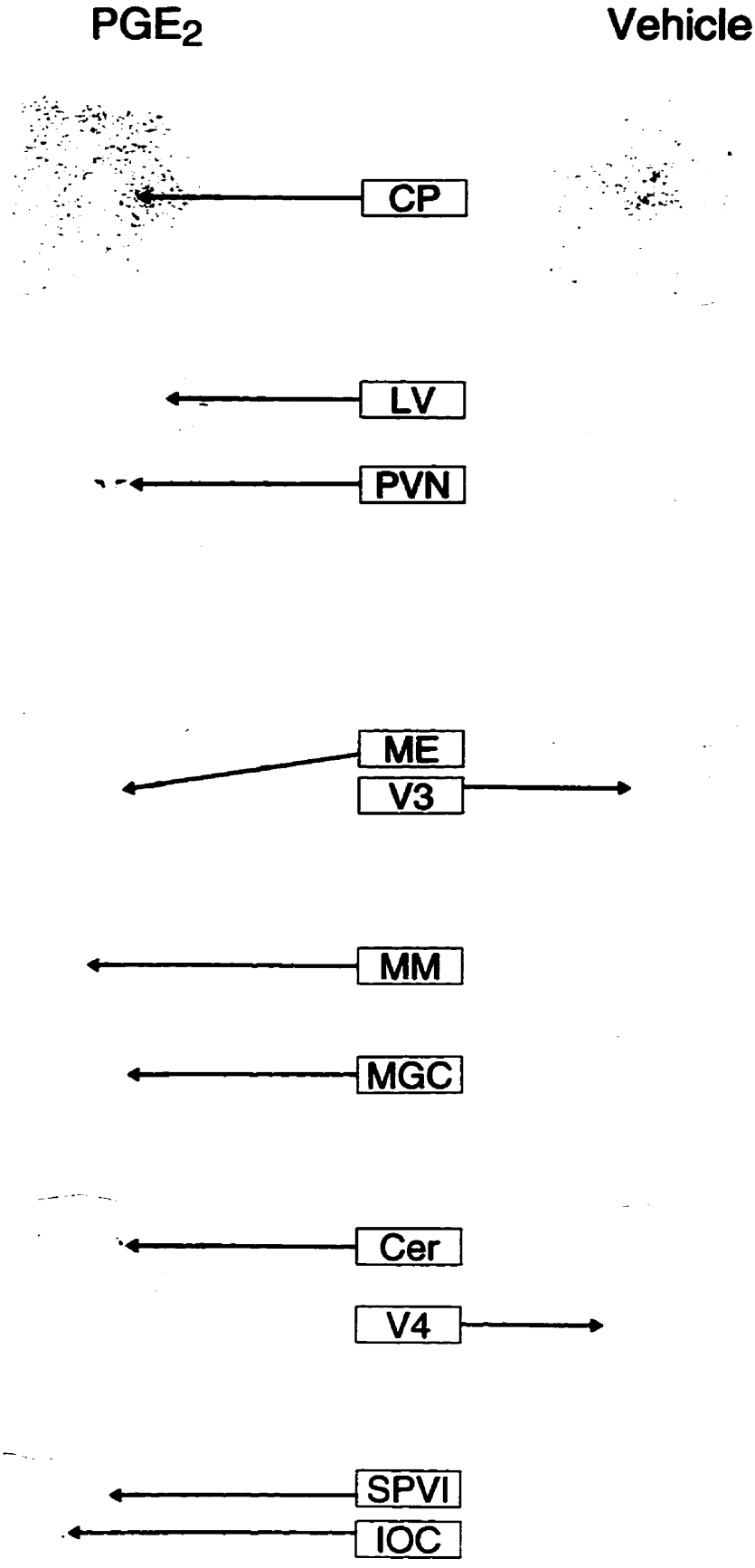

PGE2
Vehicle
CP
LV
PVN
ME
V3
MM
MGC
Cer
V4
SPVI
IOC

Figure 6:

Time-related influence of intracerebroventricular (i.c.v.) PGE_2 injection on CRF transcription (primary transcript) in the parvocellular division of the paraventricular nucleus (PVN) of the rat hypothalamus. These photos depict darkfield photomicrographs of dipped autoradiographs of hybridized 30-μm sections with rat CRF intronic probe through identical areas of the right PVN. Animals were deeply anesthetized and rapidly perfused with 4% paraformaldehyde 30 and 120 min after treatment with PGE_2 (2 μg/10 μl, left column) or the vehicle solution (right column). Note the rapid and transient induction of CRF heteronuclear (CRF hnRNA) transcript in the PVN as soon as 30 min after central administration with the PG. X25.

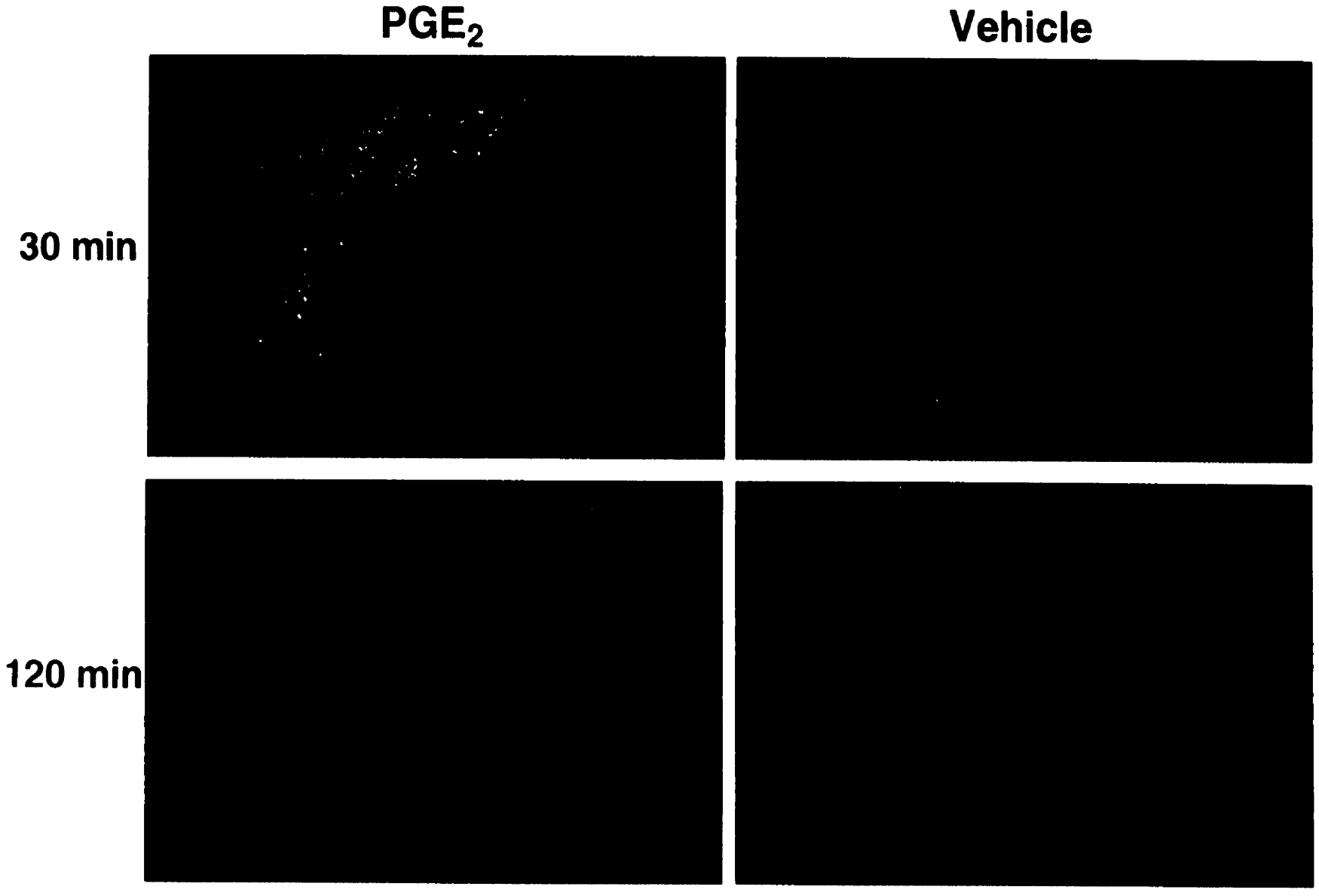

PGE2
Vehicle
30 min
120 min

Figure 7:

Representative example of the effect of central (i.c.v.) injection of PGE_2 on the expression of CRF type 1 receptor mRNA in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the rat hypothalamus. These photos depict darkfield photomicrographs of dipped autoradiographs of hybridized 30-μm sections with specific probes to hybridize rat CRF_1 receptor transcript through identical areas of the right PVN and SON. Animals were deeply anesthetized and rapidly perfused with 4% paraformaldehyde 2 h after treatments. X25.

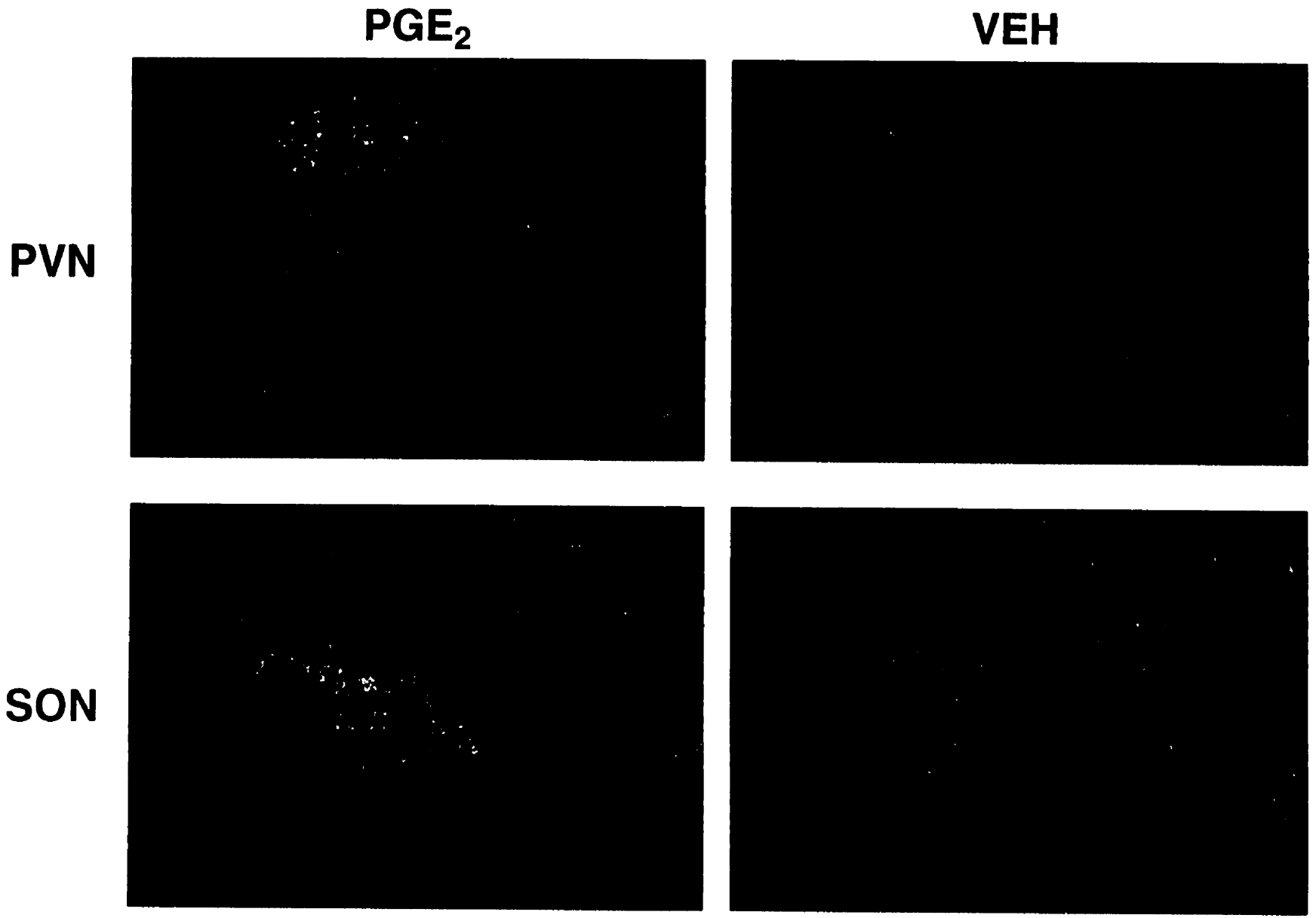
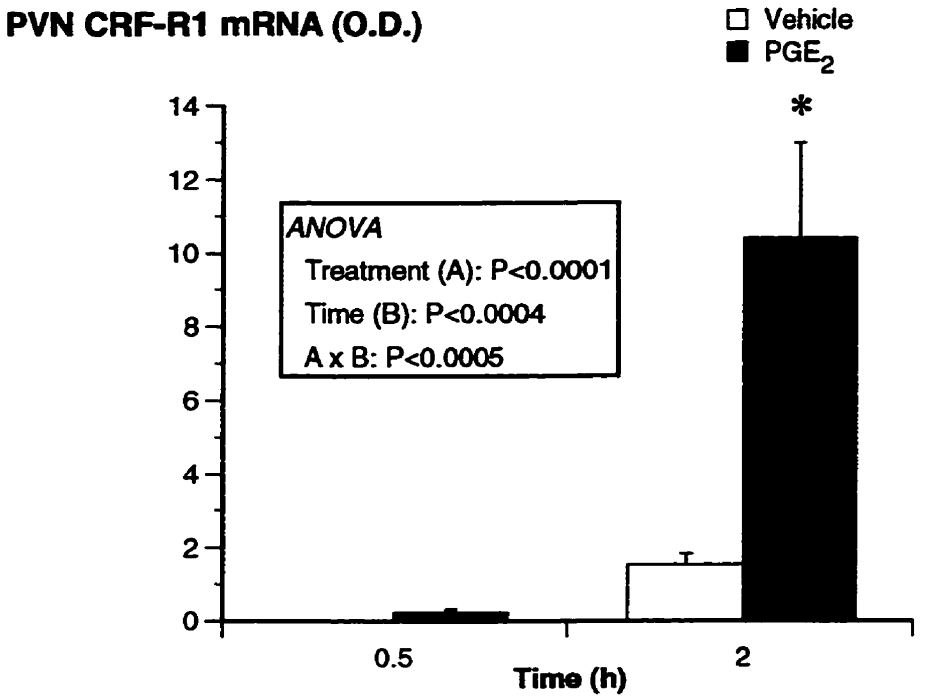
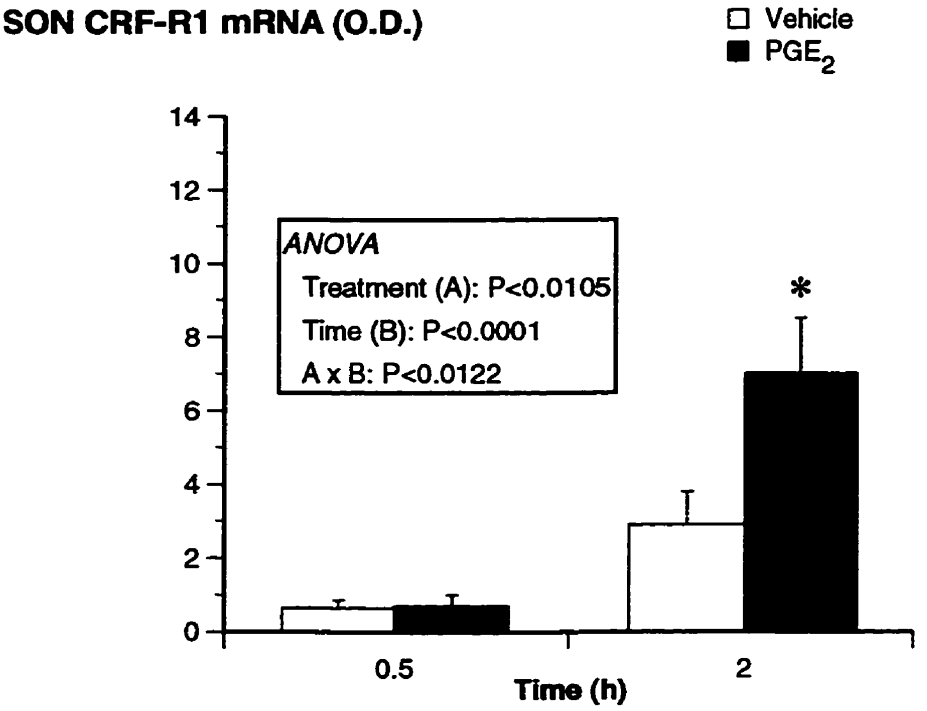

PGE2
VEH
PVN
SON

Figure 8:

Influence of intracerebroventricular (i.c.v.) injection of PGE_2 or vehicle on the average optical density (O.D., arbitrary units) for CRF_1 receptor mRNA hybridization signal in the paraventricular nucleus (PVN, top panel) and the supraoptic nucleus (SON, bottom panel) of the rat hypothalamus. The O.D. was quantified in both sides of the medial PVN and the SON using an Olympus Optical System (BX-50, BMax) coupled to a Macintosh computer (PowerPC 7100/66) and Image software (version 1.55 non-FPU, W Rasband, NIH). Results represent means ± SEM and statistical analysis was performed using a two-way analysis of variance (2 x 2) followed by a Bonferroni/Dunn post hoc test (Staview 4.01). *, Significantly different ($P < 0.05$) from all the other groups of rats. For more information on image analysis, see Material and Methods.


PVN CRF-R1 mRNA (O.D.)
Vehicle
PGE2
14
12
10
8
6
4
2
0
*
ANOVA
Treatment (A): P<0.0001
Time (B): P<0.0004
A x B: P<0.0005
0.5
2
Time (h)


SON CRF-R1 mRNA (O.D.)
Vehicle
PGE2
14
12
10
8
6
4
2
0
*
ANOVA
Treatment (A): P<0.0105
Time (B): P<0.0001
A x B: P<0.0122
0.5
2
Time (h)

CHAPITRE 5. EFFECT OF ACUTE SYSTEMIC INFLAMMATORY RESPONSE AND CYTOKINES ON THE TRANSCRIPTION OF THE GENES ENCODING CYCLOOXYGENASE ENZYMES (COX-1 AND 2) IN THE RAT BRAIN.

By

Steve Lacroix and Serge Rivest

Laboratory of Molecular Endocrinology
CHUL Research Center and
Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2

Running Title: Systemic inflammation on central COX gene transcription.

Address for Correspondence: Dr. Serge Rivest
Laboratory of Molecular Endocrinology
CHUL Research Center and
Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2
Tel: (418) 654-2296, Fax: (418) 654-2761
EMail: Serge.Rivest@crchul.ulaval.ca

The Journal of Neurochemistry, 70 (1998) 452-466

5.1 RÉSUMÉ

L'objectif de cette étude était d'étudier l'expression des gènes encodant les enzymes limitantes à la production des prostaglandines (COX-1 et 2) au cours de la réponse immunitaire de type aigu et à la suite d'injection systémique d'interleukines proinflammatoires. L'endotoxine bactérienne lipopolysaccharide (LPS; par voie intraveineuse [i.v.] ou intrapéritonéale [i.p.]) et la turpentine (par voie intramusculaire [i.m.]) furent employées à titre de différents modèles inflammatoires chez des rats adultes Sprague-Dawley (~250 g). D'autres animaux furent également sacrifiés à différents temps, suivant l'injection i.v. d'interleukin (IL)-1ß, du facteur nécrosant des tumeurs (TNF)-α ou d'IL-6. La technique d'hybridation *in situ* fut utilisée afin d'analyser les structures exprimant les ARNm encodant les protéines COX-1 et 2. Les résultats montrent que l'injection systémique de LPS a fortement stimulé la transcription du gène encodant COX-2 dans l'ensemble de la microvasculature cérébrale (fort probablement dans les cellules endothéliales), les plexus choroïdiens et les leptoméninges. De plus, l'administration i.v. de l'endotoxine LPS a aussi augmenté les niveaux d'expression du gène COX-2 dans la division parvocellulaire des noyaux paraventriculaires de l'hypothalamus. L'injection i.m. de la turpentine a stimulé la synthèse du transcrit COX-2 dans les vaisseaux sanguins de l'ensemble du cerveau. Fait intéressant, le signal observé chez tous les animaux suivait de façon parallèle l'inflammation de la patte. Des taux élevés d'ARNm de COX-2 ont été rapidement détectés dans la microvasculature du système nerveux central de rat injectés avec l'IL-1ß. De leur côté, les animaux traités avec le TNF-α ont plutôt montré une modeste, mais tout de même significative, induction du signal dans cette même structure. Par contre, l'injection i.v. d'IL-6 n'a provoqué aucun changement d'expression de l'ARNm de COX-2. Quant au gène codant COX-1, aucun traitement n'a influencé sa synthèse dans le cerveau de rats. Ces résultats suggèrent donc que certains groupes cellulaires très spécifiques, en particulier les cellules endothéliales et/ou les cellules microgliales périvasculaires-associées, sont impliqués dans la production centrale des prostaglandines au cours de la réponse inflammatoire d'origine systémique et que certaines cytokines circulantes, dont l'IL-1ß, seraient impliquées dans ce mécanisme.

5.2 ABSTRACT

The aim of this study was to investigate the influence of the acute-phase response and the proinflammatory cytokines on the transcription of the genes encoding the limiting enzymes for the production of prostaglandins (PGs), COX-1 and 2, in the rat brain. The bacterial endotoxin lipopolysaccharide (LPS, i.v. and i.p.) and turpentine (i.m.) were employed as different models of inflammation in adult male rats. Animals were also sacrificed at various times after i.v. administration of interleukin (IL)-1ß, tumor-necrosis factors alpha (TNF-α) and IL-6 and mRNAs encoding COX-1 and 2 were assayed by *in situ* hybridization histochemistry. A profound transcriptional activation of the gene encoding COX-2 was detected over blood vessels of the entire brain microvasculature, choroid plexus and leptomeninges of LPS-challenged rats. Injection of the endotoxin i.v. also increased COX-2 gene expression within parvocellular division of the hypothalamic paraventricular nucleus. Interestingly, i.m. turpentine injection stimulated transcription of COX-2 along endothelium of brain capillaries and the signal of this transcript paralleled the inflammation of the left hind limb. A robust COX-2 mRNA signal was rapidly detected in the brain microvessels of IL-1ß-injected rats, whereas TNF-α administration caused a modest but significant induction of this transcript. In contrast, i.v. injection of IL-6 did not alter genetic expression of COX-2 and none of the above described models affected the synthesis of COX-1 gene in the rat brain. These results indicate that specific cell populations, in particular vascular- and/or perivascular-associated cells, are responsible for the central production of PGs during systemic inflammation and circulating IL-1ß is likely to be a potent mediator of this response.

5.3 INTRODUCTION

Activation of the immune system is known to alter several brain functions that in return may play crucial roles in controlling appropriate systemic immune responses. This concept of bilateral interaction between the immune and neural systems is best illustrated by the stimulatory influence of proinflammatory cytokines, in particular interleukin-1ß (IL-1ß), on the activity of neurons responsible for the increase of plasma levels of glucocorticoids, which are potent immunosupressors and activators of the hepatic acute-phase proteins (APP). The production of cytokines by activated macrophages and lymphocytes in presence of foreign materials represents an essential feature of the early events of the immune activation characterizing the acute-phase response (Dinarello, 1989; Rabin *et al.*, 1990). The development of experimental models triggering some of the events occurring during this acute-phase has been very useful in better understanding the mechanisms through which infectious diseases modulate neuronal and endocrine functions. Although not an exact model of infection and inflammation, systemic administration with the bacterial endotoxin lipopolysaccharide (LPS) is an accepted mean to increase the release of a series of immunoregulatory, cytotoxic, and inflammatory molecules (including IL-1, IL-6 and tumor-necrosis factor-α [TNF-α]) from the peripheral immune system (Andersson *et al.*, 1992; Bristow *et al.*, 1991; Nathan, 1987) and therefore produces similar physiopathological outcomes that take place during sepsis (Dinarello, 1984; Ertel *et al.*, 1992; Kushner, 1982). It has been demonstrated that LPS may induce monocyte responses by interacting first with serum protein, *i.e.* LPS-binding protein (LBP) or septins, and then with a cell-surface molecule, CD14 (Wright *et al.*, 1990). Intramuscular (i.m.) injection of turpentine is a different experimental model of sterile inflammation (*i.e.* an inflammatory response developed in the absence of any microbial stimulus), which induces a local tissue damage that is responsible for the development of the systemic acute-phase response. This model produces a more restricted cytokine response (*i.e.* IL-1ß and IL-6) to acute localized inflammatory insults (Fantuzzi and Dinarello, 1996). Both LPS and turpentine inflammatory stimuli have been shown to produce a rapid increase of plasma adrenocorticotropin (ACTH) and corticosterone levels, a phenomenon dependent on the release of neuroendocrine corticotropin-releasing factor (CRF) (Fantuzzi and Dinarello, 1996; Turnbull and Rivier, 1996).

The prostaglandins (PGs) are autocrine and paracrine hormones that mediate numerous cellular, neuronal and physiological responses after induction with a variety of

substances, including inflammatory molecules and mitogens. Indeed, nonsteroidal anti-inflammatory drugs that prevent or decrease PG production can attenuate the effects of immune insults and proinflammatory cytokines on neuronal activity, neuropeptide gene transcription and many other physiological functions attributed to activation of specific cellular populations in the brain (Lacroix and Rivest, 1996; Lacroix and Rivest, 1997; Rivest and Rivier, 1995; Rivier and Rivest, 1993). Blockage of the eicosanoid cyclooxygenase pathways is able to inhibit the stimulation of CRF release by both IL-1 and 6 cytokines from *in vitro* hypothalamic explants (Lyson and McCann, 1992; Navarra *et al.*, 1991) and isolated median eminence (McCoy *et al.*, 1994). In addition, *in vivo* studies have demonstrated that inhibition of PG production is efficient in preventing the ACTH release induced by IL-1 (Katsuura *et al.*, 1988; Rivier and Rivest, 1993) and LPS-induced transcription of CRF in the paraventricular nucleus (PVN) of the hypothamus, an effect dependent on the dose and the time after the endotoxin challenge (Lacroix and Rivest, 1997). Moreover, arachidonic acid metabolism also participates in the endotoxin- and turpentine-induced adrenocortical activation in the rat (Smith *et al.*, 1994; Turnbull and Rivier, 1996). Subcutaneous injection of a dilute formalin solution into the hind paw produced spinal release of PGE_2, whereas cyclooxygenase inhibition via ibuprofen administration suppressed by 50 % the release of excitatory amino acids within the spinal dorsal horn (Malmberg and Yaksh, 1995). It has also been proposed that the mechanisms of action underlying the febrile response to endotoxin and cytokines involve the cyclooxygenase pathway and the localized diencephalic synthesis of PGs (Komaki *et al.*, 1992; Lopez-Valpuesta and Myers, 1994; Sirko *et al.*, 1989).

The exact PG subtype(s) and the site(s) of action within the brain involved in these events still remain unclear. Although various PGs have the potential to mediate the influence of immune-related factors on neuronal activation and neuroendocrine functions, a large body of evidence indicates that PG of E_2 type might be involved in several changes observed during immune challenge and treatment with cytokines (Komaki *et al.*, 1992; Navarra *et al.*, 1992; Rassnick *et al.*, 1995). Moreover, central injection of PGE_2 induces specific and selective expression of c-*fos* protein (Scammell *et al.*, 1996) and mRNA (Lacroix *et al.*, 1996) in several structures known to be activated in the brains of endotoxin-challenged rats. This raises the possibility that local production of PGE_2 is a crucial step within the CNS to mediate the action of circulating immune-related factors. The formation of PGs is initiated by the action of COX (also known as prostaglandin endoperoxide H-synthase or PGHS), which catalyses two separate reactions; the first being the oxygenation of arachidonic acid to the unstable PGG_2 by a cyclooxygenase function and the second, the subsequent reduction of

PGG_2 leading to a more stable PGH_2 by peroxidase reaction. Although constitutive expression of the isoform COX-1 was found in various cell types, mRNA and protein levels remain somewhat unchanged during inflammatory challenges and therefore believed to play housekeeping roles (DeWitt *et al.*, 1990; DeWitt and Smith, 1988). In contrast, COX-2 isoform is undetectable in most tissues under basal conditions, but marked transcriptional activation can be observed in macrophages and other cell types by the endotoxin LPS and proinflammatory cytokines (Hempel *et al.*, 1994; Jones *et al.*, 1993; Lee *et al.*, 1992). Interestingly, systemic LPS and IL-1ß injections have recently been shown to stimulate production of this enzyme within the mouse (Breder and Saper, 1996) and rat (Cao *et al.*, 1995; Cao *et al.*, 1996) brain, in particular throughout the entire microvasculature. Whether brain COX-2 gene expression constitutes a general mechanism triggered by the systemic inflammatory responses has yet to be investigated. The endotoxin LPS may cause several of its effects in binding to the cell-surface receptor CD14 or in acting directly on endothelium of brain capillaries, which is not necessarily reflecting the cascade of events produced during localized inflammatory reaction. The purpose of the present study was therefore to investigate the influence of different experimental models of acute-phase immune reaction (i.v. and i.p. endotoxin injections and i.m. turpentine insult) and the proinflammatory cytokines (IL-1ß, TNF-α and IL-6) on the regulation of genes encoding the enzymes COX-1 and COX-2 throughout the rat brain.

5.4 MATERIALS AND METHODS

5.4.1 Animals

Adult male Sprague-Dawley rats (~230-260 g b.w.) were acclimated to standard laboratory conditions (14-h light, 10-h dark cycle; lights on at 0600 and off at 2000) with free access to rat chow and water. Each rat was only used once for experimentation, and all protocols were approved by the Laval University's Animal Welfare Committee. A total of 124 rats were assigned to different protocols divided among the types of treatment and the route of administration (i.p. administration of LPS, i.v. injection of LPS, IL-1ß, TNF-α or IL-6 and i.m. turpentine injection into the left hind limb). Corresponding vehicle-treated rats were also sacrificed at different post-injection times.

5.4.2 Surgeries

Animals receiving i.v. or i.p. injections were chronically implanted with sterile cannulas. Rats were anesthetized with an i.p. injection of a mixture (100 µl/100 g b.w.) of ketamine hydrochloride (91 mg/ml) and xylazine (9 mg/ml) and implanted with a catheter into the jugular vein or into the peritoneal cavity. Catheters were made from a piece of silastic tubing (Silastic medical grade tubing, ID 0.020 in., OD 0.037 in.; Dow Corning, Midland, MI) connected to an intramedic polyethylene tubing (PE-50, Caly Adams, Parsippany, N.J.). Outlets of cannulas were placed at the level of the neck and rats were housed individually in metal cages for a recuperation period of two to five days.

5.4.3 Experimental protocols

On the day of the experiment (~0830 in the morning), the outlet portion of each catheter (i.v. or i.p.) was fixed to a truncated 27 g needle which was attached to a PE-50 tubing. These connectors were then fixed to a 1cc syringe and rats were placed individually in a quiet room for at least 2 hours before the injections.

5.4.3.1 Protocol 1: Intravenous administration of LPS

Intravenous administration of 10 µg LPS/100 g of b.w. (from *Escherichia coli*, Serotype 055:B5, Sigma, L-2880, lot #122H4025) diluted in 200 µl of sterile pyrogen-free saline or the vehicle solution was performed into the right jugular vein. The animals were conscious and freely moving at all times throughout the procedure. One and 3 hours after the treatment, animals were deeply anesthetized via an i.v. injection (100 µl) of a mixture of ketamine hydrochloride and xylazine and then rapidly perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M borax buffer (pH 9.5 at 4 °C).

5.4.3.2 Protocol 2: Intraperitoneal administration of LPS

The bacterial endotoxin LPS (50 µg/100 g b.w.) diluted in 300 µl of sterile pyrogen-free saline or the vehicle solution was infused through the chronically implanted cannula into the peritoneal cavity. The rats were conscious and freely moving at all times throughout the experimental procedure. One, 3 and 6 h after the i.p. injections, the animals were deeply

anesthetized via an i.p. administration (300 µl) of a mixture of ketamine hydrochloride and xylazine and then rapidly perfused transcardially as mentionned. This dose and the time points were selected on the basis of previous studies demonstrating strong neuronal and transcriptional activation of the genes encoding CRF and its type 1 receptor in the brain (Lacroix and Rivest, 1996; Lacroix and Rivest, 1997).

5.4.3.3 Protocol 3: Intramuscular administration of turpentine

Rats were injected into the left thigh muscles with 50 µl/100 g b.w. of turpentine (Spectrum Chemical Mfg. Corp., TU 109, CAS 8006-64-2, Gandena, CA) or 0.9% of sterile pyrogen-free saline and transcardially perfused at 1, 3, 6 and 12 h post-injection times. This dose was selected on the basis of previous studies showing evident swelling of the hind limb and profound increase of the HPA axis in rats (Turnbull *et al.*, 1997; Turnbull and Rivier, 1996). In the present case, we observed a robust inflammation 6 and 12 h after i.m. injection of turpentine, whereas no visible change was observed in vehicle-administered rats. This experimental model of sterile inflammation (*i.e.* an inflammatory response developing in the absence of any microbial stimulus) induces a local tissue damage that is responsible for the development of a systemic acute-phase response. This model also produces a more restricted cytokine response, in particular IL-1 and IL-6, to acute localized inflammatory insult.

5.4.3.4 Protocol 4: Intravenous administration of proinflammatory cytokines

Recombinant rat IL-1ß (rrIL-1ß; 187 ng/100 g b.w.; kindly provided by Dr. R. Hart, The State University of New-Jersey Rutgers, Newark, NJ), recombinant rat TNF-α (rraTNF-α; 410 ng/100 g b.w.; catalog # PRC3014, lot # 2248-02B; Biosource Int. Camarillo, CA), recombinant human IL-6 (rhIL-6; 1.2 µg/100 g b.w. [21 x 10^3 World Health Organization Units]; catalog #206-IL, lot #AI134071; R & D Systems, Minneapolis, MN) or the vehicle solution (200 µl of sterile pyrogen-free saline) was injected through the right jugular vein. These doses have been shown to cause activation of the HPA axis and/or expression of c-*fos* gene in the rat brain (Ericsson *et al.*, 1994; Sapolsky *et al.*, 1987; Sharp *et al.*, 1989; Vallières *et al.*, 1997). The animals were conscious and freely moving at all times throughout the procedure and were deeply anesthetized via an i.v. injection (100 µl) of

a mixture of ketamine hydrochloride and xylazine and rapidly perfused transcardially 1, 3 and 6 h after the injection with the cytokine or its vehicle.

5.4.4 *In situ* hybridization histochemistry

Rapidly after the transcardiac perfusions, brains were removed from the skulls, postfixed for 2 to 8 days, and then placed in a solution containing 10% sucrose diluted in 4% paraformaldehyde-borax buffer overnight at 4 °C. The frozen brains were mounted on a microtome (Reichert-Jung, Cambridge Instruments Company, Deerfield, IL) and cut into 30-µm coronal sections from the olfactory bulb to the end of the medulla. The slices were collected in a cold cryoprotectant solution (0.05M sodium phosphate buffer, pH 7.3, 30% ethylene glycol, 20% glycerol) and stored at -20 °C. Hybridization histochemical localization of each transcript was carried out on every sixth section of the whole rostro-caudal extent of each brain using ^{35}S-labeled cRNA probes. Protocols for riboprobe synthesis, hybridization, and autoradiographic localization of mRNA signal were adapted from Simmons et al. (1989). All solutions were treated with diethylpyrocarbonate (Depc) and sterilized to prevent RNA degradation. Tissue sections mounted onto poly-L-lysine-coated slides were desiccated overnight under vacuum, fixed in 4% paraformaldehyde for 30 min, and digested with proteinase K (10 µg/ml in 0.1 M tris HCl, pH 8.0, and 50 mM EDTA, pH 8.0, at 37 °C for 25 min). Thereafter, the brain sections were rinsed in sterile Depc water followed by a solution of 0.1 M triethanolamine (TEA, pH 8.0), acetylated in 0.25% acetic anhydride in 0.1 M TEA, and dehydrated through graded concentrations of alcohol (50, 70, 95, and 100 %). After vacuum drying for a minimum of 2 h, 90 µl of hybridization mixture (10^7 cpm/ml) was spotted on each slide, sealed under a coverslip, and incubated at 60 °C overnight (~15-20 h) in a slide warmer. Coverslips were then removed and the slides were rinsed in 4x standard saline citrate (SSC) at room temperature. Sections were digested by RNAse A (20 µg/ml, 37 °C, 30 min), rinsed in descending concentrations of SSC (2x, 1x, 0.5xSSC), washed in 0.1xSSC for 30 min at 60 °C (1xSSC: 0.15 M NaCl, 15 mM trisodium citrate buffer, pH 7.0) and dehydrated through graded concentrations of alcohol. After being dried for 2 h under vacuum, the sections were exposed at 4 °C to X-ray films (Kodak) for 4 days, defatted in xylene, and dipped in NTB2 nuclear emulsion (Kodak; diluted 1:1 with distilled water). Slides were exposed for 21 days, developed in D19 developer (Kodak) for 3.5 min at 14-15 °C, washed 15 sec. in water, and fixed in rapid fixer (Kodak) for 5 min. Tissues were then rinsed in running distilled water for 1-2 h,

counterstained with thionin (0.25 %), dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX.

5.4.5 cRNA probe synthesis and preparation

The pGEM4 plasmids containing the rat COX-1 and COX-2 cDNA fragments (kindly provided by Dr. K. Peri, Ste-Justine Hospital Research Center, Montreal, Canada) were linearized with *Hin*d III and *Eco*R I for the antisense and sense riboprobes, respectively. The length of the COX-1 and COX-2 cDNA fragments were respectively 143 base pairs (bp) and 176 bp consisting of nucleotides 142 to 285 and nucleotides 124 to 300 of the complete published cDNA sequence (Feng *et al.*, 1993). Radioactive cRNA copies were synthesized by incubation of 250 ng linearized plasmid in 6 mM $MgCl_2$, 40 mM Tris (pH 7.9), 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 0.2 mM ATP/GTP/CTP, [α-^{35}S]UTP, 40U RNAsin (Promega, Madison, WI) and 20U of either SP6 (COX-1 and COX-2 antisense probes) or T7 (COX-1 and COX-2 sense probes) RNA polymerase for 60 min at 37 °C. Unincorporated nucleotides were removed using ammonium-acetate method; 100 µl of DNAse solution (1 µl DNAse, 5 µl of 5 mg/ml tRNA, 94 µl of 10 mM tris/10 mM $MgCl_2$) was added, and 10 min later, a phenol-chloroform extraction was performed. The cRNA was precipitated with 80 µl of 5M ammonium acetate and 500 µl of 100% ethanol for 20 min on dry ice. The pellet was washed with 500 µl 70% ethanol, dried, and resuspended in 100 µl of 10 mM Tris/1 mM EDTA. A concentration of 10^7 cpm probe was mixed into 1 ml of hybridization solution (500 µl formamide, 60 µl 5 M NaCl, 10 µl 1 M Tris [pH 8.0], 2 µl 0.5 M EDTA [pH 8.0], 50 µl 20x Denhart's solution, 200 µl 50% dextran sulfate, 50 µl 10 mg/ml tRNA, 10 µl 1M DTT, [118 µl Depc water - volume of probe used]). This solution was mixed and heated for 5 min at 65 °C before being spotted on slides.

5.5 RESULTS

5.5.1 Systemic administrations of the bacterial endotoxin LPS

Figure 1 shows the distribution of the mRNA encoding the gene COX-2 in the rat brain. Positive hybridization signal for COX-2 transcript was detected in different regions of the brain under basal conditions. Indeed, vehicle-treated rats (right column) exhibited COX-2 expression in the basolateral nucleus of the amygdala (BLA), hippocampus (hip; fields CA1, 2 and 3 of Ammon's horn and dentate gyrus), septohippocampal nucleus (SH),

ventromedial hypothalamic nucleus (VMH), fasciola cinerea (FC), layer 2 of the piriform area (Pir), cingulate cortex and multiple layers of the cerebral cortex, including layer 2, 3 and 5. Brightfield higher magnification examination demonstrates that the basal signals were observed mainly, if not exclusively, in neuronal cells.

Systemic injection of the bacterial endotoxin LPS stimulated expression of COX-2 in various non-parenchymal structures (Fig 1, left and middle columns), but in very few nuclei of the brain and this effect was dependent on the route of administration and the time of sacrifices. Only a small group of parenchymal cells displayed notable induction of this transcript after LPS treatment. Indeed, qualitative analysis of hybridization signal on the X-ray films (data not shown) revealed that COX-2 mRNA levels in the BLA, hip, SH, FC, Pir and cerebral cortex of rats injected i.v. with LPS were not significantly different from those of the vehicle-treated animals at either 1 or 3 h post-injection times. No clear hybridization signal was observed in the parenchyma of the organum vasculosum of the lamina terminalis (OVLT)/ median preoptic area (MPOA); structures reported to produce PGs after systemic treatment with LPS (Ueno *et al.*, 1982). Signal was also hardly detectable in other immune stress-responsive regions, including the bed nucleus of the stria terminalis (BnST), supraoptic nucleus (SON), central nucleus of the amygdala (CeA), ventro-lateral medulla (VLM) and nucleus of the solitary tract (NTS). However, i.v. injection of the endotoxin significantly increased the transcription of the gene encoding COX-2 in the endocrine hypothalamus, more specifically in the paraventricular nucleus of the hypothalamus (PVN). Figure 2 shows representative photomicrographs of the expression of COX-2 mRNA in the medial PVN (panel A) 3 h after i.v. injection of LPS. Agglomeration of silver grains forming COX-2 positive cells were largely found within parenchymal elements of the parvocellular division of the PVN (Fig. 2B and C), although few, but some COX-2 expressing cells were also detected over blood vessels (bv) irrigating this endocrine nucleus (Fig. 2D). It is difficult to be certain that neurons are the cells that express COX-2 mRNA in the parenchymal PVN in response to i.v. LPS, because the possibility remains that vascular and/or perivascular-associated cells be isolated in the preparation of brain sections. The PVN is probably the densest blood supply nucleus and it is virtually impossible to cut through the nucleus without getting many isolated cells that are actually associated to blood vessels.

Systemic LPS injection strongly stimulated the transcription of the gene encoding COX-2 in various non-parenchymal structures of the brain, a phenomenon particularly robust following the i.v. route of administration. As depicted in figure 1, marked induction of

COX-2 gene was found within cells constituting brain capillaries, choroid plexus (chp) and the leptomeninges 1 and 3 h after i.v. (left column) and i.p. (middle column) LPS injection, respectively. Representative examples of such phenomena are depicted by the Fig. 3, which illustrates dark- and brightfield photomicrographs of these structures hybridized with rat COX-2 riboprobe. Microscopic analysis of slides dipped in NTB2 nuclear emulsion indicates that in addition to the entire brain microvasculature, which exhibited positive COX-2 transcript, a large number of expressing cells of the chp and leptomeninges (two structures highly vascularised) seem vascular and/or perivascular associated. In fact, most of the non-parenchymal cells expressing COX-2 mRNA (Fig. 3, bottom left panel) possess the fusiform shape normally characterizing blood vessels. Whether these cells are endothelial and/or microglial remains to be established, although perivascular microglial-associated cells have be found to be positive for COX-2 in response to systemic LPS (see discussion). The i.v. LPS treatment caused a rapid transcriptional activation of COX-2 over the entire microvasculature; hybridized signal was maximum at 1 h and declined 3 h after the i.v. administration.

The hybridization signal for COX-2 mRNA throughout the brain of i.p. LPS-injected rats was lower than the intensity generally detected following the i.v. treatment, although the pattern of distribution was quite comparable using both routes of administration (Fig. 1). Induction of the mRNA encoding COX-2 over microvasculature was more rapid in the brain of i.v.-injected rats; positive signal was observed 1 h after i.p. LPS, but peaked at time 3 h and largely vanished 6 h after i.p. administration of the bacterial endotoxin.

5.5.2 Brain COX-2 gene expression in response to acute local inflammation

Animals were injected with turpentine into the muscle of the hind limb to cause an acute localized inflammatory response. Figure 4 depicts a representative example of the distribution of the mRNA encoding the gene COX-2 in the rat brain 6 h after sterile local inflammation induced by i.m. administration of turpentine (50 µl/100 g b.w.). As described before, vehicle-treated rats exhibited COX-2 mRNA in different regions of the brain, such as the BLA, hip, SH, FC, Pir, cingulate cortex and in the cerebral cortex. Clear induction of COX-2 transcript was observed throughout the entire microvasculature of i.m. turpentine-injected brains as well as within the chp and leptomeninges. In contrast to i.v. LPS-injected rats, a low message was detected within parenchymal elements of the hypothalamic parvocellular PVN following i.m. turpentine administration. Interestingly, transcriptional activation of COX-2 in the brain paralleled the visible inflammation (swelling) caused by the

turpentine aggression. Indeed, COX-2 transcript was not induced in the brain of animals sacrificed 1 and 3 h after the insult, whereas robust expression of this gene was noted at both 6 and 12 h post-injection times when robust swelling of the left hind limb was observed. These results provide the evidence that transcriptional activation of COX-2 gene within specific non-parenchymal cells associated to blood vessels forming the brain microvasculature is a mechanism triggered by the systemic inflammatory response.

5.5.3 Proinflammatory cytokines and COX-2 gene regulation

The figure 5 shows the influence of exogenous i.v. injection of the cytokines on the distribution of the mRNA encoding the gene COX-2 in the rat brain. Rostro-caudal coronal sections of recombinant rat IL-1ß-injected rats exhibited a clear induction for COX-2 mRNA levels in most of the structures previously described following systemic LPS and turpentine treatments. Indeed, increase signals for COX-2 mRNA was observed on X-ray films in non-parenchymal cells of the blood vessels, chp and leptomeninges, whereas low expression of COX-2 transcript was detected over parenchymal cells of the PVN. Interestingly, the effect of i.v. IL-1ß injection was rapid and transient; COX-2 signal was maximal at 1h, declined 2 h after and essentially vanished at 6 h post-injection time. This time course of induction corresponds quite well with the influence of i.v. LPS injection, which suggests that the required time to activate transcription of that gene (at least within the brain microvessels) depends directly on the route of administration.

To insure that the stimulatory effects of IL-1ß on COX-2 gene expression in the rat brain was not a result of potential bacterial endotoxin contamination, an additionnal experiment was performed with i.v. injection of IL-1ß preheated to 70 °C during 30 min. Inactivation of IL-1ß by the heat, but not the bacterial endotoxin activity, failed to induce the expression of the immediate-early gene c-*fos* (marker of neuronal activity) and COX-2 in any of the IL-1ß-responsive structures confirming therefore that the effects are specific to the cytokine (data not shown).

In contrast to the stimulatory influence of IL-1ß, a large dose of IL-6 (1.2 µg/100 g b.w.) did not stimulate transcription of COX-2 gene in the brain (Fig. 5). Although positive hybridization signal was found in different endogenously-expressing sites, no difference was detected between the brains of i.v. IL-6- and vehicle-injected rats at any post-injection times. Systemic administration of recombinant rat TNF-α caused a modest but significant induction

of the mRNA encoding COX-2 within the microvasculature, a phenomenon more convincing within the blood vessels irrigating the hindbrain (Fig. 5, second column). However, some microvessels of the forebrain displayed a low but positive signal on the dipped NTB-2 emulsion slides 1 h after i.v. TNF-α injection (data not shown). The low signal on the film and the slides declined at time 3 h and essentially vanished 6 h after the challenge with TNF-α.

5.5.4 Differential effects of systemic treatments on parenchymal COX-2 transcript levels

Figure 6 shows darkfield photomicrographs of different positive brain nuclei and areas for the mRNA encoding COX-2. Although Breder and colleagues have previously reported the presence of COX-2-ir cells in the PVN of intact animals (Breder *et al.*, 1995), no convincing constitutive expression of COX-2 transcript was detected in this nucleus under basal conditions (Fig. 6, top left panel). However, i.v. LPS injection caused a robust transcriptional activation of COX-2 in this endocrine hypothalamic nucleus. The signal was particularly intense within the parvocellular neurons, but positive cells surrounding blood vessels were also found (Fig. 2). On the other hand, i.p. LPS injection stimulated COX-2 gene expression within both parenchymal and non-parenchymal cells of the PVN, although the intensity of the signal was much less intense than the one observed following the i.v. route of administration. Low but positive signal was detected in the PVN of turpentine- and IL-1ß-injected rats; positive cells were nevertheless generally associated with blood vessels, and few seemed neuronal. Systemic i.v. TNF-α and IL-6 injection did not stimulate expression of COX-2 in the hypothalamic PVN or in any other nuclei of the brain (data not shown).

Induction of COX-2 mRNA in neurons was quite limited to the hypothalamic PVN. As depicted by the figure 6, this transcript was expressed in the lateral amygdala (particularly in the basolateral nucleus, BLA) and throughout various layers of the hippocampus (Hip). In this region, the signal was particularly intense over the pyramidal cell layer of the CA3 and the dentate gyrus and no significant changes in the mRNA levels were observed following systemic challenges with the bacterial endotoxin, turpentine or the proinflammatory cytokines. In fact, endogenously-expressing parenchymal structures did not exhibit notable variations in the relative levels of COX-2 transcript. However, it is possible to detect this

gene within cells surrounding the blood microvessels irrigating these brain structures in LPS-, turpentine- and IL-1ß-challenged animals.

5.5.5 COX-1 isoform

In contrast to COX-2 that is widely distributed across the rat brain in response to immunogenic stimuli, no convincing positive hybridization signal for COX-1 transcript was detected in the brain under either basal and challenged conditions (data not shown).

5.6 DISCUSSION

The present study demonstrates that the transcription of the gene encoding COX-2, but not COX-1, is stimulated within specific cell populations of the rat brain in response to different experimental models of systemic inflammation and the proinflammatory cytokines IL-1ß and TNF-α. Indeed, peripheral i.v. and i.p. injection of the bacterial endotoxin LPS and i.m. turpentine insult caused a profound expression of COX-2 (recognized as the limiting enzyme for the production of PGs during inflammatory processes) over blood vessels of the entire brain microvasculature, choroid plexus (chp) and leptomeninges. Interestingly, brain COX-2 gene expression paralleled the visible swelling of the left hind limb, which was robust 6 and 12 h after the i.m. aggression. A robust COX-2 signal was also detected 1 h after i.v. IL-1 injection, whereas TNF-α produced a modest, although significant, induction of COX-2 mRNA within the blood vessels. On the other hand, IL-6 did not alter COX-2 expression in the rat brain and undetectable hybridization signal for the gene encoding COX-1 was observed across the rat brain under basal and challenged conditions. The present set of data provides clear evidence that COX-2 gene transcription in brain microvascular-associated cells is a mechanism triggered by the systemic inflammation and circulating IL-1ß might be a potent mediator in this process.

Stimulation of COX-2 synthesis within non-parenchymal cells of the brain microvasculature, chp and leptomeninges seems specific to the systemic inflammatory response. Indeed, the COX-2 mRNA and protein levels were reported to be upregulated in neurons, but not in blood vessels after cerebral ischemia (Nogawa *et al.*, 1997) and maximal electroconvulsive seizure (Yamagata *et al.*, 1993). In spite of the fact that signal intensity and time of induction varied with the route of administration, both i.v. and i.p. modes of LPS

injection yielded COX-2 gene expression in a pattern of distribution quite similar across the rat brain. Little is known regarding the exact mechanisms involved in the effects of the bacterial endotoxin on the genetic transcription of this enzyme in the brain and whether such event is dependent on the production of specific circulating proinflammatory cytokines. Secretion of cytokines by circulating monocytes and tissue macrophages by LPS requires a series of mechanisms in cascade; the endotoxin must reach the bloodstream to bind with the serum proteins LBP or septins. The new formed complex will bind to the membrane CD14 receptor located on mononuclear cell-surface and therefore induce the release of cytokines (Wright *et al.*, 1990). The fact that i.v. LPS injection provoked a more rapid response than the i.p. route of administration on the genetic transcription of the limiting enzyme for the PG synthesis in non-parenchymal elements might be directly related to this concept; *i.e.* the time taken by the endotoxin to form the LPS-binding protein complex and target its receptor on peritoneal macrophages and/or circulating monocytes to stimulate the production of cytokines.

The possibility remains however that LPS triggers COX-2 transcription in acting directly on brain vascular and/or perivascular-associated cells and not via a monocyte/macrophage stimulation. Activation of microvascular endothelial cells by LPS plays a major role in the pathogenesis of gram-negative bacterial sepsis and endotoxic shock and soluble CD14 is required for this response (Arditi *et al.*, 1993). Although endothelium does not express CD14, these cells respond to LPS in a soluble CD14-dependent manner in stimulating the tyrosine phosphorylation of mitogen-activated protein kinases (Arditi *et al.*, 1995). Moreover, perivascular microglial cells, which differ from parenchymal microglia, have the ability to express CD14 in response to LPS and therefore may subserve the effects of the endotoxin when injected into the circulation (Becher and Antel, 1996). Whether LPS activates directly endothelium of brain capillaries to trigger COX-2 gene transcription or such effect is mediated through a monocyte/cytokine response still remains an open question. It is nevertheless possible that the mechanisms mediating the influence of LPS differ from a route of administration to another (i.p. *Vs* i.v.).

The fact that local turpentine insult stimulated transcription of COX-2 gene within microvessels, chp and leptomeninges provides the evidence that this enzyme is regulated in the brain during the peripheral inflammatory response. This experimental model induces a local tissue damage provoking sterile inflammation, *i.e.* an inflammatory response which develops in the absence of any microbial stimulus (Fantuzzi and Dinarello, 1996). As

reviewed by these authors, this model of localized tissue damage provokes a specific induction of IL-1ß and IL-6 without any detectable IL-1α and TNF-α production, suggesting the existence of a common cascade of cytokine release, characteristic of sterile inflammation where IL-1ß and IL-6 might play a critical role (Fantuzzi and Dinarello, 1996). Interestingly, IL-1ß-deficient mice respond normally following bacterial-endotoxin LPS-induced inflammation, whereas the mutant mice exhibit an impaired acute-phase inflammatory response and are completely resistant to fever development when challenged with turpentine (Zheng *et al.*, 1995). These results highlight a central role for IL-1ß as a mediator of the acute-phase response in this particular experimental model of localized inflammation. As presented in this study, this cytokine might well play a key role in the effects of systemic inflammation on brain COX-2 gene expression; i.v. injection of recombinant rat IL-1ß caused a sharp, robust and transient expression of the mRNA encoding this enzyme in a pattern of distribution quite similar to the one observed in the brain of turpentine-challenged rats. It is likely that IL-1ß targets directly endothelium of brain capillaries to induce COX-2 gene transcription, because microvessels across the brain have been shown to express IL-1 type 1 receptor (IL-1R1) transcript (Ericsson *et al.*, 1995b).

Several pleiotropic effects of IL-1ß are mediated by IL-6, a cytokine previously identified as a B cell-stimulating factor (Poupart *et al.*, 1987). These cytokines are strongly synergistic in various immunological processes, including induction of cell proliferation and acute-phase protein synthesis by hepatocytes (Conti *et al.*, 1995; Tritarelli *et al.*, 1994) and IL-1ß is an important stimulator of IL-6 production in peripheral organs (Akira *et al.*, 1990). An important action ascribed to IL-6 is the induction of fever and several lines of evidence support the hypothesis that endotoxin-induced fever is caused by IL-6 production under the influence of IL-1ß (Chai *et al.*, 1996; Klir *et al.*, 1994). It has been proposed that the likely mechanism for the loss of response to turpentine in IL-1ß knock out mice is the lack of IL-6 production, which is the final mediator of the induction of the acute-phase response (Fantuzzi and Dinarello, 1996). However, increase in IL-6 concentration does not seem to be responsible for COX-2 genetic regulation, because in contrast to IL-1ß, a large dose of IL-6 does not alter the enzyme expression in the rat brain. This dose of IL-6 injected i.v. is however capable of stimulating the transcription of the immediate-early gene c-*fos* (index of post-synaptic cellular activation) in the sensorial circumventricular organs, bed nucleus of the stria terminalis and central nucleus of the amygdala (Vallières *et al.*, 1997). These results suggest that the proinflammatory cytokine IL-6 does not, by itself (when injected into the circulation), have the ability to trigger COX-2 gene transcription in the rat brain and the effects of systemic inflammation and circulating IL-1ß in this response are likely to be IL-6

independent. The lack of stimulatory influence of IL-6 on COX-2 gene expression has also been reported in different *in vitro* models using rat microglial (Bauer *et al.*, 1997) and primary murine astrocyte cultures (O'Banion *et al.*, 1996). This contrast with a recent study showing that COX-2 mRNA is inducible by IL-6 in normal human articular chondrocytes (Geng *et al.*, 1995). Of interest is the increase of PGE_2 synthesis causes IL-6 production, a process that implicates expression of COX-2 gene in murine models of inflammation (Hinson *et al.*, 1996), which indicates that activation of COX pathway may be involved in the IL-6 production and not necessarily vice-a-versa.

Systemic administration of a pyrogenic dose of bacterial endotoxin stimulates PGE_2-like immunoreactivity (ir) within the chp and the brain microvasculatures; PGE_2-ir staining was first detected at 1.5 h and reached a maximum at 4 h after the LPS challenge (Van Dam *et al.*, 1993). The data presented here are in agreement with this time course as COX-2 gene expression was highly stimulated as early as 1 h after i.v. LPS injection, preceding then the reported PG synthesis. Moreover, IL-1ß-ir positive cells have been detected within the chp, meninges and the luminal side of endothelium of brain capillaries in endotoxin-treated rats (Van Dam *et al.*, 1995). This result raises the possibility that internalization of circulating IL-1 may occur or IL-1 *de novo* synthesis is activated in these specific cellular populations following LPS challenge. On the other hand, IL-1R1 mapping studies by *in situ* hybridization support the concept that IL-1ß of systemic origin is responsible for most of the effects of the cytokine. IL-1R1 transcript was observed predominantly in non-parenchymal cells including the chp, leptomeninges and the brain microvasculature (Ericsson *et al.*, 1995b), while peripheral administration of IL-1ß markedly enhanced PGE_2-ir in endothelial cells of the brain blood vessels and the chp (Van Dam *et al.*, 1996). Therefore, PGE_2 synthesis through COX-2 pathway seems a good candidate to transfer the information, at the level of the brain-blood barrier, from circulating cytokines (in particular IL-1ß) to parenchymal elements during systemic inflammation.

We have faced a number of problems in the identification of the cell type(s) in the brain microvasculature expressing COX-2 transcript in response to immunogenic stimuli. Although combination of both *in situ* hybridization and immunocytochemistry techniques on the same brain sections has been quite successful for numerous mRNAs in our laboratory (Lacroix *et al.*, 1996; Rivest and Laflamme, 1995; Rivest *et al.*, 1995), COX-2 transcript was very sensitive to the immunocytochemistry procedure and essentially vanished rending evaluation of double-labeled cells barely possible. Nevertheless, dual immunocytochemical

studies have indicated that cells stained for COX-2 in response to systemic i.v. LPS injection are of perivascular microglial and meningeal macrophage types (Breder and Saper, 1996; Elmquist *et al.*, 1997). However, these authors have observed, at the edge of the blood vessels, several COX-2-immunoreactive positive cells alone and not colocalized within microglia (Elmquist *et al.*, 1997), indicating that perivascular microglia may not be the only cell type responsible for the production of PGs within the brain. The low to barely detectable immunoreactive signal for specific cells may certainly contribute to the lack of clear colocalization between COX-2 transcript or protein and other blood vessel-associated cells, such as endothelial. Be that as it may, the bacterial endotoxin has been shown to increase COX-2 mRNA and protein as well as PGE_2 in microglial cell cultures (Bauer *et al.*, 1997) and in macrophages and peripheral blood monocytes (Hempel *et al.*, 1994; Lee *et al.*, 1992). In contrast, other studies have supported the hypothesis that the non-parenchymal cells of the brain microvasculature expressing COX-2 mRNA during the acute-phase response are rather of endothelial type. Indeed, COX-2 transcription and translation and PG biosynthesis were reported to be induced in vascular endothelial cells after LPS, IL-1 and TNF treatment (Jones *et al.*, 1993). The production of PGs within endothelial cells of the brain microvessels may have different functions, such as the maintenance of vascular tone (potent vasodilatator). It is therefore possible that both endothelial and perivascular microglial-associated cells express COX-2 in response to systemic inflammation to produce specific PG subtype(s).

Systemic IL-1 injection provokes sharp increases in the levels of PGE_2 in the PVN (Watanobe and Takebe, 1994), whereas PGE_2-ir neurons were detected in the PVN of LPS-treated rats (Van Dam *et al.*, 1993). Moreover, the endocrine hypothalamus exhibits positive hybridization signal for the mRNA encoding EP_1 PGE_2 receptor subtype (Batshake *et al.*, 1995). These results and the present paper raise the possibility that local PVN production of the PG participates in the regulation of neuroendocrine functions, such as stimulation of CRF neurons and in turn the HPA axis. This seems however a simplistic view as PGE_2 receptor subtypes are constitutively expressed throughout numerous nuclei of the brain (Batshake *et al.*, 1995; Ericsson *et al.*, 1995a; Sugimoto *et al.*, 1994) and central i.c.v. PGE_2 infusion can activate several structures innervating the hypothalamic PVN (Lacroix *et al.*, 1996). Moreover, indomethacin administration within the PVN failed to prevent IL-1-induced activation of the HPA axis (Komaki *et al.*, 1992).

There are a number of lines of evidence suggesting that PGs are essential in mediating febrile response (Saper and Breder, 1992; Stitt, 1986; Stitt, 1991) and this physiological

event involves the PVN, because lesion of this hypothalamic nucleus reduces the LPS-induced fever, but not following i.c.v. injection of high doses of PGE_2 (Horn *et al.*, 1994). Pathways independent or unrelated to the PVN should therefore be considered in the generation of fever by this particular PG. In fact, the present study shows clear induction of COX-2 mRNA within blood vessels irrigating the organum vasculosum lamina terminalis (OVLT)/medial preoptic area (MPOA), a region exhibiting PG production after systemic LPS (Ueno *et al.*, 1982) and IL-1ß injection (Komaki *et al.*, 1992). High density of PGE_2-binding sites (Matsumura *et al.*, 1992; Matsumura *et al.*, 1990) and positive hybridization signal for the mRNA encoding EP3 PGE_2 receptor have also been detected in the OVLT/MPOA (Ericsson *et al.*, 1995a). Interestingly, infusion of the PG synthesis inhibitor sodium salicylate directly into the OVLT significantly prevents the fever induced by i.v. LPS treatment (Morimoto *et al.*, 1988). It is likely that stimulation of COX-2 enzyme in brain microvasculature of the OVLT/MPOA increases local PG production, which may bind to specific receptors expressed on nearby neurons to promote the fever in response to peripheral immunogenic stimuli.

In conclusion, the present study shows that systemic inflammation stimulates genetic transcription of the limiting enzyme for the PG synthesis COX-2 in a non-selective manner throughout the entire microvasculature system and that IL-1ß might play a key role as the circulating factor mediating these effects. This non-selectivity raises several questions in regard to the localized neuronal activation induced by different experimental models of inflammation and proinflammatory cytokines. It is possible that the selectivity of the neuronal response is a consequence of the fine interaction between non-parenchymal PG synthesis and expression of specific PG receptors (EP1-EP4) within parenchymal elements of different brain nuclei. Further studies are therefore essential to determine the neuroanatomical link between vascular PG synthesis and the expressed receptor(s) in neurons that are in close association to COX-2-expressing cells. This will provide clear clues on the mechanisms involved in the transfer of the information received from the circulation to the brain neuronal pathways solicited during the acute-phase response.

5.7 ACKNOWLEDGMENTS

This research was supported by the Medical Research Council (MRC) of Canada. Serge Rivest is a Medical Research Council Scholar. Steve Lacroix holds a Studentship from the National Sciences and Engineering Research Council of Canada (NSERC). We thank Dr. K. Peri (Ste-Justine Hospital Research Center, Montreal, Canada) for the generous gift of the rat COX-1 and COX-2 cDNA fragments and Dr. R. Hart (The State University of New-Jersey Rutgers, Newark, N-J) for the gift of the recombinant rat IL-1ß. The authors also thank Ms. Nathalie Laflamme for invaluable technical assistance.

5.8 REFERENCES

Akira, S., Hirano, T., Taga, T. and Kishimoto, T. (1990) Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). *FASEB J.* 4, 2860-2867.

Andersson, J., Nagy, S., Björk, L., Abrams, J., Holm, S. and Andersson, U. (1992) Bacterial toxin-induced cytokine production studied at the single-cell level. *Immunological Rev.* 127, 69-96.

Arditi, M., Zhou, J., Dorio, R., Rong, G. W., Goyert, S. M. and Kim, K. S. (1993) Endotoxin-mediated endothelial cell injury and activation: role of soluble CD14. *Infect. Immun.* 61, 3149-3156.

Arditi, M., Zhou, J., Torres, M., Durden, D. L., Stins, M. and Kim, K. S. (1995) Lipopolysaccharide stimulates the tyrosine phosphorylation of mitogen-activated protein kinases p44, p42, and p41 in vascular endothelial cells in a soluble CD14-dependent manner. *J. Immunol.* 155, 3994-4003.

Batshake, B., Nilsson, C. and Sundelin, J. (1995) Molecular characterization of the mouse prostanoid EP1 receptor gene. *Eur. J. Biochem.* 231, 809-814.

Bauer, M. K. A., Lieb, K., Schulze-Osthoff, K., Berger, M., Gebicke-Haerter, P. J., Bauer, J. and Fiebich, B. (1997) Expression and regulation of cyclooxygenase-2 in rat microgla. *Eur. J. Biochem.* 243, 726-731.

Becher, B. and Antel, J. P. (1996) Comparison of phenotypic and functional properties of immediately ex vivo and cultured human adult microglia. *Glia* 18, 1-10.

Breder, C. D., Dewitt, D. and Kraig, R. P. (1995) Characterization of inducible cyclooxygenase in rat brain. *J. Comp. Neurol.* 355, 296-315.

Breder, C. D. and Saper, C. B. (1996) Expression of inducible cyclooxygenase mRNA in the mouse brain after systemic administration of bacterial lipopolysaccharide. *Brain Res.* 713, 64-69.

Bristow, A. F., Mosley, K. and Poole, S. (1991) Interleukin-1ß production in vivo and in vitro in rats and mice measured using specific immunoradiometric assays. *J. Mol. Endocrinol.* 7, 1-7.

Cao, C., Matsumura, K., Yamagata, K. and Watanabe, Y. (1995) Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in the rat brain; its possible role in the febrile response. *Brain Res.* 697, 187-196.

Cao, C. Y., Matsumura, K., Yamagata, K. and Watanabe, Y. (1996) Endothelial-cells of the rat-brain vasculature express cyclooxygenase-2 messenger-RNA in response to systemic interleukin-1: A possible site of prostaglandin synthesis responsible for fever. *Brain Res.* 733, 263-272.

Chai, Z., Gatti, S., Toniatti, C., Poli, V. and Bartfai, T. (1996) Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. *J. Exp. Med.* 183, 311-316.

Conti, P., Bartle, L., Barbacane, R. C., Reale, M., Placido, F. C. and Sipe, J. (1995) Synergistic activation of serum amyloid A (SAA) by IL-6 and IL-1 in combination on human Hep 3B hepatoma cell line. Role of PGE2 and IL-1 receptor antagonist. *Immunol. Invest.* 24, 523-535.

DeWitt, D. L., El-Harith, E. A., Kraemer, S. A., Andrews, M. J., Yao, E. F., Armstrong, R. L. and Smith, W. L. (1990) The aspirin and heme-binding sites of ovine and murine prostaglandin endoperoxide synthases. *J. Biol. Chem.* 265, 5192-5198.

DeWitt, D. L. and Smith, W. L. (1988) Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. *Proc. Natl. Acad. Sci. USA* 85, 1412-1416.

Dinarello, C. A. (1984) Interleukin-1 and the pathogenesis of the acute-phase response. *New Eng. J. Med.* 311, 1413-1422.

Dinarello, C. A. (1989) Interleukin-1 and its biologically related cytokines. *Adv. Immunol.* 44, 153-161.

Elmquist, J. K., Breder, C. D., Sherin, J. E., Scammell, T. E., Hickey, W. F., Dewitt, D. and Saper, C. B. (1997) Intravenous lipopolysaccharide induces cyclooxygenase 2-like immunoreactivity in rat brain perivascular microglia and meningeal macrophages. *J. Comp. Neurol.* 381, 119-129.

Ericsson, A., Kovacs, K. J. and Sawchenko, P. E. (1994) A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *J. Neurosci.* 14, 897-913.

Ericsson, A., Ek, M. and Lindefors, N. (1995a) Distribution of prostaglandins E2 receptor (EP3 subtype) mRNA containing cells in the rat central nervous system. *Soc. Neurosci. Abstr.* 21, 98.

Ericsson, A., Liu, C., Hart, R. P. and Sawchenko, P. E. (1995b) Type 1 interleukin-1 receptor in the rat brain: distribution, regulation, and relationship to sites of IL-1-induced cellular activation. *J. Comp. Neurol.* 361, 681-698.

Ertel, W., Morrison, M. H., Wang, P., Ba, Z. F., Ayala, A. and Chaudry, I. H. (1992) The complex pattern of cytokines in sepsis - association between prostaglandins, cachectin, and interleukins. *Ann Surg.* 214, 141-148.

Fantuzzi, G. and Dinarello, C. A. (1996) The inflammatory response in interleukin-1ß-deficient mice: comparison with other cytokine-related knock-out mice. *J. Leukoc. Biol.* 59, 489-493.

Feng, L., Sun, W., Xia, Y., Tang, W. W., Chanmugam, P., Soyoola, E., Wilson, C. B. and Hwang, D. (1993) Cloning two isoforms of rats cyclooxygenase: differential regulation of their expression. *Arch. Biochem. Biophys.* 307, 361-368.

Geng, Y., Blanco, F. J., Cornelisson, M. and Lotz, M. (1995) Regulation of cyclooxygenase-2 expression in normal human articular chondrocytes. *J. Immunol.* 155, 796-801.

Hempel, S. L., Monick, M. M. and Hunninghake, G. W. (1994) Lipopolysaccharide induces prostaglandin H synthase-2 protein and mRNA in human alveolar macrophages and blood monocytes. *J. Clin. Invest.* 93, 391-396.

Hinson, R. M., Williams, J. A. and Shacter, E. (1996) Elevated interleukin 6 is induced by prostaglandin E2 in a murine model of inflammation: Possible role of cyclooxygenase-2. *Proc. Natl. Acad. Sci. USA* 93, 4885-4890.

Horn, T., Wilkinson, M. F., Landgraf, R. and Pittman, Q. (1994) Reduced febrile responses to pyrogens after lesions of the hypothalamic paraventricular nucleus. *Am. J. Physiol.* 267, R323-R328.

Jones, D. A., Carlton, D. P., McIntyre, T. M., Zimmerman, G. A. and Prescott, S. M. (1993) Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. *J. Biol. Chem.* 268, 9049-9054.

Katsuura, G., Gottschall, P. E., Dahl, R. R. and Arimura, A. (1988) Adrenocorticotropin release induced by intracerebroventricular injection of recombinant human interleukin-1 in rats: Possible involvement of prostaglandin. *Endocrinology* 122, 1773-1779.

Klir, J. J., McClellan, J. L. and Kluger, M. J. (1994) Interleukin-1 beta causes the increase in anterior hypothalamic interleukin-6 during LPS-induced fever in rats. *Am. J. Physiol.* 266, R1845-R1848.

Komaki, G., Arimura, A. and Koves, K. (1992) Effect of intravenous injection of IL-1ß on PGE2 levels in several brain areas as determined by microdialysis. *Am. J. Physiol.* 262, E246-E251.

Kushner, I. (1982) The phenomenon of the acute-phase response. *Ann. NY Acad. Sci.* 389, 39-48.

Lacroix, S. and Rivest, S. (1996) Role of cyclo-oxygenase pathways in the stimulatory influence of immune challenge in the transcription of a specific CRF receptor subtype in the rat brain. *J. Chem. Neuroanat.* 10, 53-71.

Lacroix, S. and Rivest, S. (1997) Functional circuitry in the brain of immune-challenged rats: partial involvement of prostaglandins. *J. Comp. Neurol.* 387, 307-324.

Lacroix, S., Vallières, L. and Rivest, S. (1996) C-fos mRNA pattern and corticotropin-releasing factor neuronal activity throughout the brain of rats injected centrally with a prostaglandin of E2 type. *J. Neuroimmunol.* 70, 163-179.

Lee, S. H., Soyoola, E., Chanmugam, P., Hart, S., Sun, W., Zhong, H., Liou, S., Simmons, D. and Hwang, D. (1992) Selective expression of mitogen-inducible cyclooxygenase in macrophages stimulated with lipopolysaccharide. *J. Biol. Chem.* 267, 25934-25938.

Lopez-Valpuesta, F. J. and Myers, R. D. (1994) Fever produced by interleukin-11 (IL-11) injected into the anterior hypothalamic pre-optic area of the rat is antagonized by indomethacin. *Neuropharmacology* 33, 989-994.

Lyson, K. and McCann, S. M. (1992) Involvement of arachidonic acid cascade pathways in interleukin-6-stimulated corticotropin-releasing factor release in vitro. *Neuroendocrinology* 55, 708-713.

Malmberg, A. B. and Yaksh, T. L. (1995) Cyclooxygenase inhibition and the spinal release of prostaglandin E2 and amino acids evoked by paw formalin injection: A microdialysis study in unanesthetized rats. *J. Neurosci.* 15, 2768-2776.

Matsumura, K., Watanabe, Y., Imai-Matsumura, K., Connolly, M., Koyama, Y., Onoe, H. and Watanabe, Y. (1992) Mapping of prostaglandin E2 binding sites in rat brain using quantitative autoradiography. *Brain Res.* 581, 292-298.

Matsumura, K., Watanabe, Y., Onoe, H., Watanabe, Y. and Hayaishi, O. (1990) High density of prostaglandin E2 binding sites in the anterior wall of the 3rd ventricle: a possible site of its hyperthermic action. *Brain Res.* 533, 147-151.

McCoy, J. G., Matta, S. G. and Sharp, B. M. (1994) Prostaglandins mediate the ACTH response to interleukin-1-beta instilled into the hypothalamic median eminence. *Neuroendocrinology* 60, 426-435.

Morimoto, A., Murakami, N., Nakamori, T. and Watanabe, T. (1988) Multiple control of fever production in the central nervous system of rabbits. *J. Physiol. Lond.* 397, 269-280.

Nathan, C. F. (1987) Secretory products of macrophages. *J. Clin. Invest.* 79, 319-326.

Navarra, P., Pozzoli, G., Brunetti, L., Ragazzoni, E., Besser, M. and Grossman, A. (1992) Interleukin-1ß and interleukin-6 specifically increase the release of prostaglandin E2 from rat hypothalamic explants *in vitro*. *Neuroendocrinology* 56, 61-68.

Navarra, P., Tsagarakis, S., Faria, M. S., Rees, L. H., Besser, G. M. and Grossman, A. B. (1991) Interleukin-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. *Endocrinology* 128, 37-44.

Nogawa, S., Zhang, F., Ross, M. E. and Iadecola, C. (1997) Cyclo-oxygenase-2 gene expression in neurons contributes to ischemic brain damage. *J. Neurosci.* 17, 2746-2755.

O'Banion, M. K., Miller, J. C., Chang, J. W., Kaplan, M. D. and Coleman, P. (1996) Interleukin-1ß induces prostaglandin G/H synthase-2 (cyclooxygenase-2) in primary murine astrocyte cultures. *J. Neurochem.* 66, 2532-2540.

Poupart, P., Vandenabeele, P., Cayphas, S., Vansnick, J., Haegeman, G., Kruys, V., Fiers, W. and Content, J. (1987) B cell growth modulating and differentiating activity of recombinant human 26-kd protein (BSF-2, HuIFN-ß2, HPGF). *EMBO J.* 6, 1219-1224.

Rabin, B. S., Cunnick, J. E. and Lysle, D. T. (1990) Stress-induced alteration of immune function. *Prog. NeuroEndocrinImmunol.* 3, 116-125.

Rassnick, S., Zhou, D. H. and Rabin, B. S. (1995) Central administration of prostaglandin E (2) suppresses in vitro cellular immune responses. *Am. J. Physiol.* 269, R92-R97.

Rivest, S. and Laflamme, N. (1995) Neuronal activity and neuropeptide gene transcription in the brain of immune-challenged rats. *J. Neuroendocrinol.* 7, 501-525.

Rivest, S., Laflamme, N. and Nappi, R. E. (1995) Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. *J. Neurosci.* 15, 2680-2695.

Rivest, S. and Rivier, C. (1995) The role of CRF and interleukin-1 in the regulation of neurons controlling reproductive functions. *Endocrine Rev.* 16, 177-199.

Rivier, C. and Rivest, S. (1993) Mechanisms mediating the effects of cytokines in neuroendocrine functions in the rat., in *Corticotropin-releasing factor, Ciba Foundation Symposium 172* (Chadwick, D. J., Marsh, J. and Ackrill, K.), pp. 204-225. John Wiley & Sons Ltd, Chichester.

Saper, C. B. and Breder, C. D. (1992) Endogenous pyrogens in the CNS: role in the febrile response. *In D. F. Swaab, M. A. Hofman, M. Mirmiran, R. David and F. W. van Leeuwen (Eds.). Prog. Brain Res.* 93, 419-429.

Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P. and Vale, W. (1987) Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* 238, 522-524.

Scammell, T. E., Elmquist, J. K., Griffin, J. D. and Saper, C. B. (1996) Ventromedial preoptic prostaglandin E2 activates fever-producing autonomic pathways. *J. Neurosci.* 16, 6246-6254.

Sharp, B. M., Matta, S. G., Peterson, P. K., Newton, R., Chao, C. and McAllen, K. (1989) Tumor necrosis factor-alpha is a potent ACTH secretagogue: comparison to interleukin-1ß. *Endocrinology* 124, 3131-3137.

Simmons, D. M., Arriza, J. L. and Swanson, L. W. (1989) A complete protocol for *in situ* hybridization of messenger RNAs in brain and other tissus with radio-labeled single-stranded RNA probes. *J. Histotechnol.* 12, 169-181.

Sirko, S., Bishai, I. and Coceani, F. (1989) Prostaglandin formation in the hypothalamus in vivo: effect of pyrogens. *Am. J. Physiol.* 256, R616-R624.

Smith, T., Hewson, A. K., Quarrie, L., Leonard, J. P. and Cuzner, M. L. (1994) Hypothalamic PGE2 and cAMP production and adrenocortical activation following intraperitoneal endotoxin injection: *in vivo* microdialysis studies in Lewis and Fischer rats. *Neuroendocrinology* 59, 396-405.

Stitt, J. T. (1986) Prostaglandin E as the mediator of the febrile response. *Yale J. Biol. Med.* 59, 137-149.

Stitt, J. T. (1991) Differential sensitivity in the sites of fever production by prostaglandin-E1 within the hypothalamus of the rat. *J. Physiol. (Lond.)* 432, 99-110.

Sugimoto, Y., Shigemoto, R., Namba, T., Negishi, M., Mizuno, N., Narumiya, S. and Ichikawa, A. (1994) Distribution of the messenger RNA for the prostaglandin E receptor subtype EP3 in the mouse nervous system. *Neuroscience* 62, 919-928.

Tritarelli, E., Greco, G., Testa, U., Belardelli, F., Peschle, C. and Proietti, E. (1994) Combined interleukin-1 beta/interleukin-6 treatment in mice: synergistic myelostimulatory activity and myelorestorative effect after cyclophosphamide-induced myelosuppression. *Cancer Res.* 54, 6469-6476.

Turnbull, A. V., Pitossi, F. J., Lebrun, J.-J., Lee, S., Meltzer, J. C., Nance, D. M., del Rey, A., Besedovsky, H. O. and Rivier, C. (1997) Inhibition of tumor necrosis factor-α action within the CNS markedly reduces the plasma adrenocorticotropin response to peripheral local inflammation. *J. Neurosci.* 17, 3262-3273.

Turnbull, A. V. and Rivier, C. (1996) Corticotropin-releasing factor, vasopressin, and prostaglandins mediate, and nitric oxyde restrains, the hypothalamic-pituitary-adrenal response to acute local inflammation in the rats. *Endocrinology* 137, 455-463.

Ueno, R., Narumiya, S., Ogorochi, T., Nakayama, T., Ishikawa, Y. and Hayaishi, O. (1982) Role of prostaglandin D2 in the hypothermia of rats caused by bacterial lipopolysaccharide. *Proc. Natl. Acad. Sci. U.S.A.* 79, 6093-6097.

Vallières, L., Lacroix, S. and Rivest, S. (1997) Influence of interleukin-6 on neural activity and transcription of the gene encoding corticotropin-releasing factor in the rat brain: an effect depending upon the route of administration. *Eur. J. Neurosci.* 9, 1461-1472.

Van Dam, A. M., Bauer, J., Tilders, F. J. H. and Berkenbosch, F. (1995) Endotoxin-induced appearance of immunoreactive interleukin-1ß in rat brain: a light and electron microscopical study. *Neuroscience* 65, 815-826.

Van Dam, A. M., Brouns, M., Man-A-Hing, W. and Berkenbosch, F. (1993) Immunocytochemical detection of prostaglandin E2 in microvasculatures and in neurons of rat brain after administration of bacterial endotoxin. *Brain Res.* 613, 331-336.

Van Dam, A. M., DeVries, H. E., Kuiper, J., Zijlstra, F. J., DeBoer, A. G., Tilders, F. J. H. and Berkenbosch, F. (1996) Interleukin-1 receptors on rat brain endothelial cells: a role in neuroimmune interaction? *FASEB J.* 10, 351-356.

Watanobe, H. and Takebe, K. (1994) Effects of intravenous administration of interleukin-1-beta on the release of prostaglandin E2, corticotropin-releasing factor, and arginine vasopressin in several hypothalamic areas of freely moving rats: estimation by push-pull perfusion. *Neuroendocrinology* 60, 8-15.

Wright, S. D., Ramos, R. A., Tobias, P. S., Ulevitch, R. J. and Mathison, J. C. (1990) CD14, a receptor for complexes of lipolysaccharide (LPS) and LPS binding protein. *Science* 249, 1431-1433.

Yamagata, K., Andreasson, K. I., Kaufmann, W. E., Barnes, C. A. and Worley, P. F. (1993) Expression of a mitogen-inducible cyclooxygenase in brain neurons: Regulation by synaptic activity and glucocorticoids. *Neuron* 11, 371-386.

Zheng, H., Fletcher, D., Kozak, W., Jiang, M., Hofmann, K., *et al.* (1995) Resistance to fever induction and impaired acute-phase response in interleukin-1ß-deficient mice. *Immunity* 3, 9-19.

Figure 1:

Representative example of the influence of endotoxin lipolysaccharide (LPS) injection on the distribution of the mRNA encoding cyclooxygenase-2 (COX-2) in the rat brain. Animals were sacrificed 1 h after intravenous (i.v.) treatment with LPS (10 μg/100 g b.w.) and 3 h following intraperitoneal (i.p.) administration of LPS (50 μg/100 g b.w.) or the vehicle solution. These rostro-caudal coronal sections (30 μm) of LPS-treated rats exhibit a positive signal on x-ray films (Biomax) for COX-2 mRNA in various structures throughout the brain, particularly over the entire microvasculature system. 4V, fourth ventricle; AQ, aqueduct; BLA, basolateral nucleus of the amygdala; bv, blood vessels; Cer, cerebellum; CP, caudate putamen; DG, dentate gyrus; Hip, hippocampus; IPN, interpeduncular nucleus; LGc, lateral geniculate complex; LV, lateral ventricle; ME, median eminence; MGv, medio-ventral geniculate nucleus; NTS, nucleus of the solitary tract; PB, parabrachial nucleus; PG, pontine gray; Pir, piriform cortex; PVN, paraventricular nucleus of the hypothalamus; VLM, ventrolateral medulla.

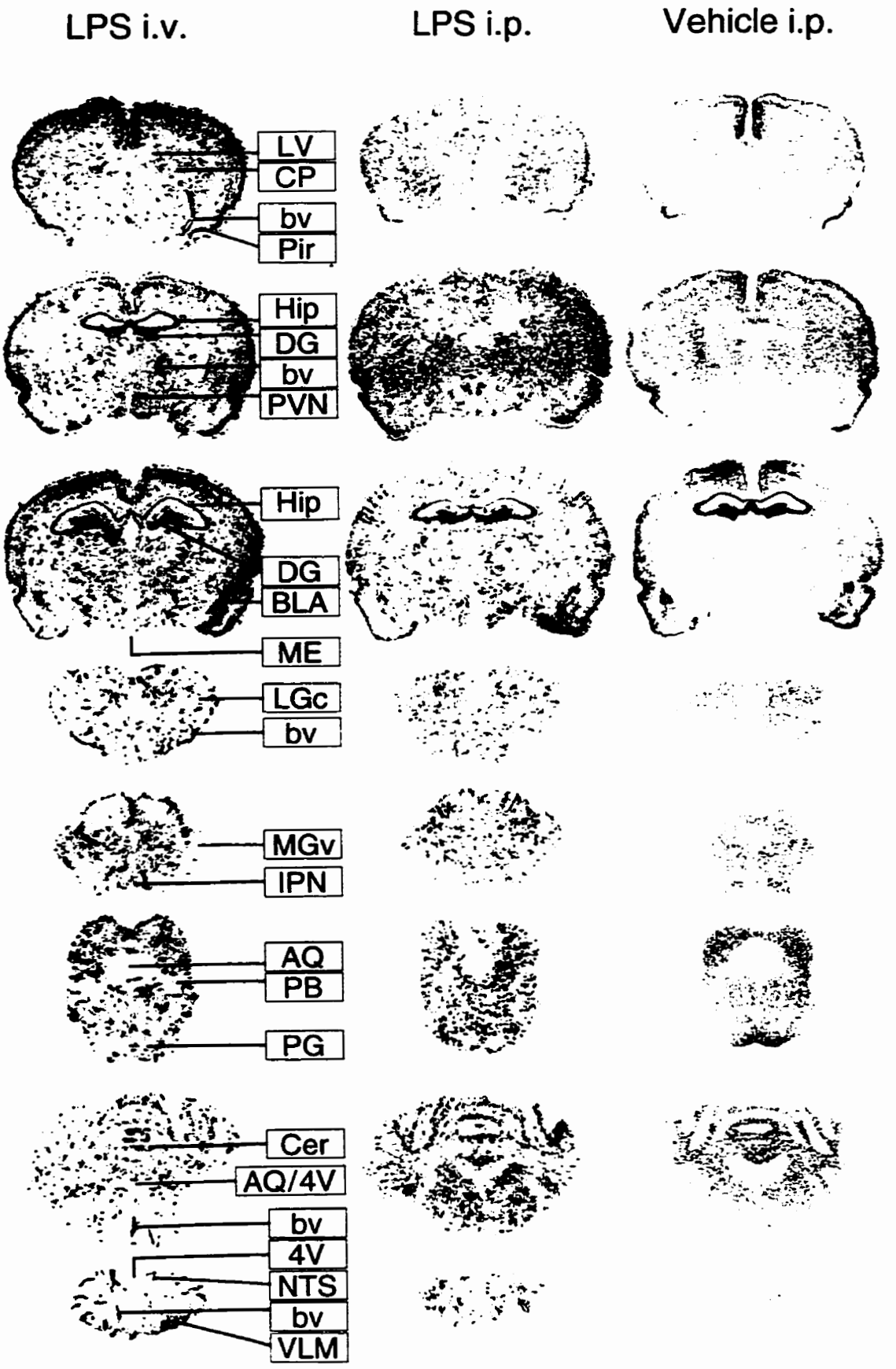

LPS i.v.
LPS i.p.
Vehicle i.p.
LV
CP
bv
Pir
Hip
DG
bv
PVN
Hip
DG
BLA
ME
LGc
bv
MGv
IPN
AQ
PB
PG
Cer
AQ/4V
bv
4V
NTS
bv
VLM

Figure 2:

High power brightfield photomicrographs showing the expression of cyclooxygenase-2 (COX-2) mRNA in the medial paraventricular nucleus of the hypothalamus (PVN; panel A) 3 h after intravenous (i.v.) injection of the bacterial endotoxin lipopolysaccharide (LPS). Note the amount of silver grains delineating several parenchymal cells expressing the gene encoding the enzyme COX-2 in the parvocellular division of the PVN (panels B and C). The possibility remains however that these cells are vascular-associated, which were isolated by the sectioning procedure. Moreover, positive cells surrounding blood vessels (bv) were also found in this endocrine nucleus (panel D). Magnification panel A, X25, Scale bar = 100 μm; others X250, Scale bar = 10 μm.

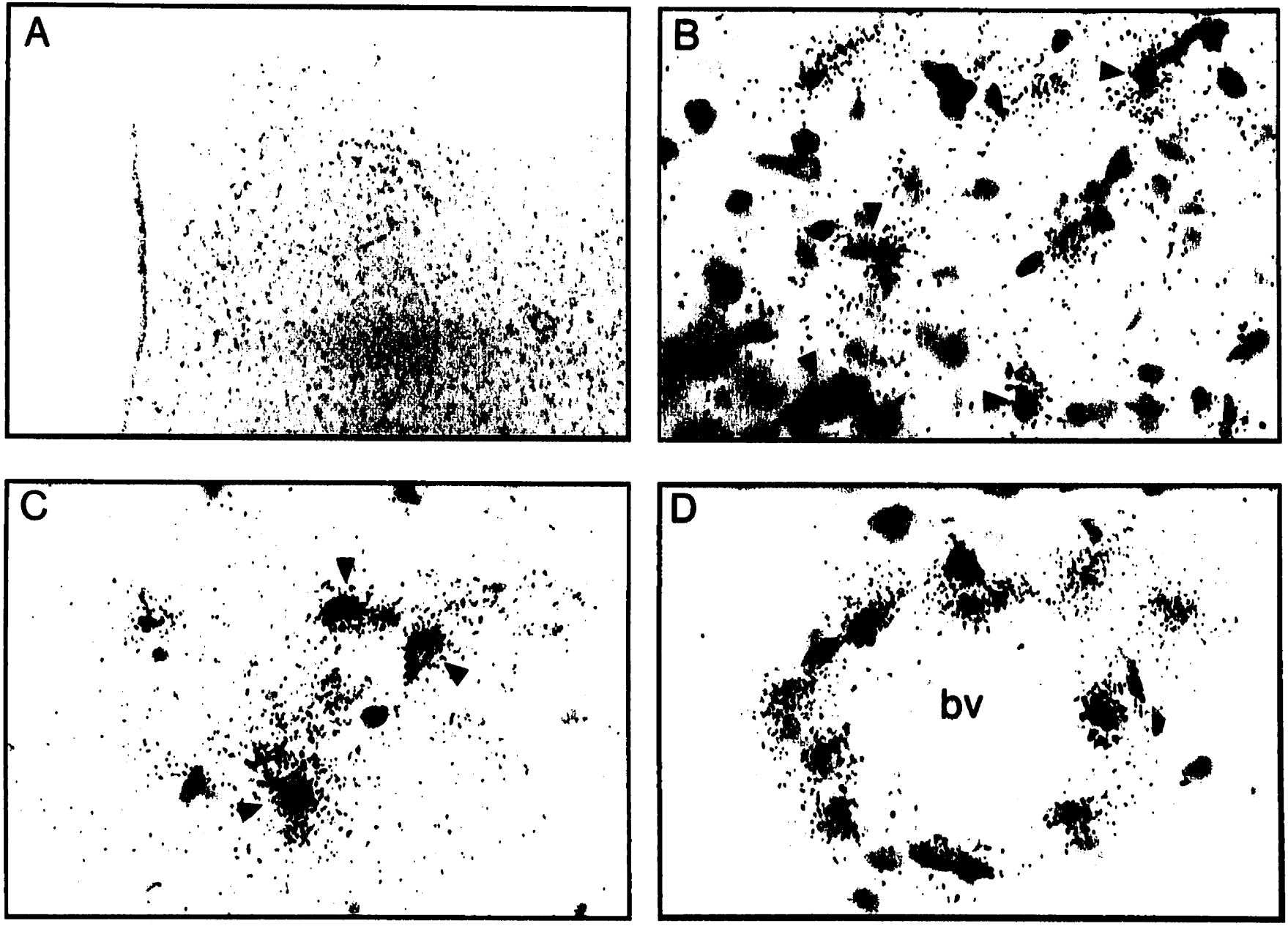

A
B
C
D
bv

Figure 3:

High power darkfield (top panels) and brightfield (bottom panels) photomicrographs showing the expression of cyclooxygenase-2 (COX-2) mRNA in blood vessels (b.v.), choroid plexus (chp), and leptomeninges of rats sacrificed 1 h after intravenous (i.v.) injection of the bacterial endotoxin lipopolysaccharide (LPS, 10 μg/100 g b.w.). Note the robust hybridization signal within non-parenchymal cells surrouding the b.v., chp and leptomeninges 1 h following the i.v. LPS challenge. Magnification top panels, X10, Scale bar = 250 μm; bottom panels, X100, Scale bar = 25 μm.

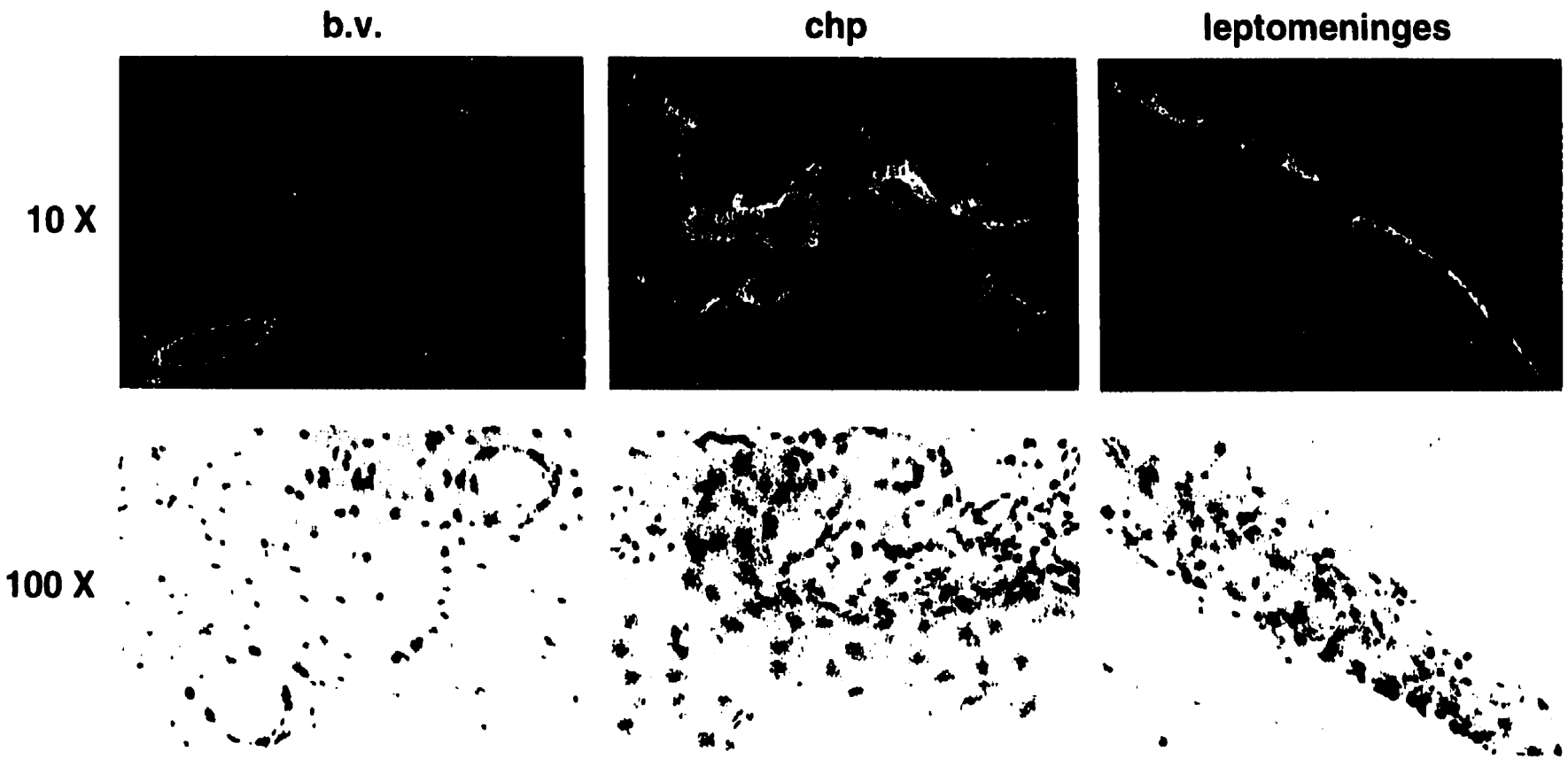

b.v.
chp
leptomeninges
10 X
100 X

Figure 4:

Representative example of the distribution of cyclooxygenase-2 (COX-2) transcript in the rat brain after sterile local inflammation induced by intramuscular (i.m.) administration of turpentine (50 µl/100 g b.w.). Animals were sacrificed 6 h after i.m. injection of turpentine or the vehicle solution in the left hind limb. These rostro-caudal coronal sections (30 µm) exhibit positive signal on x-ray films (Biomax) for COX-2 mRNA throughout the brain microvasculature and other non-parenchymal structures, including the choroid plexus and the leptomeninges of turpentine-treated rat. 4V, fourth ventricle; AQ, aqueduct; BLA, basolateral nucleus of the amygdala; bv, blood vessels; Cer, cerebellum; CP, caudate putamen; DG, dentate gyrus; DVC, dorsovagal complex; Hip, hippocampus; IPN, interpeduncular nucleus; LGc, lateral geniculate complex; LV, lateral ventricle; MGv, medio-ventral geniculate nucleus; PB, parabrachial nucleus; PG, pontine gray; Pir, piriform cortex; VLM, ventrolateral medulla.

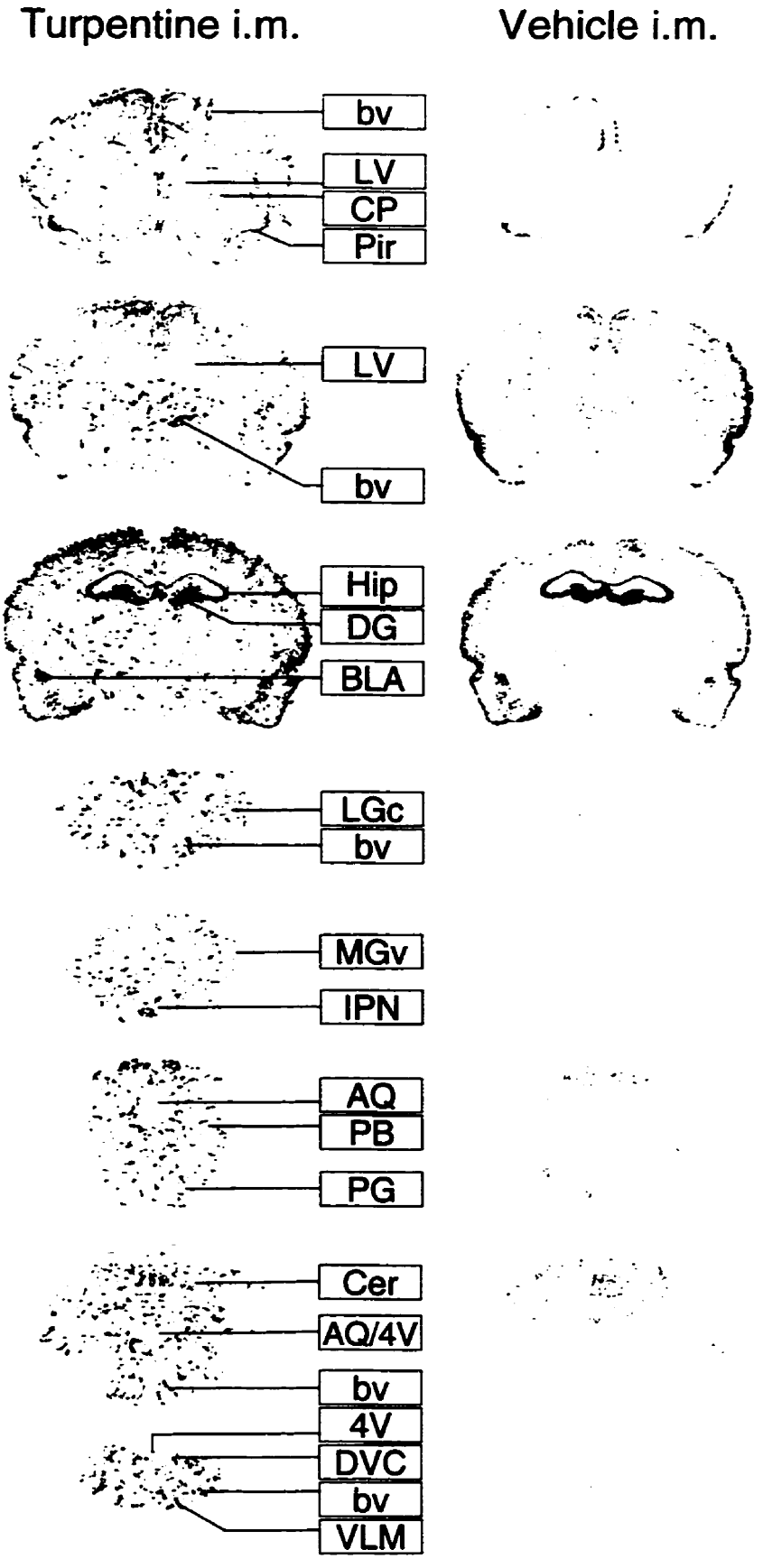

Turpentine i.m.
Vehicle i.m.
bv
LV
CP
Pir
LV
bv
Hip
DG
BLA
LGc
bv
MGv
IPN
AQ
PB
PG
Cer
AQ/4V
bv
4V
DVC
bv
VLM

Figure 5:

Influence of proinflammatory cytokines on the distribution of the mRNA encoding cyclooxygenase-2 (COX-2) in the rat brain. Animals were sacrificed 1 h after intravenous (i.v.) injection with interleukin (IL)-1ß (187 ng/100 g b.w.), tumor-necrosis factor-α (TNF-α; 400 ng/100 g b.w.), IL-6 (1.2 µg/100 g b.w.) or the vehicle solution. Rostro-caudal coronal sections (30 µm) of IL-6 treated rat did not exhibit notable induction COX-2 mRNA, whereas increase signal for this transcript can be observed on X-ray films (Biomax) over blood microvessels, choroid plexus and leptomeninges of the IL-1ß-challenged brain. On the other hand, systemic i.v. TNF-α provoked a modest and limited expression of COX-2 along some brain capillaries. 4V, fourth ventricle; AQ, aqueduct; BLA, basolateral nucleus of the amygdala; bv, blood vessels; Cer, cerebellum; CO, cochlear nucleus; CP, caudate putamen; DG, dentate gyrus; Hip, hippocampus; IPN, interpeduncular nucleus; LGc, lateral geniculate complex; LV, lateral ventricle; ME, median eminence; MGv, medio-ventral geniculate nucleus; NTS, nucleus of the solitary tract; Pir, piriform cortex; PVN, paraventricular nucleus of the hypothalamus; VLM, ventrolateral medulla.

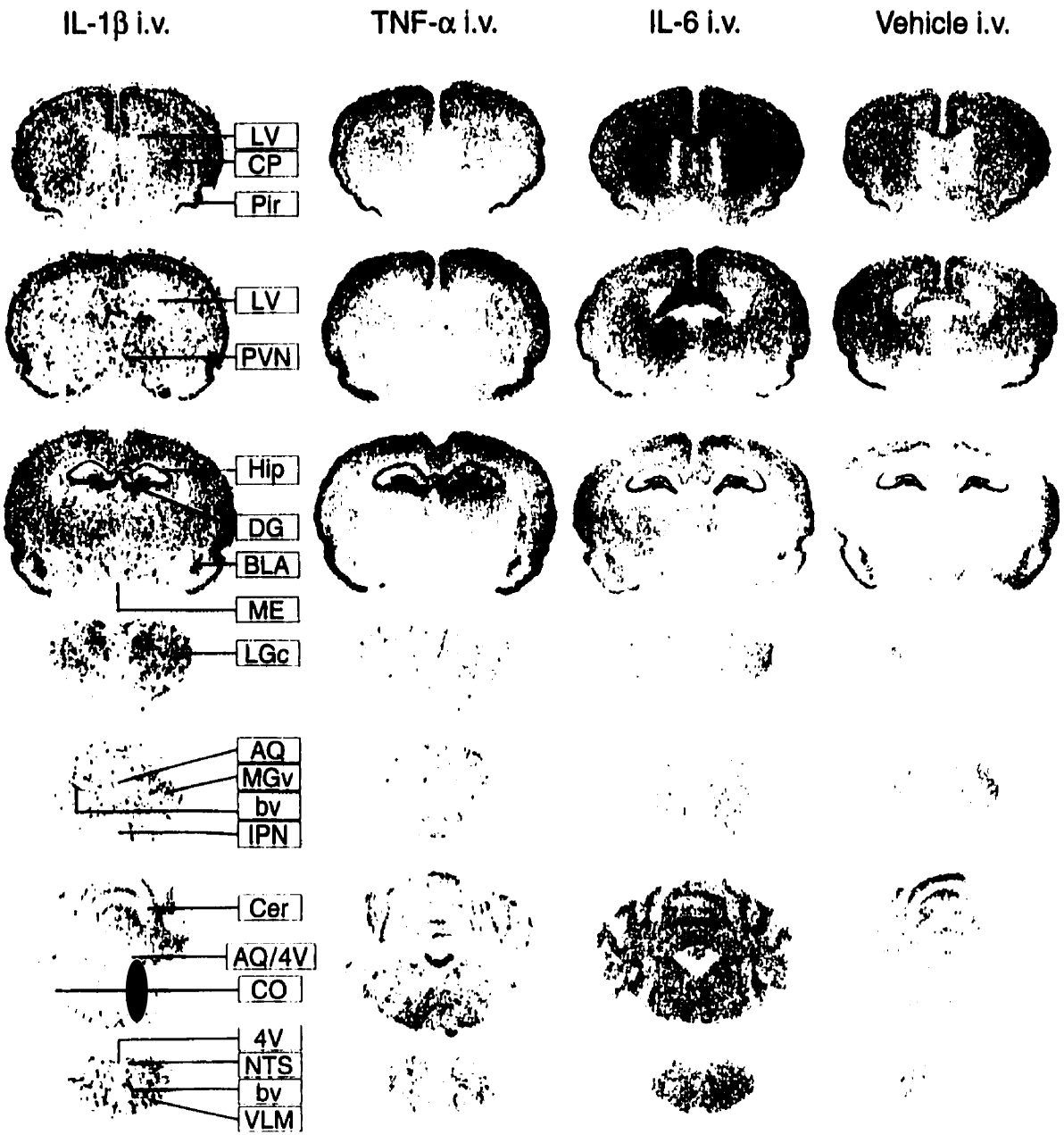
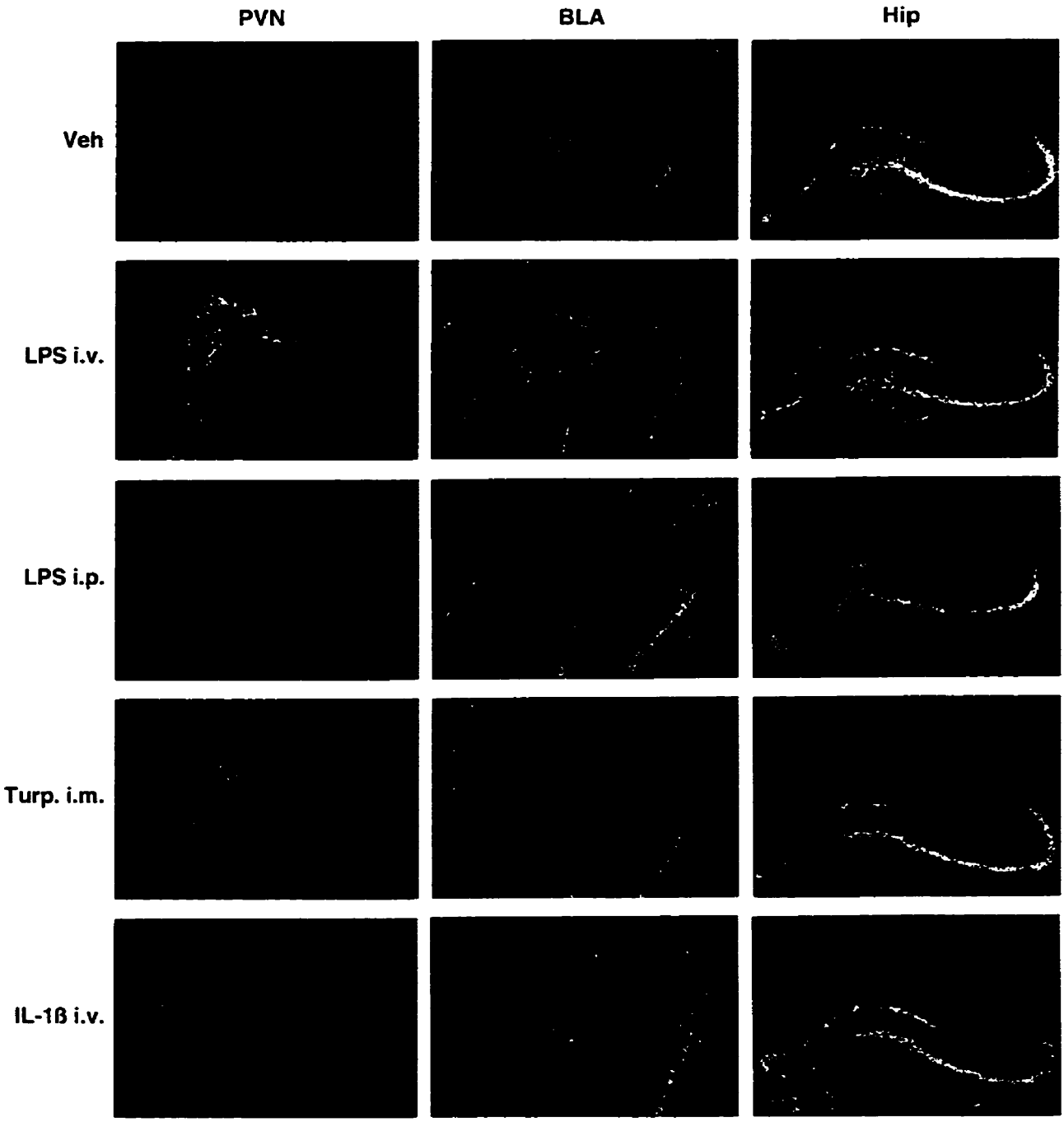

IL-1β i.v.
TNF-α i.v.
IL-6 i.v.
Vehicle i.v.
LV
CP
Pir
LV
PVN
Hip
DG
BLA
ME
LGc
AQ
MGv
bv
IPN
Cer
AQ/4V
CO
4V
NTS
bv
VLM

Figure 6:

Differential effects of intravenous (i.v.) injection of vehicle (Veh) and the bacterial endotoxin lipopolysaccharide (LPS), intraperitoneal (i.p.) LPS administration, intramuscular (i.m) turpentine (Turp.) insult and i.v. interleukin-1ß (IL-1ß) injection on the expression of COX-2 mRNA in different brain nuclei. These darkfield photomicrographs of dipped NTB-2 emulsion slides depict robust induction of COX-2 gene in the hypothalamic paraventricular nucleus (PVN, left column) of i.v. LPS-treated rats, an effect more subtle following the other challenges. This phenomenon also seems PVN selective as little changes were detected in the endogenously-expressing nuclei, including the basolateral nucleus of the amygdala (BLA, center column) and the hippocampus (Hip, right column). However, note that blood vessels irrigating these parenchymal regions display positive COX-2 mRNA signal after the immunogenic challenges, but not following i.v. vehicle injection (top panels). Magnification PVN, X25, Scale bar = 100 μm; BLA and Hip, X10, Scale bar = 250 μm.


PVN
BLA
Hip
Veh
LPS i.v.
LPS i.p.
Turp. i.m.
IL-1ß i.v.

CHAPITRE 6. THE BACTERIAL ENDOTOXIN LIPOPOLYSACCHARIDE HAS THE ABILITY TO TARGET THE BRAIN IN UPREGULATING ITS MEMBRANE CD14 RECEPTOR WITHIN SPECIFIC CELLULAR POPULATIONS.

By

Steve Lacroix*, Doug Feinstein‡ and Serge Rivest*

*Laboratory of Molecular Endocrinology
CHUL Research Center and Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2

‡Division Neurobiology
411 East 69th Street
Cornell University Medical College
New York, NY 10021

Running Title: LPS receptor expression in the rat brain.

Address for Correspondence: Dr. Serge Rivest
Laboratory of Molecular Endocrinology
CHUL Research Center and Laval University
2705, boul. Laurier
Québec, Canada
G1V 4G2
Tel: (418) 654-2296, Fax: (418) 654-2761
EMail: Serge.Rivest@crchul.ulaval.ca

Brain Pathology, in press (1998)

6.1 RÉSUMÉ

L'injection systémique de l'endotoxine bactérienne lipopolysaccharide (LPS) est un puissant modèle utilisé pour induire la production des cytokines proinflammatoires par les cellules phagocytaires tissulaires et circulantes. Il est maintenant connu, que l'action de la LPS sur les monocytes/macrophages s'effectue via l'intermédiaire d'un récepteur membranaire soit le CD14. Le but de cette étude était de vérifier l'hypothèse que le système nerveux central possède la capacité d'exprimer le gène encodant le récepteur de la LPS, permettant par la même occasion à l'endotoxine d'agir directement sur certaines populations cellulaires du cerveau afin de stimuler différentes fonctions neuronales au cours de l'endotoxinémie systémique. Pour ce, des rats mâles Sprague-Dawley (~250 g) furent sacrifiés 1, 3 et 6 et 24 h après avoir reçu une injection intraveineuse de LPS (25 µg) ou de solution témoin (salin stérile). Les cerveaux ont été coupés, du bulbe olfactif jusqu'à la médulla en tranches de 30 µm et l'ARNm du CD14 hybridé, à l'aide d'une sonde spécifique marquée au S^{35}. Les résultats montrent que l'ARNm encodant le récepteur de la LPS est exprimé de façon basale dans les méninges, le plexus choroïdien et dans l'ensemble de la microvasculature du cerveau. L'injection systémique de LPS a fortement stimulé la transcription du gène encodant CD14 dans ces trois mêmes structures ainsi que dans les organes circumventriculaires (OCVs) qui incluent: l'organe vasculaire de la lamina terminalis, l'organe subfornicale, l'éminence médiane et l'area postrema. Dans la plupart de ces régions, le signal est apparu 1 h après l'injection i.v. de LPS pour ensuite atteindre son niveau d'expression maximale à 3 h, diminuer après 6 h et disparaître au temps 24 h. Fait intéressant, un effet migratoire des cellules CD14-positives originant des OCVs vers les structures parenchymales avoisinantes a aussi été observé 3 et 6 h suivant l'administration de la LPS. En effet, plusieurs cellules positives, de taille réduite, ont été trouvées dans diverses structures parenchymales du cerveau. La combinaison de l'immunohistochimie à l'hybridation *in situ* nous indique que la grande majorité des cellules exprimant le gène CD14 au cours de la réponse immunitaire sont en réalité des cellules d'origine myéloide: des cellules microgliales dans les OCVs et le parenchyme, des macrophages dans les plexus choroidiens et les méninges et des cellules microgliales associées aux vaisseaux sanguins. Néanmoins, d'autres types cellulaires semblent posséder la capacité d'exprimer l'ARNm du CD14. Ces résultats suggèrent, pour la première fois, un rôle direct de la LPS sur certains groupes cellulaires très spécifiques du système nerveux central. Cette action directe de l'endotoxine pourrait être responsable de la transcription de différentes cytokines proinflammatoires; dans un premier temps au niveau des structures en contact direct avec la circulation sanguine, pour ensuite atteindre certains types de cellules parenchymales lors d'une septicémie plus sévère.

6.2 ABSTRACT

Systemic injection of the bacterial endotoxin lipopolysaccharide (LPS) provides a very good mean for increasing the release of proinflammatory cytokines by circulating monocytes and tissue macrophages. There is now considerable evidence that LPS exerts its action on mononuclear phagocytes via the cell surface receptor CD14. The aim of the present study was to verify the hypothesis that the brain has also the ability to express the gene encoding the LPS receptor, which may allow a direct action of the endotoxin onto specific cellular populations during blood sepsis. Adult male Sprague-Dawley rats were sacrificed 1, 3, 6 and 24 h after systemic (i.v. and i.p.) injection of LPS or the vehicle solution. Brains were cut from the olfactory bulb to the medulla in 30-µm coronal sections and mRNA encoding rat CD14 was assayed by *in situ* hybridization histochemistry using a specific ^{35}S-labeled riboprobe. The results show low levels of CD14 mRNA in the leptomeninges, choroid plexus and within blood vessels of the brain microvasculature under basal conditions. Systemic injection of the bacterial endotoxin caused a profound increase in the expression of the gene encoding CD14 within these same structures as well as in the circumventricular organs (CVOs) the organum vasculosum of the lamina terminalis, subfornical organ, median eminence and area postrema. In most of these structures, the signal for CD14 mRNA was first detected at 1 h, reached a peak at 3 h post-injection, declined at 6 h, and return to basal levels 24 h after LPS treatment. Quite interestingly, a migratory-like pattern of CD14 positive cells was observed from all sensorial CVOs to deeper parenchymal brain 3 and 6 h after LPS injection. At 6 h post-challenge, small positive cells were found throughout the entire parenchymal brain and dual-labeling procedure indicated that different cells of myeloid origin have the ability to express CD14 in response to systemic LPS. These included CVO microglia, choroid plexus and leptomeninge macrophages, parenchymal and perivascular-associated microglial cells, although specific non-myeloid cells were also positive for the LPS receptor. These results provide the very first evidence of a direct role of LPS on specific cell populations of the central nervous system, which is likely to be responsible for the transcription of proinflammatory cytokines; first within accessible structures from the blood and thereafter through scattered parenchymal cells during severe sepsis.

6.3 INTRODUCTION

Exposure to high levels of the bacterial endotoxin lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, often results in septic shock and death. Since the incidence of Gram-negative sepsis has been reported to be in constant progression during the recent years, numerous groups have begun to use systemic LPS administration as a model of infection and inflammation. Systemic LPS injection has been reported to induce changes in blood pressure, osmolarity, pain, oxygen consumption, fever, energy metabolism, multiple organ failure and different changes in the endocrine system, such as activation of the hypothalamic-pituitary-adrenal (HPA) axis. Indeed, systemic LPS injection has been shown to trigger transcription of corticotropin-releasing factor (CRF) and its type 1 receptor in the paraventricular nucleus of the hypothalamus (PVN) (1) and stimulates the release of the neuropeptide into the hypophyseal-portal vein and the adrenocorticotropic hormone (ACTH) into the general systemic circulation where it can act on distant endocrine organs, such as the adrenal glands. Increased levels of glucocorticoids, in counterpart, are potent immunosuppressors and may play a potent role in the appropriate control of the immune response. The importance of a timely release of glucocorticoids is indicated by the high mortality observed in untreated Addisonian patients (2) and in experimentally adrenalectomized animals (3-5).

Proinflammatory cytokines, including interleukin (IL)-1, IL-6 and tumor-necrosis factor (TNF)-α are considered to be the main mediators of LPS-induced neuroendocrine changes. Although the primary function of cytokines is aimed at expanding the immunologic mass and activity, cytokines also reach the general circulation and thus trigger different functions involved in the neuroendocrine-immune interaction. It is generally believed that the release of proinflammatory cytokines from LPS-stimulated monocytes/macrophages, neutrophils and lymphocytes are directly responsible for the immune acute-phase response (6) and the physiopathological outcomes that take place during sepsis (7-9). Intravenous (i.v.) injection of IL-1ß, IL-6 and TNF-α was shown to reproduce numerous of the events occurring after endotoxaemia, such as the activation of the HPA axis and expression of c-*fos* gene in the rat brain (10-17; S. Nadeau and S. Rivest, *in preparation*).

Secretion of cytokines by circulating monocytes and tissue macrophages following LPS treatment requires a series of mechanisms in cascade. The endotoxin must first reach the bloodstream to bind with the serum proteins LPS binding protein (LBP) or septins (18,

19). The new formed complex binds to CD14 receptor attached to the cell membrane of differentiated myeloid cells (principally monocytes/macrophages and also, to a lesser degree, neutrophils) and therefore induces the release of proinflammatory cytokines (20). Using different neutralizing monoclonal antibodies specific to CD14, many cellular responses to LPS concentrations sufficient to induce sepsis have been shown to be prevented *in vitro* (20-23). It has recently been reported that pretreatment of primates with antibodies directed against CD14 significantly prevented tissue damage, hypotension and the increase in plasma levels of TNF-α, IL-1ß and IL-6 (24). Transgenic mice expressing the membrane-associated human CD14 on their mononuclear phagocytic cells surface have been found to be hypersensitive to LPS, as demonstrated by their susceptibility to endotoxic shock and their mortality rate (25). In addition, transfection of human CD14 into Chinese hamster ovary fibroblasts was capable of transforming the cells from LPS nonresponders to LPS responders (26). It is not yet well understood how cell activation is triggered after binding between the LPS-LBP complex and the glycosyl-phosphatidylinositol (GPI)-anchored membrane CD14 (mCD14).

Recent studies on cytokine-related knock-out mice have clearly demonstrated that endogenous pyrogens, such as IL-1 and IL-6, are dispensable for LPS to activate the hypothalamus and HPA axis. In fact, IL-1ß -/- and IL-6 -/- mice injected with the bacterial endotoxin LPS exhibit normal levels of plasma corticosterone in comparison to their corresponding LPS-treated wild-type mice (27-29). Moreover, we have recently found a robust expression of IL-6 and TNF-α mRNA in the sensorial circumventricular organs (CVOs) and the choroid plexus of rats that received a single systemic endotoxin injection, whereas localized inflammation of the rat hindlimb and i.v. injection of different proinflammatory cytokines failed to mimic these effects (17; S. Nadeau and S. Rivest, *in preparation*). This clearly suggests that LPS has, by itself, the ability to stimulate cytokine production within the central nervous system and that activation of phagocytic cells and cytokine release of systemic origin may be a distinct and independent response. Whether the gene encoding CD14 is expressed within specific cellular populations of the brain that may allow a direct action of the bacterial endotoxin when circulating into the bloodstream has yet to be determined *in vivo*. The purpose of the present study was therefore to determine the distribution of the gene encoding CD14 and investigate the effects of systemically-injected LPS on the transcriptional regulation of its own receptor throughout the rat brain. We provide here the very first *in vivo* evidence that the bacterial endotoxin has a profound stimulatory influence on CD14 expression in both parenchymal and non-parenchymal elements of the brain, which may subserve a direct binding ability of the endotoxin to

modulate different brain functions under severe blood endotoxaemia. The physiological relevance of such phenomenon is presented and discussed.

6.4 MATERIALS AND METHODS

6.4.1 Animals

Adult male Sprague-Dawley rats (~230-260 g b.w.) were acclimated to standard laboratory conditions (14-h light, 10-h dark cycle; lights on at 0600 and off at 2000) with free access to rat chow and water. Each rat was only used once for experimentation, and all protocols were approved by the Laval University's Animal Welfare Committee. A total of 64 rats were assigned to different protocols (each corresponding to a different route of administration and a different dose of LPS; 20 and 100 μg of LPS i.v./kg of b.w. or 25 and 500 μg of LPS i.p./kg of b.w.), which were further subdivided into four post-injection times (1, 3, 6 and 24 hours following LPS administration). Paired vehicle-treated rats were also sacrificed at corresponding times after the systemic injection .

6.4.2 Surgeries

Animals receiving i.v. or i.p. injections were implanted with sterile cannulas. Rats were anesthetized with an i.p. injection of a mixture (1 ml/kg b.w.) of ketamine hydrochloride (91 mg/ml) and xylazine (9 mg/ml) and implanted with a catheter into the jugular vein or into the peritoneal cavity. Catheters were made from a piece of silastic tubing (Silastic medical grade tubing, ID 0.020 in., OD 0.037 in.; Dow Corning, Midland, MI) connected to an intramedic polyethylene tubing (PE-50, Caly Adams, Parsippany, N.J.). Outlets of cannulas were placed at the level of the neck and rats were housed individually in metal cages for a recuperation period of two to four days.

6.4.3 Experimental protocols

On the day of the experiment (~0830 in the morning), the outlet portion of each catheter (i.v. or i.p.) was fixed to a truncated 27 g needle which was attached to a PE-50 tubing. These connectors were then fixed to a 1cc syringe and rats were placed individually in a quiet room for at least 2 hours before the injections. This allows injections without

disturbing the animals and induction of genes that may be activated following the stress of handling. Intravenous (20 and 100 μg/kg diluted in 200 μl of sterile pyrogen-free saline) and intraperitoneal (25 and 500 μg/kg in 300 μl of saline) administration of LPS (from *Escherichia coli*, Serotype 055:B5, Sigma, L-2880, lot #122H4025) or the vehicle solution was performed into the right jugular vein and through the chronically implanted cannula into the peritoneal cavity, respectively. The animals were conscious and freely moving at all times throughout the procedure. One, 3, 6 and 24 hours after treatment, animals were deeply anesthetized with an i.v. (100 μl) or i.p. (400 μl) injection of a mixture of ketamine hydrochloride and xylazine and then rapidly perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M borax buffer (pH 9.5 at 4 °C). The dosage and time points were determined on the basis of previous studies demonstrating strong neuronal activation and transcriptional induction of the genes encoding CRF and its type 1 receptor in the rat brain following such treatments (30, 31). For the combination of immunocytochemistry (especially for OX-42-immunoreactive cells) to *in situ* hybridization, rats were perfused with saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate (pH: 7.2). Brains were removed from the skull, postfixed for 2 h and then placed in 20 % sucrose diluted in 4% paraformaldehyde-sodium phosphate buffer for 12-15 h.

6.4.4 Single *in situ* hybridization histochemistry

Rapidly after the transcardiac perfusions, brains were removed from the skulls, postfixed for 2 to 8 days, and then placed in a solution containing 10% sucrose diluted in 4% paraformaldehyde-borax buffer overnight at 4 °C. The frozen brains were mounted on a microtome (Reichert-Jung, Cambridge Instruments Company, Deerfield, IL) and cut into 30-μm coronal sections from the olfactory bulb to the end of the medulla. The slices were collected in a cold cryoprotectant solution (0.05M sodium phosphate buffer, pH 7.3, 30% ethylene glycol, 20% glycerol) and stored at -20 °C. Hybridization histochemical localization of each transcript was carried out on every sixth section of the whole rostro-caudal extent of each brain using ^{35}S-labeled cRNA probes. Protocols for riboprobe synthesis, hybridization, and autoradiographic localization of mRNA signal were adapted from Simmons et al. (32). All solutions were treated with diethylpyrocarbonate (Depc) and sterilized to prevent RNA degradation. Tissue sections mounted onto poly-L-lysine-coated slides were desiccated overnight under vacuum, fixed in 4% paraformaldehyde for 30 min, and digested with proteinase K (10 μg/ml in 0.1 M tris HCl, pH 8.0, and 50 mM EDTA, pH 8.0, at 37 °C for 25 min). Thereafter, the brain sections were rinsed in sterile Depc water

followed by a solution of 0.1 M triethanolamine (TEA, pH 8.0), acetylated in 0.25% acetic anhydride in 0.1 M TEA, and dehydrated through graded concentrations of alcohol (50, 70, 95, and 100 %). After vacuum drying for a minimum of 2 h, 90 µl of hybridization mixture (10^7 cpm/ml) was spotted on each slide, sealed under a coverslip, and incubated at 60 °C overnight (~15-20 h) in a slide warmer. Coverslips were then removed and the slides were rinsed in 4x standard saline citrate (SSC) at room temperature. Sections were digested by RNAse A (20 µg/ml, 37 °C, 30 min), rinsed in descending concentrations of SSC (2x, 1x, 0.5xSSC), washed in 0.1xSSC for 30 min at 60 °C (1xSSC: 0.15 M NaCl, 15 mM trisodium citrate buffer, pH 7.0) and dehydrated through graded concentrations of alcohol. After being dried for 2 h under vacuum, the sections were exposed at 4 °C to X-ray films (Kodak) for 3 days, defatted in xylene, and dipped in NTB2 nuclear emulsion (Kodak; diluted 1:1 with distilled water). Slides were exposed for 12 to 16 days, developed in D19 developer (Kodak) for 3.5 min at 14-15 °C, washed 15 sec in water, and fixed in rapid fixer (Kodak) for 5 min. Tissues were then rinsed in running distilled water for 1-2 h, counterstained with thionin (0.25 %), dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX.

6.4.5 cRNA probe synthesis and preparation

The pBlueScript SK minus plasmids containing a rat CD14 cDNA fragment of 1528 base pairs was linearized with *Sac* I and *Kpn* I for the antisense and sense riboprobes, respectively. Radioactive cRNA copies were synthesized by incubation of 250 ng linearized plasmid in 6 mM $MgCl_2$, 40 mM Tris (pH 7.9), 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 0.2 mM ATP/GTP/CTP, 200 µCi of α-^{35}S-UTP (Dupont NEN, #NEG 039H), 40U RNAsin (Promega, Madison, WI) and 20U of either T7 (CD14 antisense probe) or T3 (CD14 sense probe) RNA polymerase for 60 min at 37 °C. Unincorporated nucleotides were removed using ammonium-acetate method; 100 µl of DNAse solution (1 µl DNAse, 5 µl of 5 mg/ml tRNA, 94 µl of 10 mM tris/10 mM $MgCl_2$) was added, and 10 min later, a phenol-chloroform extraction was performed. The cRNA was precipitated with 80 µl of 5M ammonium acetate and 500 µl of 100% ethanol for 20 min on dry ice. The pellet was washed with 500 µl 70% ethanol, dried, and resuspended in 100 µl of 10 mM Tris/1 mM EDTA. A concentration of 10^7 cpm probe was mixed into 1 ml of hybridization solution (500 µl formamide, 60 µl 5 M NaCl, 10 µl 1 M Tris [pH 8.0], 2 µl 0.5 M EDTA [pH 8.0], 50 µl 20x Denhart's solution, 200 µl 50% dextran sulfate, 50 µl 10 mg/ml tRNA, 10 µl 1M

DTT, [118 µl Depc water - volume of probe used]). This solution was mixed and heated for 5 min at 65 °C before being spotted on slides.

6.4.6 Combination of immunocytochemistry with *in situ* hybridization

Immunocytochemistry was combined with the *in situ* hybridization histochemistry protocol (CD14 mRNA) to determine the types of cells that express CD14 transcript in the rat brain after systemic treatment with the bacterial endotoxin LPS. Among the antibodies selected for this study, OX-42 was used to stain the rat complement receptor type 3 on phagocytes (CD11b/c), such as monocytes, macrophages and microglia. Polyclonal antibody raised against CRF was also used to identify the cells expressing CD14 mRNA within parenchymal elements of the parvocellular division of the PVN; numerous CD14 positive cells of large diameter were observed in this division of the PVN where CRF cells are predominant. Fos immunostaining was used to determine if cells activated following acute immune challenge were able to synthesize the mCD14 receptor. Every sixth brain section was processed by using the avidin-biotin amplification bridge method with peroxidase as a substrate. Briefly, slices were washed in sterile Depc-treated 50 mM potassium phosphate-buffered saline (KPBS) and incubated at room temperature with either OX-42, CRF or Fos antibody mixed in sterile KPBS, 0.4% Triton X-100, 0.25% heparin sodium salt USP (ICN Biomedicals Inc., Aurora, OH) and 1% bovine serum albumin (fraction V, Sigma, St. Louis, MO). OX-42 monoclonal antibody raised in mouse (Cedarlane Lab. Limited, Hornby, Ont., Canada, Cat # CL042B) was diluted 1:2 500 in the solution described above. Rabbit antihuman/rat CRF serum (code PBL rc 70, 8/9/83 bleed), a generous gift from Dr. Wylie Vale (Peptide Biology Laboratory, The Salk Institute, La Jolla, CA), was used at a concentration of 1:20 000. Antiserum raised in rabbit against proto-oncogene Fos [Ab-5] (Oncogene Research Products, Cambridge, MA, Cat # PC38) was used at a concentration of 1:10 000. One to two hours after incubation with the primary antibodies (OX-42, CRF or Fos), the brain slices were rinsed in sterile KPBS and incubated with a mixture of KPBS + triton-X + heparin + biotinylated secondary antibodies (horse anti-mouse IgG for OX-42 and goat anti-rabbit IgG for CRF and Fos; 1:1 500 dilution; Vector Laboratories, CA) for 60 min. Sections were then rinsed with KPBS and incubated at room temperature for 60 min with an avidin-biotin-peroxidase complex (Vectastain ABC elite kit, Vector Laboratories, CA). After several rinses in sterile KPBS, the brain slices were reacted in a mixture containing sterile KPBS, the chromagen 3,3'-diaminobenzidine tetrahydrochloride (DAB, 0.05%), and 0.003 % hydrogen peroxide (H_2O_2).

Thereafter, tissues were rinsed in sterile KPBS, immediately mounted onto poly-L-lysine-coated slides, desiccated under vacuum for 30 min, fixed in 4% paraformaldehyde [pH 7.2] for 30 min, and digested by proteinase K (10 μg/ml in 100 mM tris HCl [pH 8.0] and 50 mM EDTA [pH 8.0]), at 37 °C for 25 min. Prehybridization, hybridization, and posthybridization steps were performed according to the above description with the difference of dehydration (alcohol 50, 70, 95, 100%), which was shortened to avoid decoloration of OX-42-, CRF-, and Fos-immunoreactive cells (brown staining). After being dried for 2 h under the vacuum, sections were exposed at 4 °C to X-ray film (Kodak) for 3 days, defatted in xylene, and dipped in NTB2 nuclear emulsion (Kodak; diluted 1:1 with distilled water). Slides were exposed for 14 to 21 days, developed in D19 developer (Kodak) for 3.5 min at 15 °C, and fixed in rapid fixer (Kodak) for 5 min. Tissues were then rinsed in running distilled water for 1 to 2 h, rapidly dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX. The presence of CD14 transcript was detected by the agglomeration of silver grains in perikarya, and OX-42, CRF or Fos immunoreactivity within the cell cytoplasm or nucleus (Fos-ir) was indicated by a brown homogeneous coloration.

6.4.7 Data analysis

Anatomical identification of brain structures was essentially based on the Swanson's atlas (33). The relative intensity of CD14 mRNA signal throughout the brain of each animal was assessed on X-ray film images and NTB-2-dipped slides and graded according to the scale of undetectable (-), low (+), moderate (++), strong (+++), or very strong signal (++++). The exact anatomical distribution of positive cells was accomplished on dipped emulsion slides under microscopic evaluation to ascertain the subcellular localization.

Determination of the double-labeled cells was performed visually for each cell exhibiting clear brown cytoplasm and a number of silver grains within the cell body that was at least five times higher than background.

6.5 RESULTS

Low to moderate basal expression of CD14 transcript was generally detected in various non-parenchymal structures of the brain, such as the choroid plexus (chp), the

leptomeninges and within blood vessels (bv) of the brain (Fig. 1, right column). Of interest is the fact that positive signal was not detected throughout the entire microvasculature of the brain, but within some bv irrigating particularly the forebrain. However, the signal in cells associated to bv increased throughout the entire brain of LPS-administered rats. Microscopic analysis of emulsion-dipped slides indicated that these non-parenchymal CD14 positive cells are likely to be of perivascular microglial-associated type; the round shape of their perikarya (in contrast to the fusiform shape of endothelial cells) is characteristic of perivascular microglia along the bv (Fig. 2, panels A, B and C). Moreover, the irregular signal across the bv does not support the hypothesis that the endothelial line of the blood-brain barrier is positive for the gene encoding CD14. Numerous studies have also reported the incapacity of the endothelial cells to express mCD14 under basal and immune-challenged conditions, while perivascular microglial-associated cells are known to display both the mCD14 protein and transcript in response to LPS. The positive signal observed across the brain microvasculature was increased 1 h after i.v. LPS, but peaked at time 3 h and declined progressively 6 and 24 h following the LPS-treatment.

I.v. and i.p. injection of the bacterial endotoxin also increased the expression of CD14 transcript in the chp and leptomeninges (Fig. 1, left and middle column). This increase of CD14 mRNA levels was apparent 1 h after the i.v. treatment, reached a maximum at 3 h post-injection (Fig. 2, panels D and E) and persisted until 24 h after LPS treatment. Meticulous analysis of emulsion-dipped slides revealed that some positive cells of the chp and leptomeninges were found surrounding bv and are likely to be perivascular microglial-associated cells. However, the high power brightfield photomicrograph (Fig. 2, panels D and E, right column) also exhibit positive CD14-hybridized cells that did not seem to be associated to the microvasculature irrigating the chp and leptomeninges. Because of the heterogeneity of the cell populations characterizing these non parenchymal structures (epithelial, ependymal, macrophage, ...), it was difficult without double-labeling procedure to clearly establish the cell type(s) that has (have) the ability to express the LPS receptor in the chp and leptomeninges of systemically endotoxin-challenged rats.

Systemic injection of LPS stimulated the expression of CD14 mRNA in a wide variety of nuclei and brain areas, an effect that was dependent on the dose and time following the LPS administration. In addition to the previously described non-parenchymal structures (chp, leptomeninges and bv), transcription of CD14 was rapidly induced in most of the CVOs, including the organum vasculosum of the lamina terminalis (OVLT), subfornical

organ (SFO), median eminence (ME), and area postrema (AP), 1 h after i.v. LPS administration. The intensity of the signal increased to its maximal level of expression at 3 h, whereas all the CVOs exhibited a reduced CD14 mRNA message at 6 h post-LPS injection. One day following the i.v. and i.p. injection of the endotoxin, the signal in the CVOs returned to the levels of control animals. A delayed response to LPS treatment was detected within different structures; a convincing expression of the mRNA encoding CD14 was observed for the first time 3 h after i.v. LPS administration in the medial preoptic area (MPOA), anterior hypothalamic area (AHA), supraoptic nucleus (SON), paraventricular nucleus of the hypothalamus (PVN), arcuate nucleus (ARC), and the nucleus of the solitary tract (NTS) (Fig. 1). Qualitative analysis also revealed that the hybridization signal for CD14 mRNA was still highly detectable 6 h after the treatment with the endotoxin in most of these areas, but completely vanished at 24 h. No clear hybridization signal was observed in these parenchymal structures under basal conditions and with the sense probe.

Examples of this time-related influence of the bacterial endotoxin on the expression of CD14 transcript is depicted by the Fig. 3, which exhibits darkfield photomicrographs of the OVLT, MPOA and AHA. The intensity of the signal for CD14 mRNA was moderate to high in the OVLT 1 h after the i.v. injection, strong and maximum at 3 h, moderate at 6 h and almost totally vanished at 24 h (Fig. 3, left column). In contrast, minimal to undetectable signal was observed in the MPOA and AHA of animals sacrificed 1 h following the i.v. administration of the endotoxin (Fig. 3, middle and right columns), respectively. Three hours after the LPS treatment, a moderate signal was found in the MPOA and the AHA. At that time, numerous scattered cells expressing CD14 mRNA were confined to those structures, while the message seemed to spread out the anatomical boundaries of the MPOA and AHA 3 h later. This was particularly obvious in animals treated with the highest doses of LPS i.v. and i.p. Animals sacrificed 24 h after the LPS injection displayed undetectable CD14 signal. These results strongly suggest that the bacterial endotoxin LPS first reaches the OVLT (an organ devoid of the blood brain barrier) to induce transcription of the gene encoding CD14 mRNA and thereafter increases CD14 mRNA levels in the regions surrounding the vascular organ.

It is very interesting to note that such phenomenon of migratory-like pattern was not only seen from the OVLT, but from all the other sensorial CVOs. Figures 4, 5 and 6 show darkfield photomicrographs of NTB-2-dipped slides hybridized with an antisense CD14 riboprobe through similar areas of the SFO, ME and AP, respectively. In the SFO and AP,

low to moderate levels of CD14 expression was noted 1 h after the i.v. injection with LPS. The pattern of expression was also quite similar in these two CVOs during the other post-injection times; a moderate but maximal signal was induced at 3 h, declined to low-to-moderate levels at 6 h and finally returned to baseline level 24 h following the systemic treatment. In the parenchymal structures adjacent to the SFO and AP, no message was observed 1 h after injection of LPS, whereas a widespread of CD14-expressing cells became apparent in the fimbria and NTS at 3 h; two structures closely related to the SFO and AP, respectively. Another clear example of migratory-like pattern is presented in figure 5; strong signal was detected in the ME 1 h after LPS administration, but at that time no labeling was observed in the adjacent ARC nuclei (C). Two h later, the ARC now exhibited moderate-to-strong CD14 levels, while the ME was entirely covered of CD14-expressing cells (D). The hybridization signal declined 6 h after the single i.v. LPS injection (E) and essentially vanished 1 day later (F).

Intravenous injection of the endotoxin significantly increased the transcription of the gene encoding CD14 in the endocrine hypothalamus, more specifically in the PVN and SON. Interestingly, the effect of LPS injection on the stimulation of CD14 transcription within the PVN and SON was slow and transient; CD14 transcript was not detected in the PVN and SON of animals sacrificed 1 h after LPS treatment (either i.v. or i.p.), whereas maximal expression of this gene took place at 3 h and essentially vanished at 6 h post i.v. injection time. Figure 7 (left column) depicts a representative example of the distribution of the mRNA encoding CD14 in these hypothalamic structures 3 h after i.v. administration of LPS. In the PVN, the signal was particularly intense within parenchymal elements of the parvocellular division beside the wall of the ventral third ventricle, which is directly in contact with the cerebrospinal fluid secreted by the chp. The high power brightfield photomicrograph of the Fig. 7 (see "insert" PVN/LPS) depicts CRF-immunoreactive (ir) perikarya of the parvocellular PVN that are positive for the LPS receptor. Few, but some, double-labeled CRF neurons were indeed found 3 h after i.v. LPS treatment (filled arrowheads), although this hypothalamic nucleus exhibited numerous single CRF-ir neurons and CD14-positive cells. In the SON, the distribution of cells expressing CD14 mRNA was more heterogeneous and several positive cells were located near the leptomeninges (Fig. 7, SON LPS). Although it is difficult to evaluate whether LPS may reach directly the endocrine hypothalamus, the possibility remains that endotoxin particles find their way through chp, leptomeninges, bv and CVOs. No constitutive expression of CD14 transcript was detected in these hypothalamic nuclei (Fig. 7, right column).

Figure 8 shows darkfield photomicrographs of an ubiquitous-like type of CD14 signal within different areas of the brain of LPS-challenged rats. Indeed, i.v. LPS treatment stimulated transcription of CD14 within parenchymal cells of the cerebral isocortex (Fig. 8A), the hippocampus (B), the tuberomammillary area (C) and the cerebellum (D). In this latter region, the signal was particularly intense over the leptomeninges and the bv, while scattered cells were found over the parenchymal granular cell layer. As described, this induction across the parenchymal brain was late, transient and dependent on the dose of the endotoxin. The lowest doses of the bacterial endotoxin injected i.v. (20 μg/kg) or i.p. (25 μg/kg) caused expression of CD14 in a more restricted pattern; *i.e.* bv, leptomeninges, CVOs and surrounding structures, whereas the highest doses (500 μg i.p./kg; 100 μg i.v./kg) induced the LPS receptor in numerous but clearly defined parenchymal regions, such as those depicted in the Figs. 1 and 8. Of interest is the fact that i.p. injection of a very high dose of LPS (2.5 g/kg) was able to provoke a robust CD14 expression across the whole brain without localized pattern 6 h after the treatment (data not shown).

6.5.1 Dual labeling data

To determine the cell types that express the gene encoding CD14 following systemic injection of LPS or vehicle solution, a combination of immunocytochemistry and *in situ* hybridization histochemistry was performed. Sections were immunoreacted against OX-42 to stain phagocytic cells (macrophages and microglia) and immediately after hybridized with an antisense rat CD14 isotopic riboprobe. OX-42-ir cells associated to bv, most likely perivascular microglia, were positive for the LPS receptor (Fig. 9A), whereas macrophages expressing CD14 mRNA were found in the chp and leptomeninges of LPS treated rats (Fig. 9B and C). As depicted in the panel D, very few (if any) Fos-ir epithelial lining cells were positive for CD14 in the leptomeninges, but double-labeled cells were detected into the subarachnoid space where fibroblasts, macrophages and endothelial cells are abundant. Since there is no clear evidence that endothelial cells are able to express CD14 receptor on their membrane surfaces and that some CD14-expressing cells were not immunoreactive to OX-42, fibroblasts are likely to be another cell type that express the LPS receptor within the subarachnoid space. It is interesting that fibroblasts has previously been shown to display high levels of CD14 (34).

The profile of distribution of the hybridized signal across the brain parenchyma of endotoxin-challenged animals along with the small size of the cells led us to believe that these CD14-positive cells were of glial type. Dual labeling with a specific antisera against OX-42 provided strong evidence that a large number of the cells that express the gene encoding LPS receptor during severe blood sepsis are of parenchymal microglial type. The Fig. 9 (panels E and F) shows examples of such phenomenon within various structures of the brain, including the ME/ARC and AP/NTS where numerous OX-42-ir cells exhibited CD14 transcript. However, not all CD14 positive cells were colocalized within OX-42-ir perikarya, suggesting that more than one type of parenchymal cells could express mCD14 during endotoxeamia. Expression of the LPS receptor has recently been reported in rat primary astrocytes (35), although we faced some technical difficulties to unravel these glial cells throughout the brain using different antisera against GFAP rending double-labeling difficult. No conclusion can therefore be reached at the moment for astrocytes.

6.6 DISCUSSION

The present study shows that specific cellular populations of the central nervous system (CNS) have the ability to express the gene encoding the membrane (m) form of CD14 LPS receptor. Under basal conditions, low levels of CD14 mRNA were detected in various non-parenchymal structures of the brain, such as the leptomeninges, the chp and within blood vessels (bv) of the brain microvasculature. Systemic injection of the bacterial endotoxin induced strong expression of CD14 transcript within these same structures as well as in many other regions, including the OVLT/MPOA, SFO, PVN, ME/ARC and the AP/NTS. In the non-parenchymal structures enumerated above and all the sensorial CVOs, the CD14 mRNA signal was rapidly detected at 1 h, reached a peak at 3 h post-injection, declined at 6 h and returned to basal levels 24 h after LPS treatment. A delayed response to LPS treatment was detected in the brain parenchyma within numerous nuclei where robust expression of the mRNA encoding CD14 was observed 3 and 6 h after the systemic challenge. Interestingly, microscopic analysis of emulsion-dipped slides revealed that CD14 positive cells spread over the anatomical boundaries of these parenchymal structures in a migratory-like pattern 6 h following i.v. LPS administration. Taken together, these results strongly suggest that the bacterial endotoxin LPS first reaches organs devoid of the blood brain barrier (BBB) to induce the transcription of its own receptor and thereafter increased CD14 biosynthesis within parenchymal structures surrounding the CVOs and then the entire brain of severely challenged animals. Using immunocytochemistry/*in situ* hybridization

double labeling, CD14 mRNA was identified within various cells of the brain, including CVO resident macrophages, parenchymal and perivascular-associated microglia as well as few CRF neurons of the hypothalamic PVN. The direct action of LPS on myeloid- and non-myeloid-derived parenchymal cells expressing mCD14 could be responsible for the modulation of numerous responses occurring during endotoxaemia, such as the production of proinflammatory cytokines of central origin and activation of the corticotroph axis for the appropriate release of glucocorticoids. Moreover, it is possible that cells expressing CD14 mRNA play a key role in the defense of the neural parenchyma against invading LPS particles penetrating the CNS via altered BBB during blood sepsis or unselective diffusion through available target structures, such as the CVOs and chp.

Systemic LPS administration is a well accepted model of infection and inflammation that activates phagocytes and stimulates the synthesis and release of cytokines into the circulation. It is believed that the secretion of these proinflammatory molecules by stimulated systemic monocytes/macrophages, neutrophils and lymphocytes is directly responsible for most of the effects of the endotoxin in the brain. Among many others, stimulation of CRF neurons and the consequent activation of the HPA axis reflected by elevated plasma glucocorticoid levels is a classical LPS response; a phenomenon of great importance in the immunosuppressive processes. Although systemic injections of IL-1ß, IL-6 or TNF-α are capable of mimicking these neuroendocrine events (10, 12, 14-16, 36, 37), proinflammatory cytokines of systemic origin may not be essential for LPS-induced activation of the HPA axis. Systemic administration of IL-1-receptor antagonist protein failed to prevent the increase of CRF mRNA in the PVN and circulating corticosterone levels (38, 39), whereas mice receiving TNF-α antibody, either alone or in combination with an IL-1-receptor antagonist protein, still exhibited robust activation of the HPA axis in response to LPS (38). It has also been demonstrated that the surge of plasma ACTH and corticosterone levels obtained after intra-arterial infusion of LPS preceded by at least 30 min measurable proinflammatory cytokines into the bloodstream (40). These results clearly indicate that cytokines of systemic origin secreted in response to LPS administration are dispensable in the early stages of HPA axis stimulation. However, the possibility remains that these cytokines contribute in the prolonged and sustained neuroendocrine responses to systemic endotoxaemia. Pretreatment with antibodies specific for either IL-1 receptor or TNF-α was indeed able to prevent LPS-induced ACTH release, but at times not earlier than 4 h post LPS challenge, whereas simultaneous administration of both antibodies diminished, but did not eliminate, the ACTH release at 2 h (41). In consequence, systemic production of cytokines may not be an essential step in the early neuroendocrine changes provoked by the bacterial

endotoxin, a fact that has been further supported in proinflammatory cytokine-deficient mice (for review, see 27).

As mentioned, significant increase of circulating levels of ACTH and corticosterone takes place as early as 15 to 30 min after systemic injection of LPS, while IL-1, IL-6 and TNF-α were not yet detected in the plasma (40). Although it has been suggested that lymphocyte-derived ACTH can play a role in triggering corticosterone release in immune-challenged animals (42), this hypothesis remains highly controversial and many studies do support the concept of neuroendocrine CRF-mediated mechanisms (43, 44). Few, but some, CRF neurons of the PVN exhibited positive CD14 signal, indicating that LPS may directly target the neurons controlling the HPA axis. However, the induction was quite late and whether systemic LPS may reach these cells has yet be to confirmed. On the other hand, it is possible that LPS stimulates accessible structures to the bloodstream, which play key roles in the activation of the neurons controlling the corticotroph axis. The ME is a good candidate and is rather well positioned to be a direct target of circulating endotoxin to modulate the HPA axis, because the region is devoid of BBB, exhibits a robust induced expression of mCD14 mRNA and contains the nerve terminals of the neurons that secrete into the infundibular system. Moreover, qualitative analysis of autoradiographic films and emulsion-dipped slides revealed that the ME is the structure that expressed the highest levels of CD14 mRNA at 1 h post-injection time. The present manuscript does not show the effects of LPS on CD14 transcription at times preceding 1 h, although it is highly possible that LPS stimulates CD14 expression in the ME within few minutes following the i.v. treatment. Circulating LPS into the bloodstream could therefore bind to its own receptor in the ME and among different functions, trigger infundibular CRF release and corticotroph axis-related hormone secretion.

Rapid induction of CD14 mRNA was also observed in the OVLT, SFO, AP, chp, leptomeninges and bv of the whole brain microvasculature 1 h after systemic (both i.v. and i.p.) LPS injection; no CD14 mRNA signal was detected in any other brain parenchymal cells at that time. These results can be explained by the fact that almost all these structures have been shown to be accessible to large molecules circulating into the bloodstream. The sensorial CVOs contain a rich vascular plexus with specialized arrangements of the bv. The tight junctions normally present between the endothelial cells are shifted in part to the ventricular surface and partly to the boundary between the CVOs and the adjacent structures explaining the diffusion of large molecules into the perivascular region (45). It is very likely

that LPS injected into the general circulation penetrates the OVLT, SFO, ME and AP tissues, which then allow the endotoxin to trigger locally the synthesis of its own receptor. The chp and leptomeninges are also recognized as being highly vascularized regions and agglomeration of silver grains forming CD14 positive cells were detected along bv irrigating these structures, although numerous isolated positive cells were also found. Since it is virtually impossible to cut through these structures without isolating cells of the microvasculature, the possibility remains that a large proportion of the cells expressing CD14 mRNA in the chp and the leptomeninges at early post-challenge times are perivascular-associated cells or macrophages and therefore in direct contact with molecules of the blood.

At fairly low doses of LPS, CD14 receptor is recognized to be essential for the release of proinflammatory cytokines (20, 21, 24, 46) and recent studies indicate that NF-κB is likely to be the key mediator through which the endotoxin provokes such cellular response. Yamamoto and coworkers have recently demonstrated that CD14-mediated LPS stimulus triggers the translocation of NF-κB and this mechanism seems dependent on the production of the second messenger diacylglycerol (47). Moreover, *in vitro* studies using antibodies recognized to block the LPS binding to its CD14 receptor, have shown a dramatic decrease of NF-κB binding activity and marked diminution of IL-1ß and TNF-α synthesis following LPS treatment in the CD14+ cell lines (21, 48). Although NF-κB induction alone may not be sufficient for the action of LPS to take place, several studies have nonetheless demonstrated that the transcription factor NF-κB is involved in mediating the transcription of genes encoding the LPS-inducible proinflammatory cytokines IL-1ß, IL-6 and TNF-α (49-57). NF-κB is normally present into the cytoplasm forming an inactive complex with an inhibitor known as I-κBα. Following extracellular stimulation by growth factors, mitogens, cellular activators, and activators of second messengers, I-κBα is phosphorylated by intracellular kinases and degraded by cytoplasmic protease releasing, by the same token, active NF-κB (58, 59). Free active NF-κB is then translocated into the nucleus where it will be able to regulate transcription of various genes, including proinflammatory cytokines. Using detection of I-κBα transcript as a marker of NF-κB activity, Quan and coworkers have recently shown a dramatic increase of the I-κBα mRNA levels in the CVOs, chp and throughout the whole brain microvasculature 1 and 2 h after i.p. LPS administration (60). In the present study, we were able to detect a rapid induction of CD14 in the CVOs, chp and bv, indicating that LPS binding to CD14 may be responsible for the transcription of IκB in the structures devoid of the BBB.

Interestingly, systemic administration of the bacterial endotoxin LPS was shown to induce transcription of the genes encoding the proinflammatory cytokines in the regions of the rat brain that display rapid induction of CD14 and I-κBα. Using *in situ* hybridization histochemistry, maximal intensity of IL-1ß mRNA were observed in the CVOs, chp, leptomeninges and bv at 2 h post-injection time (61), while cells expressing IL-6 transcript were detected 3 and 6 h after i.p. administration of the bacterial LPS (17). The proinflammatory cytokine IL-1ß is recognized to be an important stimulator of IL-6 production in peripheral organs (62), although i.v. injection of recombinant rat IL-1ß did not activate expression of IL-6 mRNA in the CNS (17). A direct action of the bacterial endotoxin on cells forming the CVOs and chp may therefore be responsible for the production of IL-6 within the brain, but the possibility remains that the IL-1ß of central origin may increase the production of IL-6. We have also recently found that systemic LPS treatment stimulates TNF-α transcription in a time frame and pattern quite similar to CD14 and I-κBα mRNA expression in the rat brain (S. Nadeau and S. Rivest, *in preparation*). It is permit to believe that the effects of LPS on the activation of CD14/NF-κB and synthesis or proinflammatory cytokines are related phenomena within specific cellular population of the brain, such as macrophages, perivascular-associated microglia and parenchymal microglial cells.

The physiological relevance of LPS-induced CD14 in the rat brain and the biosynthesis of different cytokines within the CVOs and chp has yet to be clarified. The rapid expression of CD14 and the increase of proinflammatory cytokines synthesis in the OVLT may be a central mechanism participating in the thermogenic effects of the bacterial endotoxin. The OVLT/MPOA is a crucial region involved in the control of thermoregulation and the central injection of IL-6 antibody attenuates the fever and thermogenesis induced by the endotoxin (63, 64). Abnormal fever response has also been found in IL-6-deficient mice injected with the bacterial endotoxin (65). The SFO and AP also exhibit positive signal for CD14 and proinflammatory cytokine mRNAs and cell populations expressing CD14, IL-1 and TNF-α after LPS treatment were identified as being of microglial type (66, 67; S. Nadeau and S. Rivest, *in preparation*, and this manuscript). It is conceivable that cytokines produced by microglial cells in response to LPS and/or cytokines of systemic origin bind to their receptors associated to nearby neurons, which may provide direct or indirect afferent projections to modulate specific neuronal functions. Interestingly, cells expressing mRNA for IL-1 type 1 and IL-6 subunit receptors have been localized in the CVOs (17, 68) and neural activity has been observed in the CVOs following systemic administration of IL-1ß (11, 69), IL-6 (13) and TNF-α (S. Nadeau and S. Rivest, *in preparation*).

The chp and cerebral microvasculature are also in good position to transduce the information received from systemic circulation to the brain parenchymal cells. We report here that the CD14 signal increased rapidly in cells associated to these structures after LPS treatment and microscopic analysis of emulsion-dipped slides indicated that most of the non-parenchymal CD14 positive cells are likely to be of perivascular microglial-associated and macrophage type. Indeed, the gene encoding CD14 was not uniformly expressed across the fusiform endothelium forming the internal lumen of the microvasculature and several bv exhibited only one or two CD14 positive cells, although a more uniform pattern of expressing cells were detected in other capillaries (see Fig. 2A). The cells were nevertheless not forming the internal edge of the microvasculature, a phenomenon that does not support the endothelia as being positive for the transcript. Moreover, double labeling with OX-42 provided clear evidence that perivascular-microglial associated cells have the capacity to express the gene encoding CD14 in response to systemic endotoxin challenge.

In agreement with our data, numerous studies have reported the incapacity of the endothelium to express mCD14, although these cells were shown to play a major role in the pathogenesis of gram-negative bacterial sepsis via free soluble CD14 (70-74). In fact, LPS can trigger tyrosine phosphorylation of mitogen-activated protein kinases within endothelial cells despite the lack of mCD14 (73) and this event was reported to be associated to NF-κB and cyclooxygenase enzyme 2 (COX-2) activation (60, 75). The bacterial endotoxin-induced release of proinflammatory cytokines from systemic phagocytes may also contribute to activate the endothelium of brain capillaries; IL-1 type 1 receptor mRNA is predominantly expressed in cerebral vascular endothelial cells, leptomeninges and chp (68, 76), whereas mRNAs encoding TNF-α receptors were detected in cerebrovascular endothelial cells under basal conditions (77). Moreover, we have recently reported that LPS, IL-1β and TNF-α i.v. injection caused COX-2 gene transcription in the chp, leptomeninges and along the entire brain microvasculature (75). It is conceivable that binding of proinflammatory cytokines to their receptors or a direct effect of LPS on the endothelium may induce prostaglandin synthesis that may diffuse through parenchymal elements, which in turn, modulate different brain functions occurring during endotoxaemia.

Expression of CD14 within the leptomeninges was quite robust, especially when both the pia and arachnoid matters remained intact in the tissue processing. In such cases, we observed a profound induction of the immediate-*early* gene c-*fos* within the squamous

epithelial cells lining each membrane, although very few (if any) Fos-ir lining cells were positive for CD14. However, dual-labeling Fos-ir/CD14 mRNA were detected in the subarachnoid space where numerous CD14 cells were generally found. Using an antisera against OX-42, we were able to see several macrophages that contained convincing CD14 signal (Fig. 9, panel D), but single CD14 expressing cells were also present. Their size and shape strongly suggest the fibroblasts as having the ability to express the LPS receptor. Interestingly, CD14 has recently been shown to be expressed at high density in human gingival fibroblasts and this event was shown to mediate the LPS-induced stimulation of AP-1 and NF-κB transcription factors (34). The exact role of CD14 in fibroblasts of the leptomeninges has yet to be determined, although it is possible that LPS is capable of stimulating the synthesis of cytokines within these non-myeloid-derived cells of the subarachnoid space.

Apart from its recognized role as a glycosyl-phosphatidylinositol (GPI)-anchored membrane protein that induces, in presence of LPS, activation of tyrosine kinase leading to transduction signal and cytokine gene transcription, CD14 has also been suggested to serve as an opsonic receptor for LPS-coated particles resulting in an uptake and clearance of LPS by the activated cells (74). In this regard, our data showing the biphasic regulation of CD14 in the rat brain are quite fascinating; the transcript was rapidly detected in the CVOs and the chp and dual labeling procedure indicated that most of these expressing cells were of myeloid type, including macrophages (chp) and microglial (CVOs). However, a migratory-like pattern was observed and 6 h after injection of high doses of LPS, positive cells were found over the entire brain. Although we found several parenchymal microglial cells that were positive for one of the LPS receptor, numerous CD14-expressing cells were not OX-42 immunoreactive. Non-myeloid parenchymal cells of epithelial origin, such as astrocytes and neurons, should therefore be considered and the fact that CD14 was expressed within few neuroendocrine CRF neurons supports this concept. In agreement with these data is the elegant study showing that LPS can stimulate the transcription of CD14 in non-myeloid epithelial cells of various peripheral tissues (74), a phenomenon that seems dependent on the production of cytokines from phagocytes. In the present case, it is possible that LPS targets first the myeloid cells of the CVOs and chp, which in turn may produce proinflammatory cytokines and upregulate parenchymal CD14 expression in a dose- and time-dependent manner as presented in this study. It will be crucial to determine if such mechanism is indeed taking place in the brain and to investigate its physiological relevance. One hypothesis is that CD14 expression in parenchymal cells of the CNS primes the activated cells to produce cytokines and/or prepare them to increase the clearance of the endotoxin particles that may

reach the CSF and be toxic for the neurons. This complex machinery may be of great importance to maintain the body homeostasis and protect neurons during infectious diseases and severe blood sepsis.

6.7 ACKNOWLEDGMENTS

This research was supported by the Medical Research Council (MRC) of Canada. Steve Lacroix holds a Studentship from the National Sciences and Engineering Research Council of Canada (NSERC). Serge Rivest is a Medical Research Council Scholar. The authors thank Dr. Wylie Vale (The Salk Institute, CA) for the CRF antisera and Ms. Nathalie Laflamme for invaluable technical assistance.

6.8 REFERENCES

1. Rivest, S., N. Laflamme and R.E. Nappi. 1995. Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. *J. Neurosci.* 15:2680-2695.

2. Orth, D.N., W.J. Kovacs and C.R. DeBold. 1992. The adrenal cortex. *In* Textbook of Endocrinology. J.D. Wilson and D.W. Foster, J.D. Wilson and D.W. Fosters. W. B. Saunders, Philadelphia. 418-619.

3. Edwards, C.K., L.M. Yunger, R.M. Lorence, R. Dantzer and K.W. Kelley. 1991. The pituitary gland is required for protection against lethal effects of Salmonella typhimurium. *Proc. Natl. Acad. Sci. USA* 88:2274-2277.

4. Mason, D., I. MacPhee and F. Antoni. 1990. The role of the neuroendocrine system in determining genetic susceptibility to experimental allergic encephalomyelitis. *Immunology* 70:1-5.

5. Sternberg, E.M., J.M. Hill, G.P. Chrousos, T. Kamilaris, S.J. Listwak, P.W. Gold and R.L. Wilder. 1989. Inflammatory mediator-induced hypothalamic-pituitary-adrenal axis activation is defective in streptoccocal cell wall arthritis-susceptible Lewis rats. *Proc. Natl. Acad. Sci. USA* 86:2374-2378.

6. Besedovsky, H.O. and A. Del Rey. 1996. Immune-neuro-endocrine interactions: Facts and hypotheses. *Endocr. Rev.* 17:64-102.

7. Dinarello, C.A. 1984. Interleukin-1 and the pathogenesis of the acute-phase response. *New Eng. J. Med.* 311:1413-1422.

8. Ertel, W., M.H. Morrison, P. Wang, Z.F. Ba, A. Ayala and I.H. Chaudry. 1992. The complex pattern of cytokines in sepsis - association between prostaglandins, cachectin, and interleukins. *Ann Surg.* 214:141-148.

9. Kushner, I. 1982. The phenomenon of the acute-phase response. *Ann. NY Acad. Sci.* 389:39-48.

10. Berkenbosch, F., J.V. Oers, A.D. Rey, F. Tilders and H. Besedovsky. 1987. Corticotropin-releasing factor producing neurons in the rat activated by interleukin-1. *Science* 238:524-526.

11. Ericsson, A., K.J. Kovacs and P.E. Sawchenko. 1994. A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *J. Neurosci.* 14:897-913.

12. Sapolsky, R., C. Rivier, G. Yamamoto, P. Plotsky and W. Vale. 1987. Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* 238:522-524.

13. Vallières, L., S. Lacroix and S. Rivest. 1997. Influence of interleukin-6 on neural activity and transcription of the gene encoding corticotropin-releasing factor in the rat brain: an effect depending upon the route of administration. *Eur. J. Neurosci.* 9:1461-1472.

14. Naitoh, Y., J. Fukata, T. Tominaga, Y. Nakai, T. Sunao, M. Kenjiro and H. Imura. 1988. Interleukin-6 stimulates the secretion of adrenocorticotropic hormone in conscious, freely-moving rats. *Biochem. Biophys. Res. Comm.* 155:1459-1463.

15. Bernardini, R., T.C. Kamilaris, A.E. Calogero, E.O. Johnson, M.T. Gomez, P.W. Gold and G.P. Chrousos. 1990. Interactions between tumor necrosis factor-α, hypothalamic corticotropin-releasing hormone, and adrenocorticotropin secretion in the rat. *Endocrinology* 126:2876-2881.

16. Watanobe, H. and K. Takebe. 1992. Intravenous administration of tumor necrosis factor-α stimulates corticotropin-releasing hormone secretion in the push-pull cannulated median eminence of freely moving rats. *Neuropeptides* 22:81-84.

17. Vallières, L. and S. Rivest. 1997. Regulation of the genes encoding interleukin-6, its receptor, and gp130 in the rat brain in response to the immune activator lipopolysaccharide and the proinflammatory cytokine interleukin-1ß. *J. Neurochem.* 69:1668-1683.

18. Wright, S.D., R.A. Ramos, M. Patel and D.S. Miller. 1992. Septin: A factor in plasma that opsonizes lipopolysaccharide-bearing particles for recognition by CD14 on phagocytes. *J. Exp. Med.* 176:719-727.

19. Schumann, R.R., S.R. Leong, G.W. Flaggs, P.W. Gray, S.D. Wright, J.C. Mathison, P.S. Tobias and R.J. Ulevitch. 1990. Structure and function of lipopolysaccharide binding protein. *Science* 249:1429-1431.

20. Wright, S.D., R.A. Ramos, P.S. Tobias, R.J. Ulevitch and J.C. Mathison. 1990. CD14, a receptor for complexes of lipolysaccharide (LPS) and LPS binding protein. *Science* 249:1431-1433.

21. Kitchens, R.L., R.J. Ulevitch and R.S. Munford. 1992. Lipopolysaccharide (LPS) partial structures inhibit responses to LPS in a human macrophage cell line without inhibiting LPS uptake by a CD14-mediated pathway. *J. Exp. Med.* 176:485-494.

22. Wright, S.D., R. Ramos, A. Hermanowski-Vosatka, P. Rockwell and P.A. Detmers. 1991. Activation of the adhesive capacity of CR3 on neutrophils by endotoxin: dependence on lipopolysaccharide binding protein and CD14. *J. Exp. Med.* 173:1281-1286.

23. Lee, J.D., V. Kravchenko, T.N. Kirkland, J. Han, N. Mackman, A. Moriarty, D. Leturcq, P.S. Tobias and R.J. Ulevitch. 1993. Glycosyl-phosphatidylinositol-anchored or integral membrane forms of CD14 mediate identical cellular responses to endotoxin. *Proc. Natl. Acad. Sci. USA* 1990:9930-9934.

24. Leturcq, D.J., A.M. Moriarty, G. Talbott, R.K. Winn, T.R. Martin and R.J. Ulevitch. 1996. Antibodies against CD14 protect primates from endotoxin-induced shock. *J. Clin. Invest.* 98:1533-1538.

25. Ferrero, E., D. Jiao, B.Z. Tsuberi, L. Tesio, G.W. Rong, A. Haziot and S.M. Goyert. 1993. Transgenic mice expressing human CD14 are hypersensitive to lipopolysaccharide. *Proc. Natl. Acad. Sci. USA* 90:2380-2384.

26. Golenbock, D.T., Y. Liu, M.F. H., M.W. Freeman and R.A. Zoeller. 1993. Surface expression of human CD14 in Chinese hamster ovary fibroblasts imparts macrophage-like responsiveness to bacterial endotoxin. *J. Biol. Chem.* 268:22055-22059.

27. Fantuzzi, G. and C.A. Dinarello. 1996. The inflammatory response in interleukin-1ß-deficient mice: comparison with other cytokine-related knock-out mice. *J. Leukoc. Biol.* 59:489-493.

28. Fattori, E., M. Cappelletti, P. Costa, C. Sellito, L. Cantoni, M. Carelli, R. Faggioni, G. Fantuzzi, P. Ghezzi and V. Poli. 1994. Defective inflammatory response in interleukin 6-deficient mice. *J. Exp. Med.* 180:1243-1250.

29. Zheng, H., D. Fletcher, W. Kozak, M. Jiang, K. Hofmann, C.A. Conn, D. Soszynski, C. Grabiec, M.E. Trumbauer, A. Shaw, M.J. Kostura, K. Stevens, H. Rosen, R.J. North, H.Y. Chen, M.J. Tocci, M.J. Kluger and L.H.T. Van der Ploeg. 1995. Resistance to fever induction and impaired acute-phase response in interleukin-1ß-deficient mice. *Immunity* 3:9-19.

30. Lacroix, S. and S. Rivest. 1996. Role of cyclo-oxygenase pathways in the stimulatory influence of immune challenge in the transcription of a specific CRF receptor subtype in the rat brain. *J. Chem. Neurochem.* 10:53-71.

31. Lacroix, S. and S. Rivest. 1997. Functional circuitry in the brain of immune-challenged rats: partial involvement of prostaglandins. *J. Comp. Neurol.* 387:307-324.

32. Simmons, D.M., J.L. Arriza and L.W. Swanson. 1989. A complete protocol for *in situ* hybridization of messenger RNAs in brain and other tissus with radio-labeled single-stranded RNA probes. *J. Histotechnol.* 12:169-181.

33. Swanson, L.W. 1992. Brain maps: structure of the rat brain. Elsevier, New York, NY. pp.

34. Watanabe, A., A. Takeshita, S. Kitano and S. Hanazawa. 1996. CD14-mediated signal pathway of Porphyromonas gingivalis lipopolysaccharide in human gingival fibroblasts. *Infect. Immun.* 64:4488-4494.

35. Galea, E., D.J. Reis, E.S. Fox, H. Xu and D.L. Feinstein. 1996. CD14 mediates endotoxin induction of nitric oxide synthase in cultured brain glial cells. *J. Neuroimmunol.* 64:19-28.

36. Tsagarakis, S., G. Gillies, L.H. Rees, M. Besser and A. Grossman. 1989. Interleukin-1 directly stimulates the release of corticotrophin-releasing factor from rat hypothalamus. *Neuroendocrinology* 49:98-101.

37. Sharp, B.M., S.G. Matta, P.K. Peterson, R. Newton, C. Chao and K. McAllen. 1989. Tumor necrosis factor-alpha is a potent ACTH secretagogue: comparison to interleukin-1ß. *Endocrinology* 124:3131-3137.

38. Dunn, A.J. 1992. The role of interleukin-1 and tumor necrosis factor alpha in the neurochemical and neuroendocrine responses to endotoxin. *Brain Res. Bull.* 6:807-812.

39. Kakucska, I., Y.Qi, B.D. Clark and R.M. Lechan. 1993. Endotoxin-induced corticotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus is mediated centrally by interleukin-1. *Endocrinology* 133:815-821.

40. Givalois, L., J. Dornand, M. Mekaouche, M.D. Solier, A.F. Bristow, G. Ixart, P. Siaud, I. Assenmacher and G. Barbanel. 1994. The temporal cascade of plasma level surges in ACTH, corticosterone and cytokines in endotoxin-challenged rats. *Am. J. Physiol.* 266:R164-R170.

41. Perlstein, R.S., M.H. Whitnall, J.S. Abrams, E.H. Mougey and R. Neta. 1993. Synergistic roles of interleukin-6, interleukin-1, and tumor necrosis factor in the adrenocorticotropin response to bacterial lipopolysaccharide in vivo. *Endocrinology* 132:946-952.

42. Blalock, J. and E.M. Smith. 1985. A complete loop between the immune system and neuroendocrine systems. *Fed. Proc.* 44:108-111.

43. Rivest, S. and C. Rivier. 1995. The role of CRF and interleukin-1 in the regulation of neurons controlling reproductive functions. *Endocrine Rev.* 16:177-199.

44. Rivest, S. 1995. Molecular mechanisms and neural pathways mediating the influence of interleukin-1 on the activity of neuroendocrine CRF motoneurons in the rat. *Int. J. Devl. Neurosci.* 13:135-146.

45. Oldfield, B.J. and M.J. Mckinley. 1995. Circumventricular organs. *In* The rat nervous system. G. Paxinos, G. Paxinoss. Academic Press, San Diego. 391-403.

46. Lee, J.D., K. Kato, P.S. Tobias, T.N. Kirkland and R.J. Ulevitch. 1992. Transfection of CD14 into 70Z/3 cells dramatically enhances the sensitivity to complexes of lipopolysaccharide (LPS) and LPS binding protein. *J. Exp. Med.* 175:1697-1705.

47. Yamamoto, H., K. Hanada and M. Nishijima. 1997. Involvement of diacylglycerol production in activation of nuclear factor kappa-B by a CD14-mediated lipopolysaccharide stimulus. *Biochem. J.* 325:223-228.

48. Pollack, M., C.A. Ohl, D.T. Golenbock, F. Di Padova, L.M. Wahl, N.L. Koles, G. Guelde and B.G. Monks. 1997. Dual effects of LPS antibodies on cellular uptake of LPS and LPS-induced proinflammatory functions. *J. Immunol.* 159:3519-3530.

49. Hiscott, J., J. Marois, J. Garoufalis, M. D'Addario, A. Roulston, I. Kwan, N. Pepin, J. Lacoste, H. Nguyen, G. Bensi and M. Fenton. 1993. Characterization of a functional NF-κB site in the human interleukin 1ß promoter: Evidence for a positive autoregulatory loop. *Mol. Cell. Biol.* 13:6231-6240.

50. Shimizu, H., K. Mitomo, T. Watanabe, S. Okamoto and K. Yamamoto. 1990. Involvement of a NF-kappa B-like transcription factor in the activation of the interleukin-6 gene by inflammatory lymphokines. *Mol. Cell. Biol.* 10:561-568.

51. Libermann, T.A. and D. Baltimore. 1990. Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. *Mol. Cell. Biol.* 10:2327-2334.

52. Dendorfer, U., P. Oettgen and T.A. Libermann. 1994. Multiple regulatory elements in the interleukin-6 gene mediate induction by prostaglandins, cyclic AMP, and lipopolysaccharide. *Mol. Cell. Biol.* 14:4443-4454.

53. Zhang, Y., M. Broser and W.N. Rom. 1994. Activation of the interleukin 6 gene by Mycobacterium tuberculosis or lipopolysaccharide is mediated by nuclear factors NF-IL6 and NF-kappa B. *Proc. Natl. Acad. Sci. USA* 91:2225-2229.

54. Kuprash, D.V., I.A. Udalova, R.L. Turetskaya, N.R. Rice and S.A. Nedospasov. 1995. Conserved kappa-B element located downstream of the tumor necrosis factor alpha gene distinct NF-kappa-B binding pattern and enhancer activity in LPS activated murine macrophages. *Oncogene* 11:97-106.

55. Shakhov, A.N., M.A. Collart, P. Vassalli, S.A. Nedospasov and C.V. Jongeneel. 1990. Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor alpha gene in primary macrophages. *J. Exp. Med.* 171:35-47.

56. Collart, M.A., P. Baeuerle and P. Vassalli. 1990. Regulation of tumor necrosis factor alpha transcription in macrophages: involvement of four κB-like motifs and of constitutive and inducible forms of NF-κB. *Mol. Cell. Biol.* 10:1498-1506.

57. Drouet, C., A.N. Shakhov and C.V. Jongeneel. 1991. Enhancers and transcription factors controlling the inducibility of the tumor necrosis factor-α promoter in primary macrophages. *J. Immunol.* 147:1694-1700.

58. Henkel, T., T. Machleidt, I. Alkalay, M. Kronke, Y. Ben-Neriah and P.A. Baeuerle. 1993. Rapid proteolysis of I kappa B-alpha is necessary for activation of transcription factor NF-kappa B. *Nature* 365:182-185.

59. Ghosh, S. and D. Baltimore. 1990. Activation in vitro of NF-kappa B by phosphorylation of its inhibitor I kappa B. *Nature* 344:678-682.

60. Quan, N., M. Whiteside, L. Kim and M. Herkenham. 1997. Induction of inhibitory factor κBα mRNA in the central nervous system after peripheral lipopolysaccharide administration: An *in situ* hybridization histochemistry study in the rat. *Proc. Natl. Acad. Sci. USA* 94:10985-10990.

61. Quan, N., M. Whiteside and M. Herkenham. 1998. Time course and localization patterns of interleukin-1ß messenger RNA expression in brain and pituitary after peripheral administration of lipopolysaccharide. *Neuroscience* 83:281-293.

62. Akira, S., T. Hirano, T. Taga and T. Kishimoto. 1990. Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). *FASEB J.* 4:2860-2867.

63. Blatteis, C.M., J.R.S. Hales, M.J. Mckinley and A.A. Fawcett. 1987. Role of the anteroventral third ventricle in fever in sheep. *Can. J. Physiol. Pharmacol.* 65:1255-1260.

64. Blatteis, C.M., S.L. Bealer, W.S. Hunter, Q.J. Llanos, R.A. Ahokas and T.A.J. Mashburn. 1983. Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. *Brain Res. Bull.* 11:519-526.

65. Chai, Z., S. Gatti, C. Toniatti, V. Poli and T. Bartfai. 1996. Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. *J. Exp. Med.* 183:311-316.

66. Buttini, M. and H. Boddeke. 1995. Peripheral lipopolysaccharide stimulation induces interleukin-1ß messenger RNA in rat brain microglial cells. *Neuroscience* 65:523-530.

67. Van Dam, A.M., M. Brouns, S. Louisse and F. Berkenbosch. 1992. Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of endotoxin-treated rats: a pathway for the induction of non-specific symptoms of sickness? *Brain Res.* 588:291-296.

68. Ericsson, A., C. Liu, R.P. Hart and P.E. Sawchenko. 1995. Type 1 interleukin-1 receptor in the rat brain: distribution, regulation, and relationship to sites of IL-1-induced cellular activation. *J. Comp. Neurol.* 361:681-698.

69. Brady, L., A.B. Lynn, M. Herkenham and Z. Gottesfeld. 1994. Systemic interleukin-1 induces early and late patterns of c-*fos* mRNA expression in brain. *J. Neurosci.* 14:4951-4964.

70. Arditi, M., J. Zhou, R. Dorio, G.W. Rong, S.M. Goyert and K.S. Kim. 1993. Endotoxin-mediated endothelial cell injury and activation: role of soluble CD14. *Infect. Immun.* 61:3149-3156.

71. Haziot, A., G.W. Rong, J. Silver and S.M. Goyert. 1993. Recombinant soluble CD14 mediates the activation of endothelial cells by lipopolysaccharide. *J. Immunol.* 151:1500-1508.

72. Pugin, J., C.C. Schürer-Maly, D. Leturcq, A. Moriarty, R.J. Ulevitch and P.S. Tobias. 1993. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. *Proc. Natl. Acad. Sci. USA* 90:2744-2748.

73. Arditi, M., J. Zhou, M. Torres, D.L. Durden, M. Stins and K.S. Kim. 1995. Lipopolysaccharide stimulates the tyrosine phosphorylation of mitogen-activated protein kinases p44, p42, and p41 in vascular endothelial cells in a soluble CD14-dependent manner. *J. Immunol.* 155:3994-4003.

74. Fearns, C., V.V. Kravchenko, R.J. Ulevitch and D.J. Loskutoff. 1995. Murine CD14 gene expression in vivo: extramyeloid synthesis and regulation by lipopolysaccharide. *J. Exp. Med.* 181:857-866.

75. Lacroix, S. and S. Rivest. 1998. Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. *J. Neurochem.* 70:452-466.

76. Van Dam, A.M., H.E. DeVries, J. Kuiper, F.J. Zijlstra, A.G. DeBoer, F.J.H. Tilders and F. Berkenbosch. 1996. Interleukin-1 receptors on rat brain endothelial cells: a role in neuroimmune interaction? *FASEB J.* 10:351-356.

77. Bebo, B.F.J. and D.S. Linthicum. 1995. Expression of mRNA for 55-kDa and 75-kDa tumor necrosis factor (TNF) receptors in mouse cerebrovascular endothelium: effects of interleukin-1 beta, interferon-gamma and TNF-alpha on cultured cells. *J. Neuroimmunol.* 62:161-167.

Table 1:

Hybridization signal for CD 14 mRNA in the rat brain following systemic LPS injection (25 μg i.v.).

Time post LPS / Brain Region	1 hour		3 hours		6 hours		24 hours	
	LPS	VEH	LPS	VEH	LPS	VEH	LPS	VEH
Organum vasculosum lamina terminalis	++	0/+	+++	0/+	++	0/+	0/+	—
Medial preoptic area	0/+	—	++	—	+/++	—	—	—
Supraoptic nucleus	0/+	—	+	—	0/+	—	—	—
Subfornical organ	+/++	0/+	++	0/+	+/++	0/+	0/+	0/+
Hypothalamic paraventricular nucleus	0/+	—	++	—	+	—	—	—
Arcuate nucleus	—	—	++/+++	—	0/+	—	0/+	—
Median eminence	+++	0/+	+++/++++	—	+/++	—	0/+	0/+
Area postrema	+/++	0/+	++	0/+	+/++	0/+	0/+	0/+
Choroid plexus	+/++	+	++	+	++	+	++	+
Leptomeninges	++/+++	+/++	+++	+/++	++/+++	+	++	+
Parenchymal ubiquitous-like signal	0/+	0/+	++	0/+	+/++	0/+	0/+	0/+

Legends: ++++, very strong signal; +++, strong signal; ++, moderate signal; +, low but positive signal; —, undetectable signal; LPS, lipopolysaccharide; VEH, vehicle treatment.

Figure 1:

Representative example of the influence of endotoxin lipopolysaccharide (LPS) injection on the distribution of the mRNA encoding CD14 in the rat brain. Animals were sacrificed 3 h after intravenous (i.v.; 100 µg/kg b.w.) or intraperitoneal (i.p.; 500 µg/kg b.w.) treatment with LPS or the vehicle solution. These rostro-caudal coronal sections (30 µm) of LPS-treated rats exhibit a positive signal on X-ray films (Biomax) for CD14 mRNA in various parenchymal and non-parenchymal structures of the brain. 3V, third ventricle; AQ, aqueduct; AP, area postrema; ARC, arcuate nucleus; bv, blood vessels; Cer, cerebellum; chp, choroid plexus; CP, caudate putamen; DG, dentate gyrus; LGc, lateral geniculate complex; LHA, lateral hypothalamic area; LV, lateral ventricle; ME, median eminence; MPOA, medial preoptic area; oc, optic chiasm; OVLT, organum vasculosum of the lamina terminalis; PB, parabrachial nucleus; PG, pontine gray; PVN, paraventricular nucleus of the hypothalamus; SFO, subfornical organ; SON, supraoptic nucleus; VLM, ventrolateral medulla.

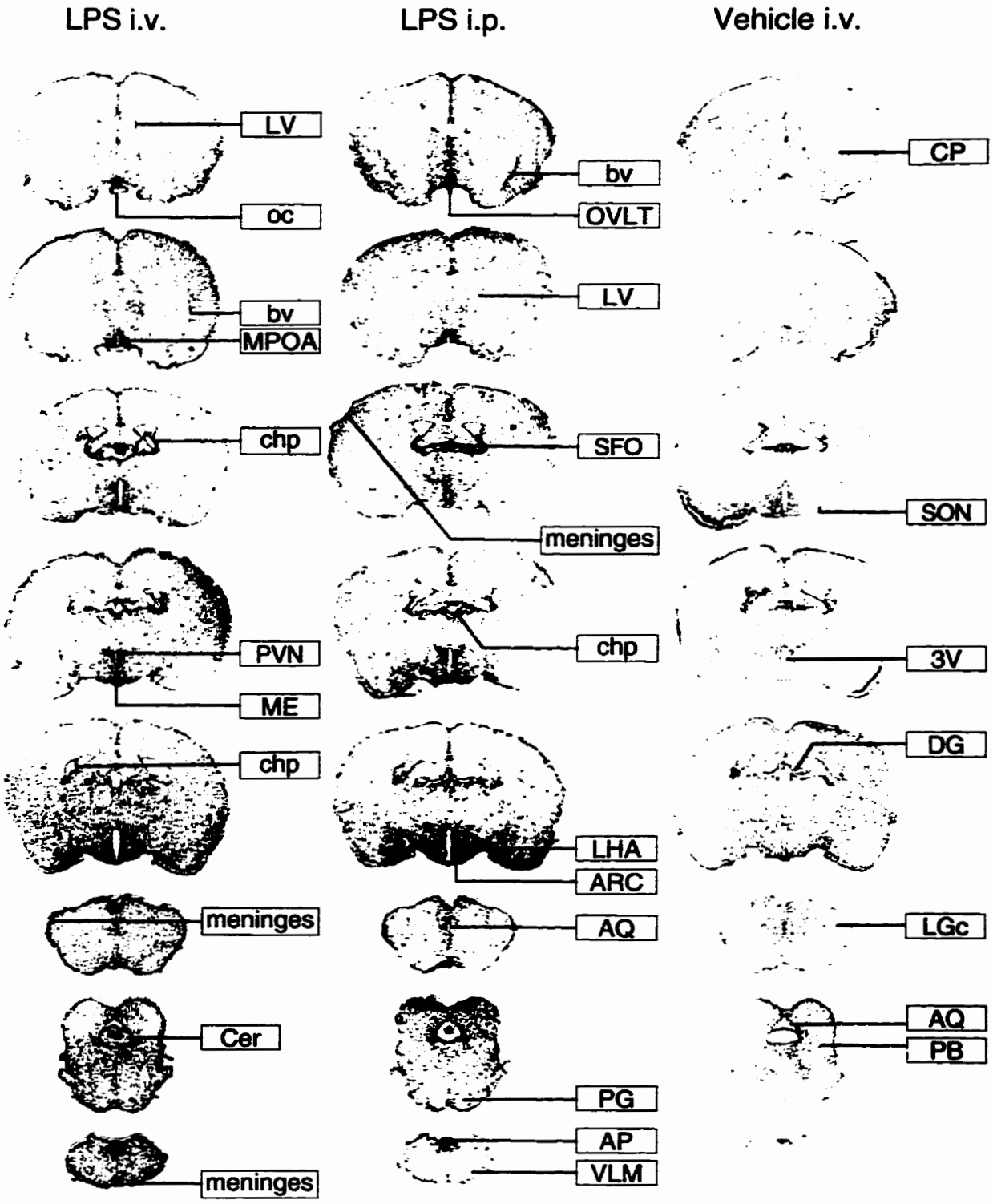

LPS i.v.
LPS i.p.
Vehicle i.v.
LV
oc
bv
MPOA
chp
PVN
ME
chp
meninges
Cer
meninges
bv
OVLT
LV
SFO
meninges
chp
LHA
ARC
AQ
PG
AP
VLM
CP
SON
3V
DG
LGc
AQ
PB

Figure 2:

Low-power darkfield (left column) and high-power brightfield (right column) photomicrographs showing the expression of CD14 mRNA in blood vessels (A, B and C), choroid plexus (D), and leptomeninges (E) of rats sacrificed 3 h after intravenous (i.v.) injection of the bacterial endotoxin lipopolysaccharide (100 μg of LPS/kg b.w.). Note the robust hybridization signal within non-parenchymal cells surrounding the blood vessels, choroid plexus and leptomeninges 3 h following the LPS challenge. Note also that CD14-positive cells in the blood vessels of LPS treated rats are round in contrast to the fusiform shape of endothelial cells and not uniformly distributed around the internal lumen of the microvasculature suggesting that they are most likely perivascular microglial-associated cells. The arrows in B and C point the blood vessels observed under brightfield at high magnification. Magnification panels A, B, C and E (left column), X25, Scale bar = 100 μm; panel D (left column), X10, Scale bar = 250 μm; right column, X100, Scale bar = 25 μm.

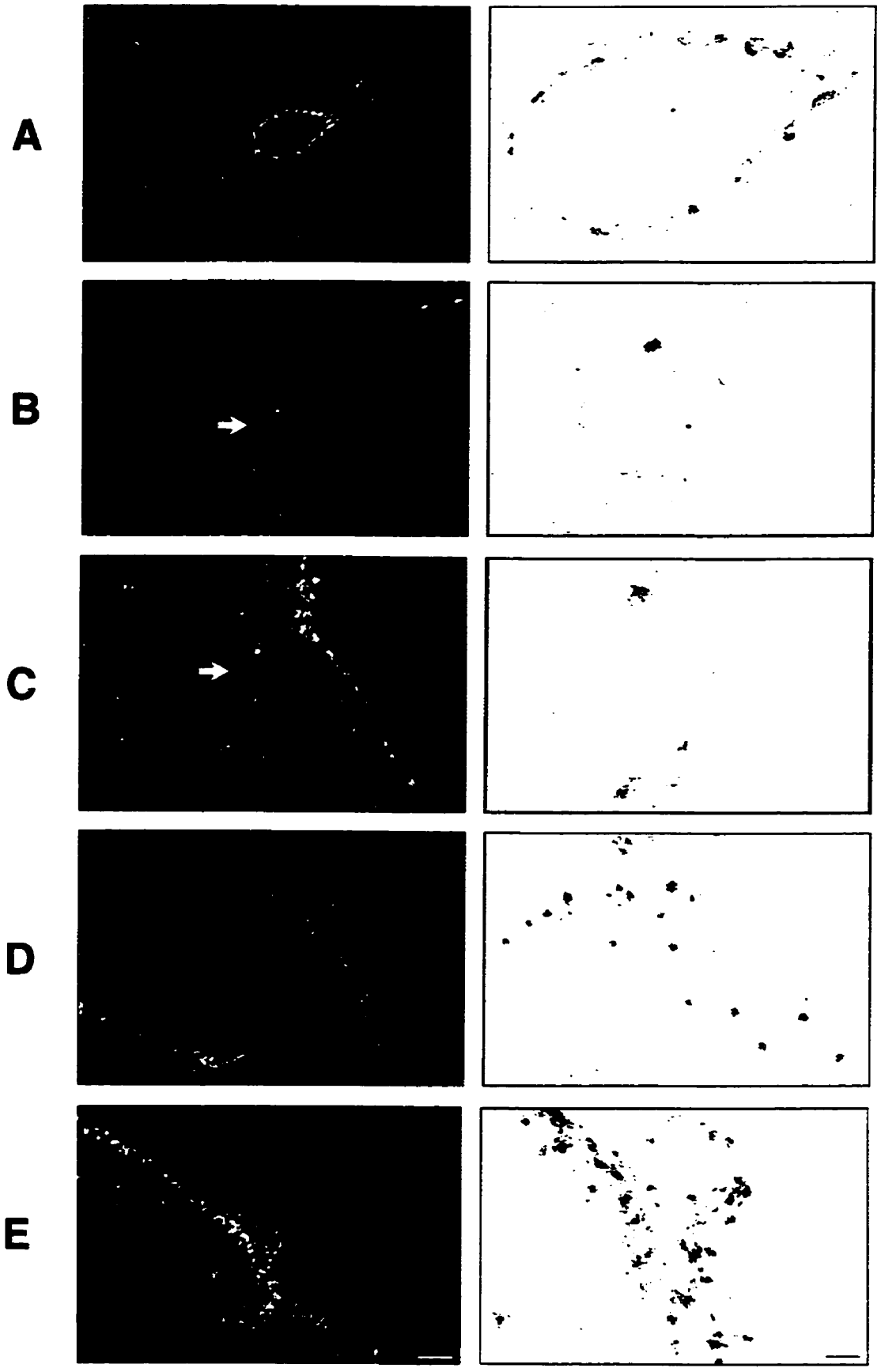

A
B
C
D
E

Figure 3:

Intravenous (i.v.) lipopolysaccharide (LPS; 100 μg/kg b.w.) caused a migratory-like pattern of CD14 expression from the organum vasculosum of the lamina terminalis (OVLT) to the medial preoptic area (MPOA) and anterior hypothalamic area (AHA). These darkfield photomicrographs were taken from dipped nuclear emulsion sections of animals sacrificed 1, 3 and 24 h after i.v. injection of LPS or vehicle solution (top panels). Note the positive hybridization signal in the OVLT as early as 1 h after LPS treatment, whereas the MPOA and AHA did not show detectable CD14 signal at this post-injection time. Three hours after LPS treatment, numerous scattered cells expressing CD14 mRNA were found in these structures, suggesting a migratory-like pattern of CD14-positive cells from the OVLT to the surrounding parenchymal brain. Magnification, X10, Scale bar = 250 μm.

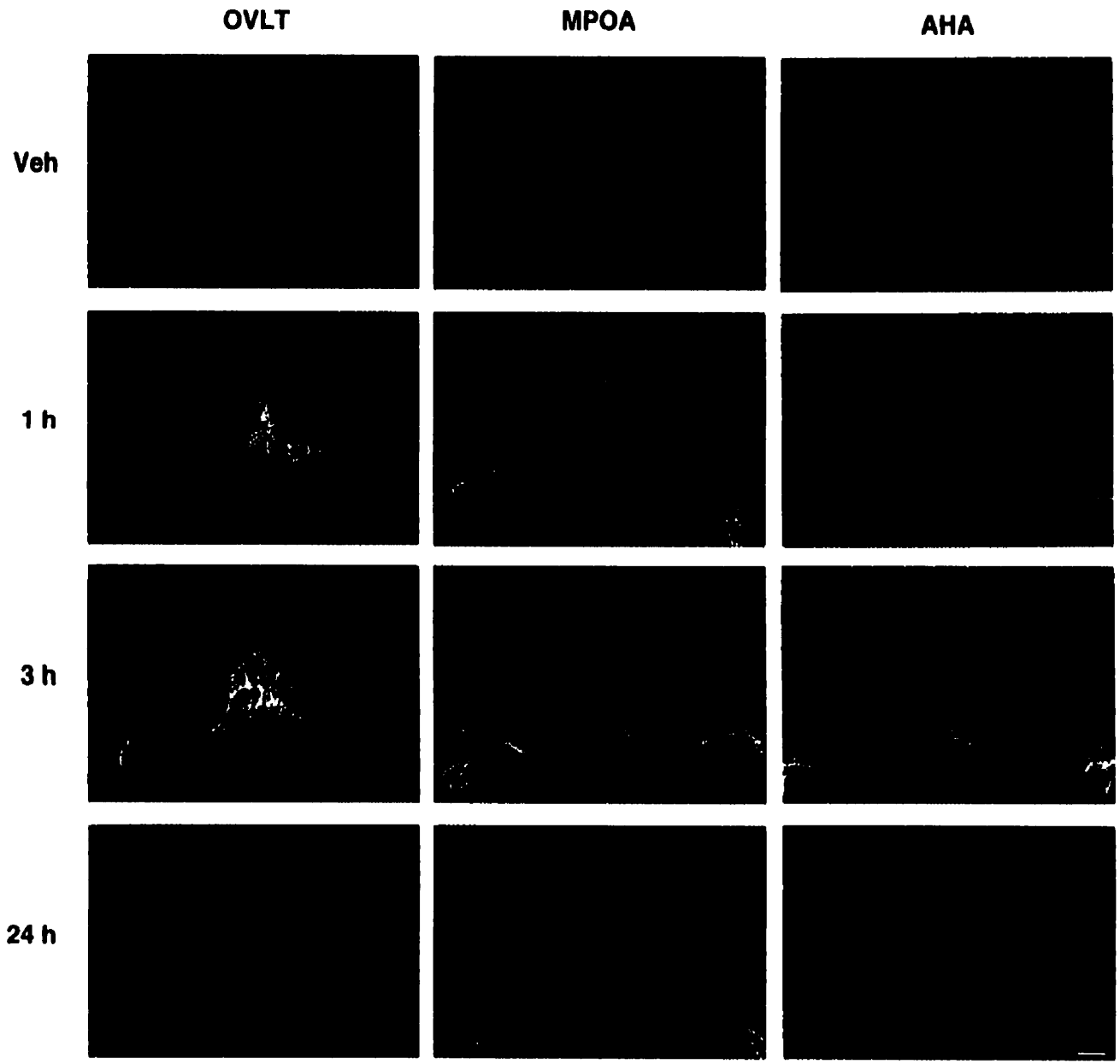

OVLT
MPOA
AHA
Veh
1 h
3 h
24 h

Figure 4:

Time-related influence of intravenous (i.v.) administration of the bacterial endotoxin lipopolysaccharide (LPS; 100 µg/kg b.w.) on the expression of CD14 mRNA through identical area of the subfornical organ (SFO). These darkfield photomicrographs of dipped NTB-2 emulsion slides depict CD14 mRNA hybridization in rats sacrificed after vehicle injection (B), or 1 h (C), 3 h (D), 6 h (E) and 24 h (F) following i.v. LPS-treatment. The signal for CD14 mRNA in the SFO was first detected at 1 h, reached a peak at 3 h post-injection, declined at 6 h, and returned to basal expression 24 h after LPS treatment. Note that some CD14-positive cells across the anatomical boundaries of the SFO became apparent at 3 h, whereas the message seemed to spread out even more 3 h later. The schema from panel A was taken from the atlas of Swanson (33). Magnification, X10, Scale bar = 250 µm.

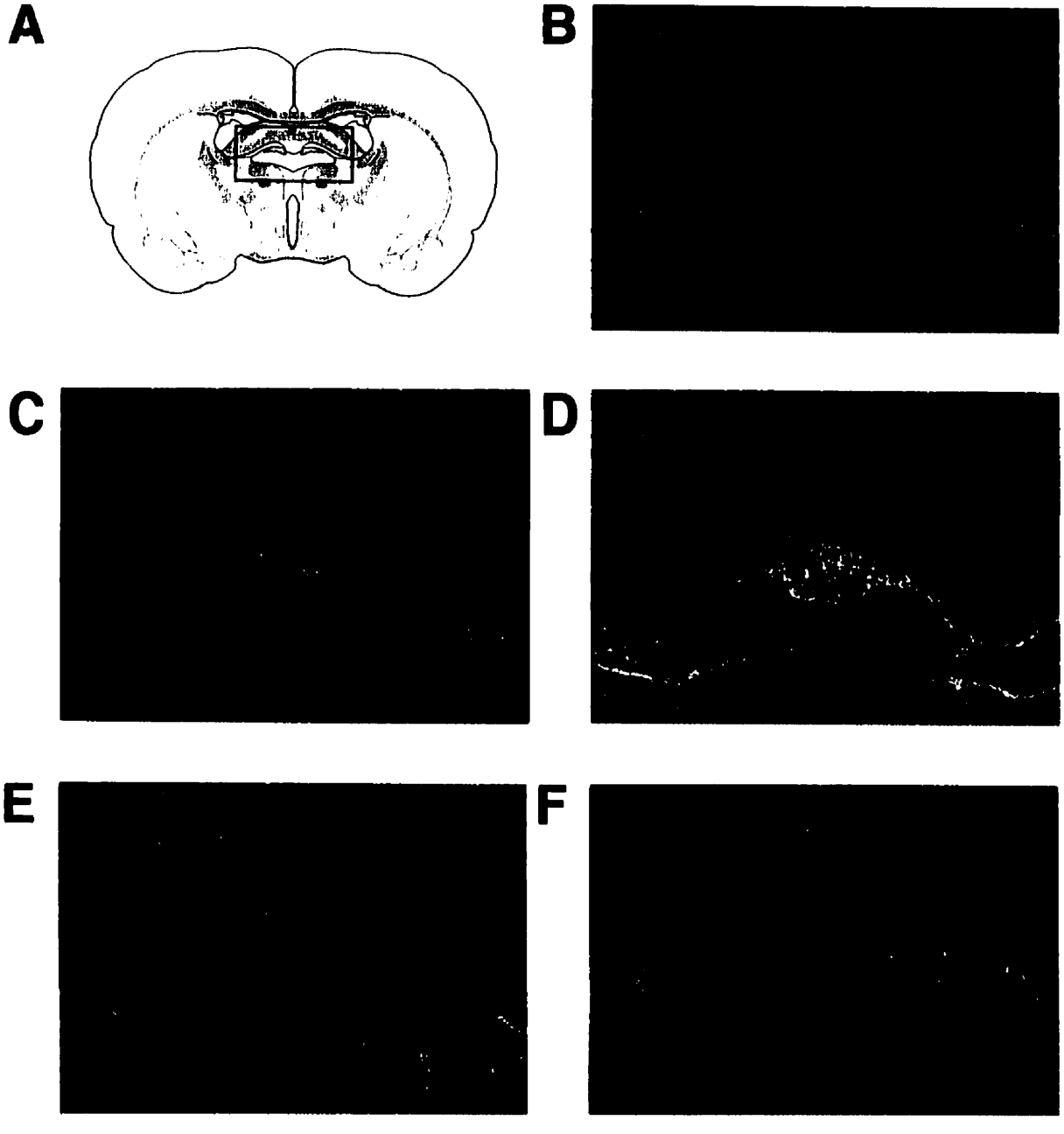

A
B
C
D
E
F

Figure 5:

Effects of systemic i.v. injection of lipopolysaccharide (LPS; 100 μg/kg b.w.) on the gene encoding CD14 in the median eminence (ME) and the arcuate nuclei (ARC) of the hypothalamus. These darkfield photomicrographs of dipped NTB-2 emulsion slides depict CD14 mRNA hybridization in rats sacrificed after vehicle injection (B) or 1 h (C), 3 h (D), 6 h (E) and 24 h (F) following i.v. LPS-treatment. The signal for CD14 mRNA in the ME was first detected at 1 h, reached a peak at 3 h post-injection, declined at 6 h and returned to basal levels 24 h after LPS treatment. Note that CD14-positive cells became apparent in the arcuate nucleus at 3 h. The schema from panel A was taken from the atlas of Swanson (33). Magnification, X25, Scale bar = 100 μm.

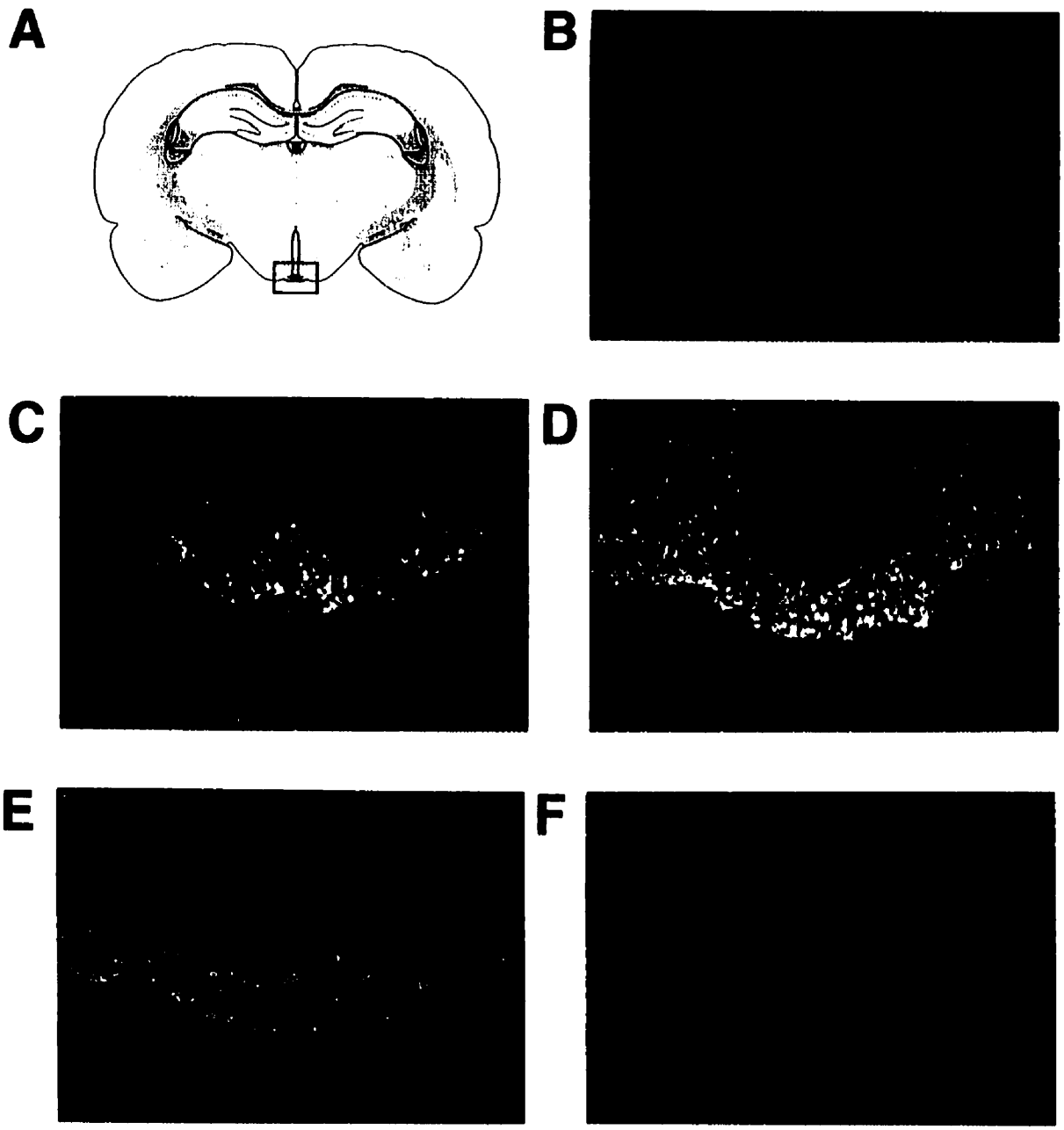

A
B
C
D
E
F

Figure 6:

Time-related influence of intravenous (i.v.) administration of the bacterial endotoxin lipopolysaccharide (LPS) on the expression of CD14 mRNA through identical area of the area postrema (AP). These darkfield photomicrographs of dipped NTB-2 emulsion slides depict CD14 mRNA hybridization in rats sacrificed under basal conditions (B) or 1 h (C), 3 h (D), 6 h (E) and 24 h (F) after the LPS challenge (100 µg/kg b.w.). The signal for CD14 mRNA in the AP was first detected at 1 h, reached a peak at 3 h post-injection, declined at 6 h and vanished 24 h following the treatment with the endotoxin. Note that CD14-positive cells became apparent in the nucleus of the solitary tract (NTS) at 3 h, whereas the message spread out over the NTS anatomical boundaries 3 h later. The schema from panel A was taken from the atlas of Swanson (33). Magnification, X10, Scale bar = 250 µm.

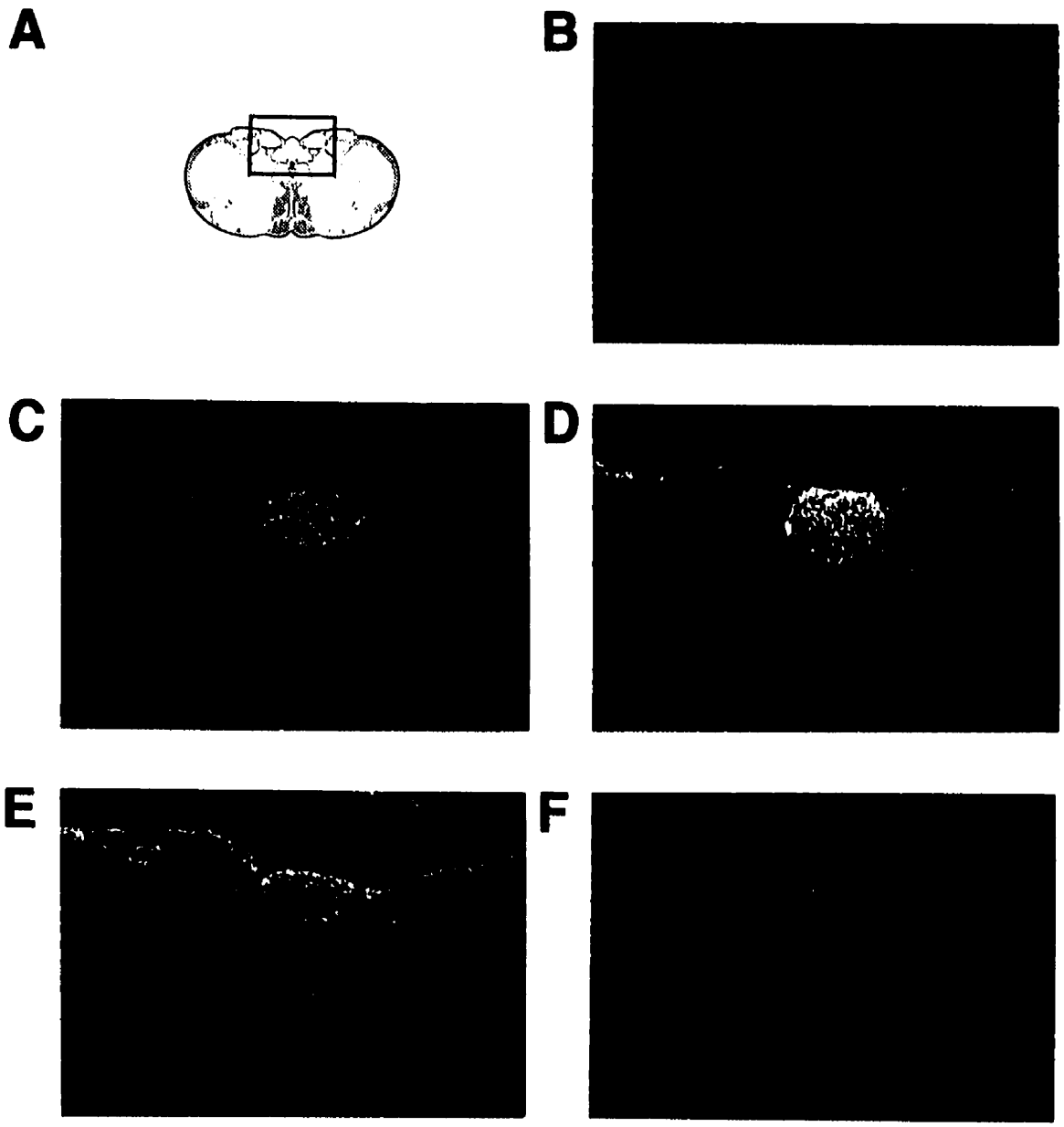

A
B
C
D
E
F

Figure 7:

Representative example of the effect of intravenous injection of LPS or vehicle solution on the expression of CD14 mRNA in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the rat hypothalamus. These darkfield photomicrographs of dipped NTB-2 emulsion slides depict positive hybridization signal for CD14 mRNA over the parvocellular division of the PVN and SON 3 h after i.v. injection of LPS (100 μg/kg b.w.). The brightfield photomicrograph inserted adjacent to the PVN of LPS-treated rats depicts examples of dual-labeled neurons immunoreactive for corticotropin-releasing factor (CRF) and expressing CD14 transcript. Note that few double-labeled neurons were found, while numerous CD14- and CRF-containing cells were not colocalized together in the hypothalamic PVN. For more details on the dual-labeling procedure, please see the Material and Methods. The schema of the right column were taken from the atlas of Swanson (33). Magnification, X25, Scale bar = 100 μm; brightfield photomicrograph insert, X250, Scale bar = 19 μm.

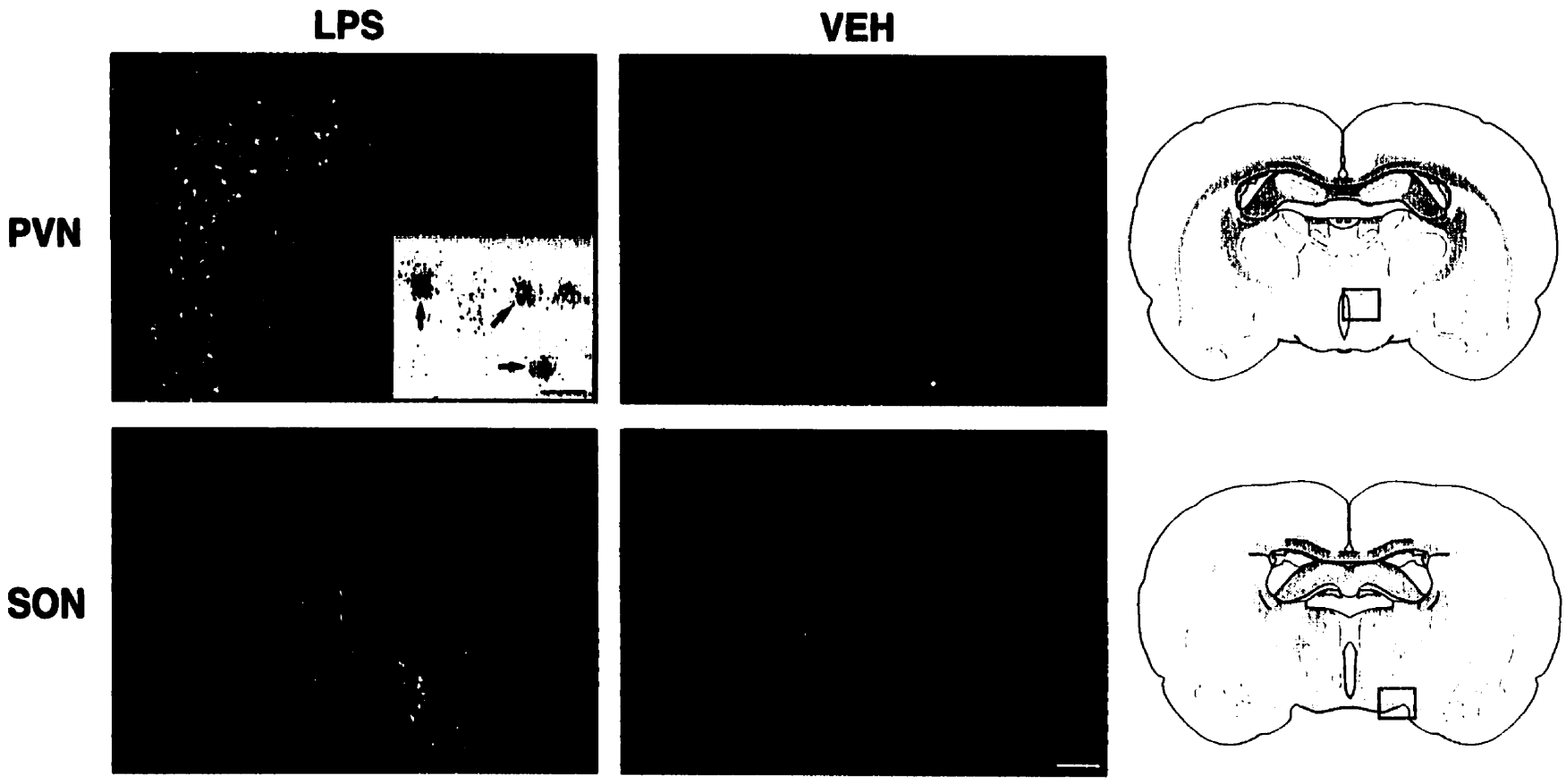

LPS
VEH
PVN
SON

Figure 8:

Representative examples of the ubiquitous-like CD14 signal within different areas of the brain of rats sacrificed 3 (panels A, C and D) and 6 (panel B) h after intravenous injection of LPS (100 μg/kg b.w.) or vehicle solution. These darkfield photomicrographs of nuclear emulsion-dipped sections show positive hybridization signal for CD14 mRNA in the cerebral isocortex (A), hippocampus (B), tuberomammillary area (C) and within the leptomeninges, blood vessel-associated cells and over the granular cell layer of the cerebellum (D). The schema of the right column were taken from the atlas of Swanson (33). Magnification panel A, X25, Scale bar = 100 μm; other panels, X10, Scale bar = 250 μm.

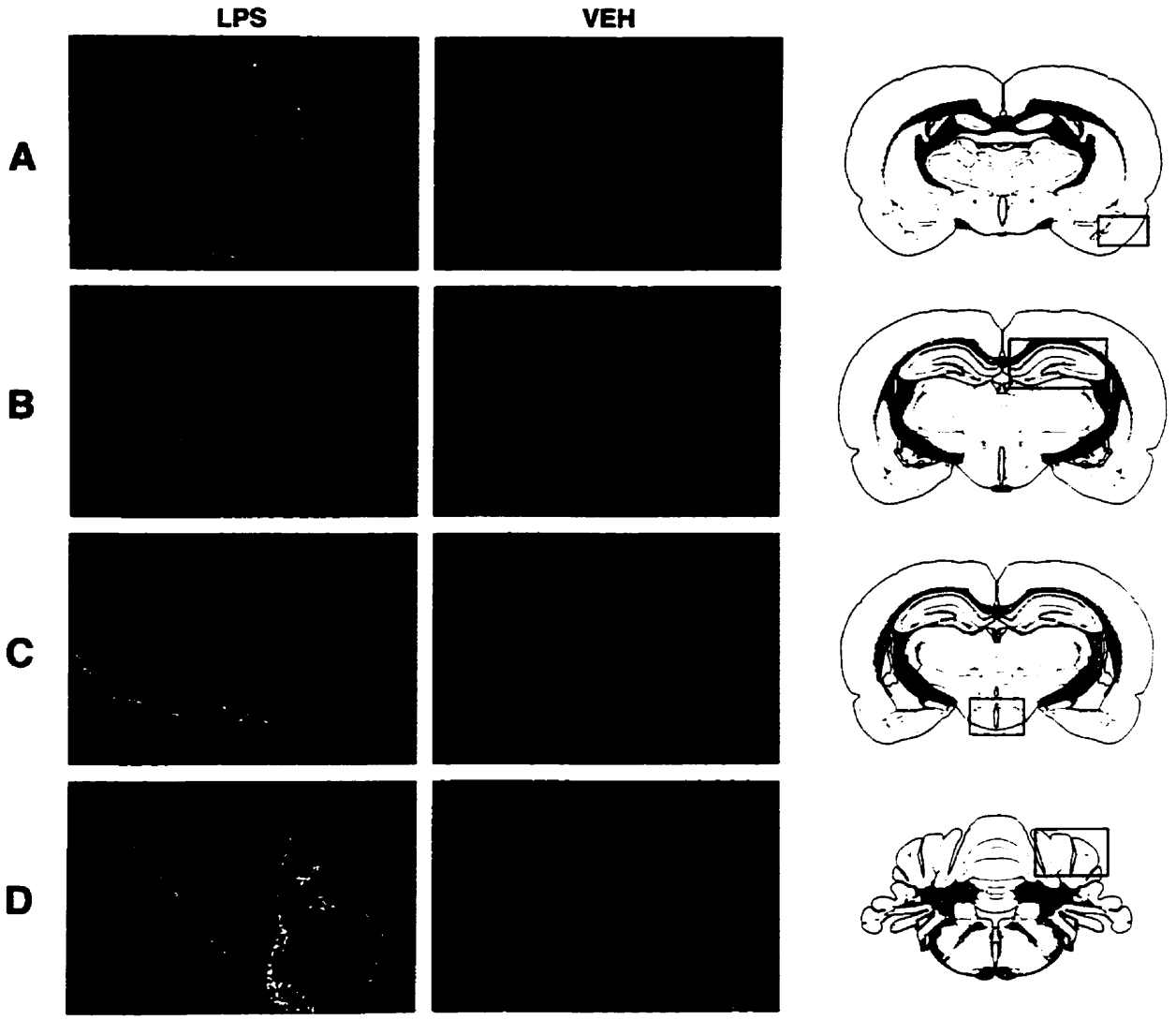
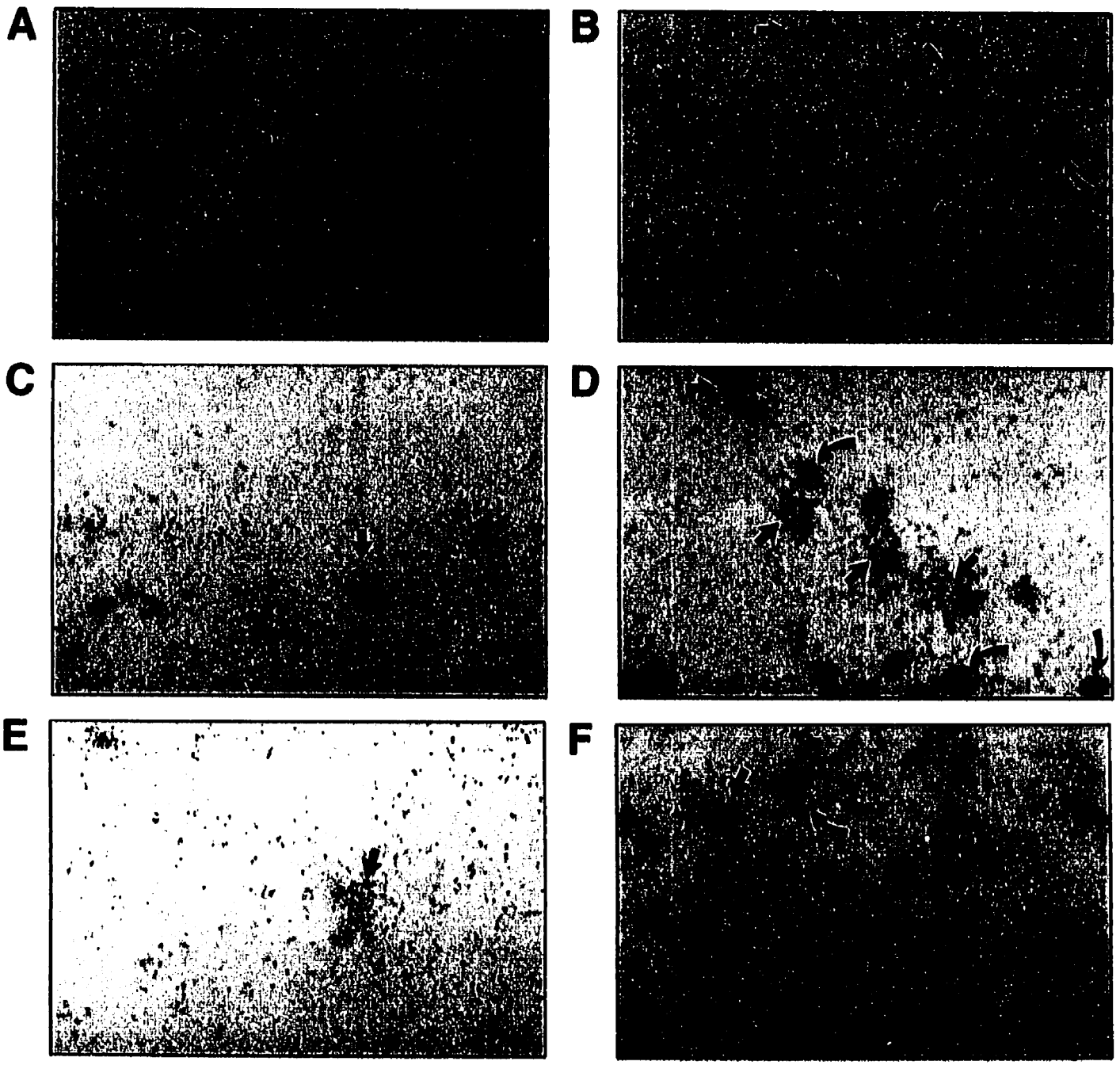

LPS
VEH
A
B
C
D

Figure 9:

Expression of CD14 mRNA within OX-42-immunoreactive (ir) cells associated to brain microvasculature (A) or found in the choroid plexus (chp; B), leptomeninges (C), median eminence/arcuate nucleus (ME/ARC; E) and area postrema/nucleus of the solitary tract (AP/NTS; F) of LPS-challenged rats. Colocalization of Fos-ir nucleus within CD14-expressing cells in the leptomeninges (particularly into the subarachnoid space) of rats treated with the bacterial endotoxin is also depicted (D). Around the blood vessels (bv), OX-42-labeled cells (most likely perivascular-associated microglia) were positive for the gene encoding the CD14 receptor. Numerous OX-42-ir macrophages expressing the LPS receptor were observed in the chp and leptomeninges of endotoxin-challenged animals. In the ME/ARC and AP/NTS, several CD14 positive cells were identified as parenchymal microglia. Note that some CD14 positive cells were not co-localized within OX-42-ir perikarya, suggesting that other parenchymal cell types may have the ability to express the LPS receptor. Filled arrowheads, OX-42-ir or Fos-ir cells expressing CD14 mRNA; curved arrowheads, OX-42-ir or Fos-ir (D) cells alone. Magnification, X250, Scale bar = 10 μm.


A
B
C
D
E
F

CHAPITRE 7. DISCUSSION GÉNÉRALE

7.1 Communication entre les systèmes immunitaire et neuroendocrinien: voies indirectes

7.1.1 Rôle des cytokines pro-inflammatoires

Dans notre première étude, nous avons tout d'abord démontré qu'une injection systémique de LPS influence l'activité cellulaire cérébrale d'une façon dose-dépendante. Nos résultats ont montré que l'injection d'une forte dose de LPS stimule de façon très intense et prolongée la transcription de l'IEG c-*fos* dans plusieurs structures du SNC du rat. Bien que l'administration d'une dose modérée de l'endotoxine ait aussi provoqué des effets similaires dans les premières heures suivant l'injection, le signal observé a rapidement disparu quelques heures plus tard dans la majorité des régions du cerveau à l'exception des OCVs. Quant à l'infusion d'une faible dose d'endotoxine, celle-ci a induit exclusivement l'expression du gène c-*fos* dans les organes circumventriculaires sensoriels. Les mécanismes exacts par lesquels l'endotoxine LPS peut influencer l'activité cellulaire dans le cerveau sont encore inconnus. Plusieurs travaux semblent suggérer que les cytokines systémiques produites en réponse à la LPS pourraient être responsables de l'induction de la transcription de c-*fos* dans des structures spécifiques du cerveau. Néanmoins, comme il sera discuté un peu plus loin, plusieurs autres intervenants tels que les médiateurs centraux (ex.: prostaglandines, cytokines) et la LPS elle-même pourraient jouer un rôle majeur. Toutefois, nous discuterons, en premier lieu, de l'influence des cytokines d'origine périphérique sur l'activité cellulaire dans le SNC.

Berkenbosch et ses collaborateurs ont rapporté que l'injection d'une faible dose de LPS provoque une augmentation des concentrations plasmatiques d'IL-6, sans toutefois affecter les niveaux circulants d'IL-1 (Berkenbosch *et al.*, 1991). Une autre étude a démontré que, bien qu'une élévation significative des concentrations plasmatiques d'IL-6 puisse être observée peu de temps après l'administration d'une faible dose de LPS, les taux sanguins d'IL-1ß demeurent toujours indétectables jusqu'à plusieurs heures après l'injection (Givalois *et al.*, 1994). Fait intéressant, l'administration i.v. d'IL-6 induit la transcription de l'ARNm du gène c-*fos* dans des structures très spécifiques du cerveau, dont les OCVs sensoriels qui incluent: l'OVLT, le SFO, l'EM et l'AP (Vallières *et al.*, 1997). Il est donc possible de spéculer que l'IL-6 d'origine systémique pourrait médier les influences d'une faible dose de LPS sur l'activité des OCVs.

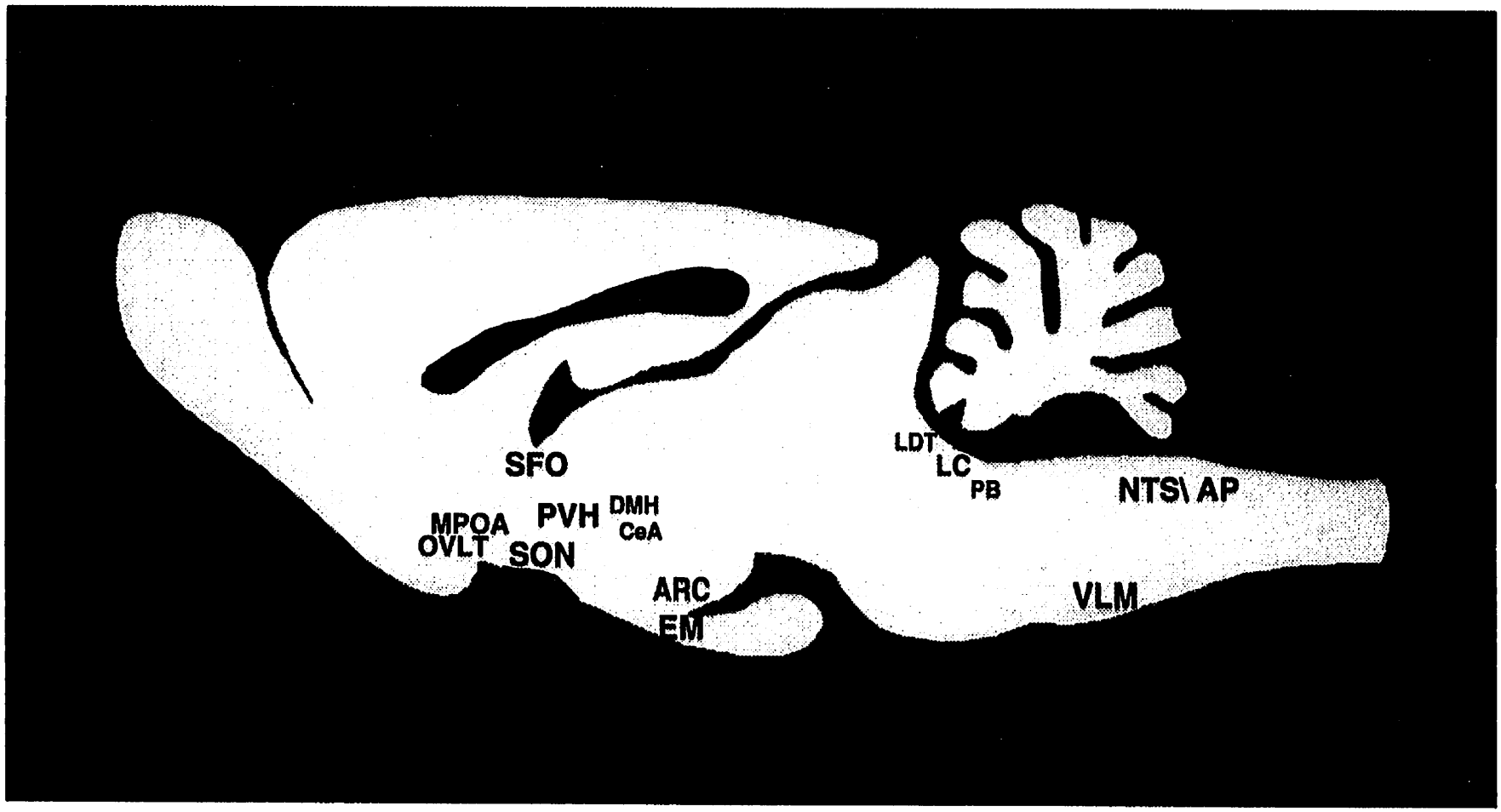


Figure 5. Illustration des sites exprimant les gènes de réponse précoce c-*fos* et NGFI-B après une administration systémique de l'endotoxine lipopolysaccharide (LPS) dans le cerveau de rats mâles. Les abréviations indiquent les régions sélectives du cerveau où l'ARNm c-*fos* fut détecté. Abréviations: AP, area postrema ARC, noyau arqué; CeA, noyau central de l'amygdale; DMH, noyau dorsomédial de l'hypothalamus; EM, éminence médiane; LC, locus coeruleus; LDT, noyau latérodorsal tegmental; MPOA, aire préoptique médiale; NTS, nucleus tractus solitarius; OVLT, organum vasculosum de la lamina terminalis; PB, noyau parabrachial; PVH, noyau paraventriculaire de l'hypothalamus; SFO, organe subfornicale SON, noyau supraoptique; VLM, région ventro-latérale médullaire.

Tel qu'illustré à la figure 5, l'administration i.p. d'une dose forte ou modérée de LPS a pour effet d'induire l'expression de c-*fos* dans plusieurs structures du SNC. Peu de temps après un traitement à la LPS (3 heures), des niveaux très élevés d'ARNm de c-*fos* ont été détectés dans de nombreux noyaux du cerveau dont notamment les OCVs, ainsi que certaines régions du tronc cérébral. Des dosages plasmatiques ont révélé que l'IL-1 peut être rapidement détectée dans le sang à des doses de LPS similaires à celles utilisées au cours de nos expériences (Berkenbosch *et al.*, 1991). De plus, des travaux ont montré que l'injection i.v. d'IL-1ß a pour effet d'induire l'expression de l'IEG c-*fos* dans les mêmes structures du cerveau, à l'exception de quelques uns des OCVs, que l'administration d'une dose forte ou modérée de LPS (Ericsson *et al.*, 1994; Rivest *et al.*, 1995). Par conséquent, il est possible qu'au cours de la réponse immunitaire de type aigu, l'IL-1 et l'IL-6 agissent en parallèle ou en série sur différentes régions du cerveau afin de stimuler l'activité cellulaire nerveuse et gliale. Toutefois, le rôle joué par chacun des sites activés dans la régulation des diverses fonctions endocriniennes et neuronales est encore mal compris. Les mécanismes sollicités par les médiateurs inflammatoires, afin d'induire l'activité de groupes cellulaires spécifiques du SNC, dont plus particulièrement les neurones endocriniens responsables du contrôle de l'axe corticotrope, demeurent eux aussi méconnus. Une deuxième partie de nos travaux consistait donc à clarifier l'hypothèse voulant que les PGs puissent médier les effets des cytokines proinflammatoires sur l'activité cellulaire cérébrale.

7.1.2 Rôle des prostaglandines

Un prétraitement à l'indométhacine n'a pas influencé l'activité transcriptionnelle de c-*fos* dans le cerveau de rats traités avec une forte dose d'endotoxine. Toutefois, le blocage des voies de la cyclooxygénase a inhibé l'expression de l'IEG dans des structures sélectives du SNC suite à un challenge immunitaire modéré. En effet, l'inhibition de la synthèse des PGs a altéré significativement l'induction de la transcription du gène c-*fos* dans des structures spécifiques du SNC incluant: l'OVLT/MPOA, les noyaux PVH et la région du VLM. La possibilité que la dose d'indométhacine utilisée n'ait pas complètement bloqué la production des PGs chez les rats traités à la LPS a bel et bien été considérée. Cependant, plusieurs études ont rapporté qu'une dose similaire pouvait prévenir efficacement les effets de la LPS et l'IL-1 sur les fonctions neuroendocriniennes (Katsuura *et al.*, 1988; McCoy *et al.*, 1994; Rivest and Rivier, 1993; Rivier and Vale, 1991; Watanabe *et al.*, 1990). Par conséquent, nos résultats suggèrent que le rôle des PGs, comme médiateurs des influences stimulatrices d'un challenge immunitaire sur la transcription centrale de l'IEG c-*fos*, dépend de la sévérité

du stress systémique. La figure 6 illustre certains des sites d'action potentiels des cytokines périphériques sur l'activité des neurones CRF des noyaux PVH. Une emphase toute particulière a été portée à l'OVLT/MPOA, aux noyaux PVH et au VLM, puisque nos résultats démontraient l'implication probable de ces deux régions dans l'activité des neurones endocriniens par des mécanismes impliquant les PGs en réponse à une dose modérée de LPS. Toutefois, il est important de noter que d'autres voies neuronales peuvent aussi participer aux effets des cytokines d'origine systémique sur l'activité des neurones CRFergiques et de l'axe HPA, celles-ci seront d'ailleurs décrites un peu plus loin (figure 7).

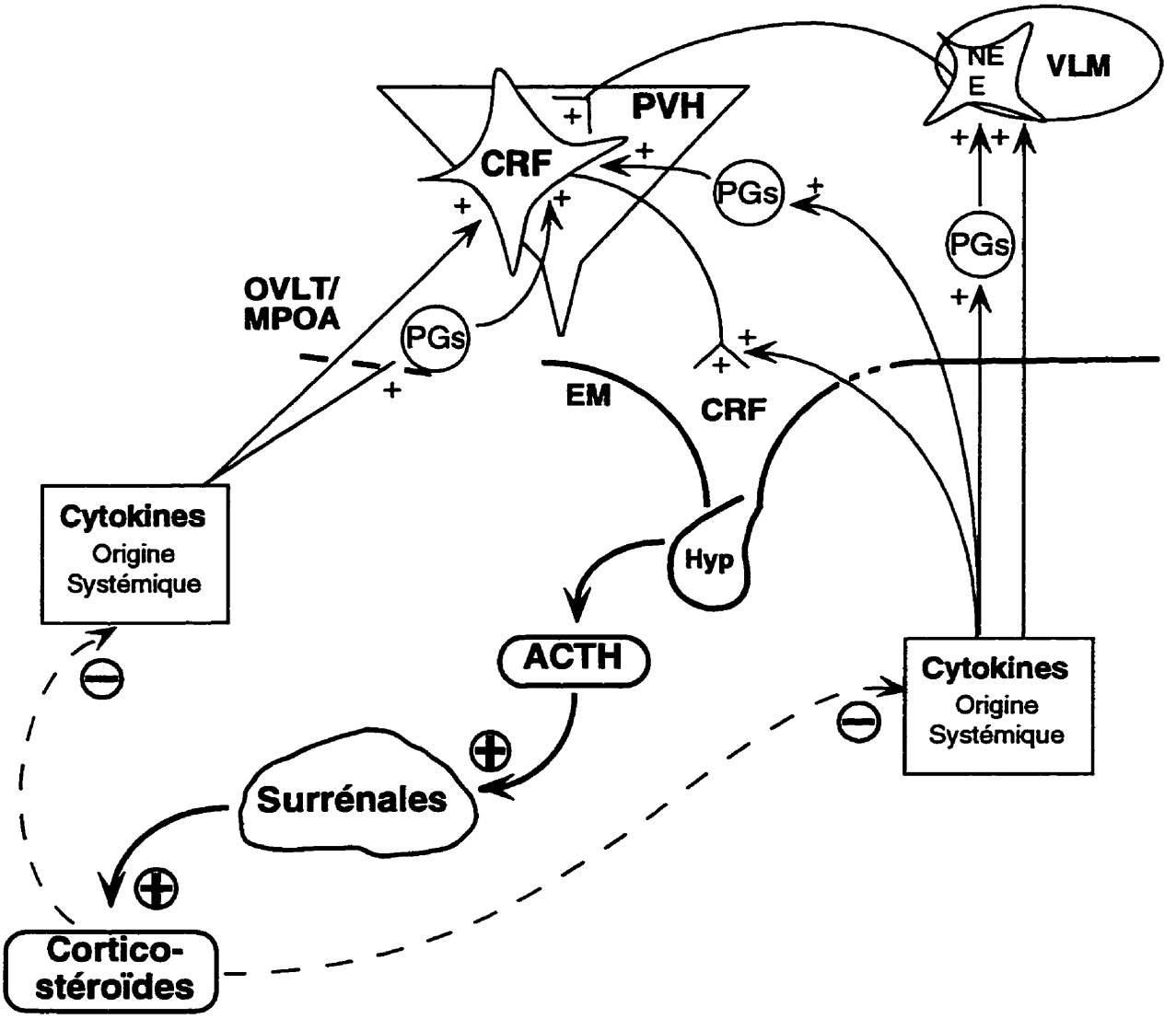


Figure 6. Sites d'action possibles des cytokines d'origine systémique sur l'activité des neurones CRF des noyaux paraventriculaires de l'hypothalamus (PVH). Abréviations: ACTH, hormone adrénocorticotrope; CRF, facteur de libération de la corticotrophine ou corticolibérine; E, groupe cellulaire adrénergique; EM, éminence médiane; Hyp, hypophyse; MPOA, région médiane pré-optique; NE, groupe cellulaire noradrénergique; OVLT, organum vasculosum de la lamina terminalis; PGs, prostaglandines; VLM, région ventro-latérale médullaire; +, stimulation; -, inhibition.

Bien que tous les mécanismes impliqués dans la communication entre les facteurs immunitaires périphériques et les neurones régulant l'activité de l'axe HPA n'aient pas encore été clairement identifiés, nos résultats semblent indiquer que la production de PGs centrales dans des régions spécifiques du SNC, tel que l'OVLT/MPOA, pourrait s'avérer essentielle. Il est donc très intéressant de constater qu'une densité très élevée des sites de liaison aux PGE_2 (Matsumura *et al.*, 1992; Matsumura *et al.*, 1990), de même que l'ARNm encodant le récepteur EP3 des PGE_2 (Ericsson *et al.*, 1995), ont été retrouvés dans la région de l'OVLT/MPOA du rat. De plus, des études neuroanatomiques ont clairement démontré que cette région possède des connections neuronales directes avec les noyaux PVH et SON (Sawchenko and Swanson, 1983). Récemment, Kovacs et Sawchenko ont d'ailleurs émis l'hypothèse qu'un des rôles de l'OVLT consisterait à détecter les variations de l'osmolarité sanguine pour ensuite transmettre ces informations aux noyaux PVH et SON (Kovacs and Sawchenko, 1993). Plus précisément, leurs études ont démontré qu'une lésion des projections descendantes originant des structures associées à la lame terminale, ou encore une lésion excitotoxique de l'OVLT, a pour conséquence d'abolir les effets stimulateurs d'une surcharge sodée sur l'expression du CRF dans l'hypothalamus endocrinien. Ainsi, il est possible que, suite à un challenge immunitaire modéré, les cytokines circulantes agissent via une circuiterie semblable afin d'induire l'activité transcriptionnelle des IEGs, du CRF et du $R\text{-}CRF_1$ dans les noyaux PVH et SON. Plusieurs groupes ont rapporté l'activation de l'OVLT/MPOA en réponse à une injection systémique d'IL-1ß (Brady *et al.*, 1994; Ericsson *et al.*, 1994), d'IL-6 (Vallières *et al.*, 1997), de TNF-α (S. Nadeau et S. Rivest, papier en préparation) ou de LPS (Hare *et al.*, 1995; Rivest and Laflamme, 1995). Par ailleurs, Scammell et ses collaborateurs ont montré qu'une microinjection des PGE_2 dans la région préoptique a pour effet d'induire la protéine Fos dans la division parvocellulaire des noyaux PVH (Scammell *et al.*, 1996). Il est aussi intéressant de noter qu'une injection systémique de l'endotoxine bactérienne LPS entraîne la production des PGs dans l'OVLT/MPOA (Ueno *et al.*, 1982), et que l'administration i.v. d'IL-1ß élève les concentrations de PGE_2 dans cette même région (Komaki *et al.*, 1992). D'autres études ont également rapporté qu'une microinjection des PGE_2 dans l'OVLT/MPOA augmente les niveaux plasmatiques d'ACTH, tandis que l'infusion d'indométhacine ou d'un antagoniste des PGs de type E dans cette même région prévient les influences stimulatrices d'une administration i.v. d'IL-1ß sur l'activité de l'axe HPA (Katsuura *et al.*, 1990). Ainsi, ces résultats suggèrent que les PGs (en particulier les PGE_2) jouent un rôle crucial dans la médiation des effets des cytokines circulantes sur l'activation de l'OVLT/MPOA, un phénomène vraisemblablement impliqué dans la stimulation des neurones responsables de moduler l'axe corticotrope.

La transcription de l'IEG *c-fos* est fortement induite dans le VLM en réponse à l'injection systémique d'IL-1ß (Ericsson *et al.*, 1994) ou de l'endotoxine bactérienne LPS (Rivest and Laflamme, 1995). Nos travaux ont démontré que, suite à un challenge immunitaire, la grande majorité des neurones activés dans le VLM sont immunoréactifs à la tyrosine hydroxylase (TH). De plus, nous avons rapporté que l'injection d'indométhacine inhibe l'activation des neurones catécholaminergiques des groupes cellulaires A1/C1 chez des rats soumis à une dose modérée de LPS. Ainsi, ces résultats indiquent que les PGs pourraient participer à l'activation des neurones A1/C1 au cours de la réponse immunitaire. Puisque les récepteurs des cytokines proinflammatoires ont été détectés dans les vaisseaux sanguins du SNC (Bebo and Linthicum, 1995; Ericsson *et al.*, 1995; Vallières and Rivest, 1997; Van Dam *et al.*, 1996), il est possible que les cytokines circulantes agissent sur leurs récepteurs respectifs au niveau de la microvasculature médullaire afin de stimuler la biosynthèse des PGs. Cette hypothèse est d'ailleurs supportée par des travaux récents rapportant que l'injection systémique des cytokines proinflammatoires ou de la LPS stimule l'activité transcriptionnelle du gène COX-2 dans le système vasculaire central (Breder and Saper, 1996; Cao *et al.*, 1995; Cao *et al.*, 1996; Lacroix and Rivest, 1998). Il est d'autant plus intéressant de constater que les transcrits encodant les récepteur EP3 (Ericsson *et al.*, 1995) et EP4 (J. Zhang et S. Rivest, papier en préparation) des PGE_2 soient exprimés dans la région du VLM. De plus, Ericsson et ses collègues ont démontré que les neurones catécholaminergiques A1/C1 activés à la suite d'une injection i.v. d'IL-1ß projettent aux noyaux PVH (Ericsson *et al.*, 1994). Enfin, des travaux ont rapporté qu'une microinjection des PGE_2 dans le VLM mime plusieurs des effets normalement observées à la suite d'une administration i.v. d'IL-1. Plus spécifiquement, l'injection intramédullaire des PGE_2 induit l'expression de la protéine Fos et la transcription du gène encodant le CRF dans les noyaux PVH (Ericsson *et al.*, 1997). Les études énumérées ci-dessus suggèrent donc l'existence d'une circuiterie additionnelle où les PGs seraient principalement responsables de moduler les diverses fonctions neuroendocriniennes au cours de la réponse immunitaire.

La circuiterie par laquelle les différents types de PGs peuvent moduler l'expression des IEGs dans des structures spécifiques du SNC demeure hypothétique. Bien que nous ayons mis l'accent jusqu'à présent sur le rôle des PGs dans l'activation de structures innervant les noyaux PVH, nous pouvons concevoir que les PGs exercent leurs actions directement au niveau de l'hypothalamus neuroendocrinien afin de moduler l'activité des neurones contrôlant l'axe HPA. Le fait qu'une injection i.v. de LPS ou d'IL-1ß provoque une augmentation des niveaux de PGE_2 dans les noyaux PVH (Van Dam *et al.*, 1993; Watanobe and Takebe, 1994), et la présence des transcrits encodant les récepteurs EP1

(Batshake *et al.*, 1995) et EP4 (J. Zhang et S. Rivest, papier en préparation) des PGE_2 dans l'hypothalamus endocrinien supporte cette hypothèse.

L'étude de la distribution du gène c-*fos* représente un excellent moyen d'identifier les structures activées au cours de la réponse immunitaire. Toutefois, il est important de prendre en considération que cet IEG n'est pas un indice parfait de l'activité transcriptionnelle des gènes encodants les facteurs neuroendocriniens. Ainsi, malgré le fait que les neurones CRF, AVP et OT des noyaux PVH et SON expriment c-*fos* suite à différentes situations de stress (Ericsson *et al.*, 1994; Rivest and Laflamme, 1995; Rivest and Rivier, 1994), seul le promoteur du gène AVP contient des éléments de réponse AP-1 reconnaissant l'hétérodimère Fos-Jun (Chan *et al.*, 1993; Seasholtz *et al.*, 1988; Thompson *et al.*, 1990). Afin d'enrayer ce problème et, par la même occasion, confirmer les sites activés à la suite d'un stress immunitaire, nous avons décidé d'utiliser un deuxième IEG indépendant de c-*fos*; i.e. NGFI-B (l'analyse combinée de c-*fos* et NGFI-B permet de détecter efficacement la très grande majorité des sites impliqués dans la cicuiterie fonctionnelle sollicitée au cours de la réponse immunitaire de type aigu). Ce facteur de transcription est particulièrement intéressant puisque les promoteurs des gènes encodant CRF, AVP et OT ont tous été reconnus pour avoir des sites de liaison pour celui-ci (Wilson *et al.*, 1991). Le fait d'utiliser NGFI-B constitue donc un avantage certain afin d'élucider les mécanismes intracellulaires régulant la transcription des neuropeptides responsables du contrôle de l'axe HPA. De cette façon, nous pouvons supposer qu'il pourrait exister une relation directe entre l'IEG et la transcription du gène ciblé. Toutefois, la possibilité demeure que la biosynthèse de ces IEGs soit une conséquence et non pas la cause de l'expression des neuropeptides responsables du contrôle de l'axe HPA (Kovacs and Sawchenko, 1994; Rivest and Laflamme, 1995). Pour ces raisons, nous avons donc entrepris de vérifier si la baisse significative des niveaux d'ARNm de c-*fos* et NGFI-B dans les noyaux PVH de rats ayant reçu un traitement combinant l'injection d'indométhacine et de LPS, était bel et bien accompagée d'une diminution de l'activité transcriptionnelle du CRF neuroendocrinien. De plus, suite aux nombreuses évidences suggérant que l'induction du R-CRF_1 dans les noyaux PVH et SON puisse être un événement directement relié la restauration des réserves en CRF durant la réponse immunitaire, le rôle des PGs comme médiateurs des influences stimulatrices des cytokines proinflammatoires sur la transcription du gène encodant le R-CRF_1 fut également analysé.

Nos travaux ont révélé que l'inhibition de l'expression de c-*fos* et NGFI-B dans les noyaux PVH était associée à une baisse dramatique des niveaux d'ARNhn du CRF; un prétraitement à l'indométhacine a complètement renversé l'induction du transcrit primaire du

CRF dans les noyaux PVH en réponse à l'administration d'une dose modérée de LPS. Bien que le blocage des voies de la cyclooxygénase n'ait pas influencé significativement les niveaux d'ARNm du R-CRF_1 dans les noyaux PVH de rats soumis à un stress immunitaire sévère, l'inhibition de la synthèse des PGs a prévenu la transcription de ce même récepteur dans les structures neuroendocriniennes d'animaux injectés i.p. avec une dose modérée ou faible de LPS. Ainsi, comme il a été rapporté précédemment pour les IEGs, il semble que les PGs jouent un rôle prédominant dans l'induction de la transcription des gènes encodant le CRF et son récepteur de type 1 suite à un challenge immunitaire modéré ou faible. Des mécanismes indépendants de la stimulation des voies de la cyclooxygénase pourraient donc être sollicités lors de situations de stress plus intenses.

7.1.2.1 Sites d'action des prostaglandines dans le système nerveux central

Les résultats discutés jusqu'à présent indiquent que les PGs jouent un rôle important comme médiateurs des effets stimulateurs de la réponse immunitaire systémique sur l'activité des neurones endocriniens et l'expression des gènes encodant le CRF et son récepteur de type 1. Nous avons mentionné que les influences des PGs semblent dépendre de plusieurs facteurs tels que la sévérité du stress, la région du cerveau affectée, le groupe cellulaire étudié, ainsi que les gènes analysés. Par conséquent, nous n'écartons pas la possibilité que les PGs puissent moduler diverses fonctions neuronales et neuroendocriniennes via des sentiers neuronaux autres que ceux impliquant l'OVLT/MPOA et le VLM. L'augmentation des concentrations en PGE_2 dans de nombreuses régions du cerveau, en réponse à une injection i.v. d'IL-1ß (Komaki *et al.*, 1992), de même que la distribution très étendue des récepteurs des PGs (Batshake *et al.*, 1995; Ericsson *et al.*, 1995; Sugimoto *et al.*, 1994), supportent cette éventualité. Afin d'éclaircir certaines de ces questions, nous avons entrepris d'étudier les effets d'une injection centrale des PGE_2 sur la distribution de c-*fos* et l'activité transcriptionnelle du CRF et son récepteur de type 1 dans le cerveau de rats. Nos études indiquent qu'un traitement central aux PGE_2 induit sélectivement l'activité cellulaire, comme le démontre l'expression rapide et transitoire du gène c-*fos*, dans plusieurs régions du SNC incluant l'OVLT, la MPOA, le SFO, les noyaux SON et PVH, les noyaux ARC, le NTS, l'AP et le VLM. Nous avons aussi observé dans la division parvocellulaire des noyaux PVH que les neurones CRF et OT étaient les deux principaux types cellulaires exprimant l'IEG. Dans la division magnocellulaire des noyaux PVH et les SON, le gène c-*fos* fut essentiellement co-localisé dans des neurones OT (très peu de neurones AVP étaient positifs

pour c-*fos*). Il est également très intéressant de noter que l'activation des neurones CRFergiques a été associée à une stimulation de l'activité transcriptionnelle du neuropeptide, comme le démontre l'expression sélective du CRFhn dans les noyaux PVH. Enfin, nos analyses ont démontré que les niveaux d'ARNm du R-CRF_1 furent augmentés dans les noyaux PVH des rats injectés i.c.v. avec des PGE_2, suggérant du même coup une implication locale du CRF dans cette structure hypothalamique sous l'influence centrale des PGs.

L'injection centrale des PGE_2 a pour effet d'induire la transcription du gène c-*fos* dans des sites presque similaires à ceux activés à la suite d'un traitement systémique à la LPS. Il est donc concevable que la biosynthèse des PGE_2 dans des structures sélectives du SNC soit une étape importante dans l'intégration centrale des informations immunitaires provenant de la périphérie. Toutefois, comme mentionné précédemment, la circuiterie par laquelle ces renseignements originant de la systémie sont acheminés vers l'hypothalamus endocrinien est encore mal comprise. La figure 7 est une illustration schématique des voies neuronales et des mécanismes qui pourraient réguler l'activité de l'axe corticotrope au cours de la réponse immunitaire. Puisqu'il est probable que les cytokines systémiques puissent agir via une circuiterie complexe sur les corps cellulaires et les fibres terminales des motoneurones CRFergiques, les mécanismes contrôlant la biosynthèse du CRF dans les noyaux PVH et le relâchement de ce dernier dans le système infundibulaire seront discutés de façon indépendante. Mentionnons qu'une attention particulière sera attribuée aux structures activées à la suite d'une injection i.c.v. des PGE_2, à l'exception de la circuiterie déjà décrite précédemment.

Outre l'OVLT, le SFO et l'AP sont deux autres structures dépourvues de barrière hémato-encéphalique qui expriment l'IEG c-*fos* à la suite d'un traitement central aux PGE_2 ou d'une injection systémique de l'endotoxine LPS. Il est intéressant de constater que de nombreux travaux ont confirmé la présence de projections originant du SFO vers les noyaux PVH; plusieurs neurones marqués ont été retrouvés dans le SFO suite à l'injection d'un traceur rétrograde dans les noyaux PVH (Johnson and Gross, 1993; Larsen and Mikkelsen, 1995; Liposits, 1993; Sawchenko *et al.*, 1993). Bien que peu ou pas de connections directes aient été décrites entre l'AP et les noyaux PVH, des études neuroanatomiques ont démontré que l'AP projette massivement vers les divisions médiales et commissurales du NTS ainsi que vers la partie latérale externe des noyaux PB (Cunningham *et al.*, 1994), deux structures

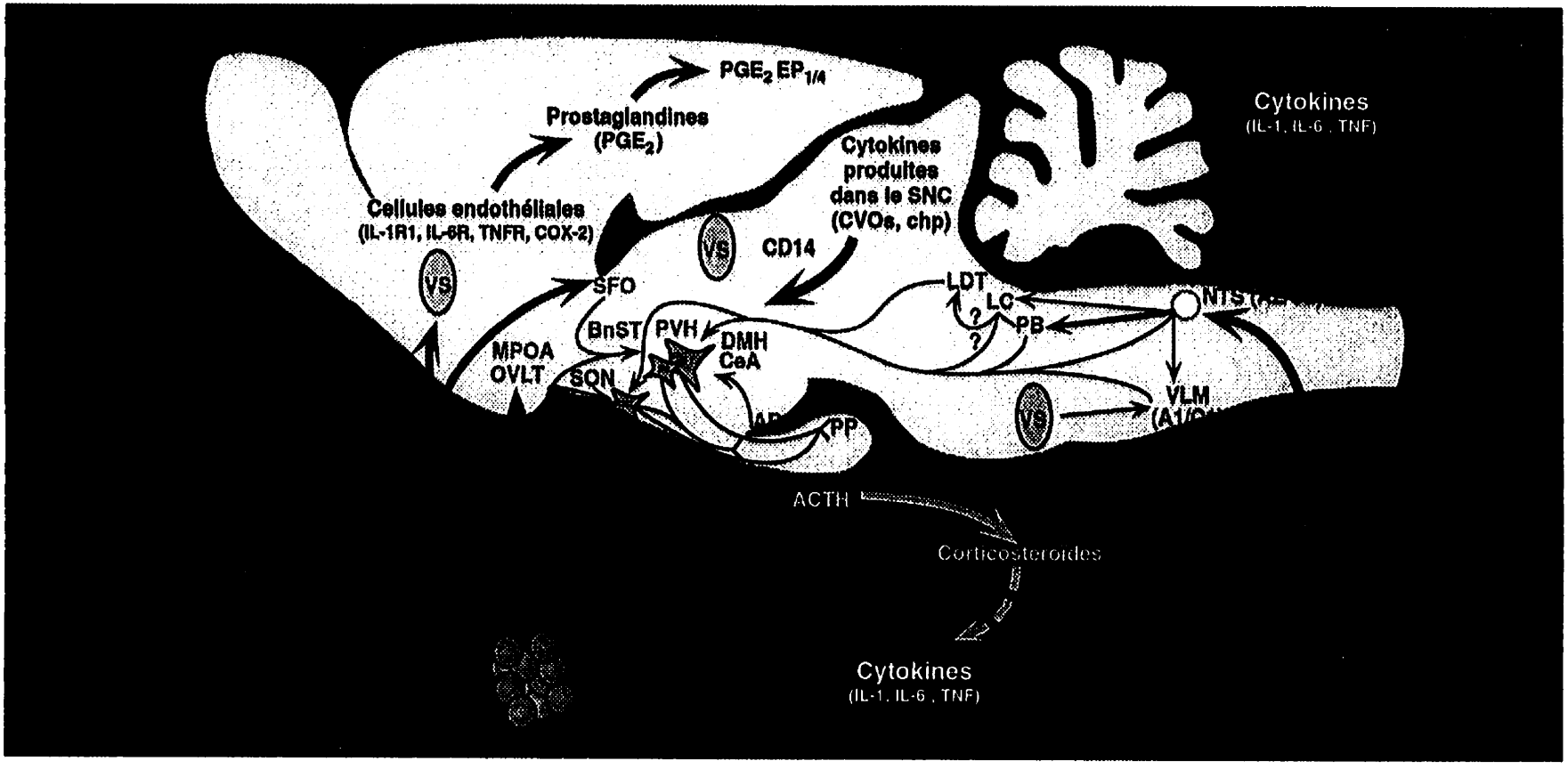


Figure 7. Circuiterie neuronale empruntée à la suite d'un traitement systémique avec l'endotoxine bactérienne LPS. Abréviations: ACTH, hormone adrénocorticotrope; AP, area postrema; ARC, noyau arqué; BnST, noyau du lit de la strie terminale; CeA, noyau central de l'amygdale; COX-2, cyclooxygénase-2; DMH, noyau dorsomédial de l'hypothalamus; EM, éminence médiane; EP, récepteur des prostaglandines de la série E; IL-1, interleukine-1; IL-6, interleukine-6; IL-1R, récepteur de l'IL-1; IL-6R, récepteur de l'IL-6; LC, locus coeruleus; LDT, noyau latérodorsal tegmental; MPOA, aire préoptique médiale; NTS, nucleus tractus solitarius; OCVs, organes circumventriculaires; OVLT, organum vasculosum de la lamina terminalis; PB, noyau parabrachial; pch, plexus choroïdiens; PGE_2, prostaglandines de type E2; PP, hypophyse postérieure; PVH, noyau paraventriculaire de l'hypothalamus (divisions parvocellulaire [pc] et magnocellulaires [mc]); SFO, organe subfornical; SON, noyau supraoptique; TNF-α, facteur nécrosant des tumeurs-alpha; TNFR, récepteur du TNF-α; VLM, région ventro-latérale médullaire; VS, vaisseaux sanguins.

ayant des projections directes avec l'hypothalamus neuroendocrinien (Swanson *et al.*, 1987). Ainsi, il semble que ces deux régions puissent influencer l'activité des neurones neuroendocriniens durant la réponse immunitaire.

Le NTS est une autre structure particulièrement intéressante puisqu'on y retrouve une très forte densité de sites de liaison des PGE_2 (Matsumura *et al.*, 1992). Toutefois, bien que Seriyama et ses collaborateurs aient rapporté que les PGs puissent faciliter les transmissions synaptiques excitatrices dans des sections du NTS chez le rat, nos travaux semblent plutôt remettre en question le rôle des PGs dans l'activation du NTS au cours de la réponse immunitaire. En effet, contrairement au VLM, un prétraitement à l'indométhacine n'a pas entraîné une diminution significative des niveaux d'ARNm de c-*fos* dans le NTS de rats traités à la LPS. Néanmoins, il est possible que le NTS influence les fonctions neuroendocriniennes via des mécanismes indépendants des PGs. Des études neuroanatomiques ont d'ailleurs démontré que ce noyau fournit les projections noradrénergiques les plus denses vers les noyaux PVH (Cunningham and Sawchenko, 1988). Il est d'autant plus intéressant de noter que, plusieurs des neurones projetant à l'hypothalamus endocrinien, expriment l'IEG c-*fos* en réponse à l'injection systémique d'IL-1ß (Ericsson *et al.*, 1994). De plus, Ericsson et ses collaborateurs ont également rapporté qu'une lésion unilatérale des voies médullaires ascendantes a pour effet d'atténuer l'augmentation des niveaux d'expression de la protéine Fos, et de l'ARNm encodant le CRF, dans les noyaux PVH de rats soumis à une injection i.v. d'IL-1ß (Ericsson *et al.*, 1994). Tel qu'illustré à la figure 7, il est possible que l'AP agisse comme site d'entrée aux cytokines d'origine systémique afin d'activer le NTS et, subséquemment, l'axe HPA. Cependant, d'autres travaux semblent plutôt supporter l'hypothèse que les cytokines périphériques puissent stimuler directement les nombreuses afférences sensitives du nerf vague. Nance et son groupe ont d'ailleurs démontré qu'une vagotomie subdiaphragmatique avait pour effet d'inhiber l'expression de la protéine Fos dans le NTS et les noyaux PVH en réponse à une injection i.p. de LPS (Wan *et al.*, 1994). Bien qu'il soit possible que les neurones activés du NTS stimulent directement les noyaux PVH, il est concevable que d'autres structures du tronc cérébral puissent relayer l'information provenant du NTS vers les neurones endocriniens ou, tout simplement, moduler eux-mêmes les influences des cytokines sur les fonctions neuroendocriniennes. Nos résultats ont montré que l'administration i.p. de l'endotoxine bactérienne LPS stimule l'expression de l'IEG c-*fos* non seulement dans le NTS, mais également dans plusieurs autres structures du tronc cérébral telles que: le LC, le LDT, les noyaux PB et le VLM. Par conséquent, il est possible d'affirmer que les voies noradrénergiques (A_1, A_2, A_6) et adrénergiques (C_1-C_2) pourraient médier certaines des

influences excitatrices des cytokines proinflammatoires d'origine systémique sur l'activité des neurones endocriniens des noyaux PVH et SON. Toutefois, il est important de prendre en considération que d'autres voies ascendantes, telles que les voies sérotoninergiques, pourraient également contribuer à l'activation des structures neuroendocriennes au cours de la réponse immunitaire. En effet, il a été démontré qu'un traitement avec un inhibiteur irréversible de la synthèse de la sérotonine, le parachlorophénylalanine (PCPA; un inhibiteur de l'enzyme tryptophane-5-hydroxylase), atténuait significativement les niveaux d'expression des gènes encodant c-*fos* et NGFI-B, de même que le CRF, dans l'hypothalamus endocrinien de rats soumis à un stress immunitaire (Laflamme *et al.*, 1998). Ces mêmes auteurs ont également rapporté que, en plus de réduire de 96% les concentrations hypothalamiques en sérotonine, l'injection de PCPA avait fortement diminué les niveaux plasmatiques de corticostérone chez les rats injectés à la LPS. Ainsi, ces travaux confirment que l'intégrité des voies sérotoninergiques est requise à l'activité cellulaire neuronale ou gliale et à l'activation de l'axe corticotrope durant la réponse immunitaire.

Bien que nos analyses ne supportent pas un rôle des PGs dans l'activation de la plupart des structures du tronc cérébral, à l'exception du VLM, il est important de prendre en considération que de nombreux facteurs ont pu modifier les données (ex.: dose de LPS utilisée). Par ailleurs, il est possible que les PGs puissent interagir avec les fibres catécholaminergiques ascendantes au niveau des noyaux PVH et, par conséquent, médier l'activation des neurones CRFergiques neuroendocriniens. Certains résultats semblent appuyer cette hypothèse; une injection de 6-hydroxydopamine (celle-ci est utilisée afin de léser sélectivement les voies catécholaminergiques) dans les noyaux PVH cause une diminution de 85% des concentrations locales en noradrénaline, en plus de réduire par 80 à 82% l'augmentation des niveaux plasmatiques en corticostérone à la suite d'une injection i.p. d'IL-1 (Chuluyan *et al.*, 1992). De plus, l'injection i.v. d'IL-1ß stimule la production des PGE_2 dans les noyaux PVH (Komaki *et al.*, 1992; Watanobe and Takebe, 1994).

Nos travaux ont montré que l'injection i.c.v. des PGE_2 stimule l'expression du gène c-*fos* dans les neurones CRFergiques, de même que la transcription du CRF et du R-CRF1 dans les noyaux PVH (Lacroix *et al.*, 1996). Rabin et ses collègues ont aussi rapporté que les PGE_2 étaient capables d'induire la sécrétion d'ACTH et de corticostérone dans le sang et de supprimer la réponse immunitaire *in vitro* (Rassnick *et al.*, 1995). Ces résultats suggèrent que la production locale des PGE_2, dans des structures sélectives du SNC, pourrait s'avérer essentielle à l'activation de la circuiterie neuronale impliquée dans la restauration et le

maintien de l'homéostasie à la suite d'une infection. Dans cette perspective, les neurones CRFergiques neuroendocriniens semblent jouer un rôle très important afin de moduler les concentrations de glucocorticoïdes nécessaires à la prévention d'une réponse immunitaire exagérée, celle-ci pouvant s'avérer néfaste pour l'organisme. Cependant, bien que de nombreuses voies neuronales semblent moduler l'expression du CRF dans les noyaux PVH, la possibilité demeure que les cytokines d'origine systémique puissent avoir une influence directe au niveau des fibres terminales CRFergiques regroupées dans l'EM. Quant aux neurones OT et AVP activés en réponse à l'injection centrale PGE_2, ils pourraient contribuer à potentialiser l'action du CRF sur la sécrétion de l'ACTH au cours d'un stress immunitaire. Rappelons que l'OT et la AVP sont de deux puissants agents sécrétagogues de l'ACTH.

7.1.2.2 Biosynthèse des prostaglandines dans le système nerveux central: sites de production

Même si les indices démontrant que les PGs produites centralement pourraient médier les effets d'un stress immunitaire sur l'activité de l'axe corticotrope se font de plus en plus nombreux, nous ne savons pas encore si la biosynthèse des PGs dans le SNC est une conséquence de la réaction inflammatoire systémique ou plutôt un effet direct de l'endotoxine LPS. Nos analyses ont montré que la transcription du gène encodant l'enzyme COX-2, mais pas COX-1, était fortement stimulée dans des populations cellulaires spécifiques du cerveau (probablement dans des cellules endothéliales) en réponse à différents modèles d'inflammation systémique ainsi qu'à la suite d'une injection i.v. d'IL-1ß ou de TNF-α. En effet, des niveaux élevés d'ARNm de COX-2 ont été détectés dans la microvasculature cérébrale, les plexus choroïdiens et les leptoméninges de rats soumis à une administration i.v. ou i.p. de LPS ou encore à une injection i.m. de turpentine. Nos travaux ont aussi rapporté que le signal observé chez tous les animaux soumis à une injection i.m. suivait de façon parallèle l'inflammation de la patte. Enfin, l'infusion i.v. d'IL-1ß ou de TNF-α, mais pas celle de l'IL-6, a fortement induit l'activité transcriptionnelle du gène COX-2 dans des structures non-parenchymales identiques à celles stimulées lors d'un stress inflammatoire.

Ces résultats constituent les premières évidences que la biosynthèse de COX-2 dans les cellules non-parenchymales de la microvasculature cérébrale, des plexus choroïdiens et des leptoméninges est un phénomène spécifique à la réponse inflammatoire systémique. Certaines études avaient, bien entendu, rapporté l'augmentation des niveaux d'ARNm de COX-2, et de la protéine correspondante, dans des neurones suite à une ischémie cérébrale

(Nogawa *et al.*, 1997) ou des convulsions (Yamagata *et al.*, 1993). Aucun de ces travaux n'avait cependant rapporté la présence du transcrit et de la protéine en question dans les vaisseaux sanguins du SNC. Les mécanismes sollicités par l'endotoxine bactérienne LPS, afin de stimuler l'activité transcriptionnelle de l'enzyme COX-2 dans le cerveau, sont encore mal connus. Bien que plusieurs des effets de la LPS soient médiés par des cytokines circulantes spécifiques, la possibilité demeure que l'endotoxine puisse induire la transcription du gène COX-2 en agissant directement sur la microvasculature cérébrale plutôt qu'en stimulant les monocytes et macrophages responsables de la sécrétion des cytokines. Il a d'ailleurs été montré que l'activation des cellules endothéliales par la LPS joue un rôle majeur dans la pathogénèse des infections aux bactéries gram-négatives. Malgré les études démontrant que l'endothélium n'exprime pas le CD14m, Arditi et son groupe ont rapporté que les cellules endothéliales peuvent répondre à la LPS via la forme soluble du CD14 (Arditi *et al.*, 1993; Arditi *et al.*, 1995). Quant aux cellules microgliales périvasculaires-associées, celles-ci ont la capacité d'exprimer le CD14m en réponse à la LPS; d'où la possibilité d'une action directe de l'endotoxine sur celles-ci (Becher and Antel, 1996).

Le fait qu'une inflammation stérile à la turpentine ait stimulé la transcription du gène COX-2 dans les vaisseaux sanguins du cerveau, les plexus choroïdiens et les leptoméninges, indique clairement que la biosynthèse de cette enzyme dans le SNC est modulée durant la réponse inflammatoire systémique. Dans cette perspective, il est intéressant de noter que l'injection i.v. d'IL-1ß a provoqué une augmentation des niveaux d'ARNm de COX-2 dans les mêmes structures que celles stimulées chez les rats soumis à un challenge à la turpentine. À l'exception de l'intensité du signal, des résultats similaires à ceux obtenus en réponse à l'administration i.v. d'IL-1ß ont été observés à la suite d'un traitement systémique au TNF-α. Nos analyses suggèrent donc que l'IL-1ß et, à un degré moindre, le TNF-α peuvent cibler la microvasculature cérébrale afin d'induire la biosynthèse de COX-2, l'enzyme limitante à la formation des PGs. Il est d'autant plus intéressant de noter que les récepteurs respectifs de ces deux cytokines ont été détectés dans la microvasculature du SNC (Bebo and Linthicum, 1995; Ericsson *et al.*, 1995).

Plusieurs évidences supportent l'hypothèse que l'IL-1ß d'origine systémique soit la principale cytokine responsable de médier la biosynthèse des PGs au cours de la réponse immunitaire. Par immunohistochimie, Van Dam et ses collègues ont détecté la présence des PGE_2 dans la microvasculature cérébrale et les plexus choroïdiens de rats traités avec une dose pyrogénique de LPS (Van Dam *et al.*, 1993). En plus d'observer les PGE_2 dans des

structures similaires à celles exprimant le gène COX-2 durant la réponse immunitaire, nous remarquons que l'apparition de l'ARNm encodant l'enzyme limitante à la synthèse des PGs précède par environ 30 minutes la formation des PGE_2. Récemment, des cellules immunoréactives à l'IL-1ß ont été trouvées dans les plexus choroïdiens, les méninges et l'endothélium formant la microvasculature du cerveau, chez des rats injectés avec l'endotoxine LPS (Van Dam *et al.*, 1995). Bien que ces résultats semblent confirmer la synthèse *de novo* d'IL-1 dans des populations cellulaires spécifiques du SNC en réponse à la LPS, il est néanmoins possible que l'anticorps utilisé permette la détection d'IL-1 circulante ayant été internalisée. Des études réalisées à l'aide de la technique d'hybridation *in situ* ont d'ailleurs montré la présence de l'ARNm encodant le récepteur de type 1 de l'IL-1 dans les structures non-parenchymales énumérées ci-dessus (Ericsson *et al.*, 1995). De plus, l'administration périphérique d'IL-1ß a fortement stimulé la production des PGE_2 dans les plexus choroïdiens ainsi que dans les cellules endothéliales du SNC (Van Dam *et al.*, 1996). Tel qu'illustré à la figure 8, les PGs synthétisées via l'action de l'enzyme COX-2 pourraient s'avérer de très bons candidats au transfert de l'information provenant des cytokines circulantes vers le cerveau. Cependant, nous ne savons pas encore si ces renseignements sont essentiels à la régulation des diverses fonctions neuronales ou endocriniennes. Nous croyons que les PGs synthétisées par la microvasculature cérébrale, en réponse aux différentes cytokines sécrétées par les cellules phagocytaires activées, diffusent dans le liquide extracellulaire afin de lier leur récepteur situé à la surface des cellules avoisinantes incluant les cellules nerveuses. Ainsi, ces neurones auraient la capacité de moduler l'axe HPA ou toute autre fonction neuronale induite au cours de la réponse immunitaire (ex.: fièvre).

7.2 Communication entre les systèmes immunitaire et neuroendocrinien: voies directes

7.2.1 Influences directes de la LPS sur le système nerveux central: sites de production du CD14

Au cours des dernières années, de nombreuses évidences (la plupart de celles-ci ont été décrites dans l'introduction générale) ont laissé sous-entendre que la LPS pourrait être directement responsable de plusieurs des réponses observées au cours de l'endotoxinémie (ex.: activation de l'axe HPA, biosynthèse des cytokines dans le SNC). Afin d'éclaircir cette question d'une importance capitale, nous avons donc entrepris, dans notre dernière étude,

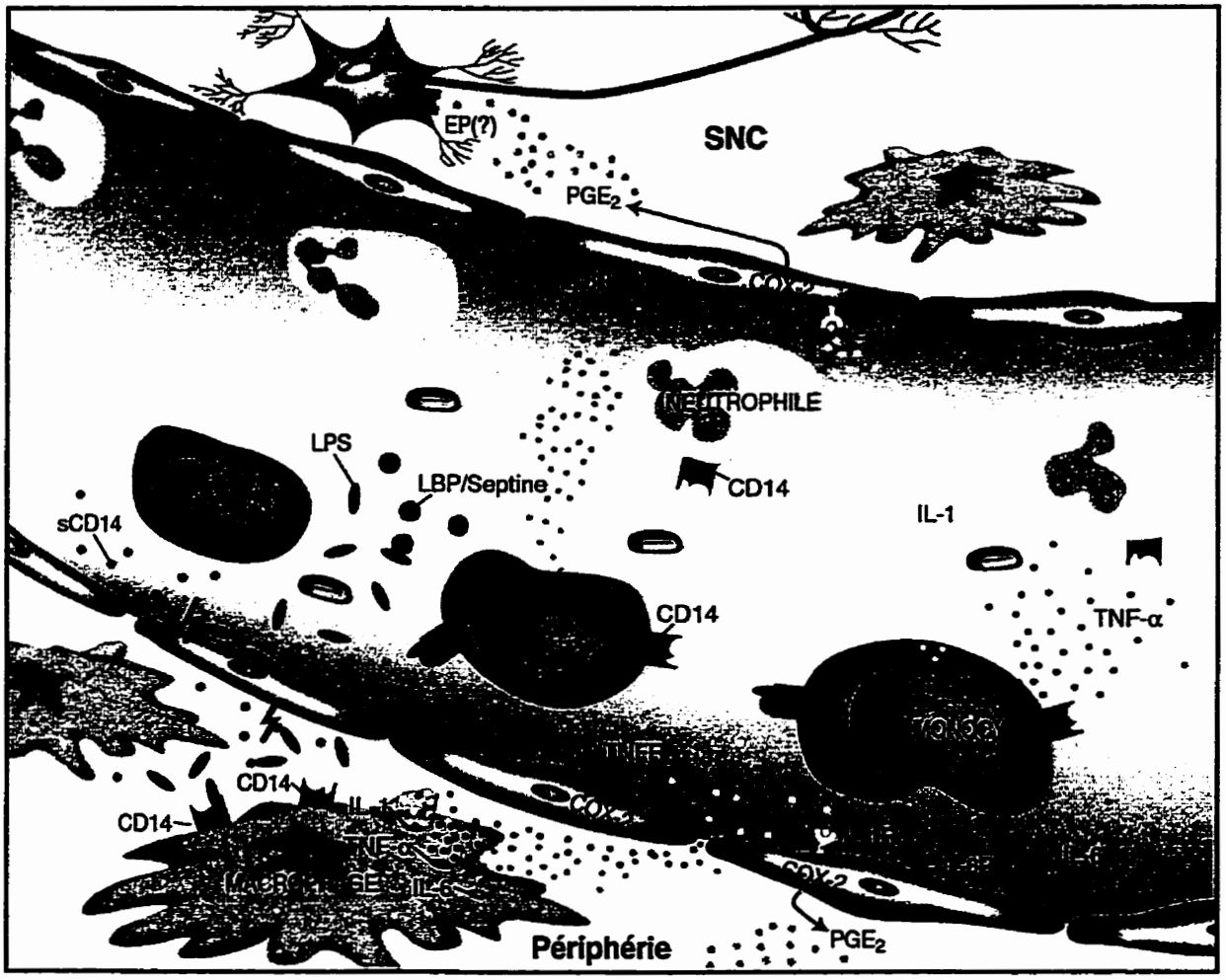


Figure 8. Mécanismes cellulaires sollicités suite à la liaison de l'endotoxine bactérienne LPS sur les cellules d'origine myéloïde. Abréviations: COX-2, cyclooxygénase-2; EP, récepteur des prostaglandines de la série E; IL-1, interleukine-1; IL-6, interleukine-6; IL-1R, récepteur de l'IL-1; IL-6R, récepteur de l'IL-6; LBP, protéine de liaison de la LPS; LPS, lipopolysaccharide; PGE_2, prostaglandines de type E2; SNC, système nerveux central; TNF-α, facteur nécrosant des tumeurs-alpha; TNFR, récepteur du TNF-α.

d'établir la distribution du récepteur CD14 dans le cerveau de rats. Nos résultats ont montré que des populations cellulaires spécifiques du SNC expriment le gène encodant la forme membranaire du récepteur CD14 de la LPS. Par HISH, des faibles niveaux d'ARNm du CD14m ont été détectés dans plusieurs structures non-parenchymales du cerveau incluant les plexus choroïdiens, les leptoméninges et la microvasculature cérébrale. L'administration systémique de l'endotoxine LPS a fortement stimulé la transcription du gène encodant le CD14m dans ces mêmes structures de même que dans plusieurs autres régions telles que: l'OVLT, la MPOA, le SFO, les noyaux PVH et ARC, l'EM, le NTS et l'AP. Une analyse plus exhaustive a révélé que la LPS induit tout d'abord le CD14m dans les structures en contact direct avec la circulation sanguine (OCVs, plexus choroïdiens, leptoméninges, vaisseaux sanguins du SNC) pour ensuite apparaître dans certains types de cellules parenchymales localisées dans les régions adjacentes aux OCVs et finalement dans l'ensemble du cerveau lors d'une septicémie plus sévère. Grâce à des études de double marquage combinant les techniques d'immunohistochimie et d'HISH, nous avons identifié que les cellules microgliales parenchymales et celles associées aux microcapillaires, de même que les macrophages et les neurones CRFergiques des noyaux PVH, étaient capables d'exprimer l'ARNm du CD14. Ainsi, ces résultats suggèrent que la LPS puisse agir directement sur le SNC, sans l'intervention des médiateurs inflammatoires/immunitaires d'origine sytémique comme les cytokines proinflammatoires, afin de moduler certaines des réponses observées durant une endotoxinémie.

Il peut sembler, à première vue, surprenant qu'une molécule à poids moléculaire élevé, telle que la LPS, puisse agir directement sur le SNC. Toutefois, la distribution du transcrit encodant le CD14 nous indique la présence du récepteur de la LPS dans des sites richement vascularisés et dépourvus de barrière hémato-encéphalique qui, par conséquent, sont facilement accessibles aux molécules circulantes. Ainsi, nous croyons que la LPS injectée dans le sang puisse pénétrer les tissus des OCVs, ou simplement atteindre les plexus choroïdiens, les leptoméninges et la microvasculature cérébrale par la circulation générale, afin de stimuler la biosynthèse de son propre récepteur. Toujours selon notre hypothèse, nous pensons que les molécules de LPS encore présentes au niveau de la systémie pourraient lier le CD14 afin d'induire la libération des cytokines pro-inflammatoires. Certains travaux ont d'ailleurs montré que la liaison de la LPS au CD14 entraîne la sécrétion des cytokines proinflammatoires (Kitchens *et al.*, 1992; Lee *et al.*, 1992; Leturcq *et al.*, 1996; Wright *et al.*, 1990), une réponse cellulaire dépendante du second messager diacylglycérol et du facteur de transcription NF-κB (Yamamoto *et al.*, 1997). En effet, des études *in vitro* ont rapporté que le prétraitement de lignées cellulaires CD14 positives, avec un anticorps capable de bloquer la

liaison entre la LPS et le CD14, provoque une baisse dramatique de l'activité de liaison de NF-κB en plus d'une diminution marquée de la synthèse de l'IL-1ß et du TNF-α en réponse à la LPS (Kitchens *et al.*, 1992; Pollack *et al.*, 1997). De plus, plusieurs travaux ont démontré que NF-κB est le facteur de transcription clé par lequel la LPS médie la transcription des gènes codant les cytokines proinflammatoires IL-1ß, IL-6 et TNF-α (Collart *et al.*, 1990; Dendorfer *et al.*, 1994; Drouet *et al.*, 1991; Hiscott *et al.*, 1993; Kuprash *et al.*, 1995; Libermann and Baltimore, 1990; Shakhov *et al.*, 1990; Shimizu *et al.*, 1990; Zhang *et al.*, 1994). Quan et ses collègues ont récemment rapporté en utilisant la détection du transcrit I-κBα comme marqueur de l'activité de NF-κB (Ghosh and Baltimore, 1990; Henkel *et al.*, 1993), une induction très forte et rapide des niveaux d'ARNm de I-κBα dans les OCVs, les plexus choroïdiens et la microvasculature cérébrale à la suite d'un challenge immunitaire à la LPS. Par ailleurs, l'administration systémique de l'endotoxine bactérienne LPS a également stimulé l'activité transcriptionnelle des gènes encodant les cytokines proinflammatoires dans ces mêmes structures du SNC (Nadeau and Rivest, 1998; Quan *et al.*, 1998; Vallières and Rivest, 1997). Il est d'autant plus intéressant de noter que l'expression de ces gènes en fonction du temps, supporte la théorie voulant que la liaison de la LPS à son récepteur pourrait être responsable de l'activation de NF-κB et, par conséquent, la biosynthèse subséquente des cytokines proinflammatoires dans des populations cellulaires spécifiques du cerveau.

Le rôle physiologique de l'expression du récepteur CD14 et de la production des différentes cytokines dans le SNC reste à clarifier. Certaines évidences semblent indiquer que l'induction rapide du CD14 dans l'OVLT, ainsi que l'augmentation subséquente de la synthèse des cytokines proinflammatoires dans cet OCV, pourraient s'avérer d'une importance capitale dans les mécanismes centraux régulant les effets thermogéniques de la LPS (Blatteis *et al.*, 1983; Blatteis *et al.*, 1987; Chai *et al.*, 1996). Il est aussi possible que la LPS stimule les OCVs afin d'activer directement ou indirectement les neurones responsables du contrôle de l'axe corticotrope. A première vue, il semblerait que la LPS puisse agir directement sur les neurones CRFergiques des noyaux PVH étant donné la présence de l'ARNm du CD14. Toutefois, il semble plutôt improbable que la LPS d'origine systémique puisse atteindre ces neurones. L'apparition du CD14 à des temps relativement tardifs dans l'hypothalamus neuroendocrinien vient d'autant plus suggérer l'intervention d'autres sites d'actions. Dans cette perspective, l'EM pourrait être un candidat privilégié étant donné sa position stratégique et la présence dans cette structure des fibres terminales CRFergiques neuroendocriniennes. De plus, nos analyses qualitatives ont révélé que l'EM est l'une des premières régions à exprimer le récepteur de la LPS en plus de renfermer les plus hauts

niveaux d'ARNm du CD14 à la suite d'une injection de LPS. Le SFO et l'AP expriment eux aussi l'ARNm du CD14 et les transcrits codant les cytokines proinflammatoires à la suite de l'administration systémique de la LPS. Les études de double marquage ont déterminé que, les cellules de l'AP et du SFO synthétisant le CD14, l'IL-1 et le TNF-α, sont en effet des cellules de type microglial (Buttini and Boddeke, 1995; Lacroix *et al.*, 1998; Nadeau and Rivest, 1998). Ainsi, nous suggérons que les cytokines sécrétées par ces cellules microgliales, en réponse à la LPS d'origine systémique, puissent lier leurs récepteurs localisés sur des neurones avoisinants. Ceux-ci pourraient donc informer, grâce à leurs projections afférentes directes ou indirectes, les structures régulant les diverses fonctions neuronales et neuroendocriniennes sollicitées au cours de l'endotoxinémie. Afin d'appuyer cette hypothèse, mentionnons des travaux démontrant que plusieurs cellules exprimant l'ARNm des récepteurs de l'IL-1 (Ericsson *et al.*, 1995), de l'IL-6 (Vallières and Rivest, 1997) ou du TNF-α (S. Nadeau et S. Rivest, papier en préparation) ont été détectées dans les OCVs dans des conditions basales ou au cours de la réponse immunitaire. De plus, l'utilisation des IEGs comme marqueur de l'activité neuronale a permis de confirmer que certains neurones des OCVs sont bel et bien stimulés lors d'une injection i.v. d'IL-1ß (Brady *et al.*, 1994; Ericsson *et al.*, 1994), d'IL-6 (Vallières *et al.*, 1997) ou de TNF-α (S. Nadeau et S. Rivest, papier en préparation). Quant aux plexus choroïdiens, aux leptoméninges et à la microvasculature cérébrale, ces structures sont elles aussi en excellente position pour transmettre aux cellules parenchymales du cerveau l'information provenant de la circulation systémique. Toutefois, puisque l'induction de la transcription du gène CD14 apparaît de façon non-sélective à travers les plexus choroïdiens, les leptoméninges et l'ensemble des vaisseaux sanguins du cerveau, il est possible que ce phénomène ne constitue, en quelque sorte, qu'une première étape dans l'élaboration de la réaction inflammatoire/immunitaire centrale. Néanmoins, il demeure probable qu'au cours de la réponse immunitaire, ces structures participent également au transfert des renseignements essentiels à la régulation des diverses fonctions neuronales et neuroendocriniennes vers les éléments du parenchyme via la sécrétion de médiateurs secondaires (ex.: prostaglandines, cytokines centrales).

Outre son rôle crucial dans la sécrétion des cytokines proinflammatoires à la suite d'une réponse immunitaire, certaines études ont récemment suggéré que le CD14 pourrait participer à la capture et l'élimination des particules exprimant la LPS à leur surface (Fearns *et al.*, 1995). Dans cette perspective, nos résultats, montrant une régulation biphasique du CD14 dans le SNC, sont très intéressants; l'ARNm du CD14 a été rapidement détecté dans des cellules d'origine myéloïde (cellules microgliales et macrophages) localisées dans les structures en contact direct avec la circulation, pour ensuite apparaître dans l'ensemble du

cerveau lors d'une septicémie sévère. Bien que nous ayons identifié plusieurs de ces cellules parenchymales comme étant de type microglial, de nombreuses cellules CD14-positives n'étaient pas immunoréactives à l'anticorps OX-42 (marqueur des cellules phagocytaires). Suite à nos travaux montrant que certains types de neurones ont la capacité d'exprimer l'ARNm du CD14, et ceux du groupe de Feinstein rapportant la présence du CD14 dans des cellules astrocytaires (Galea *et al.*, 1996), il est possible que des cellules centrales d'origine épithéliale synthétisent elles aussi, mais à des temps plus tardifs, le récepteur de la LPS au cours de l'endotoxinémie. Fearns et ses collaborateurs ont d'ailleurs démontré que la LPS a la capacité de stimuler la transcription du CD14 dans des cellules d'origine épithéliale de nombreux tissus périphériques, un effet dépendant de la production des cytokines proinflammatoires par les cellules phagocytaires (Fearns *et al.*, 1995). À la lumière de ces résultats, nous pensons que la LPS cible tout d'abord les cellules d'origine myéloïde des OCVs, plexus choroïdiens, leptoméninges et vaisseaux sanguins du SNC, qui en retour peuvent produire des cytokines (ex.: IL-1ß et TNF-α) capables d'induire la transcription du CD14 dans les cellules parenchymales. Ce phénomène fort complexe pourrait être d'une grande importance afin de maintenir l'homéostasie et protéger les neurones contre une invasion potentielle du cerveau par des corps étrangers. Ainsi, l'expression du CD14 sur les cellules parenchymales du cerveau pourrait s'avérer un élément essentiel afin d'informer les cellules activées de la nécessité de produire des cytokines, d'où le déclenchement de la réponse immunitaire centrale. Fait intéressant, une induction de la transcription des gènes encodant l'IL-1ß et le TNF-α, selon un aspect migratoire originant des OCVs vers les structures parenchymales avoisinantes, a aussi été observé quelques heures après l'administration de la LPS (Nadeau and Rivest, 1998; Quan *et al.*, 1998). Par ailleurs, il est possible que l'induction du CD14 sur les cellules parenchymales du cerveau soit plutôt requise afin de préparer ces mêmes cellules à éliminer les particules d'endotoxine qui pourraient atteindre le liquide céphalo-rachidien et s'avérer toxiques pour les neurones.

7.3 Conclusion générale et perspectives

En conclusion, nous avons démontré que les PGs médiaient certaines influences des cytokines pro-inflammatoires, ou de la LPS, sur l'activité cellulaire nerveuse ou gliale et la transcription des gènes encodant le CRF et le R-CRF_1 au cours de l'endotoxinémie. Toutefois, nos résultats ont confirmé que le rôle des PGs dépendait de plusieurs facteurs tels que la sévérité du stress, la région ou le groupe cellulaire analysé, ainsi que le gène cible étudié. En effet, l'inhibition de la synthèse des PGs a provoqué une baisse significative des

niveaux d'ARNm de c-*fos* dans des structures sélectives du SNC (OVLT/MPOA, PVH et VLM); un phénomène observé exclusivement chez les rats traités avec une dose modérée de LPS. De plus, contrairement aux résultats obtenus chez les animaux injectés avec une forte dose de LPS, le blocage des voies de la cyclooxygénase a complètement prévenu la transcription des gènes encodant le CRF et le R-CRF_1 dans les noyaux PVH de rats soumis à un stress immunitaire modéré ou faible. Il est donc probable que, lors de situations extrêmes ou critiques (ex.: suite à l'injection d'une forte dose d'endotoxine), les cytokines circulantes ou la LPS stimulent aussi des mécanismes indépendants des PGs afin de s'assurer que l'homéostasie sera bel et bien rétablie.

Dans un deuxième temps, nous avons rapporté que l'injection centrale des PGE_2 induit l'expression de c-*fos* dans plusieurs régions du cerveau reconnues pour être activées en réponse à un traitement systémique avec l'endotoxine LPS. L'infusion i.c.v. des PGE_2 a aussi fortement stimulé l'activité des neurones CRFergiques dans les noyaux PVH; un phénomène associé à une induction de l'activité transcriptionnelle des gènes codant le CRF et son récepteur de type 1. Ainsi, ces résultats suggèrent que, suite à un challenge immunitaire, les PGE_2 produites dans le SNC pourraient jouer un rôle crucial dans l'activation de plusieurs structures vraisemblablement impliquées dans la stimulation de l'axe corticotrope. Néanmoins, la possibilité demeure que d'autres types de PGs puissent médier les influences des facteurs immunitaires sur la régulation de l'axe HPA; les candidats étant les PGD_2, les PGE_1 et les $PGF_{2\alpha}$. Par conséquent, il serait très intéressant de déterminer les sites d'action potentiels des différents types de PGs, grâce entre autres à l'utilisation des IEGs et à l'étude de la distribution de leur(s) récepteur(s) respectif(s). Une autre expérience attrayante pourrait consister à administrer centralement des antagonistes spécifiques des prostanoïdes afin de définir l'influence de chaque type ou sous-type de PGs sur les fonctions neuroendocriniennes à la suite d'un stress immunitaire/inflammatoire.

Suite à nos travaux démontrant l'importance des PGs dans l'activation de l'axe HPA, nous avons identifié les sites de production des PGs dans le SNC en réponse à différents modèles infectieux ou inflammatoires. Nos analyses ont révélé la présence de l'ARNm de COX-2 dans des populations cellulaires spécifiques du cerveau, telles que la microvasculature cérébrale, les plexus choroïdiens et les leptoméninges, à la suite d'une administration systémique de LPS. L'injection i.m. de la turpentine a elle aussi fortement stimulé l'activité transcriptionnelle du gène COX-2 dans ces mêmes structures non-parenchymales, confirmant du même coup que cette enzyme puisse être synthétisée à la suite

d'une inflammation stérile localisée en périphérie. Puisque des taux élevés d'ARNm de COX-2 ont été rapidement détectés dans la microvasculature cérébrale de rat injectés avec l'IL-1ß ou le TNF-α, il est probable que ces deux cytokines d'origine systémique soient responsables de la production centrale des PGs au cours de la réponse inflammatoire d'origine systémique. Bien qu'un groupe de chercheurs ait identifié certaines des cellules exprimant l'ARNm de COX-2 comme étant des cellules microgliales associées aux microcapillaires, nos analyses histologiques semblent plutôt révéler que la grande majorité de celles-ci seraient en réalité des cellules endothéliales. Parmi nos projets futurs, nous envisageons donc d'identifier avec certitude les types cellulaires capables de synthétiser l'enzyme limitante à la synthèse des PGs. De plus, grâce à l'utilisation de souris déficientes pour le gène encodant l'IL-1ß ou le TNF-α, il nous sera possible de vérifier si ces deux cytokines pro-inflammatoires ont la capacité de stimuler les vaisseaux sanguins du cerveau, de même que les plexus choroïdiens et les leptoméninges, afin d'induire la biosynthèse de l'enzyme COX-2 et, par conséquent, des PGs centrales au cours de la réponse immune.

Enfin, nous avons démontré, dans notre dernière étude, que la LPS pouvait agir directement sur des populations cellulaires spécifiques du cerveau. Par HISH, nous avons été en mesure de détecter, dans des conditions basales, quelques cellules microgliales associées aux vaisseaux sanguins exprimant le gène CD14, de même que quelques macrophages CD14 positifs dans les plexus choroïdiens et les leptoméninges. L'injection systémique de la LPS a rapidement stimulé la transcription du CD14m dans ces mêmes structures ainsi que dans les OCVs. Quelques heures après l'administration i.v. de l'endotoxine, plusieurs cellules CD14 positives ont été observées dans les régions adjacentes aux OCVs (principalement dans les cellules microgliales) de même que dans les neurones CRFergiques des noyaux PVH. Puisque très peu d'évidences supportent l'hypothèse que la LPS puisse atteindre les noyaux PVH, il est probable qu'une circuiterie plus complexe soit sollicitée par l'endotoxine afin d'induire l'activité de l'axe corticotrope. Outre son rôle dans l'activation de l'axe HPA, possiblement via l'induction de mécanismes régulant la synthèse et la libération des cytokines et des PGs d'origine centrale, il a été suggéré qu'une des fonctions du CD14m pourrait consister à l'opsonisation des corps étrangers exprimant la LPS à leur surface membranaire. Ainsi, il est possible que, lors d'une septicémie sévère, l'expression du CD14 dans l'ensemble du cerveau ne soit qu'un mécanisme de défense contre des envahisseurs bactériens potentiels. Il serait donc très intéressant d'élucider le mécanisme par lequel les cellules du cerveau en viennent à exprimer (selon un patron temporel biphasique) le récepteur CD14m au cours de la réponse immunitaire. Nous croyons que, comme il a été démontré en périphérie, la LPS pourrait tout d'abord cibler les cellules d'origine myéloïde

localisées dans les structures en contact direct avec la circulation sanguine (OCVs, plexus choroïdiens), et ainsi induire la biosynthèse locale des cytokines pro-inflammatoires (IL-1ß et TNF-α). Toujours selon les théories émises au niveau de la périphérie, il est possible que ces mêmes cytokines stimulent (en cascade) les cellules avoisinantes pour finalement induire l'activité transcriptionnelle du CD14 dans les régions adjacentes. Afin de vérifier l'importance des cytokines d'origine centrale dans l'élaboration de ce phénomène, deux possibilités particulièrement intéressantes s'offrent à nous: 1) l'utilisation de souris IL-1ß-/-, IL-6-/- ou TNF-α-/- injectées i.v. ou i.p. avec la LPS, 2) l'infusion centrale des cytokines pro-inflammatoires.

Bref, nous espérons que les études présentées dans cette thèse de doctorat auront contribuées à élucider le rôle des PGs dans l'activation neuronale et neuroendocrinienne au cours de la réponse immunitaire systémique. De plus, nous croyons sincèrement que les projets futurs énumérés ci-dessus sauront clarifier davantage certains aspects encore mal compris des interactions entre les systèmes immunitaire et neuroendocrinien.

RÉFÉRENCES DE L'INTRODUCTION ET DE LA DISCUSSION GÉNÉRALE

Akira S, Hirano T, Taga T, Kishimoto T (1990) Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). FASEB J 4:2860-2867.

Anthonisen M, Knigge U, Kjaer A, Warberg J (1997) Histamine and prostaglandin interaction in the central regulation of ACTH secretion. Neuroendocrinology 66:68-74.

Antoni FA (1986) Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. Endocr Rev 7:351-378.

Antoni FA, Holmes MC, Jones MT (1983) Oxytocin as well as vasopressin potentiate ovine CRF *in vitro*. Peptides 4:411-415.

Arditi M, Zhou J, Dorio R, Rong GW, Goyert SM, Kim KS (1993) Endotoxin-mediated endothelial cell injury and activation: role of soluble CD14. Infect Immun 61:3149-3156.

Arditi M, Zhou J, Torres M, Durden DL, Stins M, Kim KS (1995) Lipopolysaccharide stimulates the tyrosine phosphorylation of mitogen-activated protein kinases p44, p42, and p41 in vascular endothelial cells in a soluble CD14-dependent manner. J Immunol 155:3994-4003.

Arias-Negrete S, Keller K, Chadee K (1995) Proinflammatory cytokines regulate cyclooxygenase-2 mRNA expression in human macrophages. Biochem Bioph Res Co 208:582-589.

Armstrong RC, Montminy MR (1993) Transsynaptic control of gene expression. Ann Rev Neurosci 16:17-29.

Arnold F, De Lucas Bueno M, Shiers H, Hancock DC, Evan GI, Herbert J (1992) Expression of *c-fos* in regions of the basal limbic forebrain following intracerebroventricular corticotropin-releasing factor in unstressed or stressed male rats. Neurosciences 51:377-390.

Ban E, Haour F, R.Lenstra (1992) Brain interleukin 1 gene expression induced by peripheral lipopolysaccharide administration. Cytokine 4:48-54.

Ban EM, Sarlième LL, Haour FG (1993) Interleukin-1 binding sites on astrocytes. Neuroscience 52:725-733.

Bandtlow CE, Meyer M, Lindholm D, Spranger M, Heumann R, Thoenen H (1990) Regional and cellular codistribution of interleukin-1ß and nerve growth factor mRNA in the adult rat brain. J Cell Biol 111:1701-1711.

Banks WA, Kasin AJ (1992) The interleukin-1alpha, interleukin-1beta, and interleukin-2 do not acutely disrupt the murine blood brain barrier. Int J Pharmacol 14:629-636.

Banks WA, Kastin AJ, Durnam DA (1989) Bidirectional transport of interleukin-1 alpha across the blood-brain barrier. Brain Res Bull 23:433-437.

Barbanel G, Ixart G, Szafarczyk A, Malaval F, Assenmacher I (1990) Intrahypothalamic infusion of interleukin-1ß increases the release of corticotropin-releasing hormone (CRH 41) and adrenocorticotropic hormone (ACTH) in free-moving rats bearing a push-pull cannula in the median eminence. Brain Res 516:31-56.

Bateman A, Singh A, Kral T, Solomon S (1989) The immune-hypothalamic-pituitary-adrenal axis. Endocr Rev 10:92-112.

Batshake B, Nilsson C, Sundelin J (1995) Molecular characterization of the mouse prostanoid EP1 receptor gene. Eur J Biochem 231:809-814.

Beach JE, Smallridge RC, Kinzer CA, Bernton EW, Holaday JW, Fein HG (1989) Rapid release of multiple hormones from rat pituitaries perifused with recombinant interleukin-1. Life Sci 44:1-7.

Bebo BFJ, Linthicum DS (1995) Expression of mRNA for 55-kDa and 75-kDa tumor necrosis factor (TNF) receptors in mouse cerebrovascular endothelium: effects of interleukin-1 beta, interferon-gamma and TNF-alpha on cultured cells. J Neuroimmunol 62:161-167.

Becher B, Antel JP (1996) Comparison of phenotypic and functional properties of immediately ex vivo and cultured human adult microglia. Glia 18:1-10.

Berkenbosch F, Oers JV, Rey AD, Tilders F, Besedovsky H (1987) Corticotropin-releasing factor producing neurons in the rat activated by interleukin-1. Science 238:524-526.

Berkenbosch F, Wolvers DAW, DeRijk R (1991) Neuroendocrine and immunological mechanisms in stress-induced immunomodulation. J Steroid Biochem Biol 40:639-647.

Bernardini R (1989) Arachidonic acid metabolites modulate rat hypothalamic cortocotropin-releasing hormone secretion *in vitro*. Neuroendocrinology 50:708-715.

Bernardini R, Calogero AE, Mauceri G, Chrousos G (1990) Rat hypothalamic corticotropin-releasing hormone secretion in vitro is stimulated by interleukin-1 in an eicosanoid-dependent manner. Life Sci 47:1601-1607.

Bernardini R, Kamilaris TC, Calogero AE, Johnson EO, Gomez MT, Gold PW, Chrousos GP (1990) Interactions between tumor necrosis factor-α, hypothalamic corticotropin-releasing hormone, and adrenocorticotropin secretion in the rat. Endocrinology 126:2876-2881.

Bernton EW, Beach JE, Holaday JW, Smallridge RC, Fein HG (1987) Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. Science 238:519-521.

Besedovsky H, del Rey A (1992) Immune-neuroendocrine circuits: integrative role of the cytokines. Front Neuroendocrinol 13:61-94.

Besedovsky HO, Del Rey A (1996) Immune-neuro-endocrine interactions: Facts and hypotheses. Endocr Rev 17:64-102.

Besedovsky HO, Delrey A, Klusman I, Furukawa H, Arditi GM, Kabiersch A (1991) Cytokines as modulators of the hypothalamus-pituitary-adrenal axis. J Steroid Biochem Molec Biol 40:613-618.

Blalock JE (1989) A molecular basis for bidirectional communication between the immune and neuroendocrine systems. Physiol Rev 69:1-32.

Blatteis CM, Bealer SL, Hunter WS, Llanos QJ, Ahokas RA, Mashburn TAJ (1983) Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. Brain Res Bull 11:519-526.

Blatteis CM, Hales JRS, Mckinley MJ, Fawcett AA (1987) Role of the anteroventral third ventricle in fever in sheep. Can J Physiol Pharmacol 65:1255-1260.

Brady L, Lynn AB, Herkenham M, Gottesfeld Z (1994) Systemic interleukin-1 induces early and late patterns of c-*fos* mRNA expression in brain. J Neurosci 14:4951-4964.

Breder CD, Dinarello CA, Saper CB (1988) Interleukin-1 immunoreactive innervation of the human hypothalamus. Science 240:321-324.

Breder CD, Hazuka C, Ghayur T, Klug C, Huginin M, Yasuda K, Teng M, Saper CB (1994) Regional induction of tumor necrosis factor alpha expression in the mouse brain after systemic lipopolysaccharide administration. Proc Natl Acad Sci U S A 91:11393-11397.

Breder CD, Saper CB (1996) Expression of inducible cyclooxygenase mRNA in the mouse brain after systemic administration of bacterial lipopolysaccharide. Brain Res 713:64-69.

Bristow AF, Mosley K, Poole S (1991) Interleukin-1ß production in vivo and in vitro in rats and mice measured using specific immunoradiometric assays. J Mol Endocrinol 7:1-7.

Brooks DP, Share L, Crofton JT (1986) Role of brain prostaglandins in the control of vasopressin secretion in the conscious rat. Endocrinology 118:1716-1722.

Bruce AJ, Boling W, Kindy MS, Peschon J, Kraemer PJ, Carpenter MK, Holtsberg FW, Mattson MP (1996) Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. Nat Med 2:788-794.

Bullitt E (1990) Expression of c-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat. J Comp Neurol 296:517-530.

Busbridge NJ, Grossman AB (1991) Stress and the single cytokine: interleukin modulation of the pituitary-adrenal axis. Mol Cell Endocrinol 82:C-209-C214.

Buttini M, Boddeke H (1995) Peripheral lipopolysaccharide stimulation induces interleukin-1ß messenger RNA in rat brain microglial cells. Neuroscience 65:523-530.

Buttini M, Sauter A, Boddeke H (1994) Induction of interleukin-1ß mRNA after focal cerebral ischaemia in the rat. Molec Brain Res 23:126-134.

Calogero AE, Sternberg EM, Bagdy G, Smith C, Bernardini R, Aksentijevich S, Wilder RL, Gold PW, Chrousos GP (1992) Neurotransmitter-induced hypothalamic-pituitary-adrenal axis responsiveness in inflammatory disease-susceptible Lewis rats: in vivo and vitro studies suggesting a global defect in CRH secretion. Neuroendocrinology 55:600-608.

Calvo W, Forteza-Vila J (1969) On the development of bone marrow innervation in the newborn rats as studied with silver impregnation and electron microscopy. Am J Anat 126:355-359.

Cambronero JC, Rivas FJ, Borrell J, Guaza C (1992) Interleukin-1-beta induces pituitary adrenocorticotropin secretion: evidence for glucocorticoid modulation. Neuroendocrinology 55:648-654.

Cambronero JC, Rivas FJ, Borrell J, Guaza C (1992) Role of arachidonic acid metabolism on corticotropin-releasing factor (CRF)-release induced by interleukin-1 from superfused rat hypothalami. J Neuroimmunol 39:57-66.

Cao C, Matsumura K, Yamagata K, Watanabe Y (1995) Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in the rat brain; its possible role in the febrile response. Brain Res 697:187-196.

Cao CY, Matsumura K, Yamagata K, Watanabe Y (1996) Endothelial-cells of the rat-brain vasculature express cyclooxygenase-2 messenger-RNA in response to systemic interleukin-1: A possible site of prostaglandin synthesis responsible for fever. Brain Res 733:263-272.

Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975) An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 72:3666-3670.

Castell JV, Andus T, Kunz D, Heinrich PC (1989) Interleukin-6: the major regulator of acute-phase protein synthesis in man and rat. Ann N Y Acad Sci 557:87-101.

Chai Z, Gatti S, Toniatti C, Poli V, Bartfai T (1996) Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. J Exp Med 183:311-316.

Chalmers DT, Lovenberg TW, De Souza EB (1995) Localization of novel corticotropin-releasing factor receptor (CRF_2) mRNA expression to specific subcortical nuclei in rat brain: Comparison with CRF_1 receptor mRNA expression. J Neurosci 15:6340-6350.

Champagne D, Beaulieu J, Drolet G (1998) CRFergic innervation of the paraventricular nucleus of the rat hypothalamus: a tract-tracing study. J Neuroendocrinol 10:119-131.

Chan R, Brown E, Ericsson A, Kovacs K, Sawchenko P (1993) A comparison of two immediate early genes, c-*fos* and NGFI-B, as markers for functional activation in stress-related neuroendocrine pathways. J Neurosci 13:5126-5138.

Chan RKW, Peto CA, Sawchenko PE (1995) A1 catecholamine cell group: fine structure and synaptic input from the nucleus of the solitary tract. J Comp Neurol 351:62-80.

Chan RKW, Sawchenko PE (1994) Spatially and temporally differentiated patterns of c-*fos* expression in brainstem catecholaminergic cell groups induced by cardiovascular challenges in the rats. J Comp Neurol 348:433-460.

Chang CP, Pearse RI, O'Connell S, Rosenfeld MG (1993) Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. Neuron 11:1187-1195.

Chen R, Lewis KA, Perrin MH, Vale WW (1993) Expression cloning of a human corticotropin-releasing factor receptor. Proc Natl Acad Sci (USA) 90:8967-8971.

Chuluyan HE, Saphier D, Rohn WM, Dunn AJ (1992) Noradrenergic innervation of the hypothalamus participates in adrenocortical responses to interleukin-1. Neuroendocrinology 56:106-111.

Chung IY, Benveniste EN (1990) Tumor necrosis factor-α production by astrocytes-induction by lipopolysaccharide, IFN-γ, and IL-1β. J Immunol 144:2999-3007.

Coceani F, Lees J, Bishai I (1988) Further evidence implicating prostaglandin E2 in the genesis of pyrogen fever. Am J Physiol 254:R463-R469.

Coceani F, Lees J, Dinarello CA (1988) Occurrence of interleukin-1 in cerebrospinal fluid of the conscious cat. Brain Res 446:245-250.

Collart MA, Baeuerle P, Vassalli P (1990) Regulation of tumor necrosis factor alpha transcription in macrophages: involvement of four κB-like motifs and of constitutive and inducible forms of NF-κB. Mol Cell Biol 10:1498-1506.

Conrad LCA, Pfaff DW (1975) Axonal projections of medial preoptic and anterior hypothalamic neurones. Science 190:1112-1114.

Conti P, Bartle L, Barbacane RC, Reale M, Placido FC, Sipe J (1995) Synergistic activation of serum amyloid A (SAA) by IL-6 and IL-1 in combination on human Hep 3B hepatoma cell line. Role of PGE2 and IL-1 receptor antagonist. Immunol Invest 24:523-535.

Cooper KE (1987) The neurobiology of fever: thoughts on recent developments. Annu Rev Neurosci 10:297-324.

Cross RJ, Markesbery WR, Brooks WH, Roszman TL (1984) Hypothalamic immune interactions: Neuromodulation of natural killer activity by lesioning of the anterior hypothalamus. Immunology 51:399-405.

Cunningham ET, Bohn MC, Sawchenko PE (1990) Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J Comp Neurol 292:651-667.

Cunningham ET, Miselis RR, Sawchenko PE (1994) The relationship of efferent projections from the area postrema to vagal motor and brain stem catecholamine-containing cell groups: An axonal transport and immunohistochemical study in the rat. Neuroscience 58:635-648.

Cunningham ET, Sawchenko PE (1988) Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. J Comp Neurol 274:60-76.

Cunningham ET, Wada E, Carter DB, Tracey DE, Battey JF, DeSouza EB (1992) In Situ Histochemical localisation of type I interleukin-1 receptor messenger RNA in the central nervous system, pituitary and adrenal gland of the mouse. Journal of Neuroscience 12:1101-1111.

Dantzer R (1994) How do cytokines say hello to the brain? Neural versus humoral mediation. Euro Cytokine Netw 5:271-273.

Darnell JEJ (1983) The processing of RNA. Sci Am 249:90-100.

Dascombe MJ, Milton AS (1979) Study on the possible entry of bacterial endotoxin and prostaglandin E2 into the central nervous system from the blood. Br J Pharmacol 66:565-572.

De Groote D, Zaugerle PF, Gevaert Y, Fassote MF, Beguin Y, Noiat-Pirenne F, Pirenne J, Gathy R, Lopez M, Dehart I, Igot D, Baudrihaye M, Delacroix D, Franchimont P (1992) Direct stimulation of cytokines (IL-1ß, TNF-α, IL-6, IL-2, IFN-γ and GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. Cytokine 4:239-248.

DeForge LE, Remick DG (1991) Kinetics of TNF, IL-6, and IL-8 gene expression in LPS-stimulated human whole blood. Biochem Biophys Res Commun 174:18-24.

Dendorfer U, Oettgen P, Libermann TA (1994) Multiple regulatory elements in the interleukin-6 gene mediate induction by prostaglandins, cyclic AMP, and lipopolysaccharide. Mol Cell Biol 14:4443-4454.

DeRijk R, Berkenbosch F (1992) Development and application of radioimmunoassay to detect interleukin-1 in rat peripheral circulation. Am J Physiol 263:E1092-E1098.

Derijk R, Van Rooijen N, Tilders FJH, Besedovsky HO, Del Rey A, Berkenbosch F (1991) Selective depletion of macrophages prevent pituitary-adrenal activation in response to subpyrogenic, but not to pyrogenic, doses of the bacterial endotoxin in rats. Endocrinology 129:330-338.

DeWitt DL, El-Harith EA, Kraemer SA, Andrews MJ, Yao EF, Armstrong RL, Smith WL (1990) The aspirin and heme-binding sites of ovine and murine prostaglandin endoperoxide synthases. J Biol Chem 265:5192-5198.

DeWitt DL, Smith WL (1988) Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc Natl Acad Sci USA 85:1412-1416.

Dinarello CA (1984) Interleukin-1 and the pathogenesis of the acute-phase response. New Eng J Med 311:1413-1422.

Dinarello CA (1989) Interleukin-1 and its biologically related cytokines. Adv Immunol 44:153-161.

Dinarello CA (1991) Interleukin-1 and interleukin-1 antagonism. Blood 77:1627-1652.

Dinarello CA, Cannon JG, Mancilla J, Bishai I, Lees J, Coceani F (1991) Interleukin-6 as an endogenous pyrogen: induction of prostaglandin E2 in brain but not in peripheral blood mononuclear cells. Brain Res 562:199-206.

Dinarello CA, Thompson RC (1991) Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. Immunol Today 12:404-410.

Dodds RA, Merry K, Littlewood A, Gowen M (1994) Expression of mRNA for IL1 beta, IL6 and TGF beta 1 in developing human bone and cartilage. J Histochem Cytochem 42:733-744.

Dragunow M, Faull R (1989) The use of c-*fos* as a metabolic marker in neuronal pathway tracing. J Neurosci Meth 29:261-265.

Drouet C, Shakhov AN, Jongeneel CV (1991) Enhancers and transcription factors controlling the inducibility of the tumor necrosis factor-α promoter in primary macrophages. J Immunol 147:1694-1700.

Dubuis JM, Dayer JM, Siegrist-Kaiser CA, Burger AG (1988) Human recombinant interleukin-1ß decreases plasma thyroid hormone and thyroid stimulating hormone levels in rats. Endocrinology 123:2175-2181.

Dunn AJ (1988) Systemic interleukin-1 administration stimulates hypothalamic norepinephrine metabolism paralleling the increased plasma corticosterone. Life Sci 43:429-435.

Dunn AJ (1992) The role of interleukin-1 and tumor necrosis factor alpha in the neurochemical and neuroendocrine responses to endotoxin. Brain Res Bull 6:807-812.

Dunn AJ (1993) Role of cytokines in infection-induced stress. Ann N Y Acad Sci 697:189-202.

Dupont E, Huygen K, Schandene L, Vandercruys M, Palfliet K, Wybran J (1985) Influence of *in vivo* immunosuppressive drugs on production of lymphokines. Transplantation 39:143-147.

Ebisui O, Fukata J, Murakami N, Kobayashi H, Segawa H, Muro S, Hanaoka I, Naito Y, Masui Y, Ohmoto Y, Imura H, Nakao K (1994) Effect of IL-1 receptor antagonist and antiserum to TNF-α on LPS-induced plasma ACTH and corticosterone in rats. Am J Physiol 266:E986-E992.

Edwards CK, Yunger LM, Lorence RM, Dantzer R, Kelley KW (1991) The pituitary gland is required for protection against lethal effects of Salmonella typhimurium. Proc Natl Acad Sci USA 88:2274-2277.

Elmquist JK, Breder CD, Sherin JE, Scammell TE, Hickey WF, Dewitt D, Saper CB (1997) Intravenous lipopolysaccharide induces cyclooxygenase 2-like immunoreactivity in rat brain perivascular microglia and meningeal macrophages. J Comp Neurol 381:119-129.

Elmquist JK, Saper CB (1996) Activation of neurons projecting to the paraventricular nucleus by intravenous lipopolysaccharide. J Comp Neurol 374:315-331.

Ericsson A, Ek M, Lindefors N (1995) Distribution of prostaglandins E2 receptor (EP3 subtype) mRNA containing cells in the rat central nervous system. Soc Neurosci Abstr 21:98.

Ericsson A, Kovacs KJ, Sawchenko PE (1994) A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. J Neurosci 14:897-913.

Ericsson A, Liu C, Hart RP, Sawchenko PE (1995) Type 1 interleukin-1 receptor in the rat brain: distribution, regulation, and relationship to sites of IL-1-induced cellular activation. J Comp Neurol 361:681-698.

Ericsson A, Arias C, Sawchenko PE (1997) Evidence for an intramedullary prostaglandin-dependent mechanism in the activation of stress-related neuroendocrine circuitry by intravenous interleukin-1. J Neurosci 17:7166-7179.

Ertel W, Morrison MH, Wang P, Ba ZF, Ayala A, Chaudry IH (1992) The complex pattern of cytokines in sepsis - association between prostaglandins, cachectin, and interleukins. Ann Surg 214:141-148.

Fantuzzi G, Dinarello CA (1996) The inflammatory response in interleukin-1ß-deficient mice: comparison with other cytokine-related knock-out mice. J Leukoc Biol 59:489-493.

Fattori E, Cappelletti M, Costa P, Sellito C, Cantoni L, Carelli M, Faggioni R, Fantuzzi G, Ghezzi P, Poli V (1994) Defective inflammatory response in interleukin 6-deficient mice. J Exp Med 180:1243-1250.

Fauci AS, Dale DC (1974) The effects of in vivo hydrocortisone on subpopulations of human lymphocytes. J Clin Invest 53:240-246.

Fearns C, Kravchenko VV, Ulevitch RJ, Loskutoff DJ (1995) Murine CD14 gene expression in vivo: extramyeloid synthesis and regulation by lipopolysaccharide. J Exp Med 181:857-866.

Felten DL, Felten SY, Bellinger DL, Carlson SL, Ackerman KD, Madden KS, Olschowki JA, Livnat S (1987) Noradrenergic sympathetic neural interaction with the immune system: structure and function. Immunol Rev 100:227-260.

Felten DL, Felten SY, Carlson SL, Olschoqka JA, Livnat S (1985) Noradrenergic and peptidergic innervation of lymphoid tissue. J Immunol 135 (suppl 2):755s-765.

Feng L, Sun W, Xia Y, Tang WW, Chanmugam P, Soyoola E, Wilson CB, Hwang D (1993) Cloning two isoforms of rats cyclooxygenase: differential regulation of their expression. Arch Biochem Biophys 307:361-368.

Ferrero E, Jiao D, Tsuberi BZ, Tesio L, Rong GW, Haziot A, Goyert SM (1993) Transgenic mice expressing human CD14 are hypersensitive to lipopolysaccharide. Proc Natl Acad Sci USA 90:2380-2384.

Fink T, Weihe E (1988) Multiple neuropeptides in nerves supplying mammalian lymph nodes: messenger candidates for sensory and autonomic neuroimmunomodulation? Neurosci Lett 90:39-44.

Fleshner M, Goehler LE, Hermann J, Relton JK, Maier SF, Watkins LR (1995) Interleukin-1 beta induced corticosterone elevation and hypothalamic NE depletion is vagally mediated. Brain Res Bull 37:605-610.

Fong Y, Tracey KJ, Moldawer LL, Hesse DG, Manogue KB, Kenney JS, Lee AT, Kuo GC, Allison AC, Lowry SF (1989) Antibodies to cachectin/tumor necrosis factor reduce interleukin 1ß and interleukin 6 appearance during lethal bacteremia. J Exp Med 170:1627-1633.

Fontana A, Kristense F, Dubs R, Gemsa D, Weber E (1982) Production of prostaglandins E and an interleukin-1 like factor by cultured astrocytes and C glioma cells. J Immunol 129:2413-2419.

Fontana A, Weber E, Dayer JM (1984) Synthesis of interleukin-1/endogenous pyrogen in the brain of endotoxin-treated mice: a step in fever induction? J Immunol 133:1696-1698.

Fremeau RTJ, Autelitano DJ, Blum M, Wilcox J, Roberts JL (1989) Intervening sequence-specific *in situ* hybridization: detection of the pro-opiomelanocortin gene primary transcript in individual neurons. Mol Brain Res 6:197-201.

Fried G, Terenius L, Brodin E, Efendic S, Dockray G, Fahrenkrug J, Goldstein M, Hökfelt T (1986) Neuropeptide Y, enkephalin and noradrenaline coexist in sympathetic neurons innervating the bovine spleen. Biochemical and immunohistochemical evidence. Cell Tissue Res 243:495-508.

Galea E, Reis DJ, Fox ES, Xu H, Feinstein DL (1996) CD14 mediates endotoxin induction of nitric oxide synthase in cultured brain glial cells. J Neuroimmunol 64:19-28.

Ghosh S, Baltimore D (1990) Activation in vitro of NF-kappa B by phosphorylation of its inhibitor I kappa B. Nature 344:678-682.

Gilbert MS, Payan DG (1991) Interactions between the nervous and the immune systems. Front Neuroendocrin 12:299-322.

Gillis S, Crabtree GR, Smith KA (1979) Glucocorticoid induced inhibition of T cell growth factor production. I. The effect of mitogen induced lymphocyte proliferation. J Immunol 123:1624-1631.

Giulian D, Baker TJ, Shih LCN, Lachman LB (1986) Interleukin 1 of the central nervous system is produced by ameboid microglia. J Exp Med 164:594-604.

Givalois L, Dornand J, Mekaouche M, Solier MD, Bristow AF, Ixart G, Siaud P, Assenmacher I, Barbanel G (1994) The temporal cascade of plasma level surges in ACTH, corticosterone and cytokines in endotoxin-challenged rats. Am J Physiol 266:R164-R170.

Goehler LE, Busch CR, Tartaglia N, Relton J, Sisk D, Maier SF, Watkins LR (1995) Blockage of cytokine induced conditioned taste aversion by subdiaphragmatic vagotomy: Further evidence for vagal mediation of immune-brain communication. Neurosci Lett 185:163-166.

Golenbock DT, Liu Y, H. MF, Freeman MW, Zoeller RA (1993) Surface expression of human CD14 in Chinese hamster ovary fibroblasts imparts macrophage-like responsiveness to bacterial endotoxin. J Biol Chem 268:22055-22059.

Goppelt-Struebe M (1995) Regulation of prostaglandin endoperoxidase synthase (cyclooxygenase) isozyme expression. Prostag Leukotr Ess 52:213-222.

Haour F, Ban E, Milon G, Baran D, Fillion G (1990) Brain interleukin 1 receptors: Characterization and modulation after lipopolysaccharide injection. Progress in NeuroEndocrinImmunology 3:196-204.

Harbuz MS, Rees RG, Lightman SL (1993) HPA axis responses to acute stress and adrenalectomy during adjuvant-induced arthritis in the rat. Am J Physiol 264:R179-185.

Harbuz MS, Stephanou A, Sarlis N, Lightman SL (1992) The effects of recombinant human interleukin (IL)-1 alpha, IL-1 beta or IL-6 on hypothalamo-pituitary-adrenal axis activation. J Endocrinol 133:349-355.

Hare AS, Clarke G, Tolchard S (1995) Bacterial lipopolysaccharide-induced changes in Fos protein expression in the rat brain: Correlation with thermoregulatory changes and plasma corticosterone. J Neuroendocrinol 7:791-799.

Hart BL (1988) Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 12:123-137.

Hashimoto M, Ishikawa Y, Yokota S, Goto F, Bando T, Sakakibara Y, Iriki M (1991) Action site of circulating interleukin-1 on the rabbit brain. Brain Res 540:217-223.

Havell E (1989) Evidence that tumor necrosis factor has an important role in antibacterial resistance. J Immunol 143:2894-2899.

Hedge GA (1976) Hypothalamic and pituitary effects of prostaglandins on ACTH secretion. Prostaglandins 11:293-301.

Hempel SL, Monick MM, Hunninghake GW (1994) Lipopolysaccharide induces prostaglandin H synthase-2 protein and mRNA in human alveolar macrophages and blood monocytes. J Clin Invest 93:391-396.

Henkel T, Machleidt T, Alkalay I, Kronke M, Ben-Neriah Y, Baeuerle PA (1993) Rapid proteolysis of I kappa B-alpha is necessary for activation of transcription factor NF-kappa B. Nature 365:182-185.

Herman JP, Schafer M, Thompson RC, Watson SJ (1992) Rapid regulation of corticotropin-releasing hormone gene transcription *in vivo*. Mol Endocrinol 6:1061-1069.

Herman JP, Schäfer MKH, Watson SJ, Sherman TG (1991) *In situ* hybridization analysis of arginin vasopressin gene transcription using intron-specific probes. Mol Endocrinol 5:1447-1456.

Hetier E, Ayala J, Denèfle P, Bousseau A, Rouget P, Mallat M, Prochiantz A (1988) Brain macrophages synthesize interleukin-1 and interleukin-1 mRNAs in vitro. J Neurosci Res 21:391-397.

Hibi M, Murakami M, Saito M, Hirano T, Taga T, Kishimoto T (1990) Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell 63:1149-1157.

Higgins GA, Olschowka JA (1991) Induction of interleukin-1ß mRNA in adult rat brain. Mol Brain Res 9:143-148.

Hirota H, Kiyama H, Kishimoto T, Taga T (1996) Accelerated nerve regeneration in mice by upregulated expression of interleukin (IL) 6 and IL-6 receptor after trauma. J Exp Med 183:2627-2634.

Hiscott J, Marois J, Garoufalis J, D'Addario M, Roulston A, Kwan I, Pepin N, Lacoste J, Nguyen H, Bensi G, Fenton M (1993) Characterization of a functional NF-κB site in the human interleukin 1ß promoter: Evidence for a positive autoregulatory loop. Mol Cell Biol 13:6231-6240.

Hoffman PK, Share L, Crofton JT, Shade RE (1982) The effect of intracerebroventricular indomethacin on osmotically stimulated vasopressin release. Neuroendocrinology 34:132-139.

Honda A, Sugimoto Y, Namba T, Watabe A, Irie A, Negishi M, Narumiya S, Ichikawa A (1993) Cloning and expression of a cDNA for mouse prostaglandin E receptor EP2 subtype. J Biol Chem 268:7759-7762.

Hopkins SJ, Rothwell NJ (1995) Cytokines and the nervous system I: expression and recognition. TINS 18:83-88.

Inoue M, Crofton JT, Share L (1990) Interactions between the brain renin-angiotensin system and brain prostanoids in the control of vasopressin secretion. Exp Brain Res 83:131-136.

Irie A, Sugimoto Y, Namba T, Harazono A, Honda A, Watabe A, Negishi M, Narumiya S, Ichikawa A (1993) Third isoform of the prostaglandin-E-receptor EP3 subtype with different C-terminal tail coupling to both stimulation and inhibition of adenylate cyclase. Eur J Biochem 217:313-318.

Ishikawa S, Saito T, Yoshida S (1981) The effect of prostaglandins on the release of arginine vasopressin from the guinea pig hypothalamo-neurohypophyseal complex in organ culture. Endocrinology 108:193-198.

Jacobson L, Sharp FR, Dallman MF (1990) Induction of *fos*-like immunoreactivity in hypothalamic corticotropin-releasing factor neurons after adrenalectomy in the rat. Endocrinology 126:1709-1719.

Jankovic BD, Isakovic K (1973) Neuroendocrine correlates of immune response. I. Effects of brain lesions on antibody production. Arthus reactivity and delayed type hypersensitivity in the rat. Int Arch Allergy Immunol 45:360-372.

Jankovik BD (1989) Neuroimmunodulation: facts and dilemmas. Immunol Lett 21:101-118.

Johnson AK, Gross PM (1993) Sensory circumventricular organs and brain homeostatic pathways. Faseb 7:678-686.

Johnson HM, Smith EM, Torres BA, Blalock JE (1982) Regulation of the in vitro antibody response by neuroendocrine hormones. Proc Natl Acad Sci USA 79:4171-4174.

Jones DA, Carlton DP, McIntyre TM, Zimmerman GA, Prescott SM (1993) Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. J Biol Chem 268:9049-9054.

Kakucska I, Y.Qi, Clark BD, Lechan RM (1993) Endotoxin-induced corticotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus is mediated centrally by interleukin-1. Endocrinology 133:815-821.

Kasid A, Director EP, Rosenberg SA (1989) Regulation of interleukin-6 (IL-6) by IL-2 and TNF-α in human peripheral blood mononuclear cells. Ann N-Y Acad Sci 557:564-566.

Katsuura G, Arimura A, Koves K, Gottschall PE (1990) Involvement of organum vasculosum of the lamina terminalis and preoptic area in interleukin-1ß-induced ACTH release. Am J Physiol 258:E163-E171.

Katsuura G, Gottschall PE, Dahl RR, Arimura A (1988) Adrenocorticotropin release induced by intracerebroventricular injection of recombinant human interleukin-1 in rats: Possible involvement of prostaglandin. Endocrinology 122:1773-1779.

Katsuura G, Gottschall PE, Dahl RR, Arimura A (1989) Interleukin-1ß increases prostaglandin E2 in rat astrocyte cultures: modulating effect of neuropeptides. Endocrinology 124:3125-3127.

Kawano H, Daikoku S, Shibasaki T (1988) CRF-containing neurons in the rat hypothalamus: retrograde tracing and immunohistochemical studies. J Comp Neurol 272:260-268.

Kehrer P, Turnill D, Dayer J-M, Muller AF, Gaillard RC (1988) Human recombinant interleukin-1 beta and -alpha, but not recombinant tumor necrosis factor alpha stimulate ACTH release from rat anterior pituitary cells in vitro in a prostaglandin E2 and cAMP independent manner. Neuroendocrinology 48:160-166.

Keller-Wood ME, Dallman MF (1984) Corticosteroid inhibitionof ACTH secretion. Endocr Rev 5:1-24.

Kelso A, Munck A (1984) Glucocorticoid inhibition of lymphokine secretion by alloreactive T lymphocyte clones. J Immunol 133:784-791.

Kent S, Bluthe RM, Kelley KW, Dantzer R (1992) Sickness behavior as a new target for drug development. Trends Pharmacol Sci 13:24-28.

Khansari DN, Murgo AJ, Faith RE (1990) Effects of stress on the immune system. Immunol Today 11:170-175.

Kishimoto T (1989) The biology of interleukin-6. Blood 74:1-10.

Kishimoto T, Pearse RV, Lin CR, Rosenfeld MG (1995) A sauvagine/corticotropin-releasing factor receptor expressed in the heart and skeletal muscle. Proc Natl Acad Sci USA 92:1108-1112.

Kitchens RL, Ulevitch RJ, Munford RS (1992) Lipopolysaccharide (LPS) partial structures inhibit responses to LPS in a human macrophage cell line without inhibiting LPS uptake by a CD14-mediated pathway. J Exp Med 176:485-494.

Klir JJ, McClellan JL, Kluger MJ (1994) Interleukin-1 beta causes the increase in anterior hypothalamic interleukin-6 during LPS-induced fever in rats. Am J Physiol 266:R1845-R1848.

Kluger MJ (1991) Fever: role of pyrogens and cryogens. Physiol Rev 71:93-127.

Kobayashi H, Fukata J, Murakami N, Usui T, Ebisui O, Muro S, Hanaoka I, Inoue K, Imura H, Nakao K (1997) Tumor necrosis factor receptors in the pituitary cells. Brain Res 758:45-50.

Koenig JI (1991) Presence of cytokines in the hypothalamic-pituitary axis. Prog NeuroEndocrImmunol 4:143-153.

Komaki G, Arimura A, Koves K (1992) Effect of intravenous injection of IL-1ß on PGE2 levels in several brain areas as determined by microdialysis. Am J Physiol 262:E246-E251.

Kovacs KJ, Elenkov IJ (1995) Diffencial dependence of ACTH secretion induced by various cytokines on the integrity of the paraventricular nucleus. J Neuroendocrinol 7:15-23.

Kovacs KJ, Sawchenko PE (1993) Mediation of osmoregulatory influences on neuroendocrine corticotropin-releasing factor expression by the ventral lamina terminalis. Proc Natl Acad Sci USA 90:7681-7685.

Kovacs KJ, Sawchenko PE (1994) Stress-induced transcriptional activation of the corticotropin-releasing factor gene precedes immediate-early-genes responses in the paraventricular nucleus. Soc Neurosci Abstr 20:935.

Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR (1991) TIS10, a phorbol ester tumor promoter-inducible mRNA from swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J Biol Chem 266:12866-12872.

Kuprash DV, Udalova IA, Turetskaya RL, Rice NR, Nedospasov SA (1995) Conserved kappa-B element located downstream of the tumor necrosis factor alpha gene distinct NF-kappa-B binding pattern and enhancer activity in LPS activated murine macrophages. Oncogene 11:97-106.

Kurkowski R, Kummer W, Heym C (1990) Substance P-immunoreactive nerve fibers in tracheobronchial lymph nodes of the guinea pig: Origin, ultrastructure and coexistence with other peptides. Peptides 11:13-20.

Kushner I (1982) The phenomenon of the acute-phase response. Ann NY Acad Sci 389:39-48.

Labiner DJ, Butler LS, Zhen C, Hosford DA, Shin C, McNamara JO (1993) Induction of c-*fos* mRNA in kindling seizures: complex relationship with neuronal burst firing. J Neurosci 13:744-751.

Lacroix S, Feinstein D, Rivest S (1998) The bacterial endotoxin lipopolysaccharide has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations. Brain Pathol submitted.

Lacroix S, Rivest S (1997) Functional circuitry in the brain of immune-challenged rats: partial involvement of prostaglandins. J Comp Neurol 387:307-324.

Lacroix S, Rivest S (1998) Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. J Neurochem 70:452-466.

Lacroix S, Vallières L, Rivest S (1996) C-fos mRNA pattern and corticotropin-releasing factor neuronal activity throughout the brain of rats injected centrally with a prostaglandin of E2 type. J Neuroimmunol 70:163-179.

Laflamme N, Feuvrier E, Richard D, Rivest S (1998) Involvement of serotoninergic pathways in mediating the neuronal activity and genetic transcription of neuroendocrine corticotropin-releasing factor in the brain of systemically endotoxin-challenged rats. Neurosciences in press.

Laflamme N, Rivest S (1994) Systemic endotoxin activates the genetic expression of c-*fos*, NGFI-B, and neuropeptides in selective regions of the rat brain. Proceeding of the 76th Annual Meeting of the Endocrine Society 76:514.

Larsen PJ, Mikkelsen JD (1995) The functional identification of central afferent projections conveying information of acute "stress" to the hypothalamic paraventricular nucleus. J Neurosc 15:2609-2627.

Le J, Vilcek J (1987) Biology of disease. Tumor necrosis factor and interleukin-1: cytokines with multiple overlapping biological activities. Lab Invest 56:234-238.

Lechan RM, Toni R, Clark BD, Cannon JG, Shaw AR, Dinarello CA, Reichlin S (1990) Immunoreactive interleukin-1ß localisation in the rat forebrain. Brain Research 514:135-140.

Lee HY, Herkenham M (1996) Area postrema removal abolishes stimulatory effects of intravenous interleukin-1ß on HPA axis activity and c-fos mRNA in the hypothalamic paraventricular nucleus. Soc Neurosci Abstr 22:87.

Lee JD, Kato K, Tobias PS, Kirkland TN, Ulevitch RJ (1992) Transfection of CD14 into 70Z/3 cells dramatically enhances the sensitivity to complexes of lipopolysaccharide (LPS) and LPS binding protein. J Exp Med 175:1697-1705.

Lee JD, Kravchenko V, Kirkland TN, Han J, Mackman N, Moriarty A, Leturcq D, Tobias PS, Ulevitch RJ (1993) Glycosyl-phosphatidylinositol-anchored or integral membrane forms of CD14 mediate identical cellular responses to endotoxin. Proc Natl Acad Sci USA 1990:9930-9934.

Lee SH, Soyoola E, Chanmugam P, Hart S, Sun W, Zhong H, Liou S, Simmons D, Hwang D (1992) Selective expression of mitogen-inducible cyclooxygenase in macrophages stimulated with lipopolysaccharide. J Biol Chem 267:25934-25938.

Lemay LG, Otterness I, Vander AJ, Kluger MJ (1990) In vivo evidence that the rise in plasma IL 6 following injection of fever-inducing dose of LPS is mediated by IL-1ß. Cytokine 2:199-204.

LeMay LG, Vander AJ, Kluger MJ (1990) Role of interleukin 6 in fever in rats. Am J Physiol 258:R798-R803.

Lennard DE, Eckert WA, Merchenthaler I (1993) Corticotropin-Releasing Hormone Neurons in the Paraventricular Nucleus Project to the External Zone of the Median Eminence - A Study Combining Retrograde Labeling with Immunocytochemistry. J Neuroendocrinol 5:175-181.

Leturcq DJ, Moriarty AM, Talbott G, Winn RK, Martin TR, Ulevitch RJ (1996) Antibodies against CD14 protect primates from endotoxin-induced shock. J Clin Invest 98:1533-1538.

Levin N, Shinsako J, Dallman M (1988) Corticosterone acts on the brain to inhibit adrenalectomy-induced adrenocorticotropin secretion. Endocrinology 122:694-701.

Lewin B (1980) Gene expression II. In: Eukaryotic chromosomes. (Lewin B, ed.), pp 728-864. New York: Wiley-Liss Inc.

Li H-Y, Ericsson A, Sawchenko P (1996) Distinct mechanisms underlie activation of hypothalamic neurosecretory neurons and their medullary catecholaminergic afferents in categorically different stress paradigms. Proc Natl Acad Sci USA 93:2359-2364.

Libermann TA, Baltimore D (1990) Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. Mol Cell Biol 10:2327-2334.

Liposits Z (1993) Ultrastructure of hypothalamic paraventricular neurons. Crit Rev Neurobiol 7:89-162.

Loewy AD (1990) Central autonomic pathways. In: Central regulations of autonomic functions. (Loewy AD, Spyer KM, ed.), pp 88-103. New-York: Oxford UP.

Lovenberg TW, Liam CW, Grigoriadis DE, Clevenger W, Chalmers DT, DeSouza EB, Oltersdorf T (1995) Cloning and characterization of a functional distinct corticotropin-releasing factor receptor subtype from the rat brain. Proc Natl Acad Sci USA 92:836-840.

Lucas R, Lou J, Morel DR, Ricou B, Suter PM, Grau GE (1997) TNF receptors in the microvasculatur pathology of acute respiratory distress syndrome and cerebral malaria. J Leukoc Biol 61:551-558.

Luheshi G, Miller AJ, Brouwer S, Dascombe MJ, Rothwell NJ, Hopkins SJ (1996) Interleukin-1 receptor antagonist inhibits endotoxin fever and systemic interleukin-6 induction in the rat. Am J Physiol 270:E91-E95.

Lynch EA, Dinarello CA, Cannon JG (1994) Gender differences in IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist secretion from mononuclear cells and urinary excretion. J Immunol 153:300-306.

Lyson K, McCann SM (1992) Induction of adrenocorticotropic hormone release by interleukin-6 in vivo and in vitro. Ann N Y Acad Sci 650:182-185.

Lyson K, McCann SM (1992) Involvement of arachidonic acid cascade pathways in interleukin-6-stimulated corticotropin-releasing factor release in vitro. Neuroendocrinology 55:708-713.

Ma YI, Berg-von der Emde K, Rage F, Wetsel WC, Ojeda SR (1997) Hypothalamic astrocytes respond to transforming growth factor alpha with secretion of neuroactive substances that stimulate the release of luteinizing hormone-releasing hormone. Endocrinology 138:19-25.

Maier JA, Hla T, Maciag T (1990) Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells. J Biol Chem 265:10805-10808.

Maier SF, Wiertelak EP, Martin D, Watkins LR (1993) Interleukin-1 mediates the behavioral hyperalgesia produced by lithium chloride and endotoxin. Brain Res 623:321-324.

Malarkey WB, Zvara BJ (1989) Interleukin-1ß and other cytokines stimulate adrenocorticotropin release from cultured pituitary cells of patients with Cushing's disease. J Clin Endocrinol Metab 69:196-199.

Mansi JA, Rivest S, Drolet G (1996) Regulation of corticotropin-releasing factor type 1 (CRF1) receptor messenger ribonucleic acid in the paraventricular nucleus of rat hypothalamus by exogenous CRF. Endocrinology 137:4619-4629.

Marino MW, Dunn A, Grail D, Inglese M, Noguchi Y, Richards E, A. J, Wada H, Moore M, Williamson B, Basu S, Old LJ (1997) Characterization of tumor necrosis factor-deficient mice. Proc Natl Acad Sci USA 94:8093-8098.

Martich GD, Boujoukos AJ, Suffredini AF (1993) Response of man to endotoxin. Immunobiology 187:403-416.

Mason D, MacPhee I, Antoni F (1990) The role of the neuroendocrine system in determining genetic susceptibility to experimental allergic encephalomyelitis. Immunology 70:1-5.

Mastorakos G, Chrousos GP, Weber JS (1993) Recombinant interleukin-6 activates the hypothalamic-pituitary-adrenal axis in humans. J Clin Endocrinol Metab 77:1690-1694.

Mastorakos G, Weber JS, Magiakou MA, Gunn H, Chrousos GP (1994) Hypothalamic-pituitary-adrenal axis activation and stimulation of systemic vasopressin secretion by recombinant interleukin 6 in humans: potential implications for the syndrome of innappropriate vasopressin secretion. J Clin Endocrinol Metab 79:934-939.

Matsumura K, Watanabe Y, Imai-Matsumura K, Connolly M, Koyama Y, Onoe H, Watanabe Y (1992) Mapping of prostaglandin E2 binding sites in rat brain using quantitative autoradiography. Brain Res 581:292-298.

Matsumura K, Watanabe Y, Onoe H, Watanabe Y, Hayaishi O (1990) High density of prostaglandin E2 binding sites in the anterior wall of the 3rd ventricle: a possible site of its hyperthermic action. Brain Res 533:147-151.

Matta SG, Weatherbee J, Sharp BM (1992) A central mechanism is involved in the secretion of ACTH in response to IL-6 in rats: comparison to and interaction with IL-1 beta. Neuroendocrinology 56:516-525.

McCann SM, Gonzalez CM, Milenkovic L, Karanth S, Aguila CM, Dees LW, Lyson K, Rettori V (1993) The effect of stress and infection on pituitary hormone secretion. Neuroendocrinol Lett 15:33-47.

McCoy JG, Matta SG, Sharp BM (1994) Prostaglandins mediate the ACTH response to interleukin-1-beta instilled into the hypothalamic median eminence. Neuroendocrinology 60:426-435.

McGillis JP, Hall NR, Goldstein AL (1988) Thymosin fraction 5 (TF5) stimulates secretion of adrenocorticotropin hormone (ACTH) from cultured rat pituitaries. Life Sci 42:2259-2268.

Merlie JP, Fagan D, Mudd J, Needleman P (1988) Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). J Biol Chem 263:3550-3553.

Milenkovic L, Rettori V, Snyder GD, Beutler B, McCann S, M. (1989) Cachectin alters anterior pituitary hormone release by a direct action in vitro. Proc Natl Acad Sci USA 86:2418-2422.

Moga MM, Saper CB (1994) Neuropeptide-immunoreactive neurons projecting to the paraventricular hypothalamic nucleus in the rat. J Comp Neurol 346:137-150.

Molher KM, Torrance DS, Smith CA, Goodwin RG, Stremler KE, Fung VP, Madani H, Widmer MB (1993) Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. J Immunol 151:1548-1561.

Morgan JI, Curran T (1989) Stimulus-transcription coupling in neurons: role of cellular immediate-early-genes. TINS 12:459-462.

Morgan JI, Curran T (1991) Stimulus-transcription coupling in the nervous system: Involvement of the inductible proto-oncogenes fos and jun. Annu Rev Neurosci 14:421-451.

Morimoto A, Murakami N, Nakamori T, Sakata Y, Watanabe T (1989) Possible involvement of prostaglandin E in development of ACTH response in rats induced by human recombinant interleukin-1. J Physiol 411:245-256.

Morimoto A, Murakami N, Nakamori T, Watanabe T (1988) Ventromedial hypothalamus is highly sensitive to prostaglandin E2 for producing fever in rabbits. J Physiol Lond 397:259-268.

Morimoto K, Morimoto A, Nakamori T, Tan N, Minagawa T, Murakami N (1992) Cardiovascular responses induced in free-moving rats by immune cytokines. J Physiol Lond 448:307-320.

Munck A, Guyre PM, Holbrook NJ (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev 5:25-44.

Murakami N, Watanabe T (1989) Activation of ACTH release is mediated by the same molecule as the final mediator, PGE_2, of febrile response in rats. Brain Res 478:171-174.

Mustafa MM, Ramilo O, Olsen KD, Franklin PS, Hansen EJ, Beutler B, McCracken GHJ (1989) Tumor necrosis factor in mediating experimental Haemophilus influenzae type B meningitis. J Clin Invest 84:1253-1259.

Nadeau S, Rivest S (1998) Regulation of the gene encoding tumor necrosis factor alpha (TNF-α) in the rat brain and pituitary in response to different models of systemic immune challenge. J Neurobiol submitted.

Naitoh Y, Fukata J, Shindo K, Ebisul O, Murakama N, Tominaga T, Nakay Y, Tamai S, Mori K, Kasting N, Imura H (1991) Effects of interleukins on plasma AVP and oxytocin levels in conscious, freely moving rats. Biochem Biophys Res Commun 174:1189-1195.

Naitoh Y, Fukata J, Tominaga T, Nakai Y, Sunao T, Kenjiro M, Imura H (1988) Interleukin-6 stimulates the secretion of adrenocorticotropic hormone in conscious, freely-moving rats. Biochem Biophys Res Comm 155:1459-1463.

Nakane A, Minagawa T, Kato K (1988) Endogenous tumor necrosis factor (cachectin) is essential to host resistance against Listeria monocytogenes infection. Infect Immunol 56:2563-2569.

Nakano Y, Onozuka K, Terada Y, Shinomiya H, Nakano M (1990) Protective effect of recombinant tumor necrosis factor-α in murine salmonellosis. J Immunol 144:1935-1941.

Nance DM, Hopkins DA, Bieger D (1987) Re-investigation of the innervation of the thymus gland in mice and rats. Brain Behav Immunol 1:134-137.

Nappi RE, Bonneau MJ, Rivest S (1997) C-*fos* and CRF gene transcription in the brains of endotoxin-challenged cycling female rats: A possible relevance for neuroendocrine-immunological sexual dimorphism. Neuroendocrinology 65:29-46.

Nasushita R, Watanobe H, Takebe K (1997) A comparative study of adrenocorticotropin-releasing activity of prostaglandins E_1, E_2, $F_{2\alpha}$ and D_2 in the rat. Prostag Leukotr Ess 56:165-168.

Nathan CF (1987) Secretory products of macrophages. J Clin Invest 79:319-326.

Navarra P, Pozzoli G, Brunetti L, Ragazzoni E, Besser M, Grossman A (1992) Interleukin-1ß and interleukin-6 specifically increase the release of prostaglandin E2 from rat hypothalamic explants *in vitro*. Neuroendocrinology 56:61-68.

Navarra P, Tsagarakis S, Faria MS, Rees LH, Besser GM, Grossman AB (1991) Interleukin-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. Endocrinology 128:37-44.

Neidhart M, Fluckiger EW (1992) Hyperprolactinaemia in hypophysectomized or intact male rats and the development of adjuvant arthritis. Immunology 77:449-455.

Neta R, Perlstein R, Vogel SN, Ledney GD, Abrams J (1992) Role of interleukin 6 (IL-6) in protection from lethal irradiation and in endocrine responses to IL-1 and tumor necrosis factor. J Exp Med 175:689-694.

Neuschäfer-Rube F, DeVries C, Hänecke K, Jungermann K, Püschel GP (1994) Molecular cloning and expression of a prostaglandin E2 receptor of the EP3B subtype from rat hepatocytes. FEBS Lett 351:119-122.

Nicholson C (1980) Dynamics of the brain cell microenvironnment. Neurosci Res Prog Bull 18:177-322.

Nishigaki N, Negishi M, Honda A, Sugimoto Y, Namba T, Narumiya S, Ichikawa A (1995) Identification of prostaglandin E receptor "EP2" cloned from mastocytoma cells as EP4 subtype. FEBS Lett 364:339-341.

Nogawa S, Zhang F, Ross ME, Iadecola C (1997) Cyclo-oxygenase-2 gene expression in neurons contributes to ischemic brain damage. J Neurosci 17:2746-2755.

Ohmichi M, Hirota K, Koike K, Kurachi H, Ohtsuka S, Matsuzaki N, Yamaguchi M, Miyake A, Tanizawa O (1992) Binding sites for interleukin-6 in the anterior pituitary gland. Neuroendocrinology 55:199-203.

Ojeda SR, Harms PG, McCann SM (1975) Effect of inhibitors of prostaglandin synthesis on gonadotropin release in the rat. Endocrinology 97:843-854.

Oka T, Hori T (1994) EP1-receptor mediation of prostaglandin E2-induced hyperthermia in rats. Am J Physiol 267:R289-R294.

Oldfield BJ, McKinley MJ (1995) Circumventricular organs. In: The rat nervous system (Paxinos G, ed.), pp 391-403. San Diego: Academic Press.

Ono N, Bedran-DeCastro J, McCann S (1985) Ultrashort-loop positive feedback of corticotropin (ACTH)-releasing factor to enhance ACTH release during stress. Proc Natl Acad Sci USA 82:3528-3531.

Oppenheim JJ, Gery I (1982) Interleukin 1 is more than an interleukin. Immunol Today 3:113-119.

Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK (1986) There is more than one interleukin 1. Immunol Today 7:45-56.

Orth DN, Kovacs WJ, DeBold CR (1992) The adrenal cortex. In: Textbook of Endocrinology (Wilson JD, Foster DW, ed.), pp 418-619. Philadelphia: W. B. Saunders.

Ottaway CA, Greenberg G (1984) Interaction of vasoactive intestinal peptide with mouse lymphocytes: specific binding and modulation of mitogen responses. J Immunol 132:417-423.

Parkes D, Rivest S, Lee S, Rivier C, Vale W (1993) Corticotropin-releasing factor activates c-*fos*, NGFI-B and CRF gene expression within the paraventricular nucleus of the rat hypothalamus. Mol Endocrinol 7:1357-1367.

Parsadaniantz SM, Lenoir V, Terlain B, Kerdelhué B (1993) Lack of effect of interleukins 1α and 1ß, during in vitro perifusion, on anterior pituitary release of adrenocorticotropic hormone and ß endorphin in the male rat. J Neurosci Res 34:315-323.

Partridge WM (1983) Neuropeptides and the blood-brain barrier. Annu Rev Physiol 45:73-92.

Payne LC, Obal F, Opp MR, Krueger JM (1992) Stimulation and inhibition of growth hormone secretion by interleukin-1 beta: the involvement of growth hormone-releasing hormone. Neuroendocrinology 56:118-123.

Perlstein RS, Mougey EH, Jackson WE, Neta R (1991) Interleukin-1 and interleukin-6 act synergistically to stimulate the release of adrenocorticotropic hormone in vivo. Lymphokine Cytok Res 10:141-146.

Perlstein RS, Whitnall MH, Abrams JS, Mougey EH, Neta R (1993) Synergistic roles of interleukin-6, interleukin-1, and tumor necrosis factor in the adrenocorticotropin response to bacterial lipopolysaccharide in vivo. Endocrinology 132:946-952.

Perrin M, Donaldson C, Chen R, Blount A, Berggren T, Bilezikjian L, Sawchenko P, Vale W (1995) Identification of a second corticotropin-releasing factor receptor gene and caracterization of a cDNA expressed in heart. Proc Natl Acad Sci USA 92:2969-2973.

Perrin MH, Donaldson CJ, Chen R, Lewis KA, Vale WW (1993) Cloning and functional expression of a rat brain corticotropin-releasing factor (CRF) receptor. Endocrinology 133:3058-3061.

Pollack M, Ohl CA, Golenbock DT, Di Padova F, Wahl LM, Koles NL, Guelde G, Monks BG (1997) Dual effects of LPS antibodies on cellular uptake of LPS and LPS-induced proinflammatory functions. J Immunol 159:3519-3530.

Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale WW (1994) The distribution of CRF receptor mRNA expression in the rat brain and pituitary. Proc Natl Acad Sci, USA 91:8777-8781.

Poulain P, Carette B (1974) Iontophoresis of prostaglandins on hypothalamic neurons. Brain Res 79:311-314.

Quan N, Whiteside M, Herkenham M (1998) Time course and localization patterns of interleukin-1ß messenger RNA expression in brain and pituitary after peripheral administration of lipopolysaccharide. Neuroscience 83:281-293.

Rabin BS, Cunnick JE, Lysle DT (1990) Stress-induced alteration of immune function. Prog NeuroEndocrinImmunol 3:116-125.

Rabin D, Gold PW, Margioris AN, Chrousos GP (1988) Stress and reproduction: physiologic and pathophysiologic interactions between the stress and reproductive axes. Adv Exp Med Biol 245:377-387.

Raetz CR (1990) Biochemistry of endotoxins. Annu Rev Biochem 59:129-170.

Rage F, Lee BJ, Ma YJ, Ojeda SR (1997) Estradiol enhances prostaglandin E2 receptor gene expression in luteinizing hormone-releasing hormone (LHRH) neurons and facilitates the LHRH response to PGE2 by activating a glia-to-neuron signaling pathway. J Neurosc 17:9145-9156.

Rassnick S, Zhou DH, Rabin BS (1995) Central administration of prostaglandin E (2) suppresses in vitro cellular immune responses. Am J Physiol 269:R92-R97.

Redl H, Bahrami S, Schlag G, Traber DL (1993) Clinical detection of LPS and animal models of endotoxemia. Immunobiology 187:330-345.

Regan JW, Bailey TJ, Pepperl DJ, Pierce KL, Bogardus AM, Donello JE, Fairbairn CE, Kedzie KM, Woodward DF, Gil DW (1994) Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP2 subtype. Mol Pharmacol 46:213-220.

Reimers J, Wogensen LD, Welinder B, Hejnaes KR, Poulsen SS, Nilsson P, Nerup J (1991) The pharmacokinetics, distribution and degradation of human recombinant interleukin-1ß in normal rats. Scand J Immunol 34:597-617.

Renzi PM, Flynn JR (1992) Endotoxin enhances arachidonic acid metabolism by cultured rabbit microvasculature endothelial cells. Am J Physiol 263:H1213-H1221.

Rettori V, Jurcovicova J, McCann SM (1987) Central action of interleukin-1 in altering the release of TSH, growth hormone, and prolactin in the male rat. J Neurosci Res 18:179-183.

Rivest S (1995) Molecular mechanisms and neural pathways mediating the influence of interleukin-1 on the activity of neuroendocrine CRF motoneurons in the rat. Int J Devl Neurosci 13:135-146.

Rivest S, Laflamme N (1995) Neuronal activity and neuropeptide gene transcription in the brain of immune-challenged rats. J Neuroendocrinol 7:501-525.

Rivest S, Laflamme N, Nappi RE (1995) Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. J Neurosci 15:2680-2695.

Rivest S, Rivier C (1991) Influence of the paraventricular nucleus of the hypothalamus in the alteration of neuroendocrine functions induced by intermittent footshock or interleukin. Endocrinology 129:2049-2057.

Rivest S, Rivier C (1993) Centrally injected interleukin-1ß inhibits the hypothalamic LHRH secretion and circulating LH levels via prostaglandins in rats. J Neuroendocrinol 5:445-450.

Rivest S, Rivier C (1994) Stress and interleukin-1ß-induced activation of c-*fos*, NGFI-B and CRF gene expression in the hypothalamic PVN: Comparison between Sprague-Dawley, Fisher-344 and Lewis rats. J Neuroendocrinol 6:101-117.

Rivest S, Rivier C (1995) The role of CRF and interleukin-1 in the regulation of neurons controlling reproductive functions. Endocrine Rev 16:177-199.

Rivest S, Torres G, Rivier C (1992) Differential effects of central and peripheral injection of interleukin-1ß on brain c-*fos* expression and neuroendocrine functions. Brain Res 587:13-23.

Rivier C (1993) Neuroendocrine effects of cytokines in the rat. Rev Neurosci 4:223-237.

Rivier C (1995) Influence of immune signals on the hypothalamic-pituitary axis of the rodent. Front Neuroendocrin 16:151-182.

Rivier C, Chizzonite R, Vale W (1989) In the mouse, the activation of the hypothalamic-pituitary-adrenal axis by a lipopolysaccharide (endotoxin) is mediated through interleukin-1. Endocrinology 125:2800-2805.

Rivier C, Rivest S (1993) Mechanisms mediating the effects of cytokines in neuroendocrine functions in the rat. In: Corticotropin-releasing factor, Ciba Foundation Symposium 172 (Chadwick DJ, Marsh J, Ackrill K, ed.), pp 204-225. Chichester: John Wiley & Sons Ltd.

Rivier C, Vale W (1985) Effects of corticotropin-releasing factor, neurohypophyseal peptides and catecholamines on pituitary function. Fed Proc 44:189-195.

Rivier C, Vale W (1989) In the rat, interleukin-1α acts at the level of the brain and the gonads to interfere with gonadotropin and sex steroid secretion. Endocrinology 124:2105-2109.

Rivier C, Vale W (1991) Stimulatory effect of interleukin-1 on ACTH secretion in the rat: is it modulated by prostaglandins? Endocrinology 129:384-388.

Rivier C, Plotsky PM (1986) Mediation by corticotropin-releasing factor (CRF) of adenohypophysial hormone secretion. Ann Rev Physiol 48:475-494.

Robertson RP (1995) Molecular regulation of prostaglandins synthesis implications for endocrine systems. Trends Endocrinol Metab 6:293-297.

Romero LI, Kakucska I, Lechan RM, Reichlin S (1996) Interleukin-6 (IL-6) is secreted from the brain after intracerebroventricular injection of IL-1 beta in rats. Am J Physiol 270:R518-R524.

Romero LI, Schettini G, Lechan RM, Dinarello CA, Reichlin S (1993) Bacterial lipopolysaccharide induction of IL-6 in rat telencephalic cells is mediated in part by IL-1. Neuroendocrinology 57:892-897.

Rosenbaum DM, McKenzie JD, Pettigrew LC, Yatsu FM (1989) Neurology. In: Prostaglandins in clinical practice (Watkins WD, Peterson MB, Fletcher JD, ed.), pp 211-225. New-York: Raven Press.

Rothwell NJ, Luheshi G (1994) Pharmacology of interleukin-1 actions in the brain. Adv Pharmacol 25:1-20.

Sagar SM, Sharp FR, Curran T (1988) Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science 240:1328-1331.

Sando T, Usui T, Tanaka I, Mori K, Sasaki Y, Fukuda Y, Namba T, Sugimoto Y, Ichikawa A, Narumiya S, Nakao K (1994) Molecular cloning and expression of rat prostaglandin E receptor EP2 subtype. Biochem Bioph Res Co 200:1329-1333.

Saphier D, Feldman S (1986) Effects of stimulation of the preoptic area on hypothalamic paraventricular nucleus unit activity and corticosterone secretion in freely moving rats. Neuroendocrinology 42:167-173.

Saphier D, Feldman S (1988) Iontophoretic application of glucocorticoids inhibits identified neurons in the paraventricular nucleus. Brain Res 453:183-190.

Saphier D, Ovadian H (1990) Selective facilitation of putative corticotropin-releasing factor-secreting neurons by interleukin-1. Neurosci Lett 114:283-288.

Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W (1987) Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science 238:522-524.

Sapolsky RM, Armanini MP, Packan DR, Sutton SW, Plotsky PM (1990) Glucocorticoid feedback inhibition of adrenocorticotropic hormone secretagogue release. Neuroendocrinology 51:328-336.

Sawada M, Kondo N, Suzumura A, Marunouchi T (1989) Production of tumor necrosis factor-alpha by microglia and astrocytes in culture. Brain Res 491:394-397.

Sawchenko PE, Imaki T, Potter E, Kovács K, Vale W (1993) The functional neuroanatomy of corticotropin-releasing factor. In: Corticotropin-releasing factor, Ciba Foundation Symposium 172 (Chadwick DJ, Marsh J, Ackrill K, ed.), pp 5-29. Chichester: John Wiley & Sons Ltd.

Sawchenko PE, Swanson LW (1982) The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. Brain Res Rev 4:275-325.

Sawchenko PE, Swanson LW (1983) The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. J Comp Neurol 218:121-144.

Sawchenko PE, Swanson LW (1989) Organization of CRF immunoreactive cells and fibers in the rat brain: Immunohistochemical studies. In: Corticotropin-releasing factor: basic and clinical studies of a neuropeptide. (De Souza EB, Nemeroff CB, ed.), pp 29-51. Boca Raton, FL: CRF Press.

Scammell TE, Elmquist JK, Griffin JD, Saper CB (1996) Ventromedial preoptic prostaglandin E2 activates fever-producing autonomic pathways. J Neurosci 16:6246-6254.

Schöbitz B, De Kloet ER, Holsboer F (1994) Gene expression and function of interleukin 1, interleukin 6 and tumor necrosis factor in the brain. Prog Neurobiol 44:397-432.

Schotanus K, Tilders FJ, Berkenbosch F (1993) Human recombinant interleukin-1 receptor antagonist prevents adrenocorticotropin, but not interleukin-6 responses to bacterial endotoxin. Endocrinology 133:2461-2468.

Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC, Tobias PS, Ulevitch RJ (1990) Structure and function of lipopolysaccharide binding protein. Science 249:1429-1431.

Seasholtz AF, Thompson RC, Douglas JO (1988) Identification of a cyclic adenosine monophosphate-responsive element in the rat corticotropin-releasing hormone gene. Mol Endocrinol 2:1311-1319.

Shakhov AN, Collart MA, Vassalli P, Nedospasov SA, Jongeneel CV (1990) Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor alpha gene in primary macrophages. J Exp Med 171:35-47.

Shalaby MR, Waage A, Aarden L, Espevik T (1989) Endotoxin, tumor necrosis factor-α and interleukin 1 induce interleukin-6 production *in vivo*. Clin Immunol Immunopathol 53:488-498.

Shapiro RE, Miselis RR (1985) The central neural connections of the area postrema of the rat. J Comp Neurol 234:344-364.

Sharp BM, Matta SG (1993) Prostaglandins mediate the adrenocorticotropin response to tumor necrosis factor in rats. Endocrinology 132:269-274.

Sharp BM, Matta SG, Peterson PK, Newton R, Chao C, McAllen K (1989) Tumor necrosis factor-alpha is a potent ACTH secretagogue: comparison to interleukin-1ß. Endocrinology 124:3131-3137.

Shimizu H, Mitomo K, Watanabe T, Okamoto S, Yamamoto K (1990) Involvement of a NF-kappa B-like transcription factor in the activation of the interleukin-6 gene by inflammatory lymphokines. Mol Cell Biol 10:561-568.

Shimuzu T, Yamashita A, Hayaishi O (1982) Specific binding of prostaglandin D2 to rat brain synaptic membrane. J Biol Chem 257:13570-13575.

Silverman AJ, Hoffman DL, Zimmerman EA (1981) The descending afferent connections of the paraventricular nucleus of the hypothalamus (PVN). Brain Res Bull 6(1):47-61.

Silverman AJ, Hou-Yu A, Chen WP (1989) Corticotropin-releasing factor synapses within the paraventricular nucleus of the hypothalamus. Neuroendocrinology 49:291-299.

Sims JE, Giri JG, Dower SK (1994) The two interleukin-1 receptors play different roles in IL-1 actions. Clin Immunol Immunopathol 72:9-14.

Sirko S, Bishai I, Coceani F (1989) Prostaglandin formation in the hypothalamus in vivo: effect of pyrogens. Am J Physiol 256:R616-R624.

Smith T, Hewson AK, Quarrie L, Leonard JP, Cuzner ML (1994) Hypothalamic PGE2 and cAMP production and adrenocortical activation following intraperitoneal endotoxin injection: *in vivo* microdialysis studies in Lewis and Fischer rats. Neuroendocrinology 59:396-405.

Snyder DS, Unanue ER (1982) Corticosteroids inhibit murine macrophage 1a expression and interleukin 1 production. J Immunol 129:1803-1805.

Spangelo BL, Judd AM, Isakson PC, MacLeod RM (1989) Interleukin-6 stimulates anterior pituitary hormone release *in vitro*. Endocrinology 125:575-577.

Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J (1987) Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. Proc Natl Acad Sci USA 84:2975-2979.

Stenzel P, Kesterson R, Yeung W, Cone RD, Rittenberg MB, Stenzel-Poore MP (1995) Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. Mol Endocrinol 9:637-645.

Stenzel-Poore M, Vale WW, Rivier C (1993) Relationship between antigen-induced immune activation and activation of the HPA axis in the rat. Endocrinology 132:1313-1318.

Sternberg EM, Hill JM, Chrousos GP, Kamilaris T, Listwak SJ, Gold PW, Wilder RL (1989) Inflammatory mediator-induced hypothalamic-pituitary-adrenal axis activation is defective in streptoccocal cell wall arthritis-susceptible Lewis rats. Proc Natl Acad Sci USA 86:2374-2378.

Sternberg EM, III WSY, Bernardini R, Calogero AE, Chrousos GP, Gold PW, Wilder RL (1989) A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to streptoccocal cell wall-induced arthritis in Lewis rats. Proc Natl Acad Sci USA 86:4771-4775.

Sternberg EM, Wilder RL, Gold PW, Chrousos GP (1990) A defect in the central component of the immune system-hypothalamic-pituitary-adrenal axis feedback loop is associated with susceptibility to experimental arthritis and other inflammatory diseases. Ann NY Acad Sci 594:289-292.

Stitt JT (1985) Evidence for the involvement of the organum vasculosum laminae terminalis in the febrile response of rabbits and rats. J Physiol Lond 368:501-511.

Stitt JT (1986) Prostaglandin E as the mediator of the febrile response. Yale J Biol Med 59:137-149.

Stitt JT (1991) Differential sensitivity in the sites of fever production by prostaglandin-E1 within the hypothalamus of the rat. J Physiol (Lond) 432:99-110.

Suda T, Tozawa F, Ushiyama T, Sumitomo T, Yamada M, Demura H (1990) Interleukin-1 stimulates corticotropin-releasing factor gene expression in rat hypothalamus. Endocrinology 126:1223-1228.

Suda T, Tozawa F, Ushiyama T, Tomori N, Sumitomo T, Nakagami Y, Yamada M, Shizume K (1989) Effects of protein kinase-C-related adrenocorticotropin secretagogues and interleukin-1 on proopiomelanocortin gene expression in rat anterior pituitary cells. Endocrinology 124:1444-1449.

Sugimoto Y, Namba T, Honda A, Hayashi Y, Negishi M, Ichikawa A, Narumiya S (1992) Cloning and expression of cDNA for a mouse prostaglandin E receptor EP3 subtype. J Biol Chem 267:6463-6466.

Sugimoto Y, Negishi M, Hayashi Y, Namba T, Honda A, Watabe A, Hirata M, Narumiya S, Ichikawa A (1993) Two isoforms of the EP3 receptor with different carboxyl-terminal domains. J Biol Chem 268:2712-2718.

Sugimoto Y, Shigemoto R, Namba T, Negishi M, Mizuno N, Narumiya S, Ichikawa A (1994) Distribution of the messenger RNA for the prostaglandin E receptor subtype EP3 in the mouse nervous system. Neuroscience 62:919-928.

Swanson LW, Sawchenko PE (1983) Hypothalamic integration: Organization of the paraventricular and supraoptic nuclei. Ann Rev Neurosci 6:269-324.

Swanson LW, Sawchenko PE, Lind RW, Rho JH (1987) The CRH Motoneuron: Differential peptide regulation in neurons with possible synaptic, paracrine, and endocrine outputs. Ann NY Acad Sci 512:12-23.

Swanson LW, Sawchenko PE, Rivier J, Vale WW (1983) Organization of ovine corticotropin releasing factor (CRF)-immunoactive cells and fibers in the rat brain: An immunohistochemical study. Neuroendocrinology 36:165-186.

Takeuchi K, Abe T, Takahashi N, Abe K (1993) Molecular cloning and intrarenal localization of rat prostaglandin E2 receptor EP3 subtype. Biochem Bioph Res Co 194:885-891.

Takeuchi K, Takahashi N, Abe T, Abe K (1994) Two isoforms of the rat kidney EP3 receptor derived by alternative RNA splicing: intrarenal expression co-localization. Biochem Bioph Res Co 199:834-840.

Thompson ME, Hedge GA (1978) Inhibition of corticotropin secretion by hypothalamic administration of indomethacin. Neuroendocrinology 25:212-220.

Thompson R, Seasholtz A, Douglas J, Herbert E (1990) Cloning and distribution of expression of the rat corticotropin-releasing factor (CRF) gene. In: Corticotropin-releasing factor: basic and clinical studies of a neuropeptide. (DeSouza E, Nemeroff C, ed.), pp 1-12. Boca Raton: CRC.

Thompson RC, Seasholtz AF, Herbert E (1987) The rat corticotropin-releasing hormone gene: sequence and tissue-specific expression. Mol Endocrinol 1:363-370.

Tritarelli E, Greco G, Testa U, Belardelli F, Peschle C, Proietti E (1994) Combined interleukin-1 beta/interleukin-6 treatment in mice: synergistic myelostimulatory activity and myelorestorative effect after cyclophosphamide-induced myelosuppression. Cancer Res 54:6469-6476.

Tsagarakis S, Gillies G, Rees LH, Besser M, Grossman A (1989) Interleukin-1 directly stimulates the release of corticotrophin-releasing factor from rat hypothalamus. Neuroendocrinology 49:98-101.

Turnbull AV, Dow RC, Hopkins SJ, White A, Fink G, Rothwell NJ (1994) Mechanisms of activation of the pituitary-adrenal axis by tissue injury in the rat. Psychoneuroendocrinology 19:165-178.

Turnbull AV, Pitossi FJ, Lebrun J-J, Lee S, Meltzer JC, Nance DM, del Rey A, Besedovsky HO, Rivier C (1997) Inhibition of tumor necrosis factor-α action within the CNS markedly reduces the plasma adrenocorticotropin response to peripheral local inflammation. J Neurosci 17:3262-3273.

Turnbull AV, Rivier C (1996) Corticotropin-releasing factor, vasopressin, and prostaglandins mediate, and nitric oxyde restrains, the hypothalamic-pituitary-adrenal response to acute local inflammation in the rats. Endocrinology 137:455-463.

Turnbull AV, Rivier CL (1998) Intracerebroventricular passive immunization. I. The effect of intracerebroventricular administration of an antiserum to tumor necrosis factor-alpha on the plasma adrenocorticotropin response to lipopolysaccharide in rats. Endocrinology 139:119-127.

Uehara A, Gillis S, Arimura A (1987) Effects of interleukin-1 on hormone release from normal rat pituitary cells in primary culture. Neuroendocrinology 45:343-347.

Uehara A, Gotschall PE, Dahl RR, Arimura A (1987) Interleukin-1 stimulates ACTH release by an indirect mechanisms which requires endogenous corticotropin-releasing factor. Endocrinology 121:1580-1587.

Ueno R, Narumiya S, Ogorochi T, Nakayama T, Ishikawa Y, Hayaishi O (1982) Role of prostaglandin D2 in the hypothermia of rats caused by bacterial lipopolysaccharide. Proc Natl Acad Sci USA 79:6093-6097.

Vale W, Rivier C, Yang L, Minick S, Guillemin R (1978) Effects of purified hypothalamic corticotropin-releasing factor and other substances on the secretion of adrenocorticotropin and ß-endorphin-like immunoreactivities *in vitro*. Endocrinology 103:1910-1915.

Vallières L, Lacroix S, Rivest S (1997) Influence of interleukin-6 on neural activity and transcription of the gene encoding corticotropin-releasing factor in the rat brain: an effect depending upon the route of administration. Eur J Neurosci 9:1461-1472.

Vallières L, Rivest S (1997) Regulation of the genes encoding interleukin-6, its receptor, and gp130 in the rat brain in response to the immune activator lipopolysaccharide and the proinflammatory cytokine interleukin-1ß. J Neurochem 69:1668-1683.

Vallières L, Rivest S (1998) Interleukin-6, a needed proinflammatory cytokine in the prolonged neuronal activity and transcriptional activation of corticotropin-releasing factor caused by blood endotoxemia. J Exp Med submitted.

Van Dam AM, Bauer J, Tilders FJH, Berkenbosch F (1995) Endotoxin-induced appearance of immunoreactive interleukin-1ß in rat brain: a light and electron microscopical study. Neuroscience 65:815-826.

Van Dam AM, Brouns M, Louisse S, Berkenbosch F (1992) Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of endotoxin-treated rats: a pathway for the induction of non-specific symptoms of sickness? Brain Res 588:291-296.

Van Dam AM, Brouns M, Man-A-Hing W, Berkenbosch F (1993) Immunocytochemical detection of prostaglandin E2 in microvasculatures and in neurons of rat brain after administration of bacterial endotoxin. Brain Res 613:331-336.

Van Dam AM, DeVries HE, Kuiper J, Zijlstra FJ, DeBoer AG, Tilders FJH, Berkenbosch F (1996) Interleukin-1 receptors on rat brain endothelial cells: a role in neuroimmune interaction? FASEB J 10:351-356.

van der Kooy D, Koda LY (1983) Organization of the projections of a circumventricular organ: the area postrema in the rat. J Comp Neurol 219:328-338.

Vieira LQ, Goldschmidt M, Nashleanas M, Pfeffer K, Mak T, Scott P (1996) Mice lacking the TNF receptor p55 fail to resolve lesions caused by infection with Leishmania major, but control parasite replication. J Immunol 157:827-835.

Vlaskovska M, Hertting G, Knepel W (1984) Adrenocorticotropin and ß-endorphin release from rat adenohypophysis *in vitro*: inhibition by prostaglandin E2 formed locally in response to vasopressin and corticotropin-releasing factor. Endocrinology 115:895-903.

Vlaskovska M, Knepel W (1984) Beta-endorphin and adrenocorticotropin release from rat adenohypophysis in vitro: evidence for local modulation by arachidonic acid metabolites of the cyclooxygenase and lipooxygenase pathway. Neuroendocrinology 39:334-342.

Wan W, Wetmore L, Sorensen CM, Greenberg AH, Nance DM (1994) Neural and biochemical mediators of endotoxin and stress-induced c-*fos* expression in the rat brain. Brain Res Bull 34:7-14.

Watabe A, Sugimoto Y, Honda A, Irie A, Namba T, Negishi M, Ito S, Narumiya S, Ichikawa A (1993) Cloning and expression of cDNA for a mouse EP1 subtype of prostaglandin E receptor. J Biol Chem 268:20175-20178.

Watanabe T, Morimoto A, Morimoto K, Nakamori T, Murakami N (1991) ACTH release induced in rats by noradrenaline is mediated by prostaglandin E2. J Physiol 443:431-439.

Watanabe T, Morimoto A, Sakata Y, Murakami N (1990) ACTH response induced by interleukine-1 is mediated by CRF secretion stimulated by hypothalamic PGE. Experientia 46:481-484.

Watanabe Y, Watanabe Y, Hamada K, Bommelaer-Bayt MC, Dray F, Kaneko T, Yumoto N, Hayaishi O (1989) Distinct localization of prostaglandin D2, E2 and F2 alpha binding sites in monkey brain. Brain Res 478:143-148.

Watanobe H, Sasaki S, Takebe K (1995) Role of prostaglandins E1, E2 and F2 alpha in the brain in interleukin 1 beta-induced adrenocorticotropin secretion in the rat. Cytokine 7:710-712.

Watanobe H, Takebe K (1992) Intravenous administration of tumor necrosis factor-α stimulates corticotropin-releasing hormone secretion in the push-pull cannulated median eminence of freely moving rats. Neuropeptides 22:81-84.

Watanobe H, Takebe K (1994) Effects of intravenous administration of interleukin-1-beta on the release of prostaglandin E2, corticotropin-releasing factor, and arginine vasopressin in several hypothalamic areas of freely moving rats: estimation by push-pull perfusion. Neuroendocrinology 60:8-15.

Watkins LR, Goehler LE, Relton JK, Tartaglia N, Silbert L, Martin D, Maier SF (1995) Blockage of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: Evidence for vagal mediation of immune-brain communication. Neurosci Lett 183:27-31.

Watkins LR, Maier SF, Goehler LE (1995) Cytokine-to-brain communication: a review & analysis of alternative mechanisms. Life Sci 57:1011-1026.

Watkins LR, Wiertelak EP, Goehler LE, Smith KP, Martin D, Maier SF (1994) Characterization of cytokine-induced hyperalgesia. Brain Res 654:15-26.

Weidenfeld J, Abramsky O, Ovadia H (1989) Evidence for the involvement of the central adrenergic system in interleukin-1-induced adrenocortical response. Neuropharmacology 28:1411-1414.

Weihe E, Müller S, Fink T, Zentel HJ (1989) Tachykinins, calcitonin gene-related peptide and neuropeptide Y in nerves of the mammalian thymus: interactions with mast cells in autonomic and sensory neuroimmunomodulation. Neurosci Lett 100:77-82.

Werb Z (1978) Biochemical actions of glucocorticoids on macrophages in culture. J Exp Med 147:1695-1712.

Whitnall MH (1993) Regulation of the hypothalamic corticotropin-releasing hormone neurosecretory system. Prog Neurobiol 40:573-629.

Wilson T, Fahrner T, Johnston M, Milbrandt J (1991) Identification of the DNA binding site for NGFI-B by genetic selection in yeast. Science 252:1296-1300.

Wooley PH, Dutcher J, Widmer MB, Gillis S (1993) Influence of a recombinant human soluble tumor necrosis factor receptor FC fusion protein on type II collagen-induced arthritis in mice. J Immunol 151:6602-6607.

Wright SD, Ramos R, Hermanowski-Vosatka A, Rockwell P, Detmers PA (1991) Activation of the adhesive capacity of CR3 on neutrophils by endotoxin: dependence on lipopolysaccharide binding protein and CD14. J Exp Med 173:1281-1286.

Wright SD, Ramos RA, Patel M, Miller DS (1992) Septin: A factor in plasma that opsonizes lipopolysaccharide-bearing particles for recognition by CD14 on phagocytes. J Exp Med 176:719-727.

Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC (1990) CD14, a receptor for complexes of lipolysaccharide (LPS) and LPS binding protein. Science 249:1431-1433.

Xie W, Chipman JG, Robertson DL, Erikson RL, Simmons DL (1991) Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci USA 88:2692-2696.

Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF (1993) Expression of a mitogen-inducible cyclooxygenase in brain neurons: Regulation by synaptic activity and glucocorticoids. Neuron 11:371-386.

Yamaguchi K, Hama H, Watanabe K (1997) Possible roles of prostaglandins in the anteroventral third ventricular region in the hyperosmolality-evoked vasopressin secretion of conscious rats. Brain Res 113:265-272.

Yamamoto H, Hanada K, Nishijima M (1997) Involvement of diacylglycerol production in activation of nuclear factor kappa-B by a CD14-mediated lipopolysaccharide stimulus. Biochem J 325:223-228.

Yasin SA, Costa A, Forsling ML, Grossman A (1994) Interleukin-1ß and interleukin-6 stimulate neurohypophysial hormone release in vitro. J Neuroendocrinol 6:179-184.

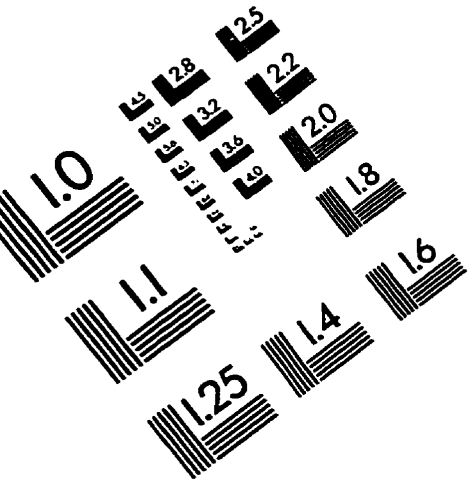
Young WS, Mezey E, Siegel RE (1986) Vasopressin and oxytocin mRNAs in adrenalectomized and Brattleboro rats: analysis by quantitative *in situ* hybridization histochemistry. Brain Res 387:231-241.

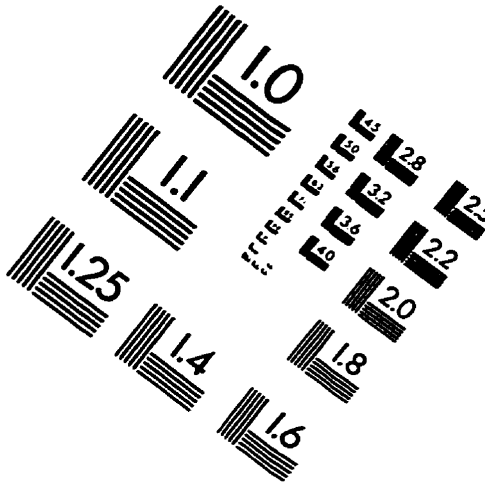
Zelazowski P, Smith MA, Gold PW, Chrousos GP, Wilder RL, Sternberg EM (1992) In vitro regulation of pituitary ACTH secretion in inflammatory disease susceptible Lewis (LEW/N) and inflammatory disease resistant Fisher (F344/N) rats. Neuroendocrinology 56:474-482.

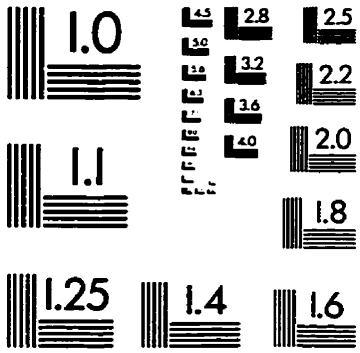
Zhang Y, Broser M, Rom WN (1994) Activation of the interleukin 6 gene by Mycobacterium tuberculosis or lipopolysaccharide is mediated by nuclear factors NF-IL6 and NF-kappa B. Proc Natl Acad Sci USA 91:2225-2229.

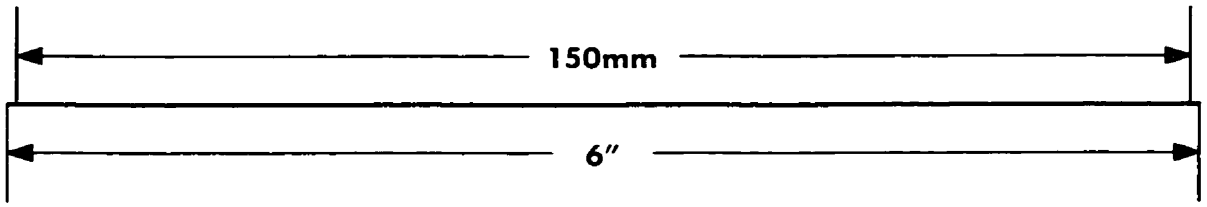
Zheng H, Fletcher D, Kozak W, Jiang M, Hofmann K, Conn CA, Soszynski D, Grabiec C, Trumbauer ME, Shaw A, Kostura MJ, Stevens K, Rosen H, North RJ, Chen HY, Tocci MJ, Kluger MJ, Van der Ploeg LHT (1995) Resistance to fever induction and impaired acute-phase response in interleukin-1ß-deficient mice. Immunity 3:9-19.

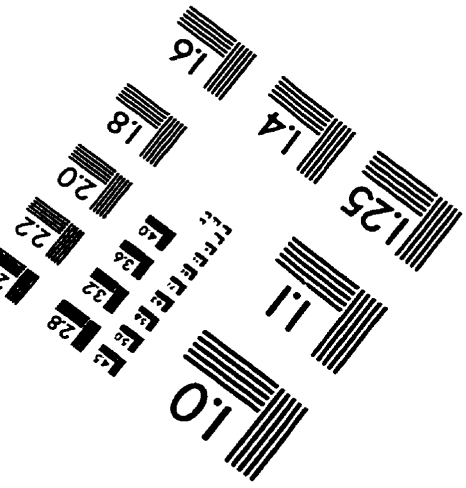
IMAGE EVALUATION TEST TARGET (QA-3)

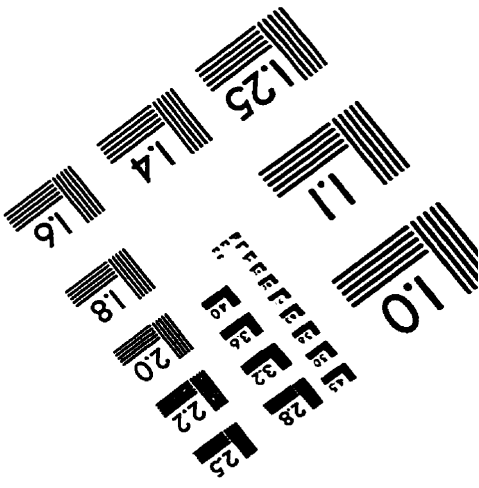












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989