# Neuropeptide Y Vascular Responses and Endothelial Function in Normals and Patients with Congestive Heart Failure

by

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## ABSTRACT

We investigated the interaction of sympathetic nervous system activity and endothelial function in the modulation of NPY receptor responsiveness in heart failure patients and normal subjects using hand vein tonometry.

NPY receptor mediated venoconstriction was found to be increased in patients with mild to moderate left ventricular dysfunction compared to normals ($p < 0.05$), while patients with severe left ventricular dysfunction had an unaltered NPY response compared to normals. Upon co-infusion of indomethacin with NPY, thus looking at the "true" NPY response, mild to moderate CHF were unaltered compared to normals, however severe CHF were significantly decreased compared to normals ($p < 0.05$). In addition, vasodilatory prostaglandins were released in response to NPY stimulation in normals rather than being a nonspecific response to vasoconstriction.

Therefore, increased sympathetic activity decreases venous NPY receptor responsiveness in severe heart failure patients and this is modulated by endothelial released substances in normal subjects.



**Keywords:** neuropeptide Y, NPY, vascular, human, hand veins, endothelium, prostaglandins, congestive heart failure, venous responses

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | -arachidonic acid |
| AC | -adenylyl cyclase |
| ACE | -angiotensin converting enzyme |
| ANOVA | -analysis of variance |
| ANP | -atrial natriuretic peptide |
| ANS | -autonomic nervous system |
| ATP | -adenosine triphosphate |
| $Ca^{2+}$ | -calcium ion |
| cAMP | -cyclic adenosine-3'5'-monophosphate |
| CHF | -congestive heart failure |
| CNS | -central nervous system |
| DAG | -diacylglycerol |
| ECG | -electrocardiogram |
| $EC_{50}$ | -concentration of agonist eliciting 50% of desired effect |
| $ED_{50}$ | -dose of agonist required to elicit 50% of desired effect |
| EDRF | -endothelium derived relaxing factor |
| $G_i$ | -inhibitory G protein |
| $G_s$ | -stimulatory G protein |
| HPB | -Health Protection Branch |
| HVD | -hand vein distention |
| $IP_3$ | -inositol-1,4,5-trisphosphate |

| | |
|---|---|
| KCl | -potassium chloride |
| L-NMMA | -$N^G$-monomethyl-L-arginine |
| LVDT | -linear variable differential transformer |
| LVEF | -left ventricular ejection fraction |
| MAP | -mean arterial pressure |
| MLCK | -myosin light chain kinase |
| $Na^+$ | -sodium ion |
| NaCl | -sodium chloride |
| NE | -norepinephrine |
| NO | -nitric oxide |
| NPY | -neuropeptide Y |
| NYHA | -New York Heart Association |
| PGE | -prostaglandin E |
| $PGF_{2\alpha}$ | -prostaglandin $F_{2\alpha}$ |
| $PGI_2$ | -prostacyclin |
| PKA | -protein kinase A |
| PKC | -protein kinase C |
| $PLA_2$ | -phospholipase $A_2$ |
| PLC | -phospholipase C |
| PNS | -parasympathetic nervous system |
| RAAS | -renin-angiotensin-aldosterone system |
| SNS | -sympathetic nervous system |

| | |
|---|---|
| TPR | -total peripheral resistance |
| $TXA_2$ | -thromboxane $A_2$ |
| 5-HT | -5-hydroxytryptamine |

**SYMBOLS**

| | |
|---|---|
| α | -alpha |
| ß | -beta |

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# CHAPTER 1

# INTRODUCTION

## 1.1 The autonomic nervous system

The nervous system is divided into two anatomical divisions: the central nervous system (CNS), which is composed of the brain and spinal cord; and the peripheral nervous system, which includes neurons located outside the brain and spinal cord. The nervous system has a high level of integration in the brain with the ability to influence processes in distant regions of the body while extensively using a negative feedback system (Katzung, 1995). The peripheral nervous system is further divided into the efferent division and the afferent division. Neurons of the efferent division carry signals away from the brain and spinal cord to the peripheral tissues, while the neurons of the afferent division bring information from the periphery to the CNS.

The efferent, or motor portion of the nervous system can be divided into 2 major subdivisions, the somatic and the autonomic divisions. The somatic division is concerned with consciously controlled functions such as movement and posture (Appenzeller, 1982). The activities of the autonomic nervous system (ANS) are not under direct conscious control. They are concerned mostly with visceral functions that are necessary for life such as cardiac output, blood flow to various organs, digestion, and maintenance of a constant internal environment, or homeostasis (Katzung, 1995). The major cellular structures innervated by the ANS are smooth muscle, cardiac muscle, glandular tissue and adipocytes (Broadley, 1996). Because our research is concerned with events associated with changes in systems not under conscious control, the specifics of the ANS are dealt with in greater detail below.

The ANS can also be divided into the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) based upon their physiological functions and their anatomy (Appenzeller, 1982). Both divisions originate in nuclei within the CNS and give rise to preganglionic efferent fibres which exit from the brain stem or spinal cord and terminate in motor ganglia (Appenzeller, 1982). Nerve impulses travel from the CNS to effector organs by the preganglionic neuron whose cell body is located within the CNS. These preganglionic neurons make a synaptic connection in ganglia, and these ganglia function to relay the nerve impulse from the preganglionic neuron to a second nerve cell called the postganglionic neuron (Katzung, 1995). The postganglionic neuron has a cell body that originates in the ganglion and terminates on effector organs. The preganglionic neurons of the sympathetic and parasympathetic efferent pathways emerge from separate locations (Netter, 1974; Gabella, 1976). The sympathetic nerves leave the spinal cord via the thoracolumbar segments, with ganglia lying in the sympathetic chain or more peripherally in the body cavities with long postganglionic fibres; the parasympathetic nerves emerge from the brain stem or the sacral segments of the spinal cord and the ganglia lie close to or within the organ of innervation, with very short postganglionic fibres (Broadley, 1996). Therefore, preganglionic fibres of the SNS tend to be short and postganglionic fibres long, while the opposite is true in the PNS. It is important to point out that the terms sympathetic and parasympathetic are anatomic ones (Katzung, 1995) and do not depend on the type of neurotransmitter released nor on the kind of effect evoked by nerve activity, whether it be excitatory or inhibitory. The SNS is concerned with the adaptation of the body to stressful situations such as trauma, fear, hypoglycemia, cold, or exercise (Mycek, 1997). Cannon (1929) described this as preparing the body for "fight or flight." The effect of sympathetic output is to increase heart rate and

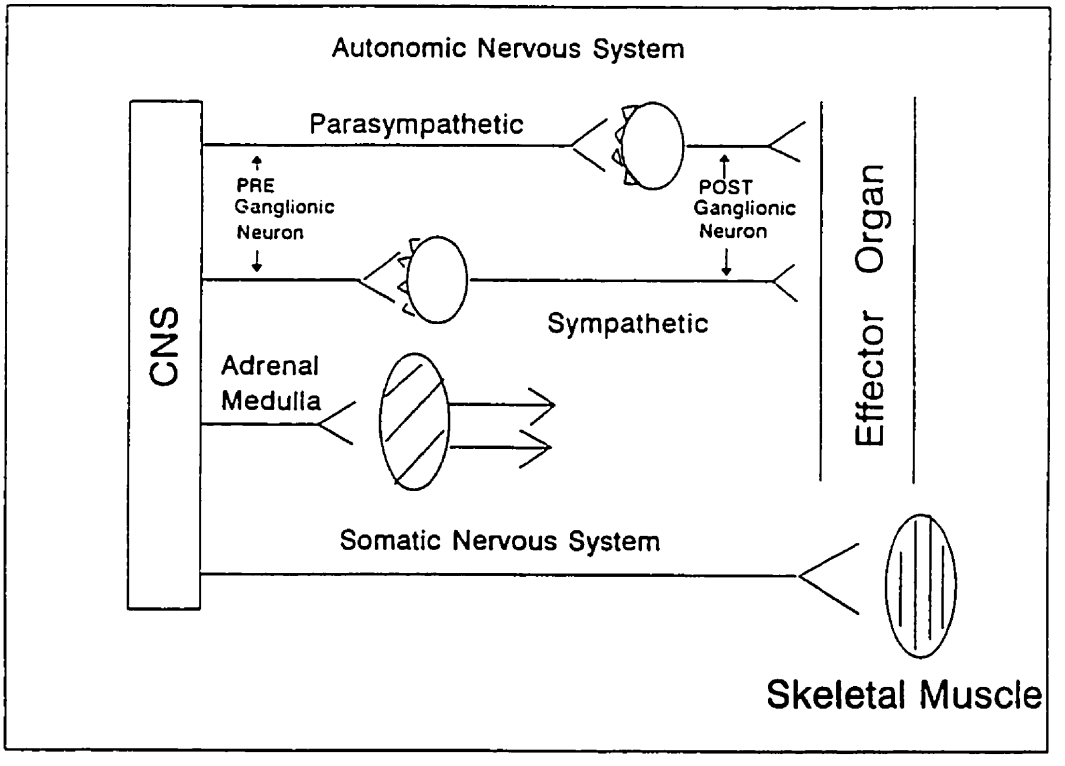


**Figure 1.1** Schematic diagram showing the linkage between the CNS and both the ANS and somatic effector cell.
Abbreviations: CNS, central nervous system; ANS, autonomic nervous system.
(Adapted from Katzung, 1995).

blood pressure, to mobilize the energy stores of the body, and to increase blood flow to skeletal muscles and the heart while diverting flow from the skin and internal organs (Mycek, 1997). Thus, the SNS is involved in a wide array of physiologic activities, but is not essential for life. In contrast, the parasympathetic division maintains essential bodily functions such as digestive processes and elimination of wastes, and is essential for life (Katzung, 1995). The parasympathetic system usually acts opposite to the sympathetic system to balance its actions and is generally dominant over the sympathetic system in "rest and digest" situations (Broadley, 1996). For example, in the heart, the rate and force of cardiac contractions are elevated by an increase in sympathetic nerve discharge in response to a stressful situation. However, raised parasympathetic nerve activity to the heart causes slowing or bradycardia.

A large number of peripheral autonomic nervous system fibres synthesize and release acetylcholine, and are therefore named cholinergic fibres. These include all preganglionic efferent autonomic fibres and the somatic motor fibres to skeletal muscle (Burnstock et al., 1992). In addition, all parasympathetic postganglionic and a few sympathetic postganglionic fibres are cholinergic, for example, sweat glands (Bevan et al., 1987) . However, most postganglionic sympathetic fibres release noradrenaline (NA) or norepinephrine (NE) and are therefore named noradrenergic or adrenergic fibres. NE has different actions in different tissues, according to the type of receptor molecule present on the responding cells. Dopamine is also released by some sympathetic fibres (Schwartz et al., 1992). It has also been realized in recent years that most autonomic nerves also release several transmitter substances or co-transmitters in addition to the primary transmitter, for example, neuropeptide Y (NPY). Each neurotransmitter exerts its effect via distinct receptors located on the effector organ, with cholinergic neurons acting through cholinoceptors and adrenergic

neurons acting via adrenoceptors. Thus, different neurotransmitters activate distinct receptors on effector organs in the PNS and the SNS, with nicotinic and muscarinic cholinoceptors in the PNS, and α and ß adrenoceptors predominant in the SNS (Korner, 1979). NPY is a neurotransmitter that is co-released with NE from the SNS where it acts at postjunctional Y1 receptors and prejunctional Y2 receptors (Wahlestedt et al., 1986 a,b). Previous research in our laboratory has focused on the family of α-adrenergic receptors in the cardiovascular system that are activated by the release of NE from postganglionic neurons of the SNS (Callow, 1995). Because NPY is a neurotransmitter that is co-released with NE from the SNS, our research interests have focused on the events associated with NPY under normal conditions and in congestive heart failure (CHF).

## 1.2 The sympathetic nervous system in cardiovascular function

### 1.2.1 Normal SNS Control of the Heart

The SNS is an important modulator of cardiovascular function by innervating several organs in the body including the heart, kidneys, and vascular smooth muscle. Sympathetic nerves to the heart leave the spinal cord at the upper 5 or 6 thoracic segments (Broadley, 1996). The preganglionic fibres then synapse within the upper 5 or 6 ganglia of the sympathetic chain and the fibres pass through these vertebral ganglia and ascend to the 3 cervical ganglia. The postganglionic fibres emerging from this area then run together and merge at the cardiac plexus along with the parasympathetic fibres. The sympathetic nerves terminate predominantly in the sinoatrial node pacemaker tissue, the atrioventricular node, and the conducting tissue (Broadley, 1996). Stimulation of the sympathetic nerves increases pacemaker activity, resulting in the speeding of the heart (positive chronotropy) and the force

of contraction increases (positive inotropy). This results in a raised cardiac output (Craig, 1986).

The vagus nerves arising from the brain medulla are the parasympathetic supply to the heart. These nerves innervate the sinoatrial node (right vagus), the atrioventricular node (left vagus) and the atrioventricular bundle (Bundle of His) (Broadley, 1996). Vagal nerve activity causes a slowing of the heart rate (negative chronotropy) and slowing of the conduction through the Bundle of His (negative dromotropy). This results in the stroke volume to be increased, but the cardiac output falls because of the decrease in heart rate (Higgins et al., 1972). Alterations in contractility of the heart, however, primarily occur via increases or decreases in SNS outflow (Appenzeller, 1982). Alterations in SNS outflow are tightly regulated by cardiovascular reflexes (e.g. baroreceptor reflex) as described below. Changes in the functioning of the heart mediated by the SNS are predominantly by the ß-adrenergic receptors (primarily the $ß_1$ receptors) (Doughty et al., 1994) which are located in the cardiac muscle cell membrane.

There are at least 3 possible sites of action for NPY within the heart. First, NPY can cause powerful coronary vasoconstriction. NPY may also act presynaptically to inhibit the release of cardiac autonomic neurotransmitters. Finally, NPY may act directly on cardiac myocytes to alter the contraction, chronotropy, and electrical conduction (Millar et al., 1994). The direct effects of NPY on cardiac contraction vary depending on the species and tissue preparation used, as negative, positive, and unaltered inotropic effects have all been reported in studies of isolated cardiac muscle strips (McDermott et al., 1993). It is well documented that systemic administration of NPY reduces cardiac output (McDermott et al., 1993).

### 1.2.2 Normal SNS control of the vasculature

In contrast to the heart, which also receives innervation from the PNS, the vascular smooth muscle is primarily under the control of the SNS and therefore the main control of total peripheral resistance (TPR) is mediated through sympathetic neurons (Barr et al., 1993). Some degree of constant vascular constriction is maintained by the continuous outflow of adrenergic impulses to the vascular smooth muscle (Barr et al., 1993). An increase in impulse outflow following SNS activation causes further constriction of the blood vessels while a decrease in impulse outflow promotes smooth muscle relaxation and vasodilation (Burnstock et al., 1992). The venous system is highly regulated by cardiovascular reflex systems, the most important one being the baroreflex system. A reduction in arterial pressure reduces baroreceptor activity resulting in increased sympathetic outflow to postganglionic adrenergic nerve fibres in the veins, causing the release of NE along with co-transmitters such as NPY and ATP (Pang, 1994). Veins are richly innervated by sympathetic nerves (Rothe, 1983). Upon increased SNS stimulation such as during physical exercise, NPY is released along with NE, which further constricts the vessel.

### 1.2.3 Normal SNS control of the kidney

The heart and kidneys are the organs principally designed to maintain the circulation. The kidneys ensure that both the quantity of blood and its oxygen-containing capacity are optimal. The endocrine cells of the juxtaglomerular apparatus are sensitive to three factors: the filtered load of $Na^+$, the blood pressure in the afferent arterioles, and inputs to the juxtaglomerular

cells from sympathetic nerves, and thus the last two factors are measures of plasma volume (Moffet et al., 1993). If the $Na^+$ concentration or the blood pressure to the kidney drops, or if sympathetic activity rises acting via the $\beta_1$ receptor, the juxtaglomerular cells increase their secretion of renin, which is a protease that catalyzes the first step of an important regulatory cascade of events (Lorenz et al., 1991). Renin cleaves a 10 amino acid fragment, angiotensin I, from angiotensinogen. Angiotensin I is then converted to the 8 amino acid peptide angiotensin II by an angiotensin converting enzyme (ACE) that resides primarily in the endothelial cells of blood vessels. Angiotensin II is a potent vasoconstrictor and it also stimulates the release of aldosterone, which increases the reabsorption of $Na^+$ by the distal tubule causing an increase in intravascular blood volume (Keeton et al., 1980). This series of neuroendocrine responses is collectively known as the renin-angiotensin-aldosterone system (RAAS).

Things that increase renal neural activity cause stimulation of renin secretion, while renal denervation results in suppression of renin secretion. For example, a reduction in renal perfusion pressure causes intrarenal redistribution of blood flow and increased reabsorption of salt and water (Katzung, 1995). In addition, the ß receptors of the juxtaglomerular cells also mediate the release of angiotensin II and aldosterone that results in a direct constriction of the blood vessels (Griendling et al.,1993) and increased sodium reabsorption which in turn results in increased intravascular blood volume which helps to restore the blood pressure to its normal set point value. Increases in MAP result in the opposite effects. Thus the RAAS is an important and fine-tuned system which maintains the appropriate levels of salt and water balance under normal circumstances. However, in disease states such as CHF, the RAAS contributes significantly to symptoms due to chronic disturbances in cardiac output, leading

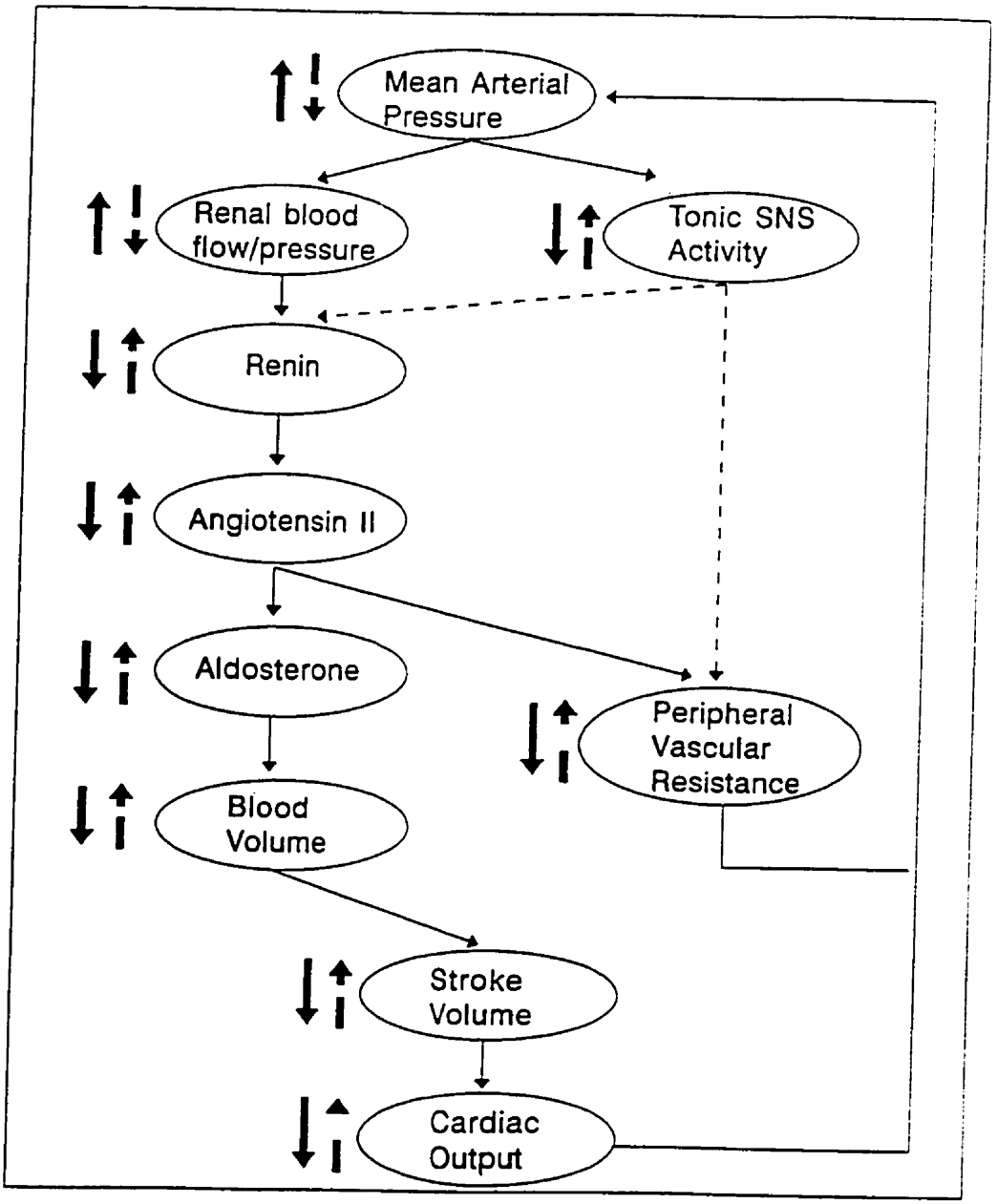


**Figure 1.2** Schematic showing the control of cardiovascular function by hormonal release via the renin-angiotensin-aldosterone system and the sympathetic nervous system.

to the progression of the disease through excessive salt and water retention and vasoconstriction (Dzau et al., 1981; Forfar, 1991). Direct microneurographic recordings have shown that renal nerve activity is increased in the rat congestive heart failure model as a result of alterations in MAP (Feng et al.,1992). Therefore, the kidney is regulated by both the autonomic system and the hormonal system which interact closely to help regulate cardiovascular function in both normal and disease states such as CHF.

Modulation of renal function by NPY can also occur at multiple levels that include indirect effects via systemic hemodynamics, the CNS, or by the modulation of release of hormones that affect renal function, and also by having direct effects on renal blood flow and tubular function. Systemic NPY administration has been reported to increase plasma levels of ANP (Baranowska et al., 1987). NPY also reduces renal blood flow in a variety of species and experimental settings (Echtenkamp & Dandridge, 1989; Modin et al., 1991; Persson et al., 1991; Bischoff et al., 1994). This is thought to occur via the $Y_1$ receptor coupling to the influx of extracellular calcium (El-Din & Malik, 1988; Oellerich & Malik, 1993). Also, in isolated rat proximal tubules, NPY has been shown to regulate $Na^+K^-$-ATPase activity (Ohtomo et al., 1994). Thus, it is known that NPY can modulate renal function *in vitro* and *in vivo* by acting on hormone release, renal perfusion, and tubular function. Therefore, NPY plays an important role in the physiological regulation of cardiovascular function, as well as in pathological conditions involving elevated arterial pressure.

### 1.2.4 Baroreceptor reflex control of SNS activity

Mean arterial pressure is tightly regulated by a group of "stretch" receptors known as the baroreceptors which are located in the carotid sinus and the aortic arch (Moffet et al., 1993).

Afferent fibres from these baroreceptor cells enter cranial nerves IX and X and ascend to the cardiovascular centre of the brainstem medulla (Moffet et al., 1993). There they synapse on interneurons that determine the outflow of impulses in parasympathetic and sympathetic pathways to the heart, sympathetic pathways to the vessels, and sympathetic activation of the adrenal medulla. Because they are stretch receptors, they are stimulated when they are distended (Donald & Shepherd, 1979). The pressure within the blood vessel is a determinant of wall tension, thus the amount of stretch to which the receptors are submitted is a function of the pressure level in the blood vessel where they are found (Donald & Shepherd, 1979). The cardiovascular integrating centre in the medulla has a "setpoint" firing pattern that corresponds to the normal mean arterial blood pressure (Taylor, 1994). If the mean arterial pressure in the carotid arteries begins to rise, increased stretch of the baroreceptors results in increased traffic in the afferent nerves connecting them with the solitary tract nucleus and as a consequence, the vagal centre and the inhibitory neurons of the vasomotor centre are activated. This results in an increase in vagal traffic to the heart while also depressing the sympathetic outflow to the heart and blood vessels (Abboud, 1979). The reverse situation occurs when the pressure falls within the blood vessels where the baroreceptors are located because the firing of the baroreceptors becomes less and the activity of the depressor neurons of the vasomotor centre and of the vagal nucleus decreases. This results in increased sympathetic activity to the heart and vessels and decreased vagal activity to the heart. This leads to constriction of the resistance vessels in muscle, kidney, splanchnic bed, and skin in order to increase total systemic vascular resistance; constriction of the splanchnic veins to maintain the appropriate preload of the heart; and an increase in heart rate and contractility (Arnold et al., 1983). Since this reflex loop involves neurons, changes occur very rapidly and

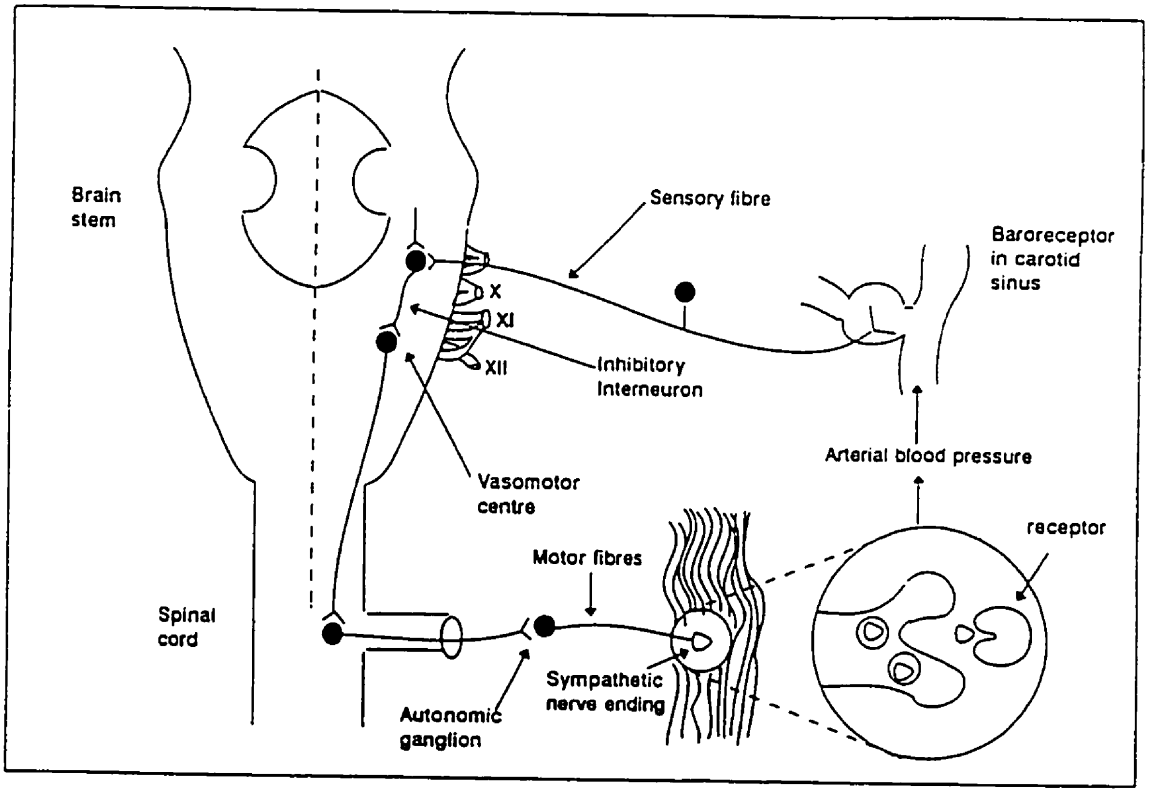


**Figure 1.3** Schematic diagram of the baroreceptor reflex arc in detail. (Adapted from Katzung, 1995).

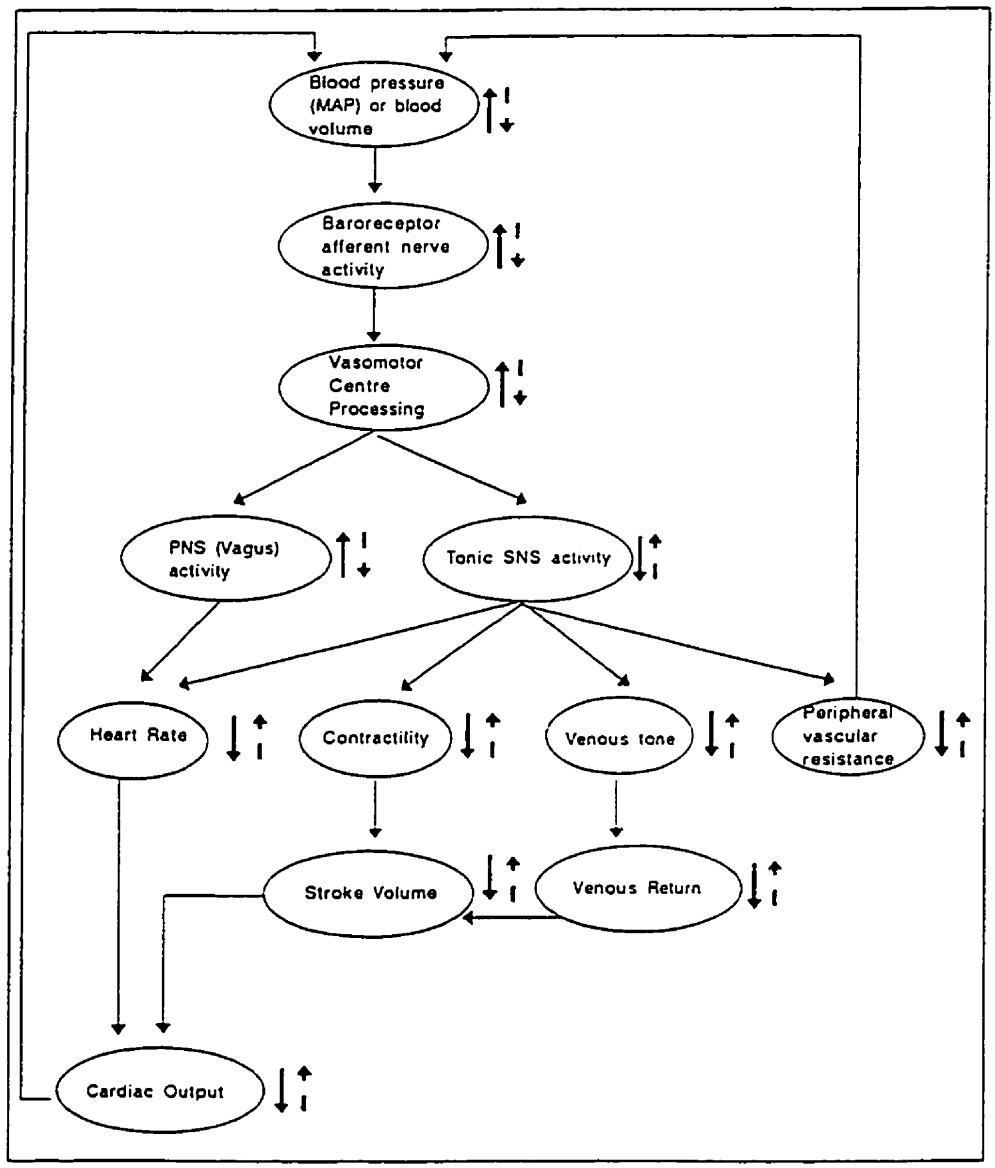


**Figure 1.4** Schematic showing sympathetic nervous system control of cardiovascular function via the baroreceptor reflex arc and the resulting responses to an increase and decrease in blood pressure or blood volume.

therefore, the baroreceptor reflexes are generally responsible for rapid, moment to moment adjustments in cardiovascular activity (Katzung, 1995). However, many cardiovascular diseases such as CHF and hypertension are associated with disturbances in the baroreceptor reflex mechanism (Burch, 1978; Ferguson et al., 1984; Cohn, 1990; Mancia, 1990; Forfar, 1991). For example, CHF causes alterations in sympathetic nerve activity, neurotransmitter release, and receptor responsiveness, which has been a source of interest in our laboratory and therefore is examined further in this thesis.

## 1.3 Neuropeptide Y receptor function and intracellular coupling

In addition to adrenergic and cholinergic nerve fibres, immunohistochemical and radioimmunoassay studies have revealed nerve fibres containing neuropeptides such as NPY (Tatemoto et al., 1982; Edvinsson et al., 1987). NPY has been identified in abundance in the central nervous system of many species, including guinea pig, rat, pig, monkey and man (Allen et al., 1983; Adrian et al., 1983; Allen et al., 1984; Dawbarn et al., 1984; Pelletier, 1984; Larhammar et al., 1987). High levels of NPY have been identified peripherally in the heart and stellate ganglion of several species, including cat, rat, mouse, guinea pig, dog, pig, and man (Lundberg et al., 1982, 1983, 1985a; Furness et al., 1983; Gu et al., 1984; Sternini and Brecha, 1985; Allen et al.,1986a,b; Dalsgaard et al., 1986; Pujeranta et al., 1986). NPY fulfills the main criteria of a neurotransmitter, since it is stored in synaptic granulae (Fried et al., 1985), is released upon electrical nerve stimulation (Lundberg et al., 1989), and acts at specific receptors (Wahlestedt et al., 1990). NPY is present in most sympathetic nerve fibres, particularly around blood vessels (i.e. perivascular fibres) (Sheikh et al., 1988; Lundberg et al., 1989; Wahlestedt et al., 1990) and in the heart and kidneys (Sundler et al.,1993). In the

ANS, it is stored with and released with NE in many sympathetic nerves, especially those distributed in the cardiovascular system, however, NPY is preferentially released by high-frequency nerve stimulation (Zukowska-Grojec & Wahlstedt, 1993). Although the adrenals of many species, including humans, costore NPY and catecholamines, it remains unclear whether adrenal NPY release is physiologically relevant, since in healthy people, plasma NPY levels under various conditions correlate with those of NE but not with those of epinephrine (Zukowska-Grojec & Wahlstedt, 1993). Ultracentrifugation studies have identified NPY in the same fraction as large dense-core vesicles containing NE. However, only about 5% of terminal axon vesicles are of this type and these appear to contain both NPY and NE. Most NE is stored separately from NPY in the same nerve endings in small, dense-cored vesicles (Fried et al.,1985; De Quidt et al., 1985). The content of NPY-immunoreactivity in sympathetically innervated tissues decreases after sympatholytic treatment with 6-hydroxydopamine or sympathectomy (Edvinsson et al., 1983; Lundberg et al., 1985b). Circulating levels of plasma NPY-immunoreactivity vary widely from low levels in humans (10 to 80 fmol/mL) to high levels in rats (1pmol/mL) (Howe et al., 1986). Plasma NPY is derived mostly from sympathetic nerves and reaches the general circulation by spillover from the synaptic cleft. The clearance and metabolism of circulating NPY are not well understood. The half-life of circulating NPY, estimated following administration of exogenous NPY in humans is approximately 20 minutes.

NPY is a 36 amino acid peptide, first purified from porcine brain (Tatemoto et al., 1982). The amino acid sequence of porcine NPY was reported in 1982 and since then, human NPY has been found to differ from it by only one amino acid, a methionine residue at position 17 in place of a leucine (Minth et al., 1984; Corder et al.,1985). NPY is structurally similar to

pancreatic polypeptide (PP) and peptide YY (PYY), which are peptides found in peripheral endocrine type cells of pancreatic and gastrointestinal tract tissue (Lin and Chance, 1974; Kimmel et al., 1975; Tatemoto et al.,1982). NPY has a tertiary structure consisting of an N-terminal polyproline helix and an amphiphilic α-helix, connected with a ß-turn, creating a hairpin-like loop, which is sometimes referred to as the PP-fold (Schwartz et al., 1990). The helices are kept together by hydrophobic interactions. There is also an amidated C-terminal end which projects away from the hairpin loop (Glover et al., 1985; Allen et al., 1987; MacKerell et al., 1989). NPY is released during stimulation of sympathetic nerves in lab animals, and in humans, plasma concentrations of NPY increase after reflex sympathetic stimulation induced by physical exercise (Morris et al., 1986; Winther et al., 1992), cold pressor testing (Morris et al., 1986), and lower body negative pressure (Kahan et al., 1992). Intravenous administration of NPY causes an increase in arterial blood pressure, local vasoconstriction, and a reduction in heart rate (Lundberg & Tatemoto, 1982). The pressor response can be attenuated by the $Ca^{2+}$ entry blocker, nifedipine, but not by adrenoceptor antagonists (Howe et al., 1986). Close intra-arterial infusions of NPY in man produced prolonged vasoconstriction and increased venous tone in the forearm. In the anaesthetized dog, it has been shown that intracoronary infusion of NPY causes a long-lasting increase in flow resistance without any change in heart rate (Aizawa et al., 1985). It has also been demonstrated that the direct perivascular microapplication of NPY around arteries, arterioles, and veins *in situ* produces a strong concentration-dependent constriction. The magnitude of the constriction was found to be similar to that produced by NE but more long lasting (Edvinsson et al., 1984).

NPY has several actions at the sympathetic neuroeffector junction. First, *in vitro* studies

have shown that NPY acts directly, presumably through its own receptor, on vascular smooth muscle by a mechanism dependent on extracellular calcium (Pernow et al., 1986b). Clarke et al. (1991) examined the effects on the human forearm vascular bed *in vivo* of intra-arterial administration of NPY and found that NPY caused a large dose-dependent reduction in blood flow very similar to that demonstrated by Pernow et al. (1988). NPY acts directly, independent of α-adrenergic receptors as demonstrated by the failure of the non-selective α-blocking agent phentolamine to diminish or abolish the constrictor action of NPY in man (Edvinsson et al., 1985). NPY also has an indirect postjunctional effect consisting of potentiation of the responses to some other agents with direct postjunctional actions, especially NE. The potentiating effects of NPY on vasoconstriction caused by nerve stimulation or NE appear to be independent of direct effects and have been observed in many studies of the action of NPY. These potentiating effects are usually seen at much lower concentrations of NPY than those necessary for any direct action (Edvinsson et al., 1983; Ekblad et al., 1984; Dahlof et al.,1985a,b; Lundberg et al., 1985; Wahlestedt et al., 1985; Wahlestedt and Hakanson, 1986). The degree of potentiation appears to vary from vessel to vessel and from species to species. Third, in vas deferens, heart, and blood vessels, it has been demonstrated that NPY acts prejunctionally to inhibit the release of NE evoked by electrical field stimulation from the sympathetic nerve terminals (Lundberg et al., 1982, 1984b; Allen et al., 1986b; Pernow et al., 1986b). In the perfused mesenteric arterial bed of the rat, NPY was shown to produce a concentration-dependent reduction in the release of NE induced by periarterial nerve stimulation (Westfall et al., 1987). Therefore, this demonstrates that NPY is involved in the regulation of NE release from adrenergic varicosities. The mechanisms behind the presynaptic regulation of NE and NPY release appear to be

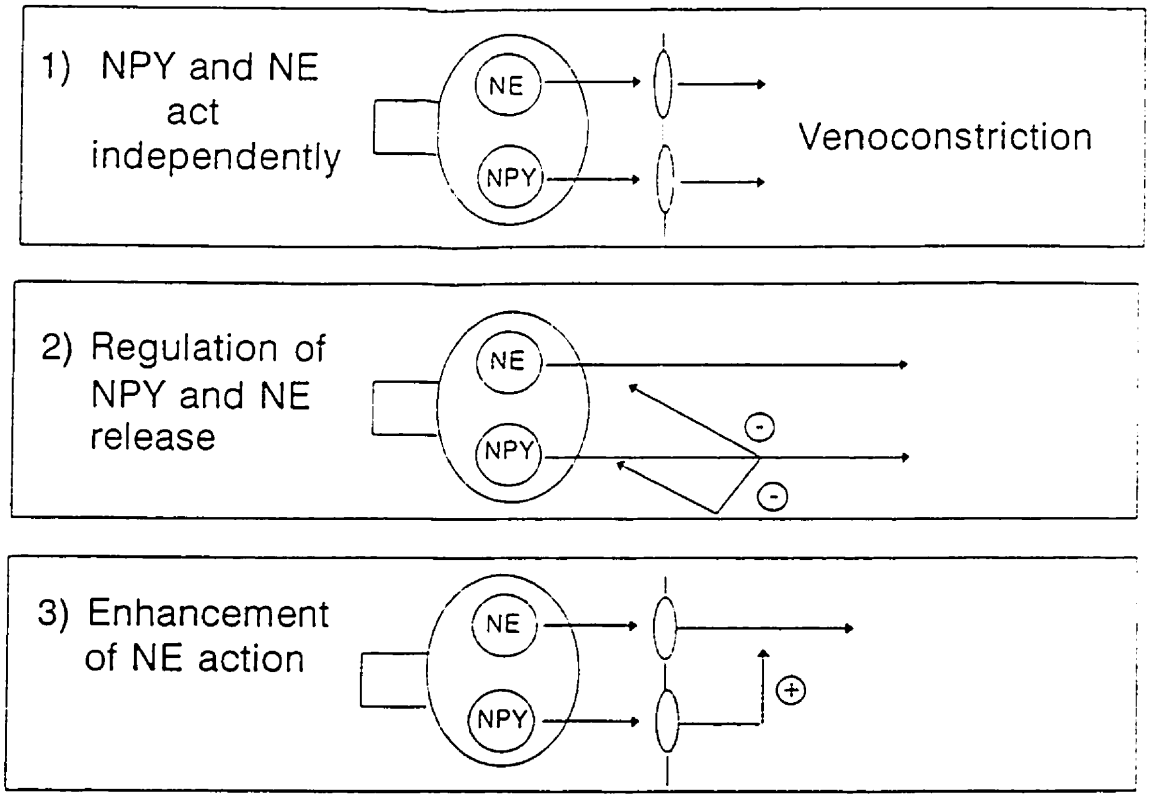


**Figure 1.5** Schematic diagram showing the 3 principal types of synaptic/junctional actions and interactions of NPY and NE. Abbreviations: NE, norepinephrine; NPY, neuropeptide Y (Adapted from Wahlestedt and Reis, 1993)

independent of $\alpha_2$-adrenoceptors, as yohimbine fails to alter the inhibitory effect of NPY on the release of NE. Similarly, the $\alpha_1$-adrenoceptor antagonist, prazosin, failed to alter the prejunctional effect of NPY.

Specific binding sites for NPY have been described in membrane preparations from brain tissue (Unden et al., 1984; Saria et al., 1985; Inui et al., 1988) and in membranes from blood vessels (Lundberg et al., 1988). In the sympathetic nervous system, 2 distinct subtypes of NPY receptors are responsible for the pre-synaptic and post-synaptic effects of the peptide (Wahlestedt et al., 1986). These receptors were named the $Y_1$ and the $Y_2$ receptors. The $Y_1$ receptors are located postjunctionally and are responsible for the direct vasoconstricting actions of NPY as well as the indirect potentiation actions of NPY. The $Y_2$ receptors in the periphery are generally considered to be localized at prejunctional sites at the sympathetic neuroeffector junction, suppressing the release of transmitters (Wahlestedt et al., 1986a,b; Westfall et al., 1990). The terms $Y_1$ and $Y_2$ were introduced to denote the receptor that required the whole NPY molecule for activation as $Y_1$ and the receptor that was selectively stimulated by the long C-terminal NPY fragments as $Y_2$ (Wahlestedt et al., 1987). However, although the $Y_1$ receptor appears to be the major vascular NPY receptor, the $Y_2$ receptor can also occur postjunctionally on vascular smooth muscle (Wahlestedt et al.,1990). The human $Y_1$ subtype gene has been cloned (Herzog et al., 1993) whereas the $Y_2$ gene has not yet been cloned. The involvement of a $Y_1$-like receptor in NPY-mediated vasoconstriction in human subcutaneous arteries and veins has been shown using antisense oligodeoxynucleotides specific for the human $Y_1$ receptor (Erlinge et al., 1993). A large number of binding studies have demonstrated the presence of NPY receptors in vascular smooth muscle (Chang & Lotti, 1988; Wahlestedt et al., 1990; Sheikh et al., 1991; Shigeri et al., 1991; Grundemar et al.,

1992). These binding sites were visualized by an autoradiographic approach at the electron-microscopic level. A small population of $Y_1$ binding sites was also detected on the vascular endothelium (Sheikh et al., 1991). NPY $Y_1$-receptor mRNA has also been detected in the human heart (Wharton et al., 1993). NPY receptors belong to the family of heptahelical G-protein-coupled receptors. Specifically, $Y_1$ receptors are linked via an inhibitory G protein ($G_i$) to adenylyl cyclase, which upon receptor activation, results in a decrease in adenylyl cyclase activity and a consequent decrease in the production of the second messenger cyclic adenosine-3',5'-monophosphate (cAMP) (Bylund, 1988). Decreased cAMP production results in smooth muscle contraction by decreasing activation of protein kinase A which, when active, phosphorylates myosin light chain kinase and decreases its affinity for calmodulin (Bouvier et al., 1987; Limbird, 1988). In contrast, stimulation of this pathway (via a stimulatory G protein, $G_s$) results in the relaxation of smooth muscle, seen during activation of vascular $ß_2$ receptors (Graham, 1990). Consequently, a decrease in the activation of protein kinase A results in increased association of myosin light chain kinase with calmodulin and increases vascular smooth muscle contraction. In addition, NPY-evoked vasoconstriction *in vivo* (Dahlof et al., 1985b; Franco-Cereceda et al., 1985; Grundemar et al., 1992) and in many but not all isolated vessels *in vitro* (Edvinsson et al., 1983; Wahlestedt et al., 1985) appears to rely in part on the influx of extracellular $Ca^{2+}$ into smooth muscle cells, since several L-channel-type $Ca^{2+}$ channel antagonists were shown to attenuate the NPY responses. To date, the vascular effects of NPY in humans *in vivo* have not been thoroughly investigated. **Therefore, our first objective was to demonstrate dose-response venoconstriction and functional postjunctional NPY receptors *in vivo* in human dorsal hand veins.**

Like many other biological messenger molecules, NPY has been suggested to be linked

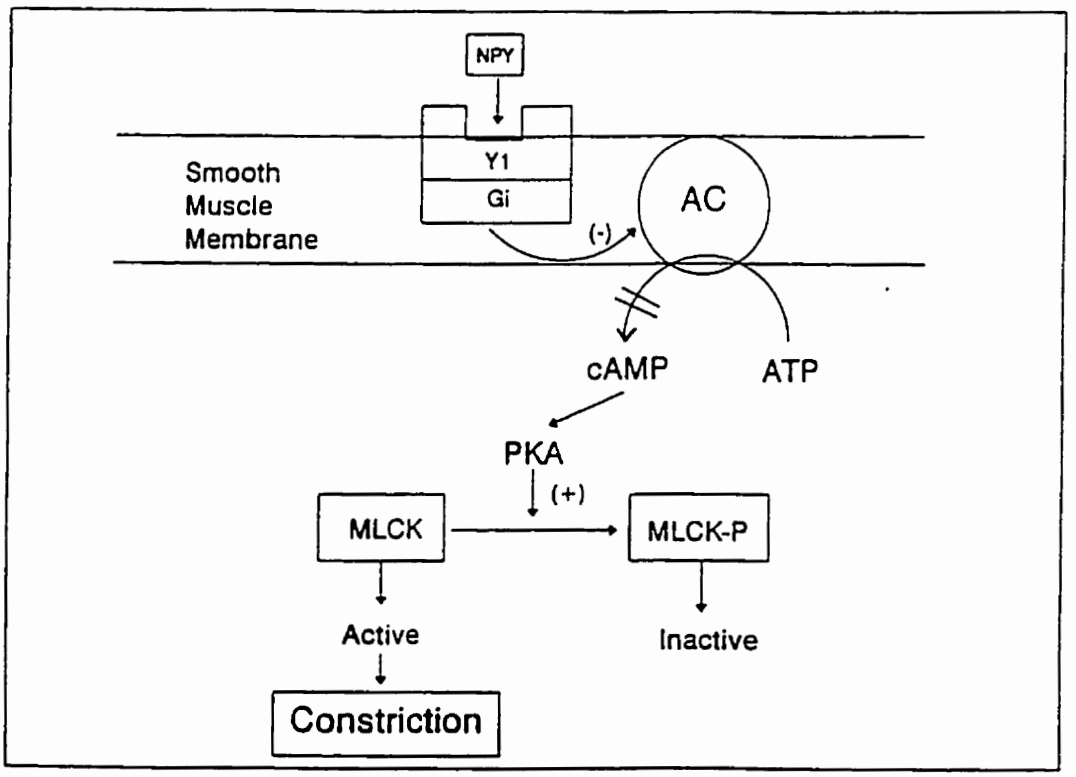


**Figure 1.6** Schematic diagram showing the signal transduction pathway of NPY receptors.
Abbreviations: NPY, neuropeptide Y; Y1, Y1 receptor; $G_i$, inhibitory G protein; AC, adenylyl cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine-3'5'-monophosphate; PKA, protein kinase A; MLCK, myosin light chain kinase; MLCK-P, phosphorylated myosin light chain kinase.
(Adapted from Graham, 1990).

with human diseases, such as congestive heart failure. In our lab, we are interested in the venous responsiveness to NPY in CHF, and this will be further described in a later section.

## 1.4 The role of the SNS in controlling venous tone

Veins are not a system of passive tubes but play an important role in the regulation of cardiac output (Webb-Peploe et al., 1968). It is estimated that the venous system contains approximately 65-75% of the blood volume and that approximately 75% of the venous blood volume is in the small veins and venules (Pang, 1994). The venous system is regulated by cardiovascular reflex systems, most importantly, the baroreflex system. A reduction in arterial pressure reduces baroreceptor activity, which increases sympathetic outflow to postganglionic adrenergic nerve fibres at the veins, resulting in the release of the transmitter NE, along with co-transmitters including NPY and ATP (Pang, 1994). Veins are richly innervated by sympathetic nerves which penetrate deep into the smooth muscle bundles of veins, unlike most arteries where postganglionic sympathetic noradrenergic nerves rarely penetrate beyond the adventitia-media junction (Wiedeman, 1963; Rothe, 1983; Milnor, 1990). Vasoconstriction results in increased pressure in the venous reservoir and consequently raises cardiac filling pressure. Filling pressure is a prime determinant of cardiac function and therefore veins play a major role in regulating cardiac output (Webb-Peploe, 1968). Under comfortable environmental conditions, there is little activity in the sympathetic nerve fibres to limb veins and thus the tone of the smooth muscle in their walls is at a minimum. Therefore, the volume of the veins and the pressure within them are determined by the viscoelastic properties of the vessel wall (Webb-Peploe, 1968). Since the venous system is the primary capacitance region in the body, an alteration of its capacity has a great influence

on venous return and cardiac output.

The major components of veins are collagen, elastin, and smooth muscle. Collagen and elastin fibres contribute mostly to passive or viscoelastic properties, while smooth muscle fibres are responsible for active tension or contractile activity (Pang, 1994). Smooth muscle cells are more abundant in superficial veins in the limbs than in deeper veins in order to oppose volume changes in response to variations in hydrostatic pressure (Shepherd & Vanhoutte, 1975). The cutaneous veins possess dense sympathetic innervation and are more reactive than deeper skeletal muscle veins to sympathetic nerve stimulation (Mellander, 1960; Tkachenko, 1971).

Veins consist of 3 layers termed the tunica intima, tunica media, and tunica adventitia. However, these layers are not as well defined as they are in arteries (Wheater et al., 1987). Veins have thinner walls than their accompanying arteries, and the lumen of the vein is usually larger than that of the arterial lumen (Ross & Romrell, 1989). The tunica intima consists of the endothelial layer and its basal lamina, and it may also have a small amount of subendothelial connective tissue with some smooth muscle cells and a thin internal elastic membrane (Ross & Romrell, 1989). The endothelial layer consists of a single layer of extremely flattened cells called endothelial cells which serve an important function as they are the source of several endothelial mediators or hormones such as $PGI_2$ and endothelium derived relaxing factor (EDRF) which is now known to be nitric oxide (NO) (Pohl et al., 1994). These endothelial derived substances are released *in vivo* upon SNS activation of vascular receptors and produce effects in the veins and arteries via hormonal cascades. These hormonal effects on cardiovascular function are of great interest to us in our lab and therefore are described more fully below. The tunica media is much thinner than in arteries and it

contains circularly arranged smooth muscle cells and collagenous fibres (Ross & Romrell, 1989). The tunica adventitia is usually thicker than the tunica media and is made up of connective tissue with many longitudinally arranged bundles of smooth muscle cells, collagenous fibres, and networks of elastic fibres (Ross & Romrell, 1989). Many veins, especially those of the limbs, contain valves that allow blood to flow in only one direction. The valves are semilunar flaps consisting of a thin connective tissue core with an endothelial surface exposed to the blood (Ross & Romrell, 1989).

Evidence supports the existence of NPY $Y_1$ receptors in veins located on the vascular smooth muscle cells of the tunica media, which mediate the interactions with neuronally released NPY via the pathways described above. The venous system is the primary capacitance region in the body, and therefore any alteration of its capacity has a great influence on venous return and cardiac output. Activation of venous $Y_1$ receptors results in profound venoconstriction which raises the pressure in the venous reservoir, increases venous return, and therefore increases cardiac output (Pernow et al., 1986a). However, venous tone should not be confused with venous return, which is the rate of blood return to the heart, and is equivalent to cardiac output at steady state conditions (Pang, 1994). Venous tone is determined by such factors as venous compliance and venous resistance which are modulated by SNS activity and circulating hormones (Rothe, 1983). Therefore, venous tone which is primarily regulated by SNS activity is an important determinant of cardiac output. However, despite the importance of the venous system in the control of blood pressure and cardiac output, and cardiovascular function in general, there are relatively few studies on the effects of alterations in venous responsiveness or venous tone, especially at the level of the receptors (Pang, 1994). In contrast, there have been numerous studies on the effects of alterations in

systemic vascular resistance. This lack of research may be due to the technical difficulties associated with studies of the venous circulation. However, advancements have been made in this area (Chapter 2) and techniques are now more available to assess changes in the tone and responsiveness of peripheral cutaneous veins. Since cutaneous veins are easily accessible for studies assessing venous responsiveness to exogenous drugs, the veins represent an ideal site for investigation of venous function and NPY receptor responsiveness.

## 1.5 Factors modulating venous responsiveness to SNS activity

### 1.5.1 The SNS in Chronic Heart Failure (CHF)

Chronic heart failure is the state when, for a variety of causes, the heart fails to perform its function of supplying an adequate amount of oxygenated blood to the tissues and organs (Sandler, 1990). CHF is one of the most devastating illnesses in North America with mild to moderate CHF patients having an average life expectancy of 5 years, and severe CHF patients having an even worse prognosis (Parmley, 1989; DiBianco, 1994). The causes of heart failure are numerous and include cardiomyopathies, ischaemic heart disease, and hypertension (Braunwald, 1987). The regulation of cardiac function is a complex combination of physiological, autonomic neurological, and endocrine responses that compensate for changes in the circulation (Sandler, 1990). It is known that SNS nerve activity is increased in patients with CHF based on evidence that urinary excretion and circulating plasma levels of NE are increased in patients with CHF, and plasma NE levels are directly related to the severity of hemodynamic dysfunction (Jackson et al., 1982; Packer, 1988). Plasma NE can be increased because of increased nerve release, reduced local reuptake by neuronal and nonneuronal mechanisms in the neuroeffector junction, and/or a reduction in the metabolism of NE by

systemic clearance mechanisms (Colucci, 1994). Studies of NE turnover kinetics based on tracer quantities of [$^3$H]-NE have demonstrated that NE spillover from both the heart and kidneys is increased in patients with heart failure (Henrich et al., 1978).

Impaired cardiac output results in the stimulation of the SNS through the ß receptors of the heart. This results in an increase in both the heart rate and the ventricular contractility in an effort to boost the cardiac output. In addition, the sympathetic stimulation in the arterioles, through stimulation of their α receptors, causes a diversion of blood away from the less important tissues such as skin, muscles and bowel, and towards the heart, kidneys and brain. This helps to preserve the blood flow to the vital organs (Colucci, 1994). Sympathetic stimulation can also affect the venous side of the circulation by increasing venous tone, which will enhance venous return to the heart and increase the preload in an effort to maintain cardiac output. The effects of increased SNS activity in heart failure may be both beneficial and detrimental. Initially, following the onset of myocardial failure, increased SNS activity plays an important role in supporting cardiovascular homeostasis. However, if there is no improvement in myocardial function, the sustained activation of the SNS may continue to be important on a long-term basis. Consequently, the degree of sympathetic activation may become excessive and result in deleterious effects (Colucci, 1994). The activity of the cardiovascular centres within the brain stem is under the influence of reflex pathways associated with a variety of mechanoreceptors and baroreceptors within the heart, blood vessels, and lungs. Increases in mean arterial pressure, or increased atrial and/or ventricular filling pressures and distention normally cause activation of cardiopulmonary and arterial baroreceptors. This results in the inhibition of SNS outflow from the cardiovascular centres and a reciprocal increase in parasympathetic nerve activity (Hirsch et al., 1987). However,

there is evidence that the baroreceptors are dysfunctional in both experimental animals and humans with heart failure (Rea & Berg, 1990). In animal models of heart failure, the sympathetic responses to increases or decreases in baroreceptor stimulation are blunted, suggesting decreased sensitivity of the baroreceptors (Wang et al., 1990). Also, in patients with CHF, several observations indicate a decrease in baroreceptor sensitivity (Hirsch et al., 1987; Rea & Berg, 1990). In normal, healthy people, reductions in arterial pressure caused by nitroprusside infusion, head-up tilt, or lower body negative pressure result in reduced venous return, arterial pressure, and the volume of the heart, and therefore cause an increase in SNS outflow. But, in patients with heart failure, the SNS response to these interventions is blunted as indicated by diminished NE, vasoconstrictor, or heart rate responses (Olivari et al., 1983; Levine et al., 1983; Kubo & Cody, 1983). In addition, infusion of the $\alpha_1$-adrenergic agonist phenylephrine, which normally causes reflex SNS withdrawal and a slowing of the heart rate, yields a blunted response in patients with heart failure (Hirsch et al., 1987). The cellular basis for baroreceptor dysfunction is not known, but may involve a number of factors such as mechanical alterations in the environment of the baroreceptor, a resetting of baroreceptor reflex pathways, or alterations in the biochemical function of the baroreceptors, such as altered $Na^+K^+$-ATPase activity (Rea & Berg, 1990; Wang et al., 1990). These abnormalities in the baroreceptors may contribute to the development of the neurohumoral excitatory state in heart failure and also interfere with the normal mechanisms for adaptation to physiological stresses such as those that may result from postural change, exercise, or emotion.

Changes in SNS activity alter other hormonal systems that are important in CHF, the main one being the RAAS. This system appears to play little physiological role under normal

conditions (Hollenberg et al., 1977; Faxon et al., 1984). However, intrarenal activation of the RAAS plays a greater role in preserving glomerular filtration when renal perfusion pressure is impaired in disease states such as CHF (Hall et al., 1977). Changes in cardiac performance that affect renal blood flow are detected by sensory receptors in the renal arterioles. This baroreceptor stimulation leads to the release of renin from the kidneys that results in the subsequent formation of angiotensin II which then acts on the efferent arterioles to increase glomerular filtration pressure in order to preserve renal function (Hirsch et al., 1987). In normal situations, excessive activation of the RAAS results in an increase in systemic and atrial pressures. This activates the baroreceptors to release atrial natriuretic peptide and to reduce the stimulation of the renal sympathetic nerves, both of which act to reduce renin release. However, in CHF, the function of the atrial and arterial baroreceptors is markedly impaired, and therefore atrial distention does not suppress renal sympathetic activity (Zucker et al., 1979). Also, the ability of atrial distention to stimulate the release of atrial natriuretic peptide is impaired in chronic CHF, as is the ability of the peptide to suppress renin release (Cody et al., 1986). Therefore, this results in most patients with heart failure having an excessive activation of the RAAS at rest or during exercise. Again, activation of the RAAS is both beneficial and deleterious in patients with chronic CHF. On the one hand, angiotensin II acts to support systemic perfusion pressures during states of renal hypoperfusion (Packer et al., 1986). On the other hand, prolonged activation of the RAAS may exert deleterious effects on ventricular function by causing systemic vasoconstriction (Francis et al., 1984). In addition, by stimulating the synthesis of aldosterone, angiotensin promotes the retention of salt and water by the kidney and the depletion of potassium and magnesium (Lee & Packer, 1986).

Because SNS activity is chronically elevated in disease states such as CHF (Francis et al., 1984; Feng et al., 1994), altered NPY receptor responsiveness may also occur. Significant elevation of sympathetic drive results in an increased level of plasma catecholamines (Cohn et al., 1984) and the extent of elevation of plasma NE is well correlated with the firing rate of the muscular sympathetic nerve (Leimbach et al., 1986). NPY is an important neurotransmitter released together with NE during physiological conditions such as physical exercise (Lundberg et al.,1985b; Pernow et al., 1986a) and pathophysiological situations of excessive increase of sympathetic tone such as CHF (Maisel et al., 1989; Hulting et al., 1990; Feng et al., 1993; Derchi et al., 1993). There is much evidence that the measurement of NPY concentrations in the circulation of humans and several animal species provides an index for sympathetic nerve activity, since circulating NPY is released mainly from sympathetic nerves. Thus, upon sympathetic stimulation, NPY overflow into the local venous effluent from organs of experimental animals, or the human heart is increased, as are systemic plasma levels in humans (Lundberg et al., 1990). Data from nerve stimulation experiments in animals (Lundberg et al., 1984a, 1986) and physiological activation of the sympathetic system in man (Lundberg et al., 1985b; Pernow et al., 1986a) suggest that NPY release is enhanced upon more intense stimuli, compared to that of NE. In CHF, there is commonly an activation of the SNS and high levels of NE have been related to a poor prognosis in chronic left ventricular failure (Chidsey et al., 1962; Thomas & Marks, 1978; Minami et al., 1983; Swedberg et al., 1984; Hasking et al., 1986). Hulting et al. (1990) also found that there is a strong relationship between high plasma NPY concentrations and moderate left heart failure. There are several reports of increased plasma levels of NPY, but also of unchanged NPY levels in CHF (Edvinsson et al., 1990; Hulting et al., 1990; Derchi et al., 1993). A study by

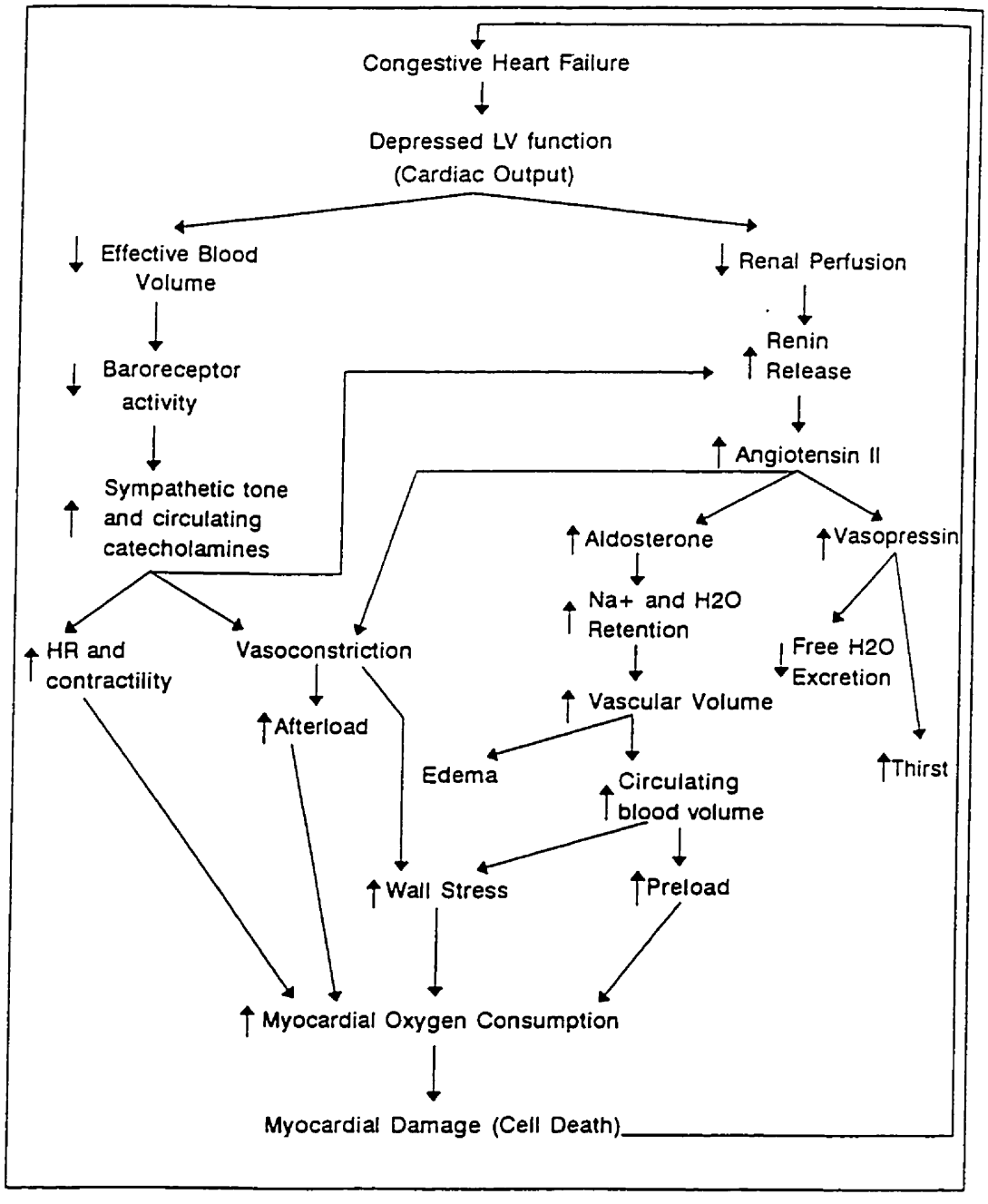


**Figure 1.7** Schematic of the vicious circle of events associated with renin-angiotensin-aldosterone system and sympathetic nervous system activation in congestive heart failure.

Valdemarsson et al. (1994) found that NPY levels were increased in cases of moderate CHF, but there was no further increase in more severe forms of CHF. Thus, the plasma level of NPY seems to be related to the activation of the SNS, but not in a way that directly gauges the severity of the degree of heart failure. In healthy volunteers, the circulating concentration of NPY-like immunoreactivity released from peripheral nerves is less than 130 pmol/L in the resting state (Lundberg et al., 1985b; Pernow et al., 1986a; Kaijser et al., 1990). A study by Feng et al. (1994) also measured the circulating concentrations of NPY and found that both the circulating concentrations of NPY and NE were significantly increased in moderate to severe forms of CHF. However, they also found that plasma concentrations of NPY correlated with plasma NE concentrations, but plasma NE concentrations alone correlated with ejection fraction and cardiac index. Thus, plasma NE concentrations seem to be a more sensitive index of cardiac dysfunction than the concentrations of NPY in CHF.

In the venous system, elevated levels of NE and NPY result in excessive venoconstriction which acts to increase venous tone and systemic venous return to further enhance the detrimental cycle that occurs in heart failure. Because of the constant activation of the α-adrenergic and $Y_1$ receptors, alterations in the responsiveness of these receptors may occur. In fact, previous research in our laboratory has shown that venous α-adrenoceptor responsiveness is decreased in severely symptomatic patients with CHF while patients with milder symptoms demonstrate increased α-receptor responsiveness (Callow, 1995). In an animal model of heart failure, it has been demonstrated that the postjunctional $Y_1$ receptors are desensitized in response to NPY activation (Feng et al., 1993). However, it is presently not known if these NPY receptors are functioning normally or are hypo- or hyper-responsive to NPY in CHF patients, in spite of the apparent importance of alterations in NPY receptors.

**Therefore, a goal of this thesis was to determine changes in NPY receptor responsiveness occurring as a result of the alterations in SNS activity which may modify receptor function and consequently cardiovascular regulation in CHF. Thus, we compared NPY receptor responsiveness in peripheral cutaneous veins of heart failure patients with normal volunteers.**

### 1.5.2 Endothelial function

The vessel intima is mainly composed of a single, flattened layer of endothelial cells, covering the luminal surface of the vessel wall. These cells form a border line separating the deeper layers of the vessel wall and the interstitial space from blood and circulating cells (Schror, 1985). The endothelium is not merely a passive, blood-compatible surface, but plays an active role in physiological processes such as homeostasis, regulation of vessel tone, and vascular permeability (Nossel & Vogel, 1982). Due to their anatomical location immediately adjacent to the bloodstream, vascular endothelial cells are an obvious target for hormones that are transported in the bloodstream (Pohl & Kaas, 1994). Interactions between circulating hormones and the endothelium play a major role in the control of vascular tone since the endothelium is able to release a number of vasoactive factors which act on the control of smooth muscle tone. Traditionally, control of peripheral vascular tone was attributed to the SNS where activation caused contraction of smooth muscle and relaxation was attributed to the reduction or withdrawal of SNS activity. However, following the observations by Furchgott & Zawadzki (1980), the endothelial cells were found to release many substances that can either contract or relax the smooth muscle. These vasoactive factors are released from the endothelial cells in response to a number of physiological stimuli including changes

in partial pressure of oxygen, stretch, the shear stress of the blood flowing across the cell surface, and circulating hormones (Miller, 1991). Therefore, the endothelial-smooth muscle complex represents a complete reflex arch containing sensory-transduction and effector elements (Miller, 1991). Many studies have revealed the existence of multiple interactions between circulating or neuronally released hormones and endothelial cells (Pohl et al., 1994). A number of hormones have been shown to elicit the release of vasoactive autacoids, pricipally $PGI_2$ and NO from the vascular endothelium that then act on the vascular smooth muscle to control its function (Pohl et al., 1994). Studies have shown that the influence of NO release by endothelial cells in the venous system in humans is minimal (Haefeli et al., 1993) and therefore, $PGI_2$ is thought to be the primary substance released by the endothelial cells in veins. $PGI_2$ is a prostanoid with potent vasodilating properties and is a potent inhibitor of platelet aggregation (Johnson et al., 1976). $PGI_2$ is synthesized by endothelial cells via the cyclo-oxygenase pathway and is believed to functionally counteract the effects of thromboxane $A_2$ released from the platelets (Moncada & Vane, 1979). A study by Vanhoutte et al. (1989) demonstrated that NE stimulated the $\alpha_2$ receptors to induce the release of $PGI_2$. Various vasoactive agonists have been shown to stimulate the release of $PGI_2$ via a receptor-dependent mechanism involving increases in intracellular $Ca^{2+}$. Studies using isolated human hand veins preconstricted with endothelin have shown that $PGI_2$ elicits a concentration-dependent relaxant effect indicating the potential of $PGI_2$ to overcome or antagonize the effects of some potent vasoconstrictors (Arner et al., 1994). In our lab, *in vivo* studies of normals, using the prostaglandin synthesis inhibitor indomethacin and several $\alpha$-adrenoceptor agonists suggested that the venous endothelium contains both $\alpha_1$ and $\alpha_2$-adrenoceptors which upon stimulation result in the release of vasodilatory prostaglandins,

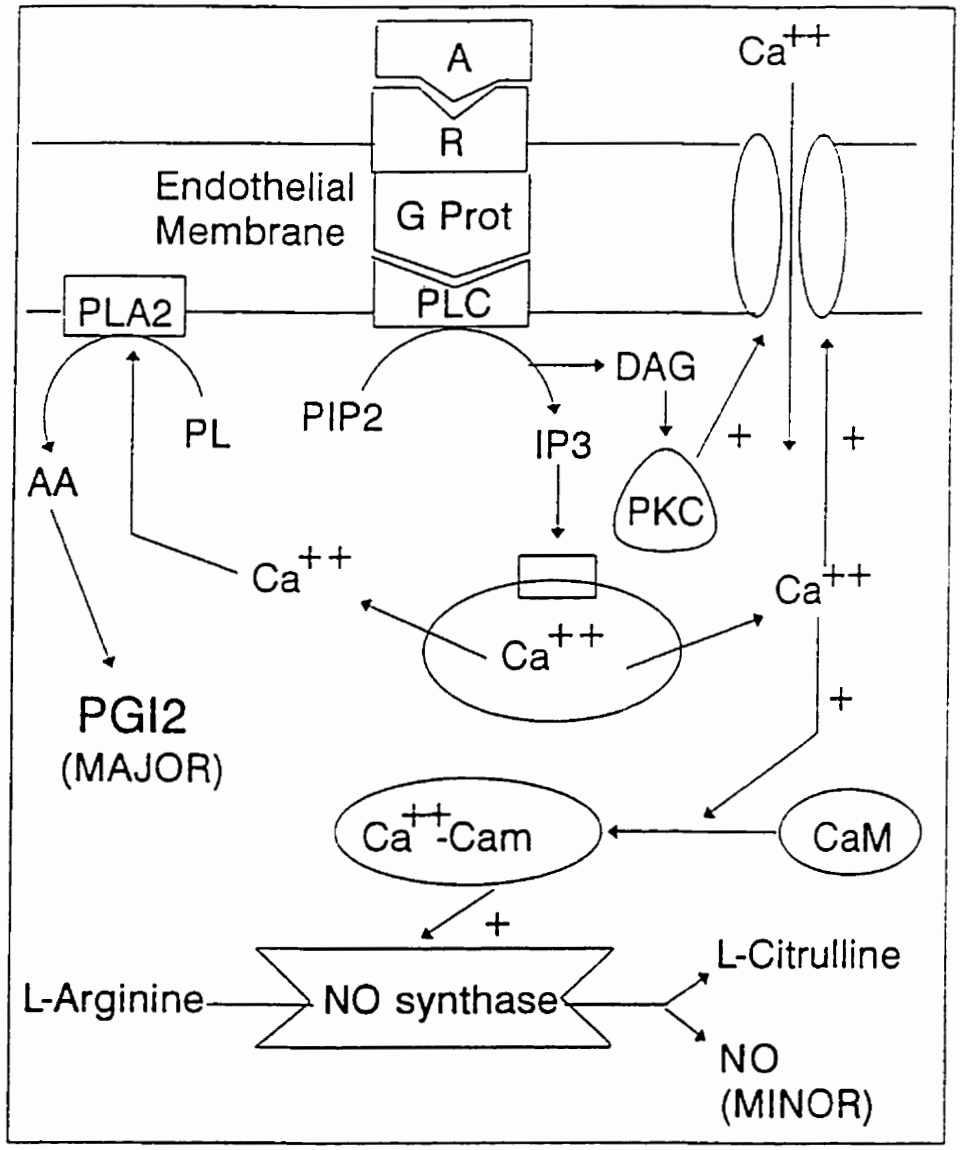


**Figure 1.8** Schematic of the major signal transduction pathways involved in agonist-induced formation of $PGI_2$ and NO in venous endothelial cells.
Abbreviations: A, agonist; R, receptor; G prot, G protein; PLC, phospholipase C; DAG, diacylglycerol; PLA2, phospholipase $A_2$; PL, phospholipids; PIP2, phosphoinositol-4',5'-bisphosphate; IP3, inositol trisphosphate; PKC, protein kinase C; AA, arachidonic acid; CaM, calmodulin; PGI2, prostacyclin; NO, nitric oxide.

most likely $PGI_2$, which antagonize the α-receptor mediated constriction of vascular smooth muscle (Callow, 1995). Consequently, endothelial released $PGI_2$ appears to play a significant role in α-adrenoceptor responsiveness to infused agonists in the venous system. NPY-Y1 receptors are present in the vascular endothelium of cultured endothelial cells and stimulation releases endothelium derived vasodilators thus producing vascular dilatation (Lind et al., 1995). A study by Kawamura et al. (1991) showed that NPY induced dose- and time-dependent stimulation of prostacyclin production in cultured porcine aortic endothelial cells. This is consistent with findings at the α-receptor and consequently it would appear that endothelial NPY receptors may also influence the NPY mediated vascular smooth muscle response. **Therefore, it was a goal of this thesis to determine whether NPY receptor activation stimulates the release of $PGI_2$, and if so, to clarify the influence that this may have on the venous responsiveness observed.**

It has recently been confirmed that endothelium dependent relaxation is decreased in the dorsal hand veins in CHF and this decrease in endothelial function may result in a decrease of $PGI_2$ synthesis in CHF (Katz, 1995). Thus, the net vasoconstrictor response to NPY may be a balance between NPY levels, receptor function and endothelial function. **Thus, an additional goal was to determine in patients with CHF, how endothelial dysfunction affects the release of $PGI_2$ upon NPY receptor stimulation, and how this affects the overall NPY responsiveness between normals and CHF patients.**

# CHAPTER 2

# METHODS

## 2.1 Introduction

The venous system does not consist of passive tubes, but rather plays a major role in the regulation of cardiac output. However, despite their importance, veins are often neglected in studies on cardiovascular pharmacology and physiology. This is probably due to the fact that most of the experimental techniques that are used on the arterial side of the circulation are not suitable for studies on veins, due to anatomical, physiological, and pathophysiological differences between these areas of the circulatory system. The important elements relating to veins are venous volume (venous tone) and venous compliance, whereas on the arterial side, arterial pressure and flow are the essential elements. However, veins offer a unique opportunity for the direct study of actions of pharmacological and physiological stimuli on a human vascular bed *in vivo* due to their easy accessibility, low intravascular pressure, their relatively thin vascular walls, and their distensibility.

Several methods have been developed over the past several years to study the direct effects of various pharmacological and physiological stimuli on superficial veins in man by assessing changes in venous compliance and venous tone.

## 2.2 Techniques for measuring venous compliance and venous tone in humans *in vivo*

In the past, the most widely used method for the *in vivo* assessment of changes in venous tone in a given peripheral region, such as the arm or leg was plethysmography. This method permits the determination of venous volume which reflects the changes in venous tone as long

as the occlusion pressure remains constant. This is done by measuring the volume increase of a limb or a segment of a limb after the occlusion of venous outflow by inflating a proximally applied occlusion cuff to a subdiastolic pressure. This method has the advantage that at the same time, arterial blood flow in the limb being investigated can also be measured. Plethysmography measures changes in the volume of a limb segment after the abrupt inflation of a proximally applied occlusion cuff to a pressure that is higher than venous but lower than diastolic pressure. Therefore, this temporarily inhibits venous outflow but does not interfere with arterial inflow and the rate of volume increase reflects arterial flow. After a period of time, the venous pressure rises to the occlusion pressure and the arterial inflow then equals the venous outflow. The first apparatus used to determine changes in limb volume was the water-filled plethysmograph. This allowed accurate measurements of changes in venous volume by measuring the increase in the volume of water that was displaced by the limb after inflating the proximally applied occlusion cuff. This method, however had several limitations because it is not easy to tighten the apparatus around the limb without interfering with venous outflow and the temperature of the water must be kept exactly the same as the limb temperature to avoid thermal effects on venous tone.

An attempt to overcome these limitations was made with the use of an air-filled collection cuff instead of the water-filled device, yet it is also limited by the fact that it requires prolonged immobilization and cooperation of the subject which may not be practical in patients who are seriously ill. Today, the most widely used plethysmographic method uses a strain-gauge around the limb (Whitney, 1953) which consists of a rubber tube filled with mercury. The strain gauge is fixed around the limb distally to the occlusion cuff and increases in the limb volume produce a lengthening of the tube causing a thinning of the mercury

column in the tube, resulting in an increase in the electrical resistance of the strain-gauge. Therefore, changes in the volume of the limb segment can easily be calculated from the electrical resistance. This method is regarded as highly accurate, but is only valid when capillary filtration is not significantly altered by the occlusion cuff (Pang, 1994). However, Schnizer et al. (1978) have shown that occlusion of venous outflow by a cuff consistently causes transcapillary fluid filtration. Thus, the plethysmography techniques, while non-invasive and relatively sensitive methods, involve several limitations that limit their use.

Another less commonly used variant of the plethysmographic technique utilizes the combination of blood pool scintigraphy with plethysmography, which measures volume changes after inflation of the occlusion cuff by determining changes in radioactivity over the limb. The technique involves the labelling of a person's own erythrocytes with $^{99m}$Tc-pertechnetate by either *in vivo* or *in vitro* means, followed by the measurement of radioactivity in a particular organ by a scintillation detector or gamma camera (Clements et al., 1981). The radioactivity detected per unit time is proportional to blood volume and is reflective of venous capacity, since a large proportion of the blood is contained in the capacitance vessels (Pang, 1994). This method offers another, less invasive method of measuring venous capacity which is highly reproducible and, unlike venous occlusion plethysmography, does not result in compression of cutaneous veins and possible capillary fluid release, since the scintillation detector does not come in contact with the body part being measured. However, there are several drawbacks of this technique, such as high operating costs, low accessibility, and the correction of background and attenuation of radioactivity are technically complex (Bell et al., 1990).

The above methodologies do not allow one to easily distinguish between the direct actions

of pharmacological or physiological stimuli on a given vein and reflex changes of venous tone secondary to effects occurring in other areas of the vascular bed. Therefore, several methodologies have been developed to look at the direct actions and interactions of pharmacological agents on venous tone in single superficial human veins *in vivo* independent of systemic reflex responses.

Burch and Murtadha (1956) first described a method to study single veins based on pressure measurements in an isolated section of a superficial human vein. This method involves isolating a segment of a superficial forearm vein from the general circulation by using externally applied wedges and a needle is inserted into this segment so that venous pressure can be recorded. After the application of physiological stimuli or the direct injection of drugs into the segment, pressure changes can then be recorded. This is one of the first techniques for studying direct drug effects on single human veins *in vivo* and is a useful technique for evaluating dose-response curves of constrictor agents such as noradrenaline and angiotensin II, and also the effects of various physiological stimuli such as a deep inspiration on venous tone. The problem with this technique, however, is that it is cumbersome and any movement of the hand interferes with the measurements.

Sicuteri et al. (1964) introduced the venoconstriction test which measures the pressure in a superficial dorsal vein of the hand or wrist before and after local infusion of a drug that produces complete constriction of the vein being investigated. This allows the volume of blood contained between the constriction and the nearest venous valves to be separated from the general circulation. This technique is similar to the single vein studies previously described by Burch and Murtadha. This method allows pressure in the segment to be measured and recorded and the venoconstrictor activity of the agent administered can be

quantified from the area under the pressure-time curve. However, the usefulness of this technique is limited as relatively high doses of the constrictor agonists must be used to produce complete venous constriction.

To overcome some of these difficulties, an attractive new method was developed by Nachev, Collier, and Robinson (1971) that relied on the measurement of changes of the diameter of the vein at a constant occlusion pressure, rather than the volume. This technique is based on the fact that, when venous pressure remains constant, changes in venous diameter directly reflect changes in venous tone. An important benefit of this technique is that it can be used to detect very small effects on venous tone and therefore very low concentrations of constrictor agonists can be used. Originally, a stereomicroscope was used to measure the diameter of superficial hand veins by marking a spot on the top of the vein with ink and determining the movement necessary to keep the dot in focus. This is a simplified approach which can be applied to any superficial limb vein and enables measurements to be obtained quickly, reproducibly, and with a minimum of discomfort to the patient. This technique is also important for studying the dilator effects of drugs by first preconstricting the vein with a constrictor agonist such as NE or 5-hydroxytryptamine. However, there are two important technical drawbacks to this optical method. Firstly, the diameter of the vein cannot be continuously recorded and secondly, even slight movements of the hand may interfere with the accuracy of the measurements, making it difficult to study ill patients who may have more difficulty remaining completely still.

Several other variations of this optical method have since been described. Aminu and Vere (1972) used a small capacitor placed on the dorsum of the hand that consisted of two plates, one placed on the skin directly over the vein of interest and the other on the skin beside the

vein. Then, movements of these plates caused by changes in venous diameter produced changes in the capacitance of the device, which was recorded. The major problem with this technique was difficulty in the fixing of the plates on the skin. White and Udwadia (1975) also modified this technique however it was extremely sensitive to even slight movements of the hand.

Aellig then developed a new technique based on Nachev's principle of the optical method to alleviate some of these problems which uses an electromechanical device called the linear variable differential transformer (LVDT) (Aellig, 1981). By mounting the device by a small tripod on the back of the hand, venous diameter at a given congestion pressure can be measured. This permits continuous recording of venous diameter, is less dependent on hand movements, and is not uncomfortable for the subjects being studied.

## 2.3 Linear Variable Differential Transformer (LVDT)

Specific details of the LVDT methodology are described fully elsewhere (Aellig, 1994a,b) and are consequently summarized for the purposes of this thesis. The LVDT method alleviates many of the problems associated with Nachev et al.'s (1971) earlier optical method, specifically because venous tone can be measured continuously and, the measuring device is placed on the dorsum of the hand, therefore small movements of the hand do not interfere with the measurements making this method ideal for restless patients who simply cannot remain motionless for long periods of time. As a result, studies can be of longer duration (often 4-5 hours) with little discomfort to the patient. Thus, clinical pharmacological studies utilize this method most often since it is useful even in severely ill patients. Another important advantage to this technique is that it allows the assessment of venous responsiveness to small

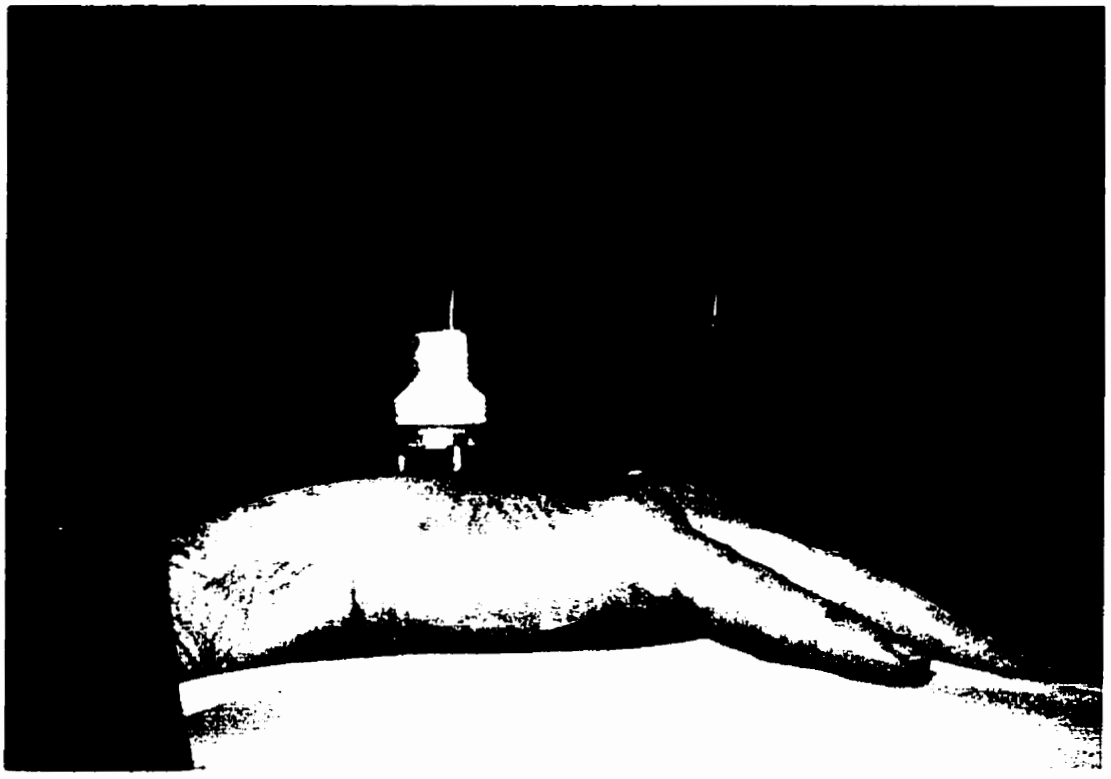

**Plate 2.1** Linear variable differential transformer in position over a superficial dorsal hand vein.

amounts of drugs in the absence of any systemic effects. It has been estimated that the dose required locally to produce a response in a single vein is about 1000 times less than that required systemically (Robinson, 1978). Therefore, much smaller doses of agonists can be administered which decreases the risk and discomfort for the patient, while still obtaining accurate results.

The LVDT is a small, lightweight, electromechanical device which can be placed directly over a superficial vein of the hand or foot with the use of a small tripod and can record continuous changes in vein diameter. The LVDT consists of a linear array of a primary coil and two secondary coils that are symmetrically spaced on a cylinder. The two secondary coils are connected in serial opposition such that the phase of the voltage of one of them differs by 180° from that of the other. A free-moving, rod-shaped magnetic core placed inside the coil assembly provides a path for the magnetic flux linking the coils. When the primary coil is energized by an external alternating current source, the voltages induced in both of the secondary coils are identical, but of opposite polarity so that the resultant voltage at the output of the transformer is zero, and it remains zero as long as this core remains in an exactly central position. However, when the core is displaced from its central position, the voltage induced in the secondary coils will become different, which will be either positive or negative, depending on the direction of the movement of the core, and the magnitude of this voltage reflects the distance the core has been moved from its central position. Therefore, the LVDT when exactly calibrated, is a displacement transducer and the resultant voltage changes are linearly proportional to the displacement of the core over the calibrated range of core movement.

One of the most useful aspects of this method is that the direct effects of drugs can be

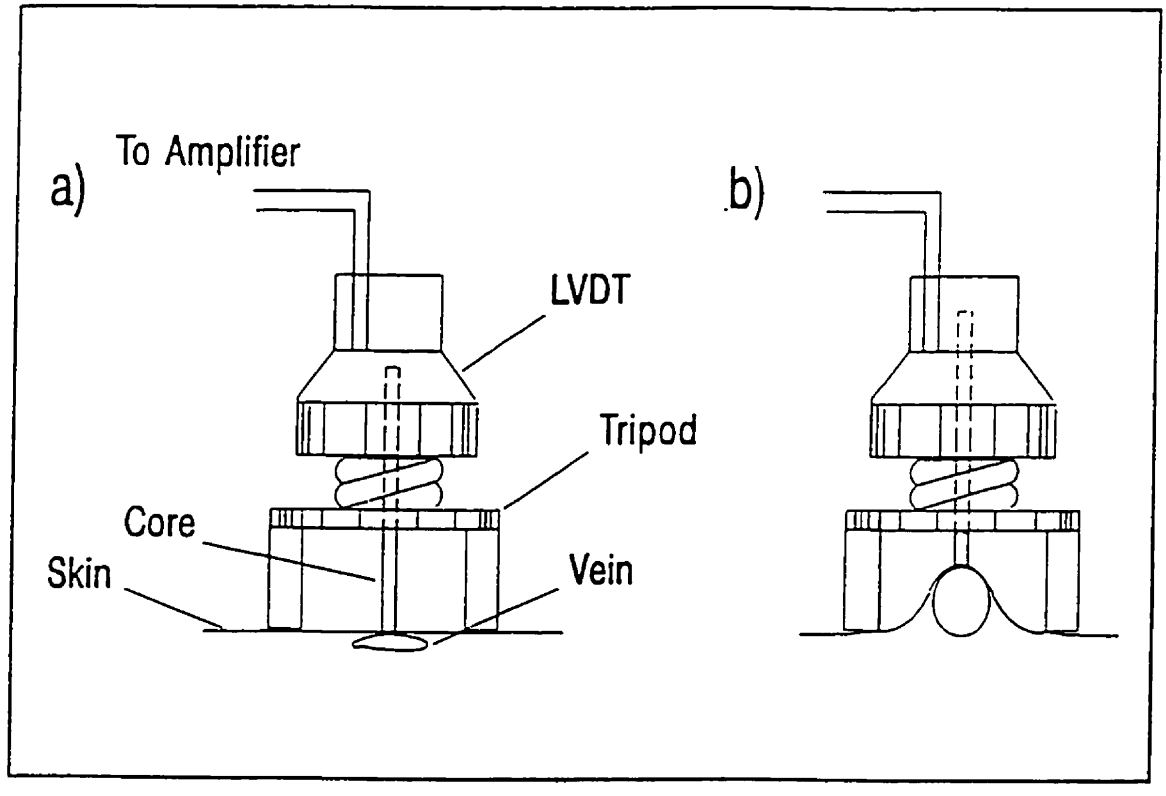


**Figure 2.1** Schematic diagram of the LVDT in position over the dorsal hand vein during: a) no applied distending pressure b) applied distending pressure of 45 mmHg.

investigated by local infusion into the vein to be studied. This is important because very low doses of these drugs can be infused into a small needle inserted into the superficial vein, thus avoiding effects on the systemic circulation and cardiovascular reflexes (Aellig, 1981; Blochl-Daum et al., 1991). Thus, the response of a specific receptor agonist represents the intrinsic receptor function in the dorsal hand vein. This may be useful for studying the effects of new drugs, where there is little experience in man. Also, because the LVDT method is able to continuously monitor changes in venous diameter, more complex studies on drug interactions are able to be observed such as studying the direct interactions between different agonists and antagonists, as well as investigation of the time course of drug effects, and correlating the pharmacological activity determined directly on human vessels *in vivo* with plasma levels (Aellig, 1994b).

Thus, the LVDT technique is very useful in studying the actions and interactions of various pharmacological and physiological stimuli *in vivo*, and is relatively non-invasive, easy to use, does not need extensive preparation, and is well tolerated by subjects. Therefore, this LVDT technique was thought to be the most suitable for the studies outlined below (Chapters 3,4,5,6,7).

### 2.3.1 Reliability and Reproducibility

Alradi and Carruthers (1985) addressed the question of the reliability and reproducibility of the LVDT technique by constructing dose-response curves to the non-selective adrenergic receptor agonist NE. Diurnal, day-to-day, intrasubject, and intersubject variability in dorsal hand vein responsiveness were investigated. NE dose-response curves were repeatedly constructed in healthy volunteers on different days and at different times of the day, and it was

found that intrasubject variability, even for data obtained on different days was low. In one subject, the doses of NE required to produce 50% constriction of hand vein diameter were 2.2 ± 0.3 ng/min in the morning, 2.3 ± 0.1 ng/min in the afternoon, and 2.3 ● 0.4 ng/min in the evening. Similar findings were found for the other subjects as well. Also, intrasubject variability was low for studies not only on the same vein, but also on different veins of either hand of the same subject. In one subject, the experiment was repeated 5 times in different veins on either hand and there was a consistent dose-response relationship, with the $ED_{50}$ being 110 ± 11.7 ng/min. Because day-to -day and diurnal variability was found to be minimal, studies of hand vein compliance can be performed either at different times throughout the day or over several days with background variability being minimal and therefore won't interfere with vein diameter measurements (Alradi and Carruthers, 1985). Aellig also found other vasoconstrictors to be equally reproducible, namely dihydroergotamine (Aellig, 1994a) and 5-hydroxytryptamine (Aellig, 1983). However, Alradi and Carruthers (1985) observed larger differences between the values obtained with NE in different subjects. The intersubject variability in the NE $ED_{50}$'s ranged from 1.85 ng/min to 160 ng/min. Again, similar variability has been observed between subjects in response to dihydroergotamine (Aellig, 1994a) and 5-hydroxytryptamine (Aellig, 1983). This wide intersubject variability in response to constrictor agonists was found to be unrelated to age, gender, or other physical characteristics such as resting heart rate, blood pressure, or race and may simply reflect physiological differences in smooth muscle contractility or endothelial function. It is important that the measurements are taken under standardized experimental conditions such as constant room temperature and a sufficiently long acclimatization to the room temperature. In addition, the inter- and intraindividual variabilities of the constrictor

agents results obtained on foot veins of the same subjects were of the same order of magnitude as those obtained on the hand veins.

Studies in pairs of monozygotic and dizygotic twins provide support for the intersubject variability seen. Luthra et al. (1991) performed a study using the LVDT technique to determine whether the venous responsiveness to locally infused NE differed between unrelated subjects and twins. They hypothesized that the variability in responsiveness to NE may be due to differences in NE disposition such as uptake, metabolism, and washout within the vein and that much of this pharmacokinetic variability might be explained by genetic influences. They found that in dizygotic twins, the variability within pairs of twins was almost as great as that observed between all twins or all unrelated subjects. However, in monozygotic twins, they exhibited almost identical responses within pairs, although variability between pairs was similar to the population at large. They also found that there was a great similarity in $ED_{50}$ (dose of NE required to produce a 50% reduction in basal vein diameter), $EC_{50}$ (plasma concentration of NE producing a 50% reduction in basal vein diameter) and clearance of NE within pairs of monozygotic twins but not within dizygotic twins. Therefore, they concluded that the majority of this variability depends on genetic differences in pharmacodynamic aspects of tissue responsiveness such as vascular adrenoceptor responsiveness, smooth muscle contractility, and endothelial function.

Therefore, the LVDT method provides a safe, minimally invasive, reliable, and reproducible means of assessing the effects of pharmacological and physiological stimuli on human dorsal hand veins *in vivo* in both health and disease states.

### 2.3.2 Limitations of the LVDT Technique

Venous tone is highly dependent on ambient temperature because superficial human veins are involved in the regulation of body temperature. Using the optical version, Nachev et al. (1971) found a positive correlation between local skin temperature and environmental temperature, as well as a positive correlation between local skin temperature and venous diameter at a congestion pressure of 45 mmHg. Environmental and skin temperature not only influence venous compliance but also have been shown to effect the venous responsiveness to constrictor agents and physiological stimuli. A study by Aellig showed that the local infusion of the partial 5-HT receptor agonist, pizotifen, produced a greater constriction of the vein when the study was carried out at a low (18°C) than at a higher (23°C) room temperature. A study by Bodelsson et al. (1990a) demonstrated an increased contractile response to 5-HT in segments of subcutaneous hand veins studied *in vitro* in organ baths when the temperature of the organ bath was lowered from 37°C to 25°C. They suggested that 5-HT receptors may be involved in cold-induced peripheral vasospasm. Also, a study by Arner and Hogestatt (1990) investigated the influence of temperature on the contractile response to NE in isolated human hand veins. They found that cooling the organ bath from 37°C to 24°C increased the NE potency by 8-fold. This was in agreement with the results of a study by Vanhoutte & Shepherd (1970) who found that the contractile responses in canine saphenous veins to both exogenously applied NE and sympathetic nerve stimulation were increased by cooling. Bodelsson et al. (1990b) then investigated the contribution of different receptor subtypes in the contractile response to NE during cooling in human hand vessels. He found that the enhanced constriction to NE was unaltered in the presence of prazosin but was abolished in the presence of yohimbine, suggesting that the increased responsiveness

upon cooling is mediated via the $\alpha_2$-adrenoceptor.

Previous studies clearly emphasize the influence that temperature has on venous responsiveness and demonstrates that the accuracy and reproducibility of the LVDT technique relies on a strict control of room and skin temperature. Therefore, it is preferable that the experiments be carried out in a temperature-controlled room (22-24°C) and subjects must be allowed sufficient time to acclimatize to the room temperature before starting the experiment (Aellig, 1994a). Studies in normal volunteers and CHF patients in our laboratory have shown that skin temperature does not change significantly over the duration of the hand vein tonometry experiments in either subject population and thus, alterations in skin temperature promoting increases or decreases in venoconstriction are not of concern to us and do not hinder the interpretation of the data under the conditions of our experiments (temperature regulated environment, suitable acclimatization period).

Emotional stimuli such as stress are potent causes of venoconstriction by reducing total systemic vascular resistance causing profound reflex venoconstriction in an attempt to maintain cardiac output during the drop in arterial pressure (Webb-Peploe et al., 1968). Thus, it is possible that the emotional state of the subjects participating in the following hand vein tonometry studies as well as the stress induced by the insertion of the needles or the hospital environment itself, may have influenced the results obtained. However, this is unlikely since all of the subjects were informed of the procedures in detail prior to giving consent and were allowed to acclimatize to their surroundings for 30 minutes prior to any measurements being taken. In addition, blood pressure and heart rate were monitored throughout all of the studies in all of the subjects and consequently any alterations of this nature would have been detected.

Also, the LVDT methodology is associated with a few technical difficulties which limited

its usefulness in a few individuals. In a few subjects, a suitable long, straight dorsal hand vein with no immediate tributaries could not be found as a result of anatomical variations in the venous network among individuals. This was more of a problem in the studies that involved the insertion of two needles into the same vein since more of the vein is required for needle insertion, leaving less vein length for the placement of the LVDT. Therefore, in a small number of individuals, the LVDT technique could not be used and therefore the study could not be carried out on those individuals. However, this problem was only experienced very rarely since the length of vein required for the needle insertion and LVDT placement is only 2-3 cm in total. Also, swelling (edema) was occasionally encountered in the hand which made the use of the LVDT technique not feasible, since accurate measurements of venous diameter cannot be made in this situation. This was typically of concern in patients with CHF due to increased Na+ and water retention which may be enhanced by the withholding of diuretic use on the study morning. However, in our studies involving CHF patients, increased peripheral edema in the hands was rarely encountered. Finally, the fact that the hand vein of interest may not be completely emptied upon deflation of the occlusion cuff even though it was inclined at 30° to the horizontal and above heart level, may influence the results obtained as this may cause differences in the measurements of absolute venous distention. However, previous research has shown that under these experimental conditions, basal venous diameter cannot be increased despite infusions of the potent venodilator nitroglycerine (Lui et al., 1996). Therefore, this limitation is of minimal concern under the experimental conditions used for the studies in this thesis.

Despite the limitations outlined above, the LVDT technique is still considered to be a reproducible and accurate method for assessing venous capacitance and venous tone relatively

non-invasively *in vivo*. It is also the simplest and most easily accessible method for measuring drug responses and allows the construction of fuller dose-response curves, in the presence or absence of antagonists, without having systemic or reflex effects. The LVDT technique is very useful not only for experiments in healthy volunteers, but also to study the interactions between various agonists and drugs given for chronic oral treatment of diseases such as hypertension or CHF.

### 2.3.3 Applicability to other vascular beds

One question frequently asked is whether veins in other areas of the body react similarly to the superficial human hand veins to the various drugs and other stimuli investigated. This question is especially important for veins in the feet because these veins are exposed to a markedly increased hydrostatic pressure when a person moves from the supine to the upright position. This was a very important question to investigate because all of the previous studies had been carried out on superficial hand veins because they are typically the most convenient for this type of study.

A study was carried out by Aellig (1990) to investigate whether veins from different areas of the body responded differently. To do so, a group of healthy male volunteers were studied using the LVDT technique and they received local infusions of NE both into hand and foot veins. Two dose-response curves were then generated to NE, one for the hand and one for the foot. The results showed that the two dose-response curves were parallel and the $ED_{50}$ doses were almost the same in both veins. Also, the slopes of the log dose-response curves for the constrictor effect of NE were virtually identical at both locations. A second study was then carried out in a group of subjects who received dihydroergotamine by systemic

intravenous administration. Again, in these subjects, the venoconstrictor effects were almost identical in the hand and foot veins in terms of both the dose required and the time course of the constrictor effect, with respect both to its onset and duration of action. Therefore, these results suggest that despite the large differences in hydrostatic pressure to which superficial hand and foot veins are exposed during orthostasis, they react quantitatively and qualitatively alike. It is important to note, however, that veins of a similar calibre were used in this study such that the diameters of the veins on the hands and feet of the subjects had to be between 0.5 and 2.0 mm at a congestion pressure of 45 mmHg. This lack of large differences in the diameter of the veins being studied may not be of importance in studies using oral drug administration since local drug concentrations in all superficial veins in the body are probably the same. However, it is of importance for experiments using local drug administration because large differences in the local drug concentrations would occur if the vein diameters were markedly different (Aellig, 1994a).

A study performed by Luthra et al. (1989) confirmed the comparable reaction of superficial hand and foot veins to constrictor agents, but more importantly, specifically addressed the potential differences in vein diameter if veins of different areas of the body are compared. To do so, they determined drug concentrations in the veins being investigated and found that $EC_{50}$ values showed a better correlation between the effects on hand and foot veins than $ED_{50}$ values. However, the determination of local vein concentrations of drugs is quite difficult to do, especially in patients who are very ill, and therefore, it is more reasonable to simply select veins of similar diameter, as suggested by Aellig (1990).

Therefore, according to these studies, hand veins appear to be representative of superficial veins in other areas of the body. Thus, results obtained on superficial hand veins can be

generalized to superficial veins of other areas of the body and this may be important for the investigation of pathophysiological states such as in patients with hyperadrenergic orthostatic hypotension. However, it is likely that the hand will still be used most often for studies using the LVDT technique since, technically, measurements can be made more easily on superficial hand veins than on other superficial veins.

Comparisons of superficial vein responsiveness to local concentrations of drugs with deep or splanchnic veins have not, to our knowledge, been investigated. Thus, further experiments are needed in order to determine whether superficial venous responsiveness is representative of these veins. This would give important information since the splanchnic veins are considered one of the most important venous beds in the body because it is highly innervated by sympathetic nerves, highly compliant, and holds approximately 20-25% of the total blood volume (Pang, 1994).

Because of differential receptor populations in various vascular beds, another question asked regarding the LVDT technique is whether the responses in the vein are representative of the overall responsiveness of the systemic vasculature? To address this question, Vincent et al. (1992) performed a study looking at the relationship between peripheral venous responsiveness using the dorsal hand vein technique and systemic vascular responsiveness by measuring blood pressure changes in response to phenylephrine in humans. They found that there was a good correlation between the doses of phenylephrine producing a blood pressure increase upon systemic administration and those doses required for local venoconstriction during direct infusion into superficial hand veins. Therefore, they concluded that responsiveness in the hand vein offers a satisfactory alternative to the use of hemodynamic changes with respect to $\alpha$-adrenoceptor responsiveness. Thus, they suggest that the dorsal

hand vein technique is a good model for investigating the systemic regulation of α-adrenergic receptors in humans. As a result of these findings, it is tempting to speculate that similar responsiveness would be seen in the splanchnic and deep veins since anatomically, these veins are much more similar to superficial veins than arteries.

Therefore, the LVDT technique is an easily accessible method of investigating changes in venous capacitance and venous tone to constrictor agonists *in vivo*, and the results obtained may be representative of the general vasculature.

### 2.3.4 General protocol for present studies

All studies were approved by the University of Western Ontario Committee for Health Sciences Research involving human subjects and all subjects gave written, informed consent (see Appendix). Participants withheld alcohol and caffeine-containing beverages for at least 12 hours prior to the experiments but were permitted to have a light breakfast on the morning of the study. Studies were carried out in a quiet, temperature controlled (23-24°C) Vascular Function Laboratory in the morning. Upon arrival, subjects were asked to empty their bladder to avoid reflex activation of the SNS and then remained in a supine position throughout the duration of the experiment. Several CHF patients had head and shoulders slightly elevated in order to avoid dyspnea. The hand was placed on a padded, inclined board angled at 30° to the horizontal with the shoulder comfortably supported to allow for rapid, enhanced emptying of the veins after cuff deflation. This elevation is consistent with all previous experiments using this technique (Aellig, 1994a) and minimizes resting venous tone as the vein is in a collapsed position when the cuff is deflated. A standard sphygmomanometer cuff was placed on the upper arm of the limb being studied and

connected to a manually activated Hokanson Rapid Cuff Inflator (Issaqua, Washington).

Subjects were covered with a blanket to avoid any cold-induced venoconstriction and the cuff was inflated to 45 mmHg to cause venous distention so that the veins become visible. Then, a dorsal hand vein suitable for measurements was selected (long, straight section with no immediate tributaries) and a small butterfly needle (E-Z Infusion Set, Becton Dickinson Vascular Access, Utah) was inserted into the vein in a proximal direction. A 0.9% NaCl solution was then infused into the vein using a Harvard Infusion Pump (Model 2400-003, Harvard Apparatus, Mass.) and the subject was allowed to acclimatize to the surroundings for 30 minutes.

During this equilibration period, the LVDT (Schaevitz, Type 025 MHR) was placed with the aid of a small tripod on the summit of the vein of interest such that the lightweight, movable central core was approximately 10 mm proximal to the tip of the butterfly needle. Care was taken to ensure that none of the tripod legs was situated over any other veins on the hand, and that the hand was properly positioned such that the LVDT was in a vertical position in order to allow unhindered movement of the core upon inflation of the proximally applied occlusion cuff to 45 mmHg. The fingers were then covered with a cloth and lightly taped down to avoid cold-induced venoconstriction and small finger movements.

The LVDT was connected to a Schaevitz modular LVDT (HVC-2) signal conditioner (LHSC - University Campus biomedical engineering department) which amplified the signal from the LVDT and the resultant voltage change was recorded (via a pen deflection) on a potentiometric recorder (LKB 2210 Bromma recorder, Sweden).

Several measurements of hand vein distention (HVD) were made by inflation of the occlusion cuff during 0.9% NaCl infusion to ensure a stable baseline. Measurements of HVD

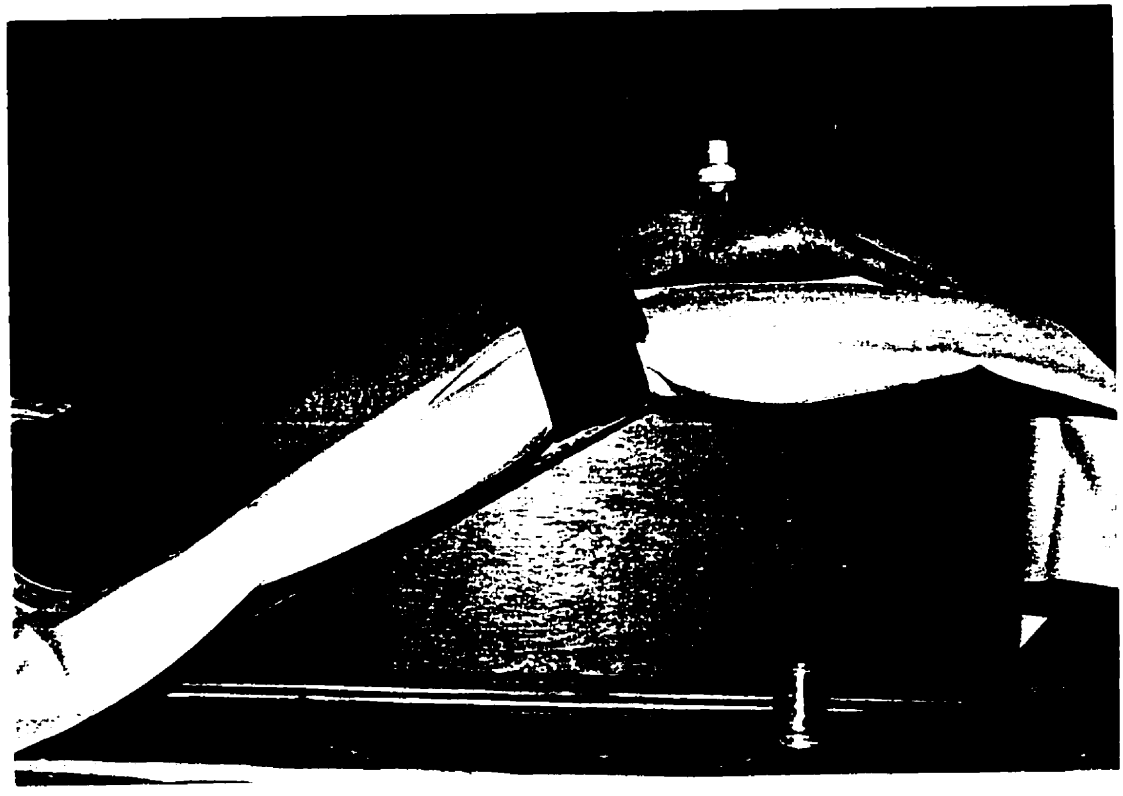

**Plate 2.2** Experimental setup showing elevation of hand to 30° above horizontal.

were taken from the point of cuff inflation to the plateau level of the peak and the average distention achieved during 0.9% saline infusion regarded as 100% dilation or maximum HVD (referred to as the control distention). After baseline measurements were taken, the saline syringe was replaced by a syringe containing freshly prepared drug and cumulative graded infusions were begun through the needle in the hand vein. Each dose of drug was infused for 5 minutes with the cuff inflated at the 3rd minute and deflated at the 5th minute of each 5 minute interval which allowed for measurement of any changes in venous distention as a result of the drug. Following deflation of the occlusion cuff, the next higher concentration of drug was infused and subsequent measurements were taken the same as previously described. Drug infusions continued until the full range of infusions was completed or a stable maximum venoconstriction was achieved. Upon completion of the infusions, the LVDT was removed from the hand and was calibrated using a micrometer manufactured by the Mitutoyo Company, Japan and computer assisted cumulative dose-response curves were constructed using a non-linear curve fitting analysis (GraphPad Inplot 4.0, Graphpad Software, San Diego, California). Absolute vein diameter was calculated by dividing the average control distention (mm) by the measured calibration value (mm). Subsequent measurements of HVD were then expressed as a percentage of the control HVD and the values were subtracted from 100 in order to convey the data in terms of percent venoconstriction.

Throughout the entire duration of each study, heart rate and blood pressure were continuously monitored non-invasively in the contralateral arm using a semi-automated BP recorder (Dinamap 846SX, Critikon, Tampa, Florida). All experiments incorporated safety limits such that the experiment would be terminated if the blood pressure rose above 160/90 mmHg or fell below 80/60 mmHg or if the heart rate rose above 120 beats/min or fell below

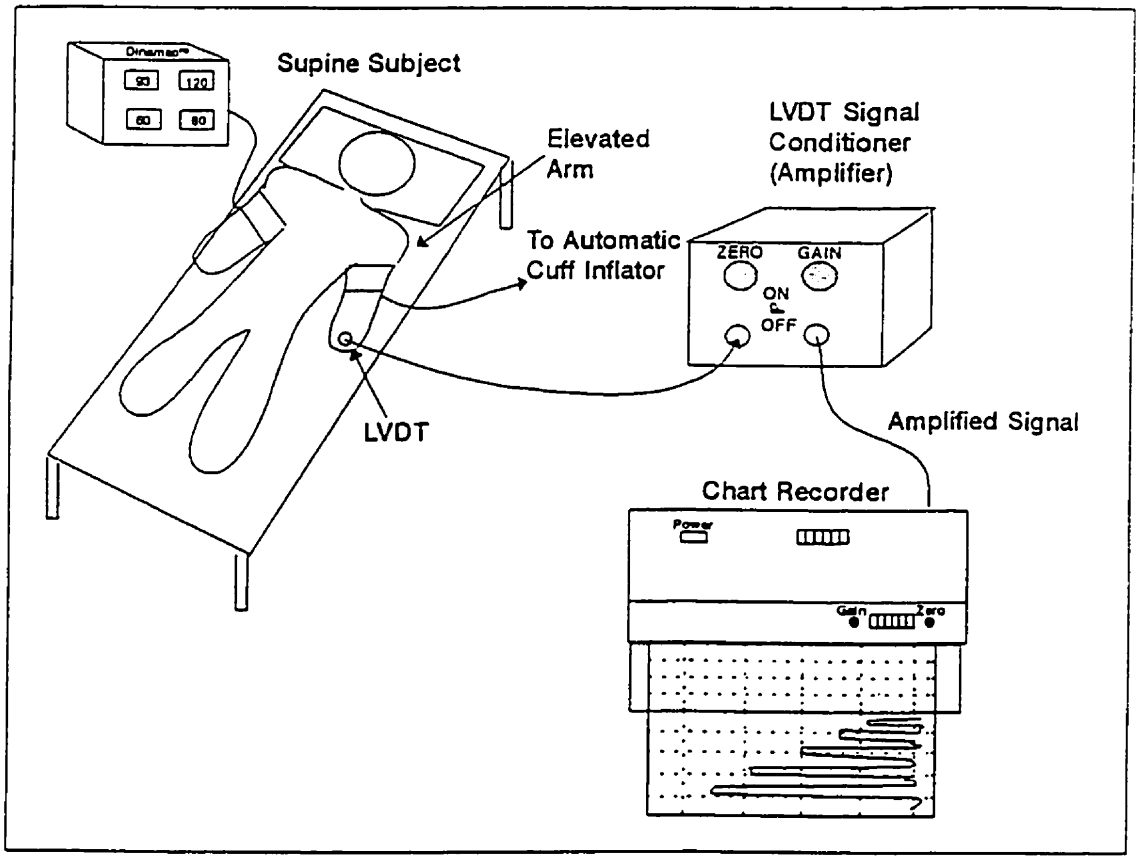


**Figure 2.2** Schematic diagram showing the equipment connections used during the LVDT measurements

40 beats/min. No significant alterations in blood pressure or heart rate were observed in any of the studies outlined below and none had to be terminated for safety reasons. Also, a small temperature probe (YSI 409B, VWR Scientific of Canada Ltd) was placed on the dorsum of the hand on each study day and was attached to a thermometer with digital readout display to monitor skin temperature.

### 2.3.5 Drugs

NPY (GMP, Peninsula Laboratories, CA) which was approved by FDA, USA for study in humans was initially diluted to 50 μg/ml with 0.5% albumin saline to help avoid loss of NPY in the filtering process. It was then passed through a low protein binding millipore filter (Millex, GV) for sterilization before further serial dilution in 0.9% NaCl in glass bottles on the study morning using sterile technique and infused into a dorsal hand vein through glass syringes. NPY sticks to plastic, therefore glass bottles and syringes were used to minimize the amount of NPY lost.

Indomethacin (Indocid PDA, i.v., Merck Frosst Canada Inc.; 3μg/min) was freshly prepared by serial dilution in 0.9% NaCl on each study morning using sterile technique. The dose of indomethacin selected was based on the effective concentration range (0.3 - 3.0 μg/ml) for indomethacin that is outlined in Goodman and Gilman (1987). This dose of indomethacin has previously been used in this laboratory and has been shown to effectively block the release of vasodilatory prostaglandins. Preparation of indomethacin in 0.9% NaCl resulted in a stable solution.

$PGF_{2\alpha}$ (Dinoprost, Upjohn, Canada, i.v.; 1-2048 ng/min), acetylcholine (Miochol, CIBA Vision, Canada, i.v.; 0.01-1.0 nmol/min) and sodium nitroprusside (Nipride, Hoffman-La

Roche, Canada, i.v.; 0.3-10 nmol/min) were freshly prepared by serial dilution in 0.9% NaCl on the study morning using sterile technique.

### 2.3.6 Measurement of Plasma Norepinephrine

Thirty minutes after insertion of a 20 gauge Jelco winged catheter (Critikon, Inc., Tampa, Florida) into the opposite arm to that of LVDT measurements and before drug infusions, 8 mL of blood were drawn into a cooled syringe and transferred to an ice cold centrifuge tube containing heparin, sodium metabisulfite (0.2 M), and EDTA (0.2 M). The blood sample was centrifuged (3000 g x 10 min at 4°C) for separation of plasma. The catecholamines were extracted according to the method of Anton and Sayre (1962) and assessed by reverse phase high performance liquid chromatography with electrochemical detection (Hjelmdahl et al., 1979). The detection limit of norepinephrine was 25 pg/mL. The intra- and inter-assay coefficients of variation were 3% and 8% respectively. Special thanks to the Endocrinology Laboratory at London Health Sciences Centre - Victoria Campus for analyzing the plasma norepinephrine levels.

### 2.3.7 Measurement of Plasma NPY

Under the same conditions as previously mentioned, 7 ml of venous blood were drawn into a centrifuge tube containing EDTA. The blood sample was centrifuged (3000 g x 10 min at 4°C) and the plasma was then isolated. Plasma concentrations of NPY-like immunoreactivity were measured by radioimmunoassay according to the method of Theodorsson-Norheim et al. (1985). The sensitivity of the method was around 10 pmol/L in a 0.5 ml plasma sample. Inter- and intra-assay coefficients of variation were 7% and 5%, respectively. Special thanks

to Dr. Lars Edvinsson at the University Hospital of Lund, Sweden for analyzing the plasma NPY levels.

### 2.3.8 Data Analysis and Statistics

Dose-response curves (semi-logarithmic) were constructed for NPY, $PGF_{2\alpha}$, acetylcholine, and sodium nitroprusside using a non-linear curve fitting programme (GraphPad Inplot 4.0 software package, H.J. Motulsky, San Diego, CA). Since venoconstriction to NPY in most cases did not reach 50%, maximum venoconstriction occurring at the highest dose of NPY was assessed and calculated as a percent of the control distention. The subjects were ranked according to age from youngest to oldest and divided into tertiles. The maximum venoconstriction for each tertile was expressed as mean ± standard error. Linear regression analysis was performed on the data. One-way analysis of variance (ANOVA) followed by post-hoc analysis using Tukey's procedure on significant main effects was used to compare the differences in plasma NE and NPY levels, basal hand vein diameter, MAP, and heart rates between the groups of subjects. Two-way ANOVA (BMDP,5V) was used to compare the differences in dose-response curves to NPY among the tertiles and NPY and $PGF_{2\alpha}$ among the groups of normals and CHF patients. Repeated measures ANOVA was used to compare the dose-response curves to NPY and $PGF_{2\alpha}$ obtained in the presence of saline placebo with the dose-response curves to NPY and $PGF_{2\alpha}$ in the presence of indomethacin. A two-tailed p value less than 0.05 was considered to be of statistical significance.

# CHAPTER 3

# NEUROPEPTIDE Y RESPONSIVENESS IN THE DORSAL HAND VEINS OF NORMAL SUBJECTS

## 3.1 Introduction

### 3.1.1 Background Information

The sympathetic nervous system is important in the regulation of cardiovascular function. Recently, a regulatory peptide, neuropeptide Y (NPY) was found to be co-released with norepinephrine (NE) from sympathetic nerve endings during times of increased sympathetic nerve activity (Ekblad et al., 1984; Lundberg et al., 1986). Although NE is considered a major sympathetic neurotransmitter and a primary mediator of cardiovascular functions, the role of NPY is not yet well defined.

Upon sympathetic nerve stimulation, NPY is coreleased with NE where it acts at postjunctional Y1 receptors to produce potent vasoconstriction by a mechanism dependent on extracellular calcium in both *in vitro* and *in vivo* experiments and prejunctional Y2 receptors to inhibit the presynaptic release of both NE and NPY (Dahlof et al., 1985a; Pernow and Lundberg, 1989). In humans, plasma concentrations of NPY increase after reflex sympathetic stimulation induced by physical exercise (Morris et al., 1986; Winther et al., 1992), cold pressor testing (Morris et al., 1986), and lower body negative pressure (Kahan et al., 1992). Considering the abundance and potent actions of NPY in the cardiovascular system, and a high degree of conservation of the peptide structure in several mammalian species (Larhammar et al., 1987), NPY appears to be an important cardiovascular regulatory factor.

### 3.1.2 Rationale

NPY constricts arteries and veins by a direct, receptor-mediated action on vascular smooth muscle. However, not all blood vessels are responsive to NPY. In many large blood vessels, such as the human mesenteric (Lundberg et al., 1985b), rabbit pulmonary artery (Wahlestedt et al., 1985), and rat aorta (Mabe et al., 1987), NPY is without a constrictive effect. However, small human (Clarke et al., 1987) and canine (Komaru et al., 1990) coronary arteries are extremely sensitive to NPY-induced direct vasoconstriction more so than large arteries. Large veins, unlike large arteries are quite sensitive to the vasoconstrictive effects of NPY. For example, the guinea-pig iliac vein (Wahlstedt et al., 1986b) and vena cava (Grundemar, 1991) are among the most sensitive vascular preparations. It appears that the order of sensitivity of blood vessels to the vasoconstrictor effects of NPY parallels their dependency on extracellular calcium for the contractile response (Zukowska-Grojec & Wahlstedt, 1993). Specific, high affinity NPY-binding sites were found in membrane preparations of blood vessels (Chang & Lotti, 1988) and on cultured vascular smooth muscle cells (Zukowska-Grojec et al., 1992). It has been demonstrated that the direct perivascular application of NPY around arteries, arterioles, and veins *in situ* produces a strong concentration-dependent constriction (Edvinsson et al., 1984). Clarke et al. (1991) examined the effects of intra-arterial administration of NPY on the human forearm vascular bed *in vivo* and found that NPY caused a large dose-dependent reduction in blood flow very similar to that demonstrated by Pernow et al. (1988). Thus, NPY has been shown to have direct constrictor effects in many human vessels, however, to date the vascular effects of NPY in human veins *in vivo* have not been

thoroughly investigated. Therefore, we chose the LVDT methodology to investigate the vasoconstrictor effects of NPY in human dorsal hand veins *in vivo*. Previously in our lab, the α-adrenergic mediated responses were studied using the same technique and therefore it was of interest to us to investigate the effects of NPY since it is known to be costored and coreleased with NE.

### 3.1.3 Hypothesis

NPY receptors are present in the dorsal hand veins of normal subjects and produce dose-dependent venoconstriction.

## 3.2 Methods

*Subjects*

Twenty-four normal, healthy subjects (10 males, 14 females), aged 20-72 years participated in the study. All subjects were normotensive, non-smokers, not taking any vasoactive medications, and in good general health as assessed by medical history, physical examination, and ECG. The subjects avoided consuming alcohol and caffeine containing beverages for at least 12 hours prior to the study but were permitted a light breakfast on the morning of the study.

*Study Design*

Hand vein tonometry was employed using the LVDT technique as outlined in Chapter 2, to measure the local venous responsiveness to NPY in the absence of systemic or reflex responses.

On the study morning, a short (1.9 cm) 25-gauge butterfly needle (Butterfly-Abbocath)

was inserted into a suitable dorsal hand vein with a long straight section and no visible tributaries. Normal saline (0.9%) was infused through the needle at 0.4 ml/min for 30 minutes. During this equilibration period, several measurements of hand vein distention at 45 mmHg were made to ensure a stable baseline and the average was taken as the control distention. Thirty minutes after insertion of an i.v. needle and before any drug infusions, a blood sample (15 ml) was drawn for the measurements of plasma catecholamines and NPY. Following this, sequential graded local 5 minute i.v. infusions of human NPY (25, 50, 100, 200, 500, 1000, 2000 pmol/min) were commenced to constrict the dorsal hand vein and cumulative dose-response curves were constructed with an upper arm occlusion cuff inflated for the last 2 minutes of each dose step.

## 3.3 Results

Figure 3.1 demonstrates a typical tracing of dorsal hand vein distention during intravenous administration of NPY in a young normal subject. Resting arterial pressure and heart rate were not significantly altered over the duration of the study (Figure 3.2). Skin temperature remained constant during each study ($31.3 \pm 0.3°C$) and did not vary between subjects (Figure 3.3).

Graded local infusions of NPY induced dose-dependent venoconstriction in all subjects studied except for one female, with a mean maximal venoconstriction of $45.3 \pm 5.3\%$ (Figure 3.4), however it appeared that the younger subjects in this group of normals achieved a greater maximum venoconstriction to NPY than the older subjects. This led us to believe that perhaps there is an age-related responsiveness to NPY in dorsal hand

veins.

We ranked the subjects in order from youngest to oldest and divided them into tertiles. Tertile 1 consisted of subjects 20-40 years of age, tertile 2 included 41-55 years of age, and tertile 3 included 57-72 years of age. When the mean dose-response curves were plotted for the 3 tertiles, tertile 3 was significantly decreased from tertiles 1 and 2 ($p<0.05$) (Figure 3.5). The maximum venoconstriction to NPY expressed as the mean $\pm$ sem was 65.1 $\pm$ 7.0, 46.5 $\pm$ 9.4, and 24.4 $\pm$ 4.8 for tertiles 1, 2, and 3 respectively (Figure 3.6). The maximum venoconstriction in tertile 3 was significantly ($p<0.01$) decreased from tertile 1. This demonstrates that as age increases, the venous responsiveness to NPY decreases. To examine the age-related hypothesis more closely, the maximum venoconstriction to NPY was plotted vs. age in Figure 3.7. This resulted in the maximum venoconstriction to NPY being significantly and negatively correlated with the age of the normal subjects ($r=-0.63$, $p<0.01$).

Plasma norepinephrine levels were elevated in older subjects (tertile 3) when compared to younger subjects (tertile 1) ($p<0.05$) but plasma NPY levels were not significantly different between the groups, however there was a trend of increased levels with increasing age (Table 3.1). Basal vein diameter at 45 mmHg venous occlusion pressure during 0.9% saline infusion was not significantly different between the tertiles (Table 3.2).

## 3.4 Discussion

In the present study, we have demonstrated that NPY produces dose-dependent vasoconstriction in human dorsal hand veins *in vivo*. A recent paper by Peduzzi et al.

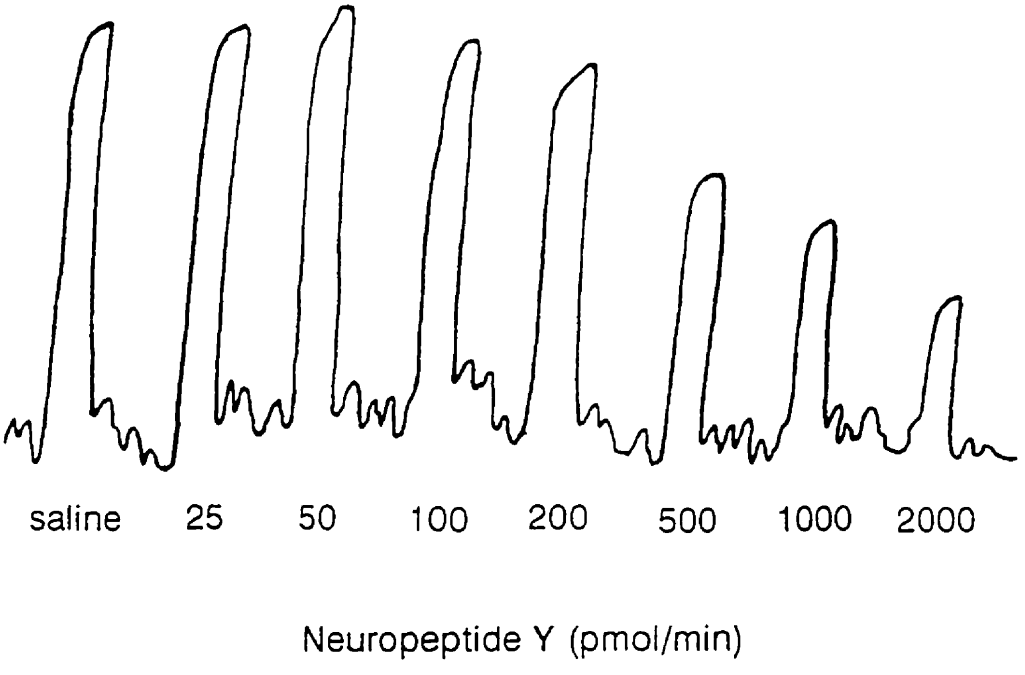


**Figure 3.1** Sample original tracing of dorsal hand vein distention during intravenous administration of NPY in a 20 year old normal subject.

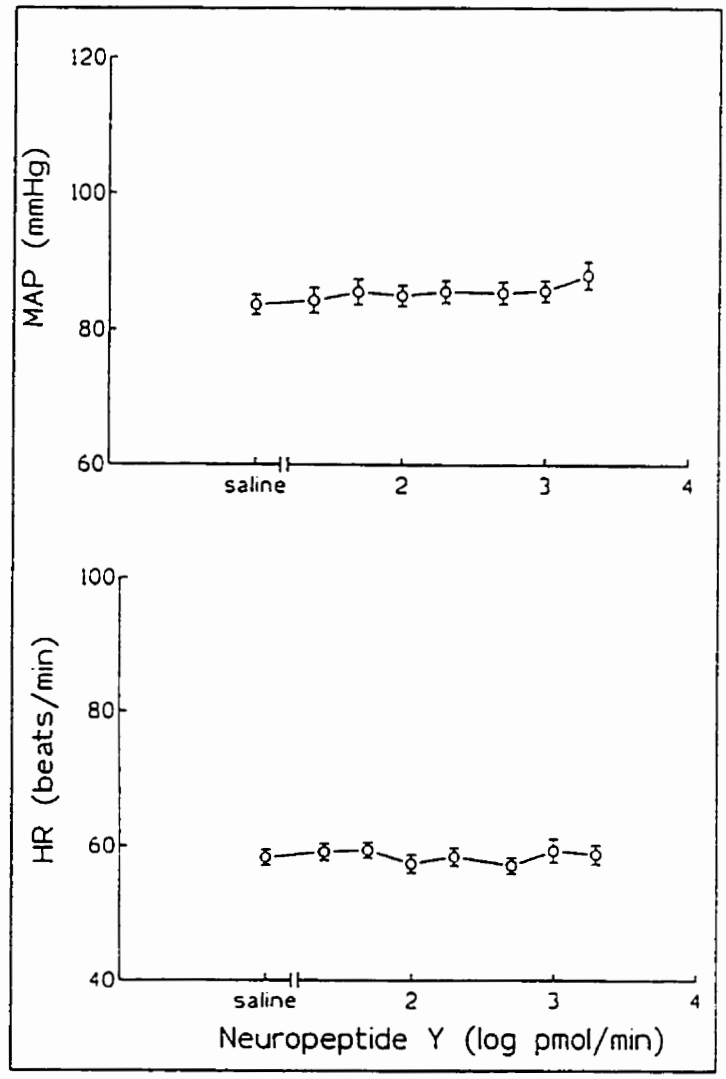


**Figure 3.2** Average changes in mean arterial pressure and heart rate during infusion of NPY over the course of hand vein tonometry experiments in normal subjects

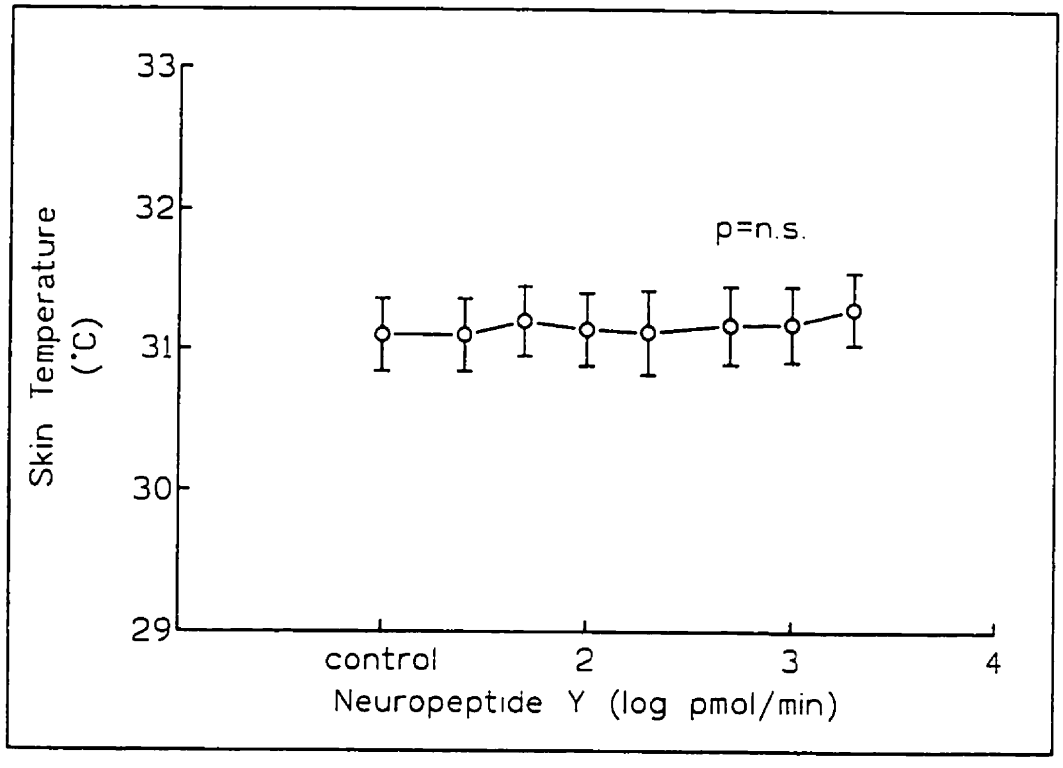


**Figure 3.3** Average changes in hand skin temperature during infusion of NPY over the course of hand vein tonometry experiments in normal subjects.

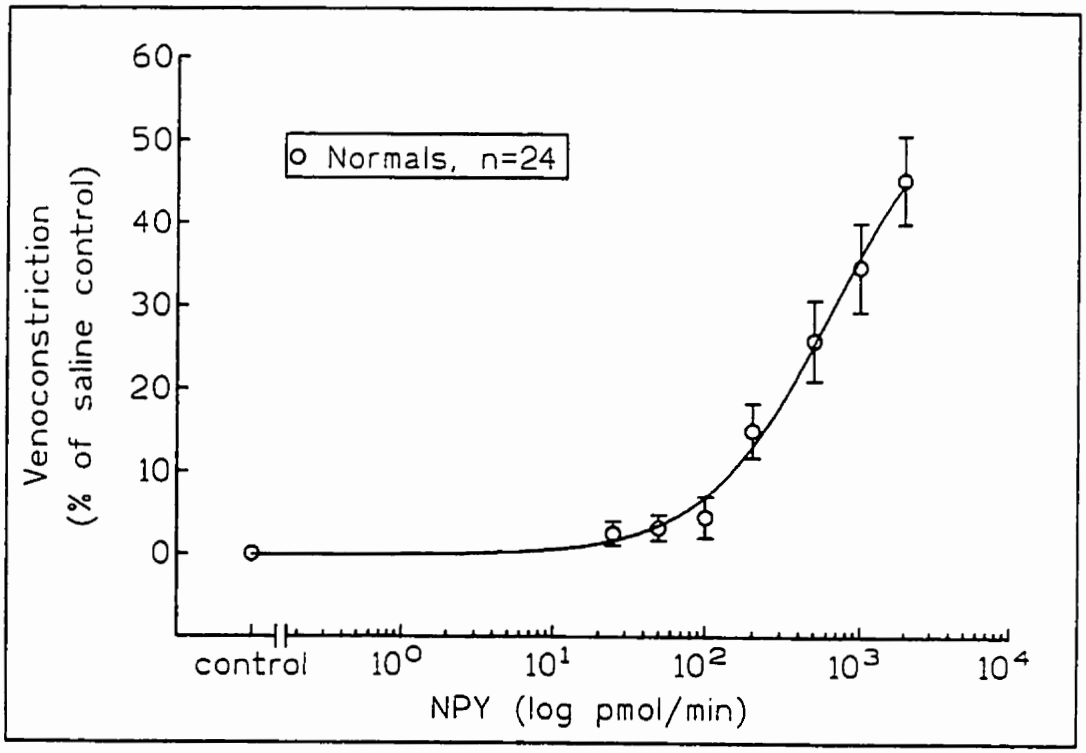


**Figure 3.4** Average response to NPY in the dorsal hand veins of normal subjects.

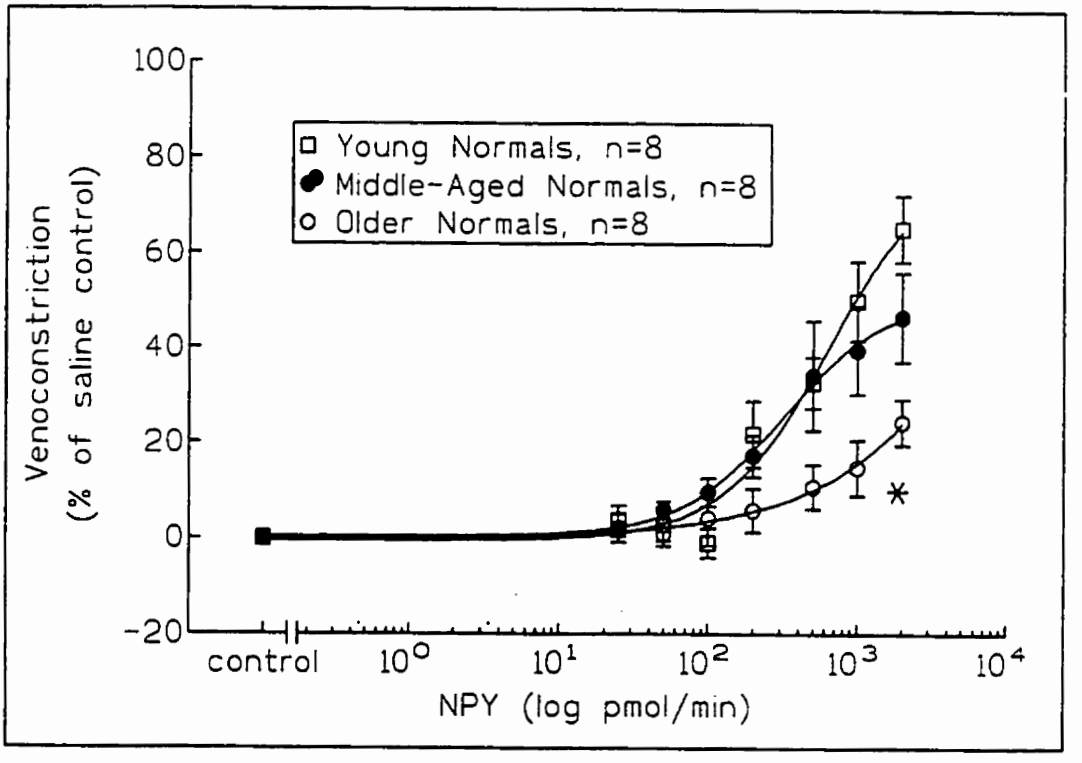


**Figure 3.5** Average response to NPY in the dorsal hand veins of young, middle, and older normal subjects.
*$p<0.05$ older vs. young and middle-aged subjects.

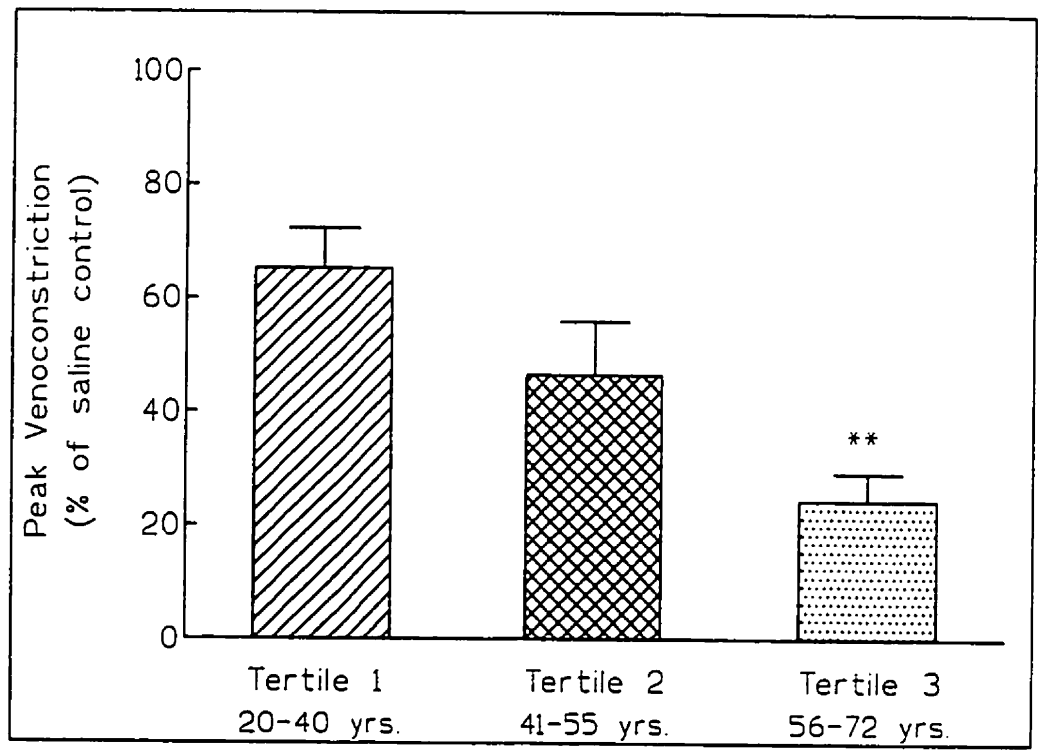


**Figure 3.6** Histogram showing the average maximal venoconstriction obtained in response to NPY in tertile 1 (20-40 yrs.), tertile 2 (41-55 yrs.), and tertile 3 (57-72 yrs.) in normal subjects. **$p<0.01$ vs. tertile 1.

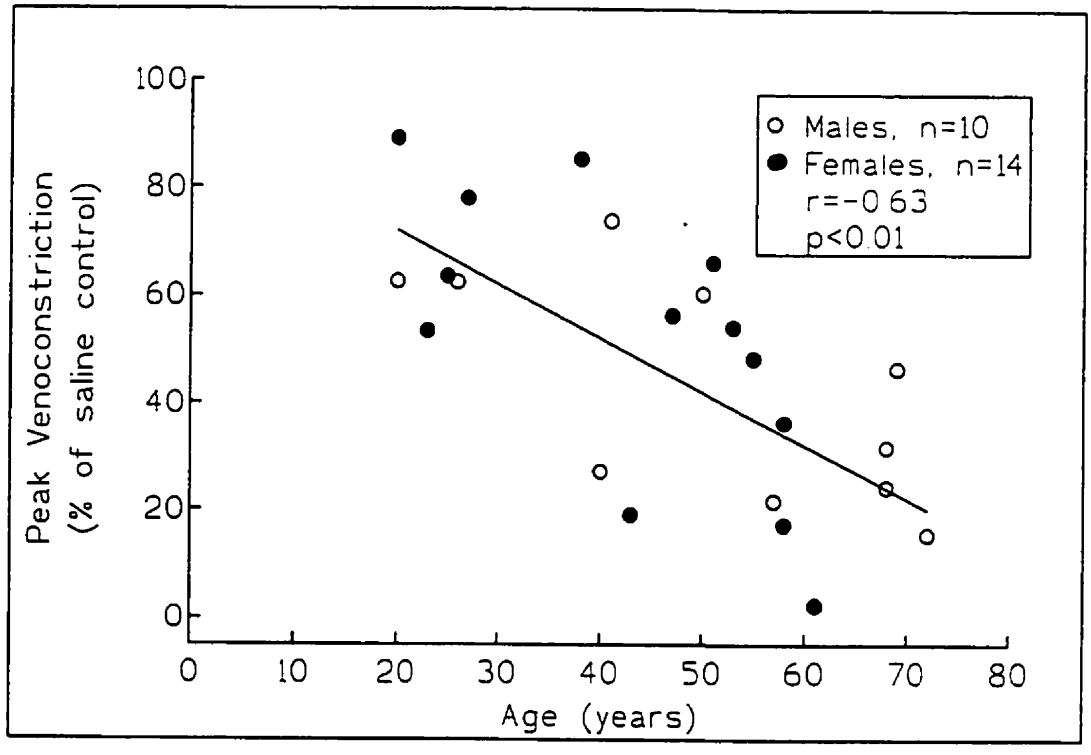


**Figure 3.7** Correlation between maximum venoconstriction to NPY and age in normal subjects.

**Table 3.1 Basal plasma norepinephrine and NPY levels, arterial pressure and heart rate**

| Groups | Mean Arterial Pressure (mmHg) | Heart Rate (bpm) | Plasma Norepinephrine (pmol/L) | Plasma NPY (pmol/L) |
|---|---|---|---|---|
| Tertile 1 (n=8) | 78.7±0.2 | 57.7±0.6 | 745±112 | 118±5 |
| Tertile 2 (n=8) | 89.4±0.6** | 60.9±0.3 | 1153±192 | 125±6 |
| Tertile 3 (n=8) | 88.5±0.8** | 56.8±0.3 | 1515±337* | 130±6 |

Results given as mean ± sem, *p<0.05, **p<0.01 vs. Tertile 1.

**Table 3.2 Basal vein diameter and maximum vein constriction to NPY**

| Groups | Basal Vein Diameter (mm) | NPY Maximum Constriction (% Control) |
|---|---|---|
| Tertile 1 (n=8) | 0.88±0.08 | 65.1±7.0 |
| Tertile 2 (n=8) | 1.02±0.18 | 46.5±9.4 |
| Tertile 3 (n=8) | 1.18±0.19 | 24.4±4.8** |

Results given as mean ± sem, **p<0.01 vs. Tertile 1.

(1995) also demonstrated the vasoconstrictor effect of NPY in human dorsal hand veins *in vivo*. They studied young, healthy subjects using doses of NPY similar to those that we used and found that the infusion of endogenous NPY caused potent vasoconstriction, verifying what we found. In one subject in our study, NPY had no effect on hand vein compliance. In the study by Peduzzi et al. (1995), they also found that a few of the subjects studied demonstrated no response to infused NPY and concluded that interindividual variability in human NPY responses is large. The lowest 2 doses of NPY infused in this study result in plasma concentrations of NPY that are similar to what is found in the plasma of people after physical exercise or in CHF patients with increased SNS activation. Therefore, these lowest doses of NPY infused are at the physiological level (Peduzzi et al., 1995) The finding that NPY produces dose-dependent venoconstriction in dorsal hand veins is an important one because the cutaneous venous system contains a large fraction of the total intravascular blood volume, and thus small changes in the compliance and tone of these veins caused by NPY may greatly alter the blood distribution and volume load of the heart (Peduzzi et al., 1995). In the study by Peduzzi et al. (1995), they did not study older normal subjects. We found that the venous responsiveness to NPY in older normal subjects was decreased significantly from the young subjects and concluded that ageing affects the responsiveness of dorsal hand veins to NPY in normal subjects.

Major structural changes in the heart and peripheral vasculature play a dominant role in the development of cardiovascular disorders such as myocardial infarction, CHF, and hypertension which have an increased incidence in the elderly. However, functional rather

than structural alterations that occur with ageing are also of importance but are less well studied and understood. Studies have shown that vascular reactivity to vasoconstrictor and/or vasodilator agents can increase, decrease, or show no change during ageing. There have been many studies demonstrating a specific decrease in ß-adrenoceptor-mediated vascular relaxation. It has been shown that chronotropic cardiac responsiveness and vascular smooth muscle relaxation of both arteries and veins to the ß-adrenoceptor agonist isoprenaline are reduced with age in man (Pan et al., 1986; Van Brummelen et al., 1981; Vestal et al., 1979). The effect of ageing on α-adrenoceptor function has been less well studied and the studies that have been done show conflicting results (Klein et al., 1990). Also, many of the studies of α-adrenoceptor-mediated function have failed to distinguish between the $\alpha_1$ and $\alpha_2$ subtypes. Hyland & Docherty (1985) demonstrated *in vitro*, in the human saphenous vein an alteration with age in the responsiveness of postjunctional $\alpha_2$, but not of prejunctional $\alpha_2$ or postjunctional $\alpha_1$ adrenoceptors. In the present study, we have demonstrated *in vivo* for the first time that venous vascular NPY receptor function in humans is decreased with increasing age, consistent with previous findings demonstrating that ß- and $\alpha_2$-adrenoceptor mediated responses are decreased with increasing age.

NPY is known to cause potent vasoconstriction by activating an intracellular signalling cascade similar to $\alpha_2$ receptors. NPY acts at the postsynaptic Y1 receptor where it activates a G protein coupled mechanism. Specifically, NPY receptors are linked via an inhibitory G protein ($G_i$) to adenylyl cyclase which, upon receptor activation, results in a decrease in adenylyl cyclase activity and a consequent decrease in the production of the

second messenger cAMP (Aakerlund et al., 1990; Herzog et al., 1992). Decreased cAMP production results in smooth muscle contraction by decreasing activation of protein kinase A which, when active, phosphorylates myosin light chain kinase and decreases its affinity for calmodulin (Bouvier et al., 1987; Limbird, 1988). Stimulation of the $\alpha_2$ receptor acts via the same intracellular pathway by activating the $G_i$ protein to decrease the activation of adenylyl cyclase and the production of cAMP. Stimulation of the ß receptors activates the $G_s$ protein to stimulate the activation of adenylyl cyclase and the production of cAMP. Therefore, the $\alpha_2$, ß, and NPY receptors all function through the adenylyl cyclase/cAMP pathway and their functions have all been shown to be decreased with increasing age. Thus, it appears that the decreased responsiveness may be the result of improper coupling of the receptor/G protein complex to adenylyl cyclase or a decreased functioning of the adenylyl cyclase. One study involving healthy subjects aged 19 to 79 found that vascular relaxation induced by the ß-adrenoceptor agonist, isoproterenol, in human hand veins was markedly reduced with increasing age (Pan et al., 1986). However, no differences in responsiveness were found in these subjects to local infusions of the $\alpha_1$-adrenoceptor agonist, phenylephrine or the smooth muscle relaxant, nitroglycerine. Therefore, Pan et al. (1986) concluded that ageing is associated with a specific decrease in ß-adrenoceptor-mediated vascular relaxation. The mechanisms underlying the age-related decline in ß-adrenoceptor responsiveness remain unclear. Studies using human lymphocytes in man suggest a decline in affinity of the ß-adrenoceptor agonists with a reduced isoprenaline-stimulated adenylyl cyclase activity, but no change in receptor numbers (Abrass & Scarpace, 1981; Feldman et al., 1984).

A study by Bedarida et al. (1995) investigated histamine receptor-mediated vasodilation in human hand veins with ageing and found that the $H_2$-receptor-mediated pathway, which is dependent on cAMP appears to be blunted with ageing, whereas the $H_1$-receptor-mediated pathway, which is dependent on the endothelium appears to be conserved. This is in agreement with a possible impairment of the adenylyl cyclase/cAMP pathway in ageing. This may also be the case for NPY receptor function. However, responses to other cAMP-dependent venodilators, namely prostaglandin $E_1$ and adenosine have been shown to be preserved in human veins *in vivo* (Hiremath et al., 1989; Ford et al., 1992). Therefore, this suggests that the age-related decline in vascular response is highly specific to certain agonists and does not reflect a generalized age-associated loss in responsiveness to adenylyl cyclase coupled receptors. However, this study does show that an additional cAMP-dependent pathway is blunted with ageing in addition to histamine, ß- and $\alpha_2$-adrenoceptor agonists.

In these subjects, the plasma norepinephrine levels were significantly increased in the older group of subjects compared to the young group (tertile 3 vs. tertile 1). This is consistent with previous findings which demonstrated that plasma norepinephrine concentrations are increased with advancing age in resting healthy subjects (Christensen, 1986). In contrast to this, the plasma NPY levels in these subjects were not significantly different between the older and younger groups. This is consistent with a study by Hetland et al. (1991) who found that under resting conditions, plasma NPY concentration is unchanged with advancing age. Thus, the decreased NPY responsiveness does not appear to be a result of increased NPY receptor stimulation.

We have demonstrated that the venous responsiveness to exogenously infused NPY is decreased with increasing age in man. Our studies do not address the molecular mechanism for the loss in response to NPY. However, studies in animals have demonstrated that cAMP production is blunted in vessels from older animals (Cohen & Berkowitz, 1974) and thus it is possible that ageing affects the NPY receptor-adenylyl cyclase coupling pathway in some way.

This decreased responsiveness may play a beneficial role by counterbalancing the increased sympathetic nerve activity observed in many of the cardiovascular disorders associated with increasing age. The evaluation of the importance of NPY in such physiologic and pathologic conditions will require the development of specific inhibitors of human NPY. There has very recently been the development of a specific NPY-Y1 receptor antagonist available for human use developed by Peninsula Laboratories (January, 1997).

This is an important finding for future studies in which we will compare NPY responsiveness in CHF patients to normal controls. Because we know that as age increases, NPY responsiveness in the dorsal hand vein decreases, and most CHF patients are older, it will be very important to only study age-matched subjects in order to accurately compare the 2 groups.

A limitation of using NPY in this study is that we were unable to achieve full dose response curves. We infused the highest concentration of NPY that we had available to infuse, however it was not enough to achieve complete dose response curves. It is possible that we are losing some of the NPY in the syringes due to it sticking to the sides, and

therefore we would not be actually infusing the amount that we report that we are. Thus, the doses that we infused are estimated doses, rather than actual doses. However, we used glass bottles and syringes to help overcome that problem since NPY sticks much less to glass than it does to plastic. Infusing each dose of NPY appears to be long enough because in several subjects, we infused saline for several minutes after the highest dose of NPY was infused, and a greater amount of constriction was not achieved. This demonstrates that the 5 minute interval was a sufficient period of time to achieve the constriction caused by each dose of NPY.

## Summary

We have demonstrated that NPY produces dose-dependent vasoconstriction in human dorsal hand veins *in vivo*. In addition, we have shown that the NPY responsiveness is decreased with increasing age. This decreased responsiveness may be beneficial in counterbalancing the increased sympathetic nerve activity observed in many of the cardiovascular disorders associated with increasing age.

# CHAPTER 4

# NEUROPEPTIDE Y RESPONSIVENESS IN THE DORSAL HAND VEINS OF CONGESTIVE HEART FAILURE PATIENTS

## 4.1 Introduction

### 4.1.1 Background Information

Heart failure is the state when, for whatever cause, the heart fails to perform its function of supplying an adequate amount of oxygenated blood to the tissues and organs. Cardiac contractility is depressed in most patients with heart failure, which may be the result of an absolute loss of myocardial fibres secondary to necrosis, reduced myocardial blood flow and ischemic dysfunction, or it may be a result of myocardial hypertrophy, as a result of a pressure or volume overload with decreased systolic contractile function and diastolic compliance (Vatner et al. 1990). Ventricular dysfunction activates neuroendocrine "compensatory" mechanisms to maintain perfusion pressure to vital organs, which later become detrimental and contribute to the progression of the syndrome. Increased SNS activation, as shown by increased plasma levels of norepinephrine resulting in increased preload and afterload, have been directly correlated with disease severity and mortality in heart failure because they result in a vicious circle of events which may further depress heart function (Abboud et al. 1979; Francis et al. 1984; Hasking et al. 1986; Leimbach et al. 1986). NPY is a sympathetic cotransmitter which is costored and coreleased with NE, and mediates vasoconstriction independently of catecholamines. Several studies have reported increased plasma NPY levels in patients with ischemic heart disease (Maisel et al. 1989; Franco-Cereceda et al. 1990), especially those with advanced

congestive heart failure (Hulting et al. 1990). Thus, NPY may be an additional neuroendocrine factor that has increased release in heart failure, further adding to the detrimental series of events.

### 4.1.2 Rationale

Altered NPY receptor responsiveness may occur in disease states, such as congestive heart failure (CHF), where sympathetic nervous system activity is chronically elevated (Francis et al. 1984; Feng et al. 1994). Significant elevation of sympathetic drive results in an increased level of plasma catecholamines (Cohn et al. 1984) and the extent of elevation of plasma NE is well correlated with the firing rate of the muscular sympathetic nerve (Leimbach et al. 1986). NPY is an important neurotransmitter released together with NE during physiological conditions such as physical exercise (Lundberg et al. 1985b; Pernow et al. 1986a) and pathophysiological situations of excessive increase of sympathetic tone such as CHF (Feng et al. 1993; Derchi et al. 1993; Maisel et al. 1989; Hulting et al. 1990). Functional postjunctional NPY receptors have been demonstrated in the deep veins of the forearm (Pernow et al. 1987) and in dorsal hand veins of normal subjects (Chapter 3), but no study has investigated vascular NPY responsiveness in patients with CHF. It has previously been shown that venous $\alpha$-adrenoceptor responsiveness is decreased in severely symptomatic patients with CHF while patients with milder symptoms demonstrate increased $\alpha$-receptor responsiveness (Arnold et al. 1997). Since severe CHF is characterised by increased plasma catecholamine levels (Francis et al. 1984; Feng et al. 1994), it is not surprising that $\alpha$-receptor responsiveness is decreased within that specific population. In an animal model of heart failure, it has been demonstrated that the

postjunctional Y1 receptors are desensitized in response to NPY activation (Feng et al. 1993). It is presently not known if these receptors are functioning normally or are hypo- or hyper-responsive to NPY in CHF patients.

### 4.1.3 Hypothesis

NPY receptors are present in the dorsal hand veins of patients with CHF, but the responsiveness to NPY in the dorsal hand veins is decreased in these patients.

## 4.2 Methods

*Subjects*

Thirty patients (24 males and 6 females) with physical signs and clinical symptoms of chronic systolic heart failure were studied (Table 4.1). The clinical diagnosis of these patients was attributed to either coronary heart disease or non-ischemic dilated cardiomyopathy and none had evidence of valvular heart disease or hypertrophic cardiomyopathy by echocardiography. Patients did not have unstable angina, hypertension or a history of recent myocardial infarction (within 3 months of study). In all patients, left ventricular ejection fraction (LVEF) was assessed by radionuclide angiogram within at least 6 months prior to the study date with no significant cardiac event or change in clinical symptoms in the intervening period. All subjects were NYHA functional class II-IV by clinical history and had stable symptoms at the time of study. Patients with LVEF greater than 20% but less than 40% (LVEF > 20%) were classified as mild to moderate LV systolic dysfunction (16 patients), while patients with LVEF less than or equal to 20% (LVEF≤20%) were classified as severe LV systolic dysfunction (14 patients). The NYHA

**Table 4.1 Characteristics of Patients with Congestive Heart Failure and Age-Similar Normal Controls**

| Groups | Age (yrs.) | Sex (Male/Female) | LVEF (%) | NYHA Class |
|---|---|---|---|---|
| Normal (n=17) | 55±2 (40-72) | 8/9 | not measured | not applicable |
| LVEF>20% (n=16) | 62±3 (37-82) | 14/2 | 31.4±2.0 | II-III |
| LVEF≤20% (n=14) | 63±3 (45-75) | 10/4 | 15.1±0.7 | III- IV |

Results given as mean ± sem.

Abbreviatons: LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

classification system is a method to classify CHF patients into 1 of 4 groups depending on their physical symptoms. Class I consists of patients with cardiac disease but without resulting limitation of physical activity. Class II consists of patients with cardiac disease resulting in slight limitation of physical activity. Class III consists of patients with cardiac disease resulting in marked limitation of physical activity, and Class IV consists of patients with cardiac disease resulting in the inability to carry on any physical activity without discomfort. Cohn et al. (1993) have shown a sharp increase in mortality in patients with LVEF $< 20\%$, indicating increased severity of CHF. Vasoactive agents such as angiotensin-converting enzyme (ACE) inhibitors and long-acting nitrates were withheld for 5 times their plasma half-life before the study. Patients taking long-acting ACE inhibitors (ie. lisinopril or enalapril) were switched one week prior to the study to the short-acting ACE inhibitor captopril (half-life approximately 4-5 hours) at an equivalent dose, since withholding ACE inhibitors for longer than 24 hours could prove detrimental to the patients' conditions. Diuretics, digoxin and all other medications were held on the study morning to avoid acute drug effects. Patients taking calcium channel antagonists or beta-blockers within one month prior to the study were excluded from participating.

Seventeen age-similar ($55 \pm 2$ yrs) normal subjects (8 males, 9 females) with no evidence of heart disease as assessed by medical history, physical examination, and electrocardiogram, were studied to compare to the heart failure patients. All normal subjects were non-smokers, did not have a history of hypertension and none were on any vasoactive medications. Three of the CHF patients and 3 normal subjects were postmenopausal, taking hormone replacement therapy, and therefore any effects that this

therapy may have should be equally balanced between the groups.

*Study Design*

Hand vein tonometry was employed using the LVDT technique as outlined in Chapter 2, to measure the local venous responsiveness to NPY in the absence of systemic or reflex responses.

On the study morning, a short (1.9 cm) 25-gauge butterfly needle (Butterfly-Abbocath) was inserted into a suitable dorsal hand vein with a long straight section and no visible tributaries. Normal saline (0.9%) was infused through the needle at 0.4 ml/min for 30 minutes. During this equilibration period, several measurements of hand vein distention at 45 mmHg were made to ensure a stable baseline and the average taken as the control distention. Thirty minutes after insertion of an i.v. needle and before any drug infusions, a blood sample (15 ml) was drawn for the measurements of plasma catecholamines and NPY. Following this, sequential graded local 5 minute i.v. infusions of human NPY (25, 50, 100, 200, 500, 1000, 2000 pmol/min) were commenced to constrict the dorsal hand vein and cumulative dose-response curves were constructed with an upper arm occlusion cuff inflated for the last 2 minutes of each dose step.

## 4.3 Results

Prior to NPY infusion, both groups of CHF patients had a significant elevation in heart rate compared to normal controls ($p<0.05$ for moderate CHF, $p<0.01$ for severe CHF) though mean arterial pressure under resting conditions was not significantly different from

**Table 4.2 Mean Arterial Pressure, Heart Rate, Basal Plasma Norepinephrine and NPY Levels, and Basal Hand Vein Diameter**

| Groups | Mean Arterial Pressure (mmHg) | Heart Rate (bpm) | Plasma Norepinephrine (pmol/L) | Plasma NPY (pmol/L) | Basal Vein Diameter (mm) |
|---|---|---|---|---|---|
| Normal (n=17) | 88±1 | 59±1 | 1324±181 | 127±3 | 1.13±0.12 |
| LVEF>20% (n=16) | 91±3 | 70±3* | 1860±450 | 146±4* | 1.01±0.10 |
| LVEF≤20% (n=14) | 88±2 | 81±4** | 4890±1550** | 148±5* | 1.01±0.15 |

Results given as mean ± sem, *p<0.05, **p<0.01 vs. Normal.
Abbreviations: LVEF, left ventricular ejection fraction; NPY, Neuropeptide Y.

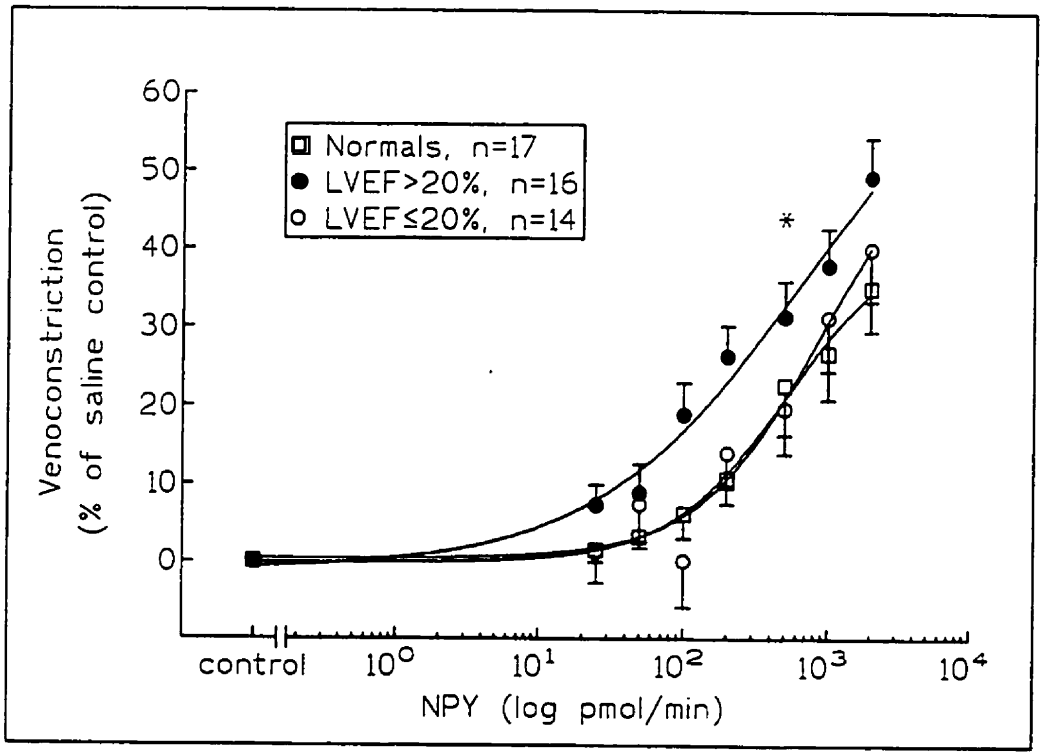


**Figure 4.1** Average response to NPY in the dorsal hand veins of CHF patients with LVEF>20%, LVEF≤20%, and Normal controls.
*$p<0.05$ LVEF > 20% vs. Normal controls.

normal controls (Table 4.2). Plasma NE levels were markedly elevated in patients with severe CHF when compared to normal controls but were not significantly altered in CHF patients with mild to moderate CHF. Plasma NPY levels were elevated in patients with severe and mild to moderate CHF when compared to normal controls (Table 4.2). No significant changes in blood pressure or heart rate were seen in any groups throughout the duration of the experiment.

Basal vein diameter at 45 mmHg venous occlusion pressure during 0.9% saline infusion, in normals and patients with CHF, were not significantly different (Table 4.2). Graded infusion of NPY induced dose-dependent venoconstriction in all subjects studied. The mean dose-response curve for NPY in patients with mild to moderate CHF (LVEF>20%) was significantly shifted to the left compared to that of age similar normal controls ($p<0.05$). However, the dose-response curve for NPY in patients with severe CHF (LVEF≤20%) was not significantly altered compared to that of normal controls, but was significantly to the right of that for mild to moderate CHF ($p<0.05$) (Figure 4.1).

## 4.4 Discussion

In the present study, we have demonstrated *in vivo* for the first time that venous vascular NPY receptor function is increased in patients with mild to moderate CHF compared to age-similar normal controls. While venous NPY receptor responsiveness in patients with severe CHF was not decreased compared to age-similar normal controls, this response was significantly decreased compared to patients with mild to moderate CHF. CHF has been thoroughly investigated with regard to both hemodynamic effects and

alterations of neurohumoral activity, however less is known about alterations at the vascular receptor level. This novel finding in patients with mild to moderate CHF may suggest that NPY receptor function is an important mediator and contributes to increased vasoconstriction in the early stages of CHF.

CHF patients in the present study all had depressed left ventricular function with LVEF ranging from 12-39% and had stable symptoms. In patients with severe CHF, plasma NE levels and basal heart rate were significantly increased indicating strong sympathetic activation. Plasma NPY levels were also significantly increased in this group of patients, although the increase was less than that seen in plasma NE levels. The NPY levels exhibited little variation, in contrast to the NE levels which were spread over quite a wide range. The normal plasma level of NPY is less than 130 pmol/L and the CHF groups had NPY plasma levels slightly higher than that. This demonstrates that although the NPY plasma levels are elevated in CHF, they are not elevated a great deal more than normal levels. The plasma samples were blinded when they were analysed, and therefore no bias was introduced. In patients with mild to moderate CHF, although the plasma NE level was not increased compared to normal controls, basal heart rate was significantly increased compared to age-similar normal subjects suggesting some degree of sympathetic activation. Plasma NPY levels were again elevated in these patients to the same degree as the severe CHF patients. There are several reports of increased plasma levels of NPY (Edvinsson et al., 1990; Hulting et al., 1990; Valdemarsson et al., 1991; Derchi et al., 1993; Hauser et al., 1996) but also of unchanged NPY levels in CHF (Dubois-Rande et al., 1992). Hauser et al. (1996) found that resting plasma levels of NPY are high in CHF patients

compared to normals, however they found that norepinephrine and epinephrine plasma levels correlated well, but NPY levels did not correlate with either norepinephrine or epinephrine levels. In the present study, these levels did not correlate either. In a study by Dubois-Rande et al. (1992), patients with chronic stable heart failure were found to have high NE plasma levels, but the NPY levels were the same as in control subjects. The reason for these differences in NPY plasma levels may be due to different patient populations being studied. Once NPY is released, it is resupplied by axonal transport (Fried et al., 1985; Lundberg et al., 1986). This is likely to limit the amount of NPY available for terminal release when compared with NE which is stored in nerve endings and obtained from both local synthesis and reuptake. This may explain the lack of increased NPY plasma levels but increased NE levels. Thus, despite some synergistic effects and co-localization in nerve terminals, the relationship between the release of NPY and NE in humans is complex, with NPY levels decreasing, not changing, or increasing in various situations. NPY appears to play a relatively minor role in the minute-to-minute maintenance of cardiovascular homeostasis, its role being more of a second line of defence that comes into play in situations of greater or more prolonged stress.

To avoid the influence of vasoactive agents on the vascular NPY responses, angiotensin-converting enzyme (ACE) inhibitors and long-acting nitrates were withheld for 5 times their plasma half-life before the study. Since more than 97% of the drug is eliminated after 5 half-lives, the potential acute influence of these agents should be negligible. We chose the dorsal hand vein tonometry technique as it is an *in vivo* method that allows us to construct dose-response curves to very small amounts of agonists such as

NPY but does not induce systemic changes of arterial pressure and confounding reflex alterations (Blochl-Daum et al., 1991). In the present study, basal mean arterial pressure was unaltered in the CHF patients, but heart rate was significantly increased in both mild to moderate, and severe CHF patients compared to normal subjects. Upon infusing increasing doses of NPY, no significant changes in arterial pressure or heart rate were observed. Thus, the responses observed to NPY in the present study represent local vascular responsiveness and intrinsic receptor activity. A difficulty that we had with these experiments was that we were unable to construct complete dose-response curves with the NPY and therefore were unable to compare $ED_{50}$ values. In order to obtain a more complete dose-response curve, we would need to infuse higher doses of NPY, however we did not have access to increased amounts of NPY and therefore we infused the highest dose that we were able to.

One would expect that upon chronic sympathetic activation, postjunctional receptors may undergo downregulation or desensitization of the receptor function. A classic example of this is the downregulation of myocardial ß-adrenergic receptors in CHF (Bristow et al. 1986). In the present study, we clearly demonstrated that vascular NPY receptor responsiveness in the dorsal hand veins was significantly increased in patients with mild to moderate CHF compared to normal controls. The mechanism of this increased NPY receptor responsiveness in mild to moderate CHF is not known, but it is most likely not due to receptor upregulation since sympathetic activity and NPY release are not decreased in these patients (Feng et al. 1994; Francis et al. 1984). We have recently demonstrated similar functional changes in vascular α-adrenoceptors in the dorsal hand

veins in patients with mild to moderate CHF (Arnold et al. 1995). Evidence from experimental models of early stages of heart failure have also shown the same functional changes in vascular α-adrenoceptors. In a pacing-induced heart failure model in the dog, increased $\alpha_1$-adrenoceptor responsiveness *in vitro* has been reported in isolated pedal arteries 3-4 weeks after rapid pacing (Forster & Armstrong, 1990). A study by Bergdahl et al. (1995) looked at the contractile response to NPY in CHF by ligating the left coronary artery in the rat resulting in a myocardial infarction with the subsequent development of CHF. They found that the strongest contractile response to NPY was seen in the iliac vein of CHF rats compared to sham rats, indicating the possibility of increased sensitivity to NPY, however it was not statistically significant.

Increased vascular responsiveness to NE has been observed in thoracic aorta in rats with myocardial infarction one week after coronary artery ligation (Teerlink et al. 1994b). Moreover, the increased vascular response to NE in rats with myocardial dysfunction is due to a decreased endothelial function (Teerlink et al. 1994a). Recent studies have shown that endothelium-dependent relaxation is decreased in patients with CHF (Kubo et al., 1991). We have also demonstrated that endothelium-dependent relaxation in the dorsal hand vein is decreased in mild to moderate CHF (See Chapter 5). NPY receptors are present in the vascular endothelium and stimulation of these NPY receptors releases endothelium-derived vasodilators and induces vasorelaxation (Lind et al. 1995). The decreased endothelial function in CHF may result in a decreased release of endothelium-derived vasodilators by NPY receptor stimulation, and therefore cause an increased vascular NPY receptor response in patients with mild to moderate CHF. In addition, in

patients with severe CHF, the "true" NPY response may actually be decreased compared to normals since in normals with a functional endothelium, vasodilatory compounds such as prostacyclin ($PGI_2$) or EDRF (NO) may be released from the endothelium and act to antagonize the constriction of the vascular smooth muscle by NPY. This hypothesis was therefore subsequently investigated (See Chapter 5).

**Summary**

We have demonstrated that venous vascular NPY responsiveness is increased in patients with mild to moderate CHF compared to age-similar normals, while NPY responsiveness in severe CHF patients is not different from normal controls. This increased responsiveness in moderate CHF may in fact be due to decreased endothelial function and will be investigated further in Chapter 5.

# CHAPTER 5

# NEUROPEPTIDE Y VASCULAR RESPONSES AND ENDOTHELIAL FUNCTION IN NORMALS AND CONGESTIVE HEART FAILURE PATIENTS

## 5.1 Introduction

### 5.1.1 Background Information

In the previous study investigating the *in vivo* responsiveness of NPY receptors in patients with congestive heart failure (CHF) and age-similar normal subjects, we demonstrated that the dose-response curve for mild to moderate CHF patients was significantly shifted to the left compared to both normal subjects and severe CHF patients (See Chapter 4). However, upregulation of the NPY receptors in moderate CHF seems unlikely since plasma NPY levels were not decreased in these patients. It has become increasingly evident that the vascular endothelium plays a major role in the regulation of vascular tone through the release of many vasoactive hormones including nitric oxide, endothelins, endothelium derived hyperpolarizing factor, and prostaglandins (PG) that have a large number of effects both locally and systemically (Miller, 1991). Studies using isolated human hand veins preconstricted with endothelin have shown that $PGI_2$ elicits a concentration-dependent relaxant effect indicating the potential of $PGI_2$ to overcome or antagonize the effects of some potent vasoconstrictors (Arner et al., 1994). In addition, it was previously shown in our lab that $\alpha_1$ and $\alpha_2$ receptor stimulation results in the release of vasodilatory prostaglandins that are effectively able to counteract the venoconstriction caused by $\alpha$-receptor stimulation. Thus, it is possible that NPY receptor stimulation also results in the release of vasodilatory prostaglandins which are able to counteract the

venoconstriction. It has been demonstrated in *in vitro* studies that the infusion of vasoconstrictor hormones such as angiotensin II, vasopressin, and NE stimulates prostaglandin synthesis, which is able to counteract peripheral vasoconstriction (Zusman and Keiser, 1977; Shebuski and Aiken, 1980). These studies suggest that vasodilator prostaglandins, such as $PGI_2$ and $PGE_2$ play a critical part in preserving circulatory function in vasoconstrictive states. It has also been demonstrated in porcine aortic endothelial cells that NPY stimulates the synthesis of prostacyclin, by measuring the levels of 6-keto-$PGF_{1\alpha}$, a stable derivative of $PGI_2$ (Kawamura et al., 1991).

### 5.1.2 Rationale

In our laboratory, *in vivo* studies of normals, using the prostaglandin synthesis inhibitor indomethacin and several α-adrenoceptor agonists suggest that the venous endothelium contains both $\alpha_1$- and $\alpha_2$-adrenoceptors which upon stimulation result in the release of vasodilatory prostaglandins, most likely $PGI_2$, which antagonize the α-receptor mediated constriction of vascular smooth muscle (Callow et al., 1997). Consequently, endothelial released $PGI_2$ appears to play a significant role in α-receptor responsiveness to infused agonists in the venous system. NPY-Y1 receptors are present in the vascular endothelium of cultured endothelial cells and stimulation releases endothelium derived vasodilators thus producing vascular dilatations (Lind et al., 1995). This is consistent with findings at the α-receptor and consequently it would appear that endothelial NPY receptors may also influence the NPY mediated vascular smooth muscle response. Since it has recently been confirmed that endothelium dependent relaxation is decreased in the dorsal hand veins in

CHF, this decrease in endothelial function may result in a decrease of $PGI_2$ synthesis in CHF (Katz, 1995). Thus the net vasoconstrictor response to NPY may be a balance between NPY levels, receptor function and endothelial function. The current studies were designed to clarify the role of endothelial dysfunction in modulating sympathetic vascular responses to NPY in normals and CHF patients. The importance of these studies is in their ability to address mechanisms of sympathetic and endothelial function *in vivo*.

### 5.1.3 Hypotheses

NPY receptor stimulation results in the liberation of vasodilatory prostaglandins, most likely $PGI_2$, which, when blocked by indomethacin, will result in a significant shift to the left of the dose-response curve obtained in response to NPY infusion in normal subjects.

It is further hypothesized that, in patients with CHF and endothelial dysfunction, the dose-response curve will not be significantly altered by indomethacin administration.

Upon infusion of the endothelium-dependent vasodilator acetylcholine, in $PGF_{2\alpha}$ preconstricted veins, it is hypothesized that the vasodilation will be decreased in patients with CHF due to endothelial dysfunction.

Upon infusion of the endothelium-independent vasodilator, sodium nitroprusside, similar dose-dependent vasodilation will occur in both normal subjects and CHF patients.

## 5.2 Methods

*Subjects*

Twenty-two patients (18 males, 4 females) with clinical and physical signs of chronic

systolic heart failure were studied (Table 5.1). The clinical diagnosis of these patients was attributed to either coronary heart disease or non-ischemic dilated cardiomyopathy. None had evidence of valvular heart disease or hypertrophic cardiomyopathy by echocardiography. Patients did not have unstable angina, hypertension, or a history of a recent myocardial infarction (i.e. within 3 months of study). Subjects were NYHA functional class II-IV by clinical history and all had stable symptoms at the time of study. In all patients, left ventricular ejection fraction (LVEF) was assessed by radionuclide angiogram within at least 6 months prior to the study date with no significant cardiac event or change in clinical symptoms in the intervening period. Patients were divided into 2 groups according to their resting LVEF. Those with LVEF greater than 20% but less than 40% (LVEF $>$ 20%) were classified as moderate LV systolic dysfunction (11 patients), and those with LVEF less than or equal to 20% (LVEF $\leq$ 20%) were classified as severe LV systolic dysfunction (11 patients). Vasoactive agents such as angiotensin-converting enzyme (ACE) inhibitors and long-acting nitrates were withheld for 5 times their plasma half life before the study. Digoxin, diuretics, and all other medications were withheld on the study morning. Patients taking calcium channel antagonists or beta-blockers within one month prior to the study were excluded from participating.

Eleven age-similar normal subjects (8 males, 3 females) with no evidence of heart disease as assessed by medical history, physical examination, and electrocardiogram, were studied for comparison to the CHF patients. All normal subjects were non-smokers, did not have a history of hypertension and none were on any vasoactive medications. Subjects with a known history of sensitivity to aspirin (due to the potential cross-sensitivity to

**Table 5.1 Characteristics of Patients with Congestive Heart Failure and Age-Similar Normal Controls**

| Group | Age (yrs.) | Sex (Male/Female) | LVEF (%) | NYHA Class |
|---|---|---|---|---|
| Normal (n=11) | 54±3 (43-71) | 8/3 | not measured | not applicable |
| LVEF>20% (n=11) | 67±2 (52-82) | 10/1 | 28.1±1.2 | II-III |
| LVEF≤20% (n=11) | 64±4 (45-81) | 8/3 | 18.0±0.8 | III-IV |

Results given as mean ± sem.

Abbreviations: LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

indomethacin) or indomethacin were excluded from participating in the study.

*Study Design*

Hand vein tonometry was employed using the LVDT technique as outlined in Chapter 2, to measure the local venous responsiveness to NPY in the absence and presence of indomethacin, in the absence of systemic or reflex responses.

The study was carried out on three separate days with less than 10 days separating each day. On the first study morning, two short (1.0 cm) 27-gauge butterfly needles (Butterfly-Abbocath) were inserted in a proximal direction less than 1 cm apart into a suitable dorsal hand vein with a long straight section and no visible tributaries. Normal saline (0.9%) was infused through both needles at 0.2 ml/min for 30 minutes. During this equilibration period, several measurements of hand vein distention at 45 mmHg were made to ensure a stable baseline and the average taken as the control distention. Thirty minutes after insertion of an i.v. needle and before any drug infusions, a blood sample (15 ml) was drawn for the measurement of plasma catecholamines and NPY. Following this, the saline syringe connected to the distal needle was replaced with a syringe randomly assigned to contain either 0.9% NaCl (as a placebo) or the prostaglandin synthesis inhibitor indomethacin (mixed in saline) and was also administered at a rate of 0.2 ml/min. Once this infusion was begun, it was given continuously throughout the remainder of the experiment. Fifteen minutes later, sequential graded infusions of NPY were administered through the second needle in conjunction with the saline placebo or indomethacin infusion and measurements were made as described in Chapter 2.

On the second study morning, two 27 gauge butterfly needles were again inserted into

a suitable dorsal hand vein. The sequential infusions of NPY were then repeated, in conjunction with the appropriate co-infusion of either 0.9% NaCl or indomethacin (whichever was not used on the first day) and measurements of venous distention repeated.

On the third study morning, two 27 gauge butterfly needles were again inserted into a dorsal hand vein of the same hand as previously described. Again a 0.9% saline infusion was started for 30 minutes in both needles. Following this, the vein was preconstricted to approximately 50% with $PGF_{2\alpha}$ (0.2 ml/min) (256-1024 ng/min - the dose required varied from person to person) through the most distal needle. The dose range chosen for the infusion of $PGF_{2\alpha}$ to obtain a 50% constriction was determined by constructing full dose response curves in several subjects and taking the dose range that produced 50% constriction in these subjects. After 15 minutes, sequential graded infusions of acetylcholine (0.01, 0.03, 0.1, 0.3, 1.0 nmol/min) were administered (through the second needle) in conjunction with the $PGF_{2\alpha}$ and measurements were made as described in Chapter 2. Following this, the acetylcholine syringe was replaced with saline to wash out the acetylcholine, while keeping the $PGF_{2\alpha}$ infusion running throughout. Once the response returned to the preconstricted level, sequential graded infusions of sodium nitroprusside (0.3, 0.625, 1.25, 2.5, 5.0, 10 nmol/min) were administered (through the second needle) in conjunction with the $PGF_{2\alpha}$ and measurements were made as described in Chapter 2.

## 5.3 Results

In all 3 groups, the initial baseline venous diameter during indomethacin co-infusion

was not significantly different from saline placebo (Table 5.2). Graded infusions of NPY induced dose-dependent venoconstriction during co-infusion of both saline placebo and indomethacin, in all subjects studied. In the normal subjects, the mean dose-response curve obtained during indomethacin infusion was significantly ($p<0.05$) shifted to the left compared with the saline placebo (Figure 5.1). In the mild to moderate and severe CHF patients, the mean dose-response curve obtained during indomethacin infusion was not significantly different from the saline placebo (Figures 5.2 and 5.3). Figure 5.4 shows the mean dose-response curves to NPY in the presence of indomethacin for the 3 groups of subjects. The dose-response curve obtained in the severe CHF group of patients was significantly decreased ($p<0.05$) from the moderate CHF group and the normal subjects.

To test endothelial function in these subjects, the veins were preconstricted with $PGF_{2\alpha}$ and then dilated with the endothelial-dependent vasodilator, acetylcholine and the endothelial-independent vasodilator, sodium nitroprusside. Six normal subjects, 4 moderate CHF, and 5 severe CHF patients were tested for endothelial function. Acetylcholine produced vasodilation in the normal subjects with a maximum response of $36.0 \pm 9.3\%$, whereas the moderate CHF patients had a maximum dilation of $8.3 \pm 15.0$, and the severe CHF patients had a maximum dilation of $-2.0 \pm 10.9$ (Figure 5.5). Both CHF groups showed constriction at higher doses of acetylcholine. In contrast, sodium nitroprusside produced vasodilation in all 3 groups with maximum dilation reaching $91.1 \pm 15.7\%$ in normals, $58.5 \pm 18.5\%$ in moderate CHF, and $81.1 \pm 15.4\%$ in severe CHF (Figure 5.6).

**Table 5.2 Mean Arterial Pressure, Heart Rate, Basal Plasma Norepinephrine and NPY Levels, and Basal Hand Vein Diameter**

| Groups | Mean Arterial Pressure (mmHg) | Heart Rate (bpm) | Plasma NE (pmol/L) | Plasma NPY (pmol/L) | Basal Vein Diameter (mm) (Saline Placebo) | Basal Vein Diameter (mm) (Indo) |
|---|---|---|---|---|---|---|
| Normal (n=11) | 90±3 | 61±2 | 1491±262 | 125±5 | 0.92±0.11 | 1.08±0.11 |
| LVEF>20% (n=11) | 96±4 | 69±4* | 1534±183 | 138±5 | 0.84±0.16 | 0.97±0.15 |
| LVEF≤20% (n=11) | 91±4 | 71±5** | 3793±809** | 140±10 | 0.95±0.13 | 1.00±0.11 |

Results given as mean ± sem, *p<0.05, **p<0.01 vs. Normal.
Abbreviations: LVEF, left ventricular ejection fraction; NE, norepinephrine; NPY, Neuropeptide Y.

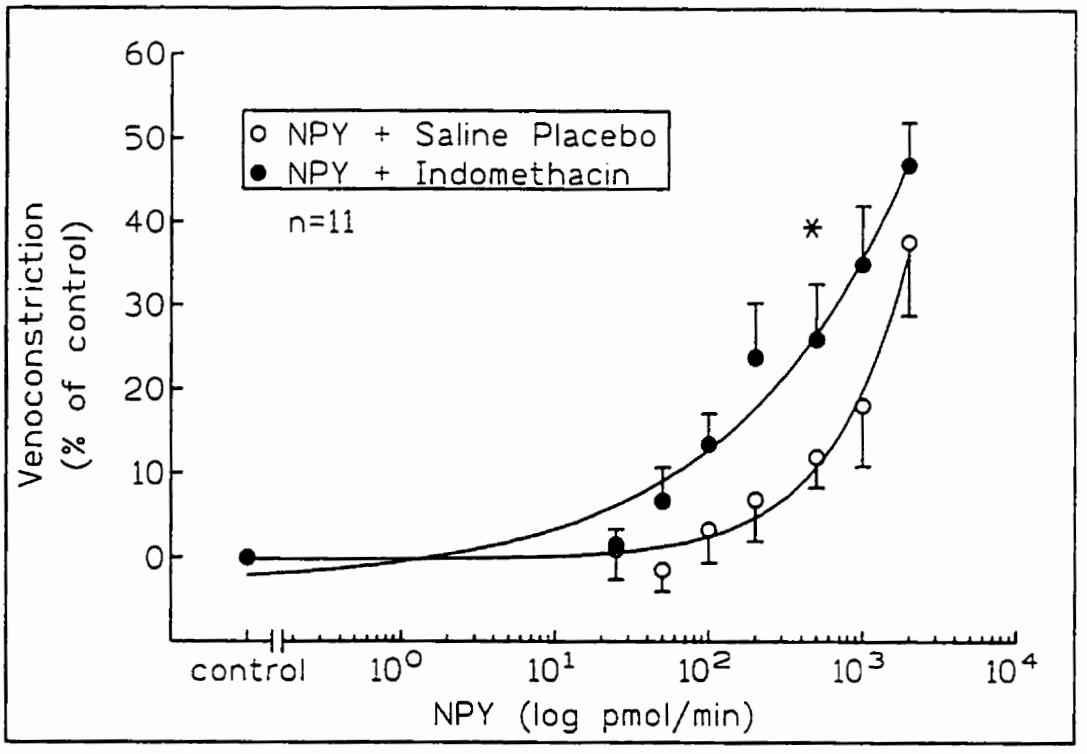


**Figure 5.1** Average response to NPY in the dorsal hand veins of age-similar normal subjects in the presence and absence (saline placebo) of indomethacin.
*$p<0.05$ vs. saline placebo.

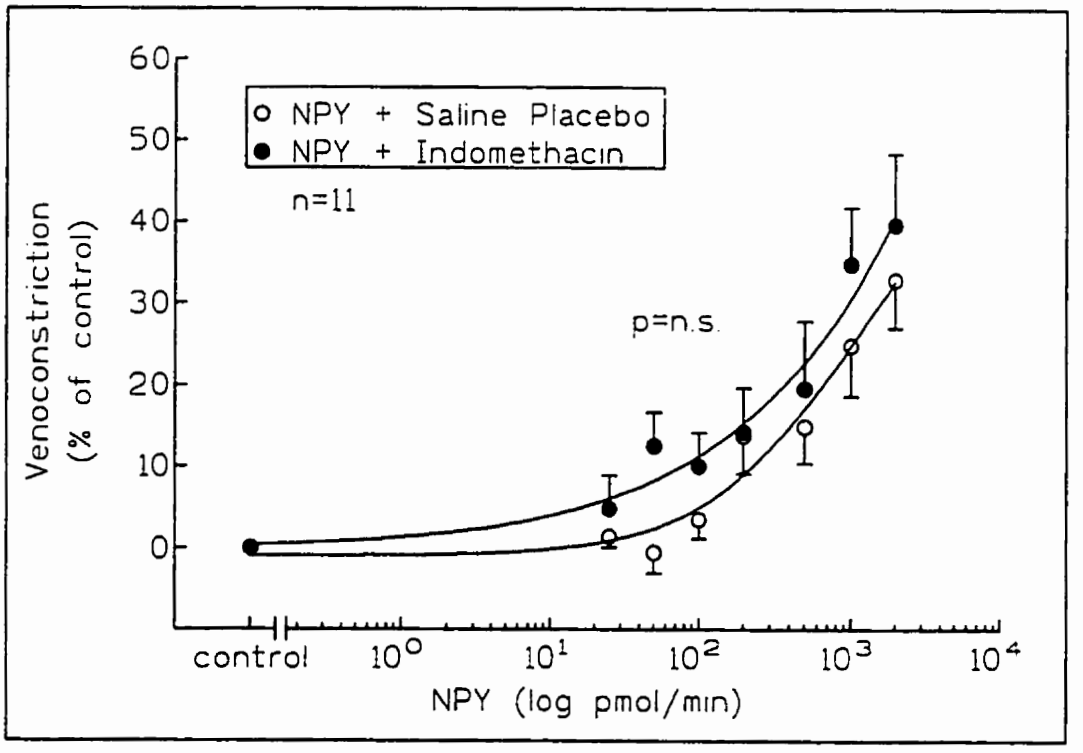


**Figure 5.2** Average response to NPY in the dorsal hand veins of mild to moderate CHF patients, LVEF >20% in the presence and absence (saline placebo) of indomethacin.

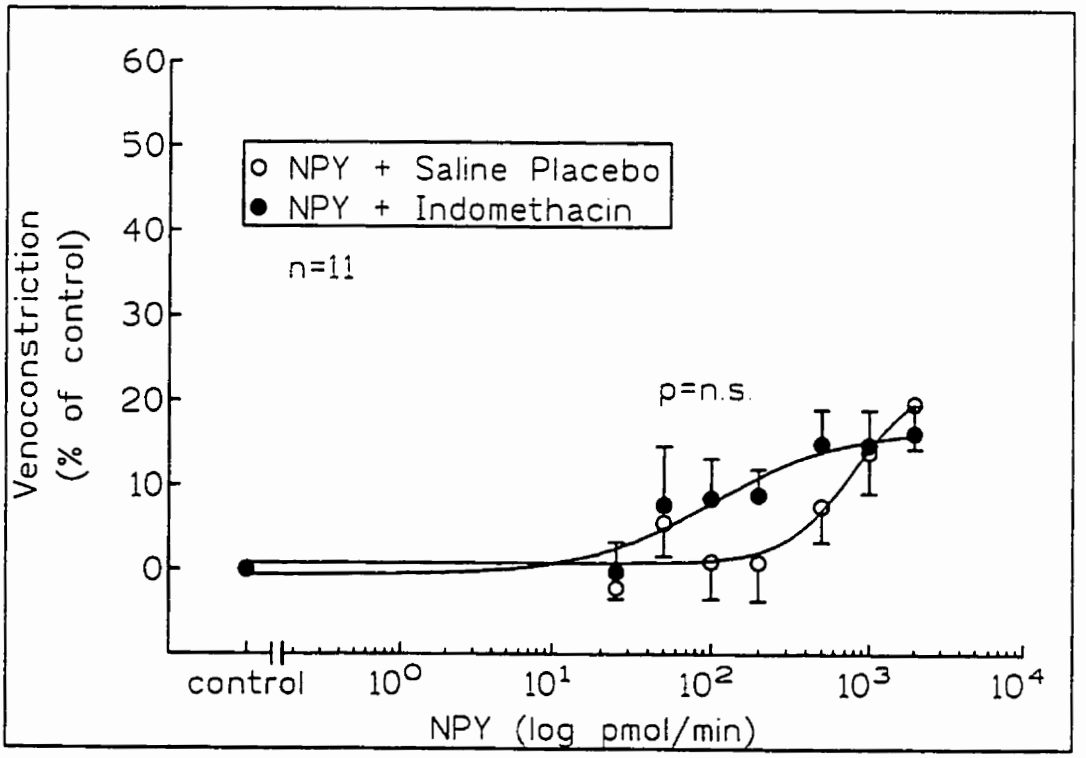


**Figure 5.3** Average response to NPY in the dorsal hand veins of severe CHF patients, LVEF≤20% in the presence and absence (saline placebo) of indomethacin.

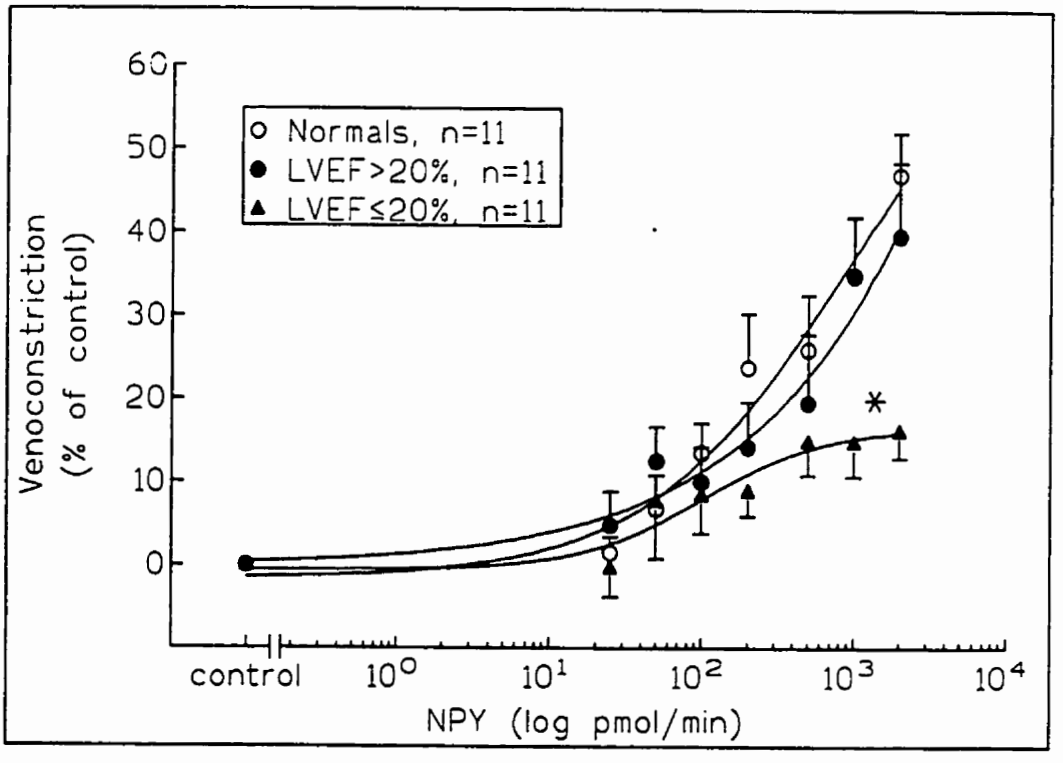


**Figure 5.4** Average response to NPY in the dorsal hand veins of age-similar normal subjects, mild to moderate CHF patients, and severe CHF patients in the presence of indomethacin.
*$p<0.05$ LVEF≤20% vs. normals and LVEF>20%.

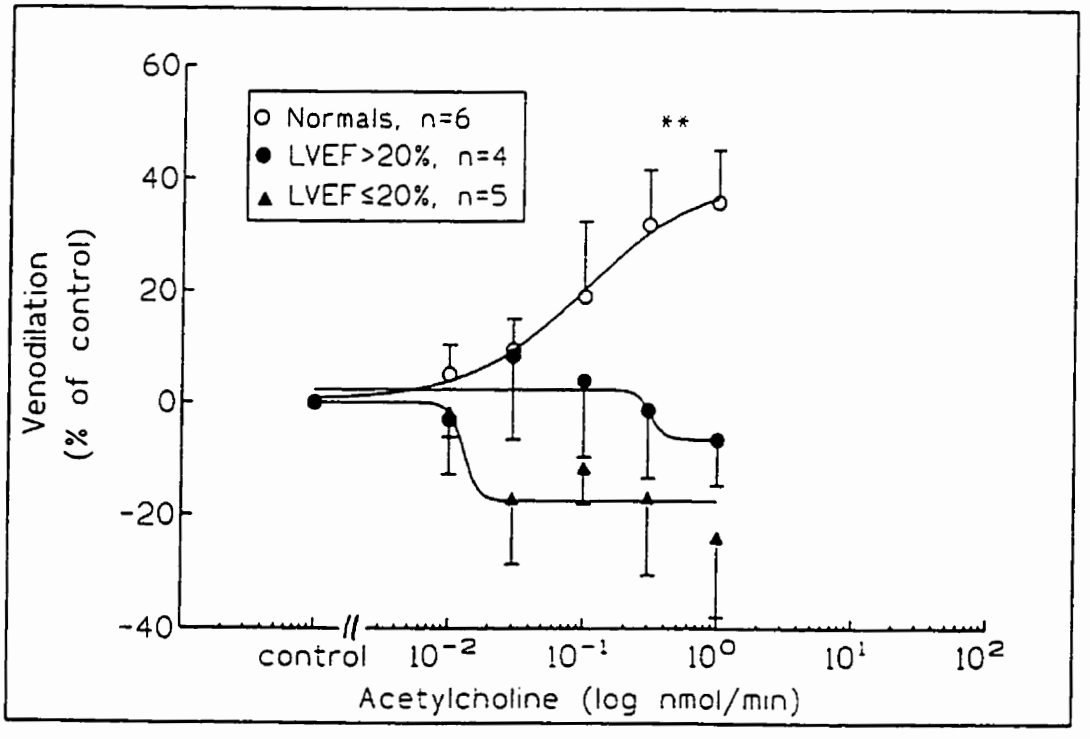


**Figure 5.5** Average response to acetylcholine in the dorsal hand veins of age-similar normal subjects, mild to moderate CHF patients, and severe CHF patients.
*$p<0.01$ Normals vs CHF.

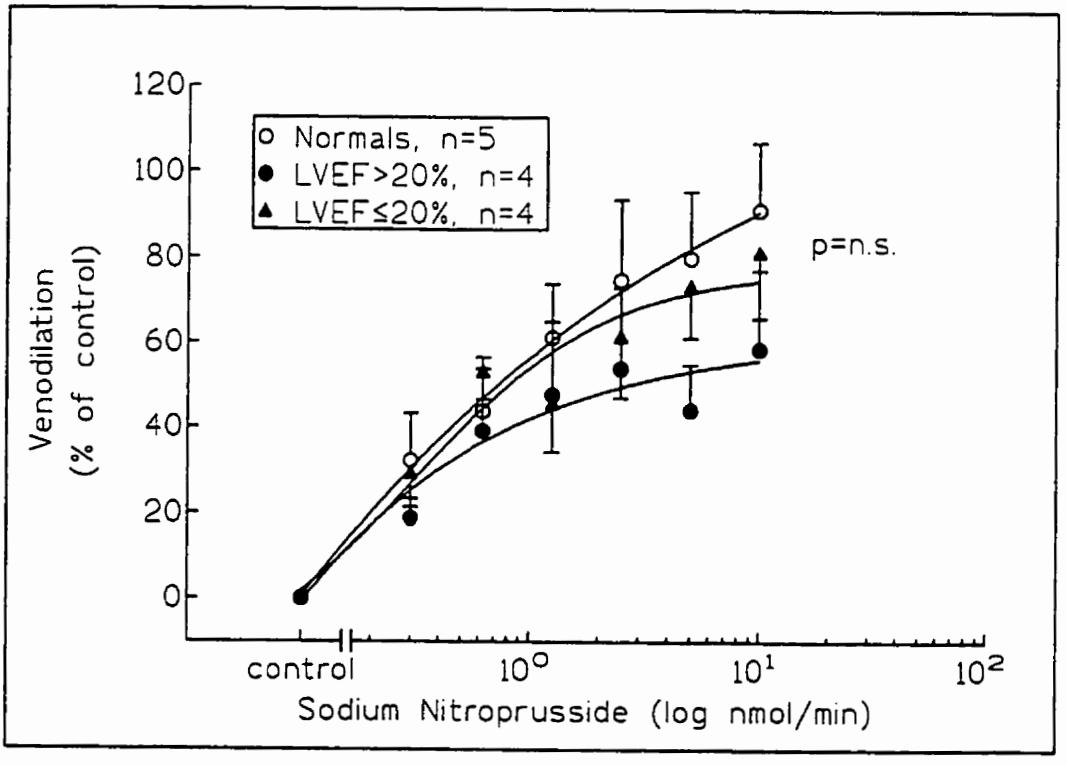


**Figure 5.6** Average response to sodium nitroprusside in the dorsal hand veins of age-similar normal subjects, mild to moderate CHF patients, and severe CHF patients.

## 5.4 Discussion

The main objective of this study was to determine whether vasodilatory prostaglandins, the most prominent in blood vessels being $PGI_2$ (Moncada et al., 1977; Rang and Dale, 1991), influence the NPY-mediated venoconstrictive effects on superficial dorsal hand veins of CHF patients and age-similar normal subjects. Using sequential graded intravenous infusions of NPY in conjunction with the prostaglandin synthesis blocker indomethacin, we demonstrated that the venoconstriction elicited by NPY was significantly increased over that obtained during co-infusion of saline placebo in the normal subjects studied. Pre-infusion of indomethacin for 15 minutes while taking repeated measurements of baseline venous distention demonstrated no significant differences from the control distentions during saline placebo. This suggests that indomethacin was not simply activating the NPY receptors resulting in an additive effect with NPY, but was more likely to be enhancing the venoconstrictive effects of NPY by removing the opposing vasodilatory influence of prostaglandins produced upon NPY stimulation. This suggests that NPY is also activating NPY receptors other than those located on the vascular smooth muscle, resulting in the production and release of vasodilatory prostaglandins such as $PGI_2$. Early studies by Moncada et al. (1977) demonstrated that the endothelial cells are the major site of $PGI_2$ synthesis and thus it is likely that NPY receptors are present on endothelial cells. The vascular smooth muscle has also been shown to produce small quantities of $PGI_2$ (Vane et al., 1995) and therefore we cannot exclude the possibility that the vascular smooth muscle NPY receptors are causing the release of prostaglandins. However, there is less evidence for vascular smooth muscle release of significant amounts

of vasodilator prostaglandins. This study provides indirect evidence for the existence of NPY receptors on the vascular endothelium resulting in vasodilatory prostaglandins being released upon NPY stimulation.

Our present results found *in vivo* are in agreement with previous results found *in vitro* (Kawamura et al., 1991; Lind et al., 1995). Upon pre-infusion of indomethacin in the present study, the measurements of venous distention were not different from those obtained during saline pre-infusion. This would suggest that resting basal production of vasodilatory prostaglandins is low. Thus it appears that the stimulation of the NPY receptors results in the production of sufficient amounts of vasodilatory prostaglandins to effectively counteract the venoconstrictor effect of NPY. This has been demonstrated both *in vitro* and *in vivo*. It would have been helpful to measure local venous $PGI_2$ levels in this study before and after NPY stimulation, however the necessarily small i.v. needles used in the study prevented us from being able to obtain a sufficient blood sample from which to accurately measure $PGI_2$ levels in the dorsal hand vein. In addition, $PGI_2$ is highly unstable, and therefore we would need to measure the more stable metabolite, 6-keto-$PGF_{1\alpha}$. However, this is also quite difficult to measure and therefore may give inaccurate results. Thus, we cannot be certain that we are not simply blocking basal prostaglandin levels that are present before NPY stimulation and this is addressed subsequently in Chapter 7.

Indomethacin is a non-specific inhibitor of prostaglandin synthesis, however it remains the best available blocker of prostaglandin synthesis because aspirin and other NSAIDS such as ibuprofen are currently not available for intravenous use in humans in Canada.

The LVDT methodology is a useful technique in that it is able to accurately assess the dorsal hand vein responses to oral antagonists such as aspirin, however we wanted to study the local vascular responses to various agonists without systemic effects to confound the results. By administering a systemic drug such as oral aspirin, there is an increased potential of systemic reflexes coming into play that would make the results difficult to interpret. It is also difficult to ensure adequate inhibition when using oral antagonists whereas indomethacin can be infused locally throughout the duration of the study to ensure continuous inhibition. To ensure that adequate inhibition is maintained with an oral antagonist, we would need to administer a suitable agonist over a range of doses to ensure blockade. Following this, we would need a suitable washout period to ensure that the agonist was completely gone so as to not confound the results. It would be difficult to be sure that there were not still lingering effects of the test agonist, even after the washout period. It would also be difficult to ensure that the inhibition is maintained over the entire study because the antagonistic effects may wear off over the course of the study. In addition, this study was also performed on severely ill CHF patients, and administration of oral antagonists may not be practical in these patients because of the potential to exacerbate the patient's condition.

An additional problem with using indomethacin is that it does not specifically block $PGI_2$ production, but rather it blocks the production of all prostaglandins via the inhibition of the cyclooxygenase enzyme. Because of this, several possible alternative effects of indomethacin may influence the NPY receptor function and the results of the present study. First, the shift in the NPY curve in the normal subjects studied may be due to the

inhibition of the production of several vasodilatory prostaglandins such as $PGI_2$, PGE, and $PGE_2$. However, work by Moncada et al. (1977) has shown that $PGI_2$ is the major product released in veins, and thus the contribution of other prostaglandins is probably quite small. In addition, indomethacin may also be blocking constrictor prostaglandins. However, if we were blocking the production of a significant amount of constrictor prostaglandins, we would expect to see a shift of the NPY curve to the right rather than the left. Therefore, either there are not any constrictor prostaglandins being released, or there are a greater amount of vasodilatory prostaglandins being released such that when blocked, they outweigh the constrictor ones. Second, it has been reported that there are interactions between the cyclooxygenase enzyme and nitric oxide (NO) (Salvemini et al., 1993). In this study, it was shown *in vitro* that NO was able to activate cyclooxygenase resulting in an increased release of prostaglandins such as $PGI_2$ and $PGE_2$. These results may mean that the shift in the NPY curve may be due to the release of NO which would in turn act to release prostaglandins by interacting with the cyclooxygenase enzyme, and this would then be blocked by the indomethacin. However, this is quite unlikely as research by Haefeli et al. (1993) has shown that the role of NO is minimal in human hand veins *in vivo*.

A study by Haynes et al. (1993) investigated whether or not local vascular production of nitric oxide or prostacyclin regulates the venoconstriction induced by the endothelium-derived peptide, endothelin-1, *in vivo* in man. They found that inhibition of nitric oxide production did not affect endothelin-1-induced venoconstriction, but there was substantial potentiation of endothelin-1-induced venoconstriction after pretreatment with 600 mg

aspirin. This suggests that there is little or no stimulated production of nitric oxide in human veins, but there is endothelial production of prostacyclin which attenuates responses to endothelin-1 in human veins *in vivo*. In man, there is evidence to suggest that there is heterogeneity between arterial and venous beds in the production of NO. It has been shown that brachial artery administration of L-NMMA leads to substantial forearm venoconstriction (Vallance et al., 1989a), whereas L-NMMA alone has no effect on dorsal hand vein size (Vallance et al., 1989b). This suggests that there is differential basal production of NO in the resistance and capacitance beds of man.

The functional findings of the present study suggest that vascular smooth muscle NPY receptor function is influenced by the release of vasodilatory prostaglandins, most likely $PGI_2$, from endothelial cells upon simultaneous stimulation of endothelial NPY receptors. Because $PGI_2$ release secondary to NPY stimulation appears to be an important finding *in vivo* in normal subjects, we then assessed this in CHF patients.

This finding that $PGI_2$ release significantly antagonizes the venoconstrictive effects of NPY in normal subjects may help to explain the results previously found in which the NPY venoconstrictor response was increased in mild to moderate CHF compared to normals (See Chapter 4). In the present study, we have shown that vasodilatory prostaglandins are released in sufficiently large amounts to antagonize the vasoconstrictive effects of NPY in normal subjects. We also found in the present study that in mild to moderate CHF, and severe CHF patients, indomethacin did not significantly increase the venoconstriction caused by NPY. This suggests that sufficient amounts of vasodilatory prostaglandins are not being released in these CHF patients from the endothelium to

antagonize the NPY venoconstrictor effects. Several researchers have demonstrated that in CHF, the endothelium is dysfunctional, which may explain the decrease in the synthesis and release of vasodilatory prostaglandins (Luscher et al., 1993; Teerlink et al., 1994; Drexler et al., 1994). Therefore, in the groups of subjects studied in this chapter, when taking into account the vasodilatory effect of the prostaglandins and just looking at the "true" NPY response, the group of mild to moderate CHF patients response is not different from the normals, but the response of the patients with severe CHF is significantly decreased from the normals (Figure 5.4).

It appears that restoring endothelial function and vasodilatory PG release may play an important role in the treatment of CHF patients by preventing excessive constriction of peripheral vessels and resulting in a decrease in preload. The decreased NPY receptor response in severe CHF may also help to protect against excessive peripheral vasoconstriction due to the increased SNS activity that occurs in severe CHF.

Angiotensin converting enzyme (ACE) inhibitors are a widely used treatment of CHF and have vascular protective effects. ACE is an enzyme that is located in the endothelial cell membrane, where it converts angiotensin I to the potent vasoconstrictor angiotensin II. An additional property of ACE is that it breaks down bradykinin, which is a potent vasodilator, to inactive products (Luscher et al., 1993). Bradykinin activates endothelial receptors resulting in the formation of $PGI_2$ and NO. Therefore, ACE inhibitor therapy may have additional benefits because it not only inhibits angiotensin II formation, but it also increases local levels of bradykinin, resulting in increased $PGI_2$ and NO levels (Busse et al., 1993; Ito et al., 1995; Zhu et al., 1995). A study by Silberbauer et al. (1982)

showed the attenuation of the acute hypotensive effect of captopril in normotensive and hypertensive subjects after cyclooxygenase inhibition. An additional study by Nishimura et al. (1989) showed that after cyclooxygenase inhibition, the peripheral vasodilator effect of captopril in CHF patients was blunted, and the ACE-inhibitor-induced increase in plasma prostanoids was suppressed. Therefore ACE inhibitor therapy appears to be a valuable therapy for restoring normal endothelial function and $PGI_2$ synthesis since, in CHF patients, the stimulated endothelial release of vasodilatory prostaglandins appears to be diminished. However, almost all of the patients studied were already taking ACE inhibitors as part of their daily drug therapy. Since it is thought that ACE inhibitors are able to restore endothelial function by inhibiting the breakdown of bradykinin, we might have expected these CHF patients to have a shift in the NPY dose response curve in the presence of indomethacin. However, most of these patients were also taking 325 mg of aspirin daily as part of their medications. Thus, it is quite possible that the aspirin was blocking the cyclooxygenase and counteracting the effects of the ACE inhibitors. There were 2 patients in this study who were taking ACE inhibitors, but were not taking aspirin, and they had a shift in their dose response curve to NPY. It is possible in these 2 subjects, that the ACE inhibitors were in fact restoring their endothelial function. A study by Nakamura et al. (1994) found that the inhibition of angiotensin-converting enzyme potentiates endothelium-dependent vasodilation in the peripheral vasculature of patients with mild chronic heart failure, but that this is diminished after pretreatment with 500 mg of aspirin. The patients in the present study were on 325 mg of aspirin per day, and therefore we cannot be sure if this is enough to be blocking the cyclooxygenase enzyme

or not. Further studies need to be pursued in order to determine whether 325 mg aspirin is enough to block cyclooxygenase. Most CHF patients are treated with aspirin and therefore we studied these patients under real-life conditions.

The endothelium has been shown to exert a profound influence on the contractile state of the underlying vascular smooth muscle. Much evidence suggests that the vascular endothelium functions abnormally in heart failure. Several studies have demonstrated impaired relaxation in response to endothelium-dependent vasodilators in the peripheral and coronary circulation in heart failure. Kubo et al. (1991) measured the forearm blood flow during the intraarterial administration of the endothelium-dependent dilator methacholine in patients with heart failure and normal subjects. Increases in flow in response to methacholine were significantly depressed in patients with heart failure compared with normal control patients. In contrast, flow responses to the endothelium-independent dilator, nitroprusside were similar in the 2 groups. In another study by Katz et al. (1992), using transcutaneous Doppler ultrasonography to determine lower limb arterial blood flow velocity, acetylcholine and nitroglycerin were infused in the femoral artery of heart failure patients and normal age-matched subjects. Again, patients with heart failure had no response to acetylcholine in contrast to normal control patients, suggesting an endothelial defect in the peripheral microcirculation of the human. They found that endothelium-dependent responses were similarly impaired regardless of the etiology of heart failure. Drexler et al. (1992) studied the microvascular responses in the human forearm using high resolution A-mode and Doppler ultrasonography. They infused acetylcholine and nitroglycerine in heart failure patients and normals and found that the

acetylcholine responses were impaired in patients with heart failure, whereas responses to nitroglycerine were preserved.

In the present study, we determined that the normals had a functioning endothelium because infusion of acetylcholine resulted in venodilatation, however the CHF patients studied showed endothelial dysfunction because infusion of acetylcholine resulted in very little dilatation and even constriction in some subjects. It has been demonstrated that the response of blood vessels to exogenous acetylcholine depends on a balance between a direct vasoconstrictor action and an indirect, endothelium-dependent, vasodilator action (Collier and Vallance, 1990). In intact vessels, dilatation usually predominates due to the release of nitric oxide and prostaglandins. Once the endothelium is removed, the response to acetylcholine is usually constrictor due to a direct action on the vascular smooth muscle. It was found that in superficial veins, the maximum degree of acetylcholine-induced dilatation is in the order of 30% (Collier and Vallance, 1990). Therefore, in the present study it is normal that we only achieved 36% maximum dilatation in the normal subjects. A study by Vallance et al. (1989) showed that acetylcholine produced dilatation in human hand veins *in vivo*, and acetylcholine is known to cause dilatation by releasing nitric oxide. They also found that the venoconstriction induced by NE did not seem to be accompanied by much or any release of nitric oxide since L-NMMA had no effect on the action of NE. It is possible that acetylcholine is able to release nitric oxide in veins to produce dilatation, however other agonists such as NE and NPY release little or no nitric oxide, but rather release vasodilatory prostaglandins. There is also evidence that acetylcholine stimulates prostaglandin release, resulting in vasodilation (Tyagi et al., 1996). Thus, it is possible

that acetylcholine is causing dilatation of the veins by stimulating the release of vasodilatory prostaglandins. A study by Haefeli et al. (1993) tested whether spontaneously released nitric oxide modulates the venous tone in humans by studying the influence of methylene blue, an inhibitor of NO-mediated activation of guanylate cyclase, on the dose-response relationship of the $\alpha_1$-adrenergic agonist phenylephrine. They found that unlike in studies using vessels obtained from arterial beds in animals where coadministration of methylene blue substantially increased phenylephrine's potency (Martin et al., 1985; Vinet et al., 1991), there was no difference in efficacy and potency of phenylephrine after pretreatment with methylene blue in their studies using veins, thus demonstrating that NO is not released in veins in response to α-receptor stimulation.

Sodium nitroprusside is a potent, rapid-acting nitrovasodilator used clinically in hypertensive emergencies and heart failure. It is thought to produce its vasorelaxant action by releasing nitric oxide at or in the vascular smooth muscle cell, independent of the endothelium. The generated nitric oxide then activates soluble guanylate cyclase, catalyzing cGMP accumulation and resulting in relaxation (Murad, 1986; Ignarro, 1989). We infused the endothelium-independent vasodilator, sodium nitroprusside to ensure that the lack of vasodilation in CHF patients with acetylcholine was due to endothelial dysfunction, and not due to the inability of the vascular smooth muscle to dilate. Nitroprusside caused dose-dependent dilatation in all subjects studied, thus confirming that the lack of vasodilatation seen in the CHF patients was due to endothelial dysfunction.

$PGF_{2\alpha}$ was used to preconstrict the veins, rather than phenylephrine or NE because phenylephrine and NE have been shown to stimulate the release of vasodilatory

prostaglandins. Since we preconstricted the veins in order to look at dilatation, it would not be appropriate to preconstrict with an agonist that releases vasodilators. Therefore, we preconstricted with $PGF_{2\alpha}$ since it is a nonadrenergic agonist and thought not to stimulate the release of vasodilatory prostaglandins.

## 5.5 Summary

We have shown that the venoconstriction caused by stimulation of vascular smooth muscle NPY receptors is markedly increased during co-infusion of indomethacin in normal subjects. This increased responsiveness provides evidence for vasodilatory prostaglandin release, most likely $PGI_2$, secondary to NPY receptor stimulation. The vasodilatory prostaglandins are released in sufficient amounts to significantly antagonize the agonist-induced venoconstriction, and suggests one hypothesis that there exists a second population of NPY receptors located on the vascular endothelial cells, which mediate this prostaglandin release. Furthermore, CHF is associated with endothelial dysfunction resulting in a decreased release of vasodilatory prostaglandins and greater venoconstriction to NPY because there are no opposing vasodilators being released. Thus, NPY receptor responsiveness is determined by a balance between vascular smooth muscle and endothelial effects. In mild CHF, venoconstriction to NPY is exaggerated due to endothelial dysfunction. In severe CHF, the degree of downregulation of NPY receptors may be underestimated due to the effects of the endothelium.

# CHAPTER 6

# IS THE ALTERED NEUROPEPTIDE Y RESPONSIVENESS A RECEPTOR RELATED OR NONSPECIFIC VASCULAR SMOOTH MUSCLE DEFECT?

## 6.1 Introduction

### 6.1.1 Background Information

CHF is characterized by increased sympathetic nervous system activation with direct evidence of an increased muscular sympathetic outflow by microneurographic recording of muscle sympathetic nerve activity in patients with CHF (Leimbach et al., 1986). Significant elevation of sympathetic drive results in an increased level of plasma catecholamines which may result in excessive venoconstriction with increased venous tone and systemic venous return (Cohn et al., 1984). Evidence supports the existence of adrenergic receptors, particularly the $\alpha_1$ and $\alpha_2$ subtypes, located on venous smooth muscle cells (Blochl-Daum et al., 1991). Constant activation of these receptors in CHF may lead to an alteration in their responsiveness. It has been demonstrated in a rat model of heart failure induced by myocardial infarction, that vascular $\alpha_1$- and $_2\alpha$ -adrenoceptor responsiveness is decreased and $\alpha_2$-adrenoceptors of mesenteric arteries are downregulated (Feng et al., 1996). We have also found in our laboratory that in patients with severe CHF, *in vivo* $\alpha_1$- and $\alpha_2$-adrenoceptor responsiveness in the dorsal hand veins is decreased (Callow, 1995). Plasma NE levels were significantly elevated in these subjects, consistent with increased SNS activation. We have also recently found that NPY receptor responsiveness in severe CHF patients is decreased compared to normals when the "true" venoconstrictor response is examined (See Chapter 5). The decreased NPY receptor

response in patients with severe CHF relative to normal subjects may suggest a decreased vascular NPY receptor function in severe CHF.

### 6.1.2 Rationale

Although these findings suggest that the altered α and NPY receptor responsiveness seen in CHF are due to an alteration at the specific receptors, we could not be sure that the decreased responsiveness is not due to a nonspecific change at the smooth muscle level since changes in the blood vessels have long been recognised (Zelis and Flaim, 1982). In *in vitro* studies, it is known that the constrictor response to KCl in rat aortas is unchanged between normals and CHF (Feng et al., 1996) but KCl is unsuitable for use in humans. $PGF_{2\alpha}$ causes vascular smooth muscle constriction independent of the α receptor as demonstrated by DuCharme and Weeks (1966) in the unanesthetized rat after ganglion blockade and after pretreatment with reserpine. Although flow mediated vascular responsiveness is decreased in humans with CHF, there has been little published regarding nonadrenergic mediated vasoconstriction *in vivo*. If the decreased responsiveness to α agonists and NPY in severe CHF is in fact due to altered receptor responsiveness and not due to a nonspecific change in the smooth muscle, then the responsiveness to the venoconstrictor $PGF_{2\alpha}$ will not be significantly different in CHF patients compared to normals.

### 6.1.3 Hypothesis

*In vivo* venoconstriction to $PGF_{2\alpha}$ is unaltered in CHF patients compared to age-similar normal subjects, suggesting that the decreased NPY responsiveness previously seen

(Chapter 5) is due to a receptor-related effect rather than a non-specific vascular smooth muscle effect.

## 6.2 Methods

*Subjects*

Twelve patients (10 males, 2 females) with clinical and physical signs of chronic systolic heart failure were studied (Table 6.1). The subjects were divided according to LVEF, the same as previously described in Chapter 5. Subjects met the same criteria and their medications were held the same as described in Chapter 5.

Six age-similar normal subjects (4 males, 2 females) with no evidence of heart disease as assessed by medical history, physical examination, and electrocardiogram, were studied for comparison to the CHF patients. All subjects were non-smokers, did not have a history of hypertension and no subjects were on any vasoactive medications.

*Study Design*

Hand vein tonometry was employed using the LVDT technique as outlined in Chapter 2, to measure the local venous responsiveness to $PGF_{2\alpha}$ in the absence of systemic or reflex responses.

On the study morning, a short (1.9 cm) 25-gauge butterfly needle (Butterfly-Abbocath) was inserted into a suitable dorsal hand vein with a long straight section and no visible tributaries. Normal saline (0.9%) was infused through the needle at 0.4 ml/min for 30 minutes. During this equilibration period, several measurements of hand vein distention at 45 mmHg were made to ensure a stable baseline and the average taken as the control

**Table 6.1 Characteristics of Patients with Congestive Heart Failure and Age-Similar Normal Controls**

| Groups | Age (yrs.) | Sex (Male/Female) | LVEF (%) | NYHA Class |
|---|---|---|---|---|
| Normal (n=6) | 63±4 (52-73) | 4/2 | not measured | not applicable |
| LVEF>20% (n=6) | 65±4 (52-82) | 5/1 | 28.8±1.8 | II-IV |
| LVEF≤20% (n=6) | 62±4 (47-73) | 5/1 | 18.0±1.0 | II-IV |

Results given as mean ± sem.

Abbreviations: LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

distention. Thirty minutes after insertion of an i.v. needle and before any drug infusions, a blood sample (15 ml) was drawn for the measurement of plasma catecholamines and NPY. Following this, sequential graded local 15 minute i.v. infusions of $PGF_{2\alpha}$ (1, 4, 16, 64, 256, 512, 1024 ng/min) were commenced to constrict the dorsal hand vein and cumulative dose-response curves were constructed with an upper arm occlusion cuff inflated for the last 2 minutes of each dose step.

## 6.3 Results

The group of severe CHF patients had a significant increase in heart rate compared to normal controls ($p < 0.05$), and while the group of moderate CHF patients also had a slightly elevated heart rate compared to normals, it was not significant. Mean arterial pressure was not significantly different between CHF patients and normal controls (Table 6.2). Plasma NE levels were elevated in patients with severe CHF when compared to normal controls but were not significantly altered in mild to moderate CHF patients. Plasma NPY levels demonstated a trend of increased levels in the CHF patients compared to normal controls, but not significantly (Table 6.2). No significant changes in blood pressure or heart rate were seen in any groups throughout the duration of the experiment.

Basal vein diameter at 45 mmHg venous occlusion pressure during 0.9% saline infusion, in normals and patients with chronic heart failure, were not significantly different (Table 6.2). Graded infusion of $PGF_{2\alpha}$ induced dose-dependent venoconstriction in all subjects studied. The mean dose-response curve for $PGF_{2\alpha}$ was not significantly different in mild to moderate and severe CHF patients from the normal controls (Figure 6.1).

**Table 6.2 Mean Arterial Pressure, Heart Rate, Basal Plasma Norepinephrine and NPY Levels, and Basal Hand Vein Diameter**

| Groups | Mean Arterial Pressure (mmHg) | Heart Rate (bpm) | Plasma NE (pmol/L) | Plasma NPY (pmol/L) | Basal Vein Diameter (mm) |
|---|---|---|---|---|---|
| Normal (n=6) | 96±5 | 59±3 | 1073±160 | 131±5 | 1.04±0.21 |
| LVEF>20% (n=6) | 92±4 | 67±7 | 1541±184 | 136±6 | 1.29±0.28 |
| LVEF≤20% (n=6) | 92±5 | 77±6* | 3085±680** | 139±9 | 1.12±0.22 |

Results given as mean ± sem, *p<0.05, **p<0.01 vs. Normal.
Abbreviations: LVEF, left ventricular ejection fraction; NE, norepinephrine; NPY, Neuropeptide Y.

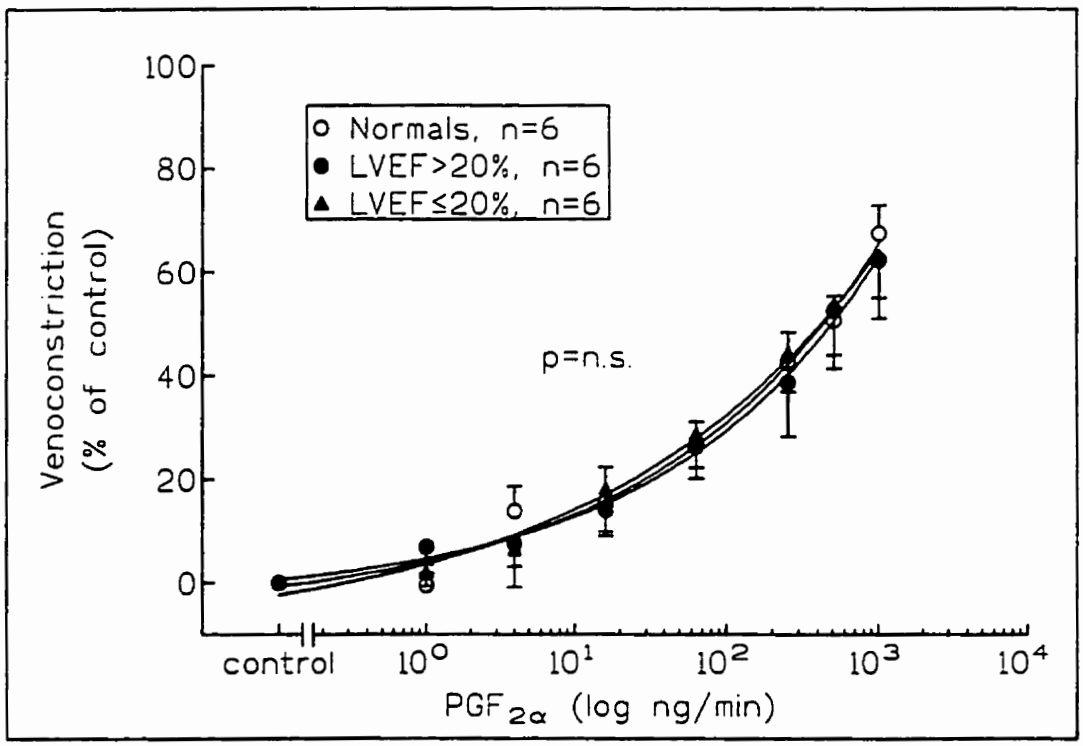


**Figure 6.1** Average response to the non-adrenergic venoconstrictor, $PGF_{2\alpha}$, in the dorsal hand veins of age-similar normal subjects, mild to moderate CHF patients, and severe CHF patients.

## 6.4 Discussion

The main objective of this study was to determine whether the decreased NPY responsiveness previously seen in severe CHF patients was due to a receptor-related defect or a non-specific vascular smooth muscle defect. Using sequential graded intravenous infusions of $PGF_{2\alpha}$, we demonstrated that the venoconstrictor response to $PGF_{2\alpha}$ was not significantly different between CHF patients and normal controls, thus suggesting that the decreased responsiveness is not a non-specific defect of vascular smooth muscle. In a previous study (Chapter 4), we demonstrated that NPY responsiveness was increased in mild to moderate CHF patients compared to normal controls, and that NPY responsiveness in patients with severe CHF was unchanged compared to normals but significantly decreased compared to mild to moderate CHF patients. Subsequently, (Chapter 5), we demonstrated that when the "true" NPY venoconstrictor response is determined without opposing vasodilation from prostaglandin release, the NPY responsiveness in mild to moderate CHF patients is unaltered from normal subjects but that the NPY responsiveness in severe CHF patients is significantly decreased compared to normals possibly due to downregulation of NPY receptor responsiveness. This decreased venous NPY receptor responsiveness may serve to attenuate venoconstriction in patients with severe CHF where chronic sympathetic activation exists.

It has been shown that the activity of $PGF_{2\alpha}$ in the rat is not mediated through an alteration of sympathetic vasoconstrictor activity since $PGF_{2\alpha}$ activity persists after the acute administration of the ganglion-blocking agent, pentolinium, and the chronic administration of the catecholamine-depleting agent, reserpine (DuCharme et al., 1968).

The same study also demonstrated that the effect of $PGF_{2\alpha}$ on vascular volume in the dog was not affected by the alpha adrenergic blocking agent, phenoxybenzamine. Mark et al. (1971) have demonstrated that saphenous venomotor responses to $PGF_{2\alpha}$ in dogs are not dependent on the integrity of the sympathetic nervous system. In that study, the use of phentolamine, reserpine, and hexamethonium did not reduce responses to $PGF_{2\alpha}$. Therefore, they concluded that saphenous venomotor responses to $PGF_{2\alpha}$ are direct effects which do not result from the stimulation of alpha-adrenergic receptors, liberation of catecholamines, or activation of sympathetic reflexes.

A variety of non-adrenergic, non-NPY agonists, including angiotensin II, and endothelin, could be used to further demonstrate that the decreased responsiveness to NPY in severe CHF is not due to a vascular smooth muscle defect. However, $PGF_{2\alpha}$ is the only one of these agonists that is approved for intravenous use in humans in Canada and therefore was the only agonist used in this study.

There is little information published regarding the constrictor effects of $PGF_{2\alpha}$ *in vivo*. However, Robinson et al. (1973) studied the effects of local infusions of $PGF_{2\alpha}$ in superficial hand veins of man. They found that $PGF_{2\alpha}$ caused a dose-dependent constriction of the vein when infused over the dose range of 100 - 500 ng/min. They also found that the constrictor effect developed and waned slowly and therefore they needed to infuse each dose for 15 minutes. They found that the venoconstrictor effect of $PGF_{2\alpha}$ in man is similar to its effects on capacitance vessels in the dog hind-limb (Greenberg and Sparks, 1969) and the canine pulmonary vein (Hyman, 1969). Because of these findings, we chose to infuse each dose of $PGF_{2\alpha}$ for 15 minutes, whereas the other studies in this

thesis, using NPY, infused each dose for 5 minutes. By doing so, we could be sure that we were infusing the agonist into the vein for a sufficient amount of time to see its full effects. We chose the highest dose to be twice as much as previously infused in order to get a more complete dose response curve than what they had previously achieved. Despite this, we did not achieve full dose-response curves though no systemic effects were observed. $PGF_{2\alpha}$ is normally given at a dose of 40 mg for systemic effects and it has been estimated that the dose required to produce a response in a single vein is about 1000 times less than that required systemically (Robinson, 1978). In the present study, the amount of $PGF_{2\alpha}$ that was infused over the duration of the study was 28 $\mu$g (1400 times smaller than that required for a systemic effect) and thus future studies should be able to infuse higher doses.

Thromboxane $A_2$ ($TXA_2$) and prostaglandins are cyclo-oxygenase metabolites of arachidonic acid that constrict or dilate the vasculature, thus playing an important role in the regulation of the local circulation. $PGF_{2\alpha}$ is a potent constrictor of vascular smooth muscle and has been shown to do so in cerebral, coronary, renal, and mesenteric arteries of various mammals (Ellis et al., 1977; Toda and Miyazaki, 1978; Hayashi et al., 1986). Hanasaki and Arita (1989) found that there are 2 binding sites for prostanoids in rat cultured aortic vascular smooth muscle cells. There is a binding site for $TXA_2$ and a common binding site for prostaglandins. $PGF_{2\alpha}$ is known to act through $PGF_{2\alpha}$-specific processes to cause contraction by stimulating $IP_3$ formation and subsequent $Ca^{2+}$ mobilization (Smith et al., 1988). It is thought that $PGF_{2\alpha}$ functions via a receptor coupled to an $N_p$-like guanine nucleotide regulatory protein as well as acting at thromboxane $A_2$

receptors (Smith et al., 1988). A study by Dorn et al. (1992) found that the pharmacologic blockade of vascular smooth muscle $TXA_2$ receptors prevented the contraction induced by $PGF_{2\alpha}$, thus demonstrating that $PGF_{2\alpha}$ acts by binding to the thromboxane receptor. There is no evidence to date that thromboxane receptors function abnormally in CHF. NPY and $PGF_{2\alpha}$ act at different receptors coupled to different intracellular second messenger systems, therefore the defect could be at the NPY receptor level or at one of the intracellular levels such as a defect in the functioning of cAMP or adenylyl cyclase.

## 6.5 Summary

We have shown that the venoconstriction caused by infusion of the non-adrenergic, non-NPY mediated venoconstrictor, $PGF_{2\alpha}$ is unchanged in mild to moderate and severe CHF patients compared to age-similar normal subjects. Thus, this provides evidence that the decreased responsiveness to NPY described previously in severe CHF (Chapter 5) is not a result of a vascular smooth muscle defect, but rather is due to an NPY receptor related defect.

# CHAPTER 7

# ARE VASODILATORY PROSTAGLANDINS RELEASED UPON NPY RECEPTOR STIMULATION OR ARE THEY RELEASED AS A NONSPECIFIC RESPONSE TO VASOCONSTRICTION?

## 7.1 Introduction

### 7.1.1 Rationale

*In vivo*, we have provided evidence for vasodilatory prostaglandin release secondary to NPY receptor stimulation in sufficient quantities to significantly antagonize the agonist-induced venoconstriction. This finding has important implications in diseases with endothelial dysfunction such as CHF, hypertension, hypercholesterolemia, and diabetes.

In order to confirm that the vasodilatory prostaglandins are being liberated specifically through NPY receptor stimulation and are not always present in the background or released as a nonspecific response to smooth muscle constriction, we used a nonadrenergic, non-NPY venoconstrictor ($PGF_{2\alpha}$) in the absence and presence of indomethacin, in order to determine if this also results in a shift of the curve to the left. $PGF_{2\alpha}$ causes vascular smooth muscle constriction independent of the $\alpha$ receptor as demonstrated by DuCharme and Weeks (1966). If the liberation of vasodilatory prostaglandins is in fact due to NPY receptor stimulation and is not present prior to agonist addition, then the responsiveness to the venoconstrictor $PGF_{2\alpha}$ will not be significantly different in the absence and presence of the prostaglandin synthase inhibitor, indomethacin in normal subjects.

### 7.1.2 Hypothesis

$PGF_{2\alpha}$ stimulation does not result in the release of vasodilatory prostaglandins and its dose-response curve for venoconstriction will not be significantly shifted to the left in the

presence of indomethacin, an inhibitor of PG production.

## 7.2 Methods

*Subjects*

Ten normal subjects (4 males, 6 females), aged 39.6 $\pm$ 7.5 yrs. participated in the study. All subjects had no previous history of cardiac disease, had normal ECG's and blood pressure and were non-smokers not on any vasoactive medications. Subjects avoided consuming caffeine and alcohol containing beverages for at least 12 hours prior to the study but were permitted a light breakfast on the morning of the study and all emptied their urinary bladders prior to commencing the study. Subjects with a known history of sensitivity to aspirin (due to the potential cross-sensitivity to indomethacin) or indomethacin were excluded from participating in the study.

*Study Design*

LVDT methodology was employed to measure changes in venous diameter in response to $PGF_{2\alpha}$ stimulation using the identical protocol to that described in Chapter 5. Following initial baseline measurements of venous distention, an i.v. infusion of either saline placebo or indomethacin (randomly determined) was administered (0.2 ml/min) through the most distal butterfly needle, while increasing doses of $PGF_{2\alpha}$ were subsequently given (0.2 ml/min) through the most proximal needle. On the second study day, increasing concentrations of $PGF_{2\alpha}$ were again administered in conjunction with either the saline placebo or indomethacin (whichever was not given on the first day) with measurements taken as described in the general protocol (Chapter 2).

## 7.3 Results

Initial baseline venous diameter during indomethacin co-administration was not significantly different from saline placebo (Table 7.1). Graded infusions of $PGF_{2\alpha}$ induced dose-dependent venoconstriction during co-infusion of both saline placebo and indomethacin, in all subjects studied. The mean dose-response curve obtained during indomethacin infusion was not significantly different compared with saline placebo (Figure 7.1).

Resting blood pressure and heart rate were not significantly different between study days (Table 7.1), and were not significantly altered throughout the duration of the studies in the subjects.

## 7.4 Discussion

The main objective of this study was to determine whether vasodilatory prostaglandins influence the $PGF_{2\alpha}$-mediated venoconstrictive effects on superficial dorsal hand veins of normal subjects. Using sequential graded intravenous infusions of $PGF_{2\alpha}$ in conjunction with the prostaglandin synthesis blocker indomethacin, we demonstrated that the venoconstriction elicited by $PGF_{2\alpha}$ was not significantly increased over that obtained during co-infusion of saline placebo in the normal subjects studied. Thus, this demonstrates that basal release of vasodilatory prostaglandins is very low or negligible and that their release is not stimulated upon $PGF_{2\alpha}$ stimulation. $PGF_{2\alpha}$ is known to be a nonadrenergic vasoconstrictor (described in Chapter 6) and therefore this study demonstrates that in the previous studies with NPY or other adrenergic agonists, we were

**Table 7.1 Mean Arterial Pressure, Heart Rate, and Basal Hand Vein Diameter**

| Groups | Mean Arterial Pressure (mmHg) | Heart Rate (bpm) | Basal Vein Diameter (mm) |
|---|---|---|---|
| Saline Placebo (n=10) | 84±4 | 64±3 | 0.62±0.1 |
| Indomethacin (n=10) | 82±4 | 65±3 | 0.61±0.1 |

Results given as mean ± sem.

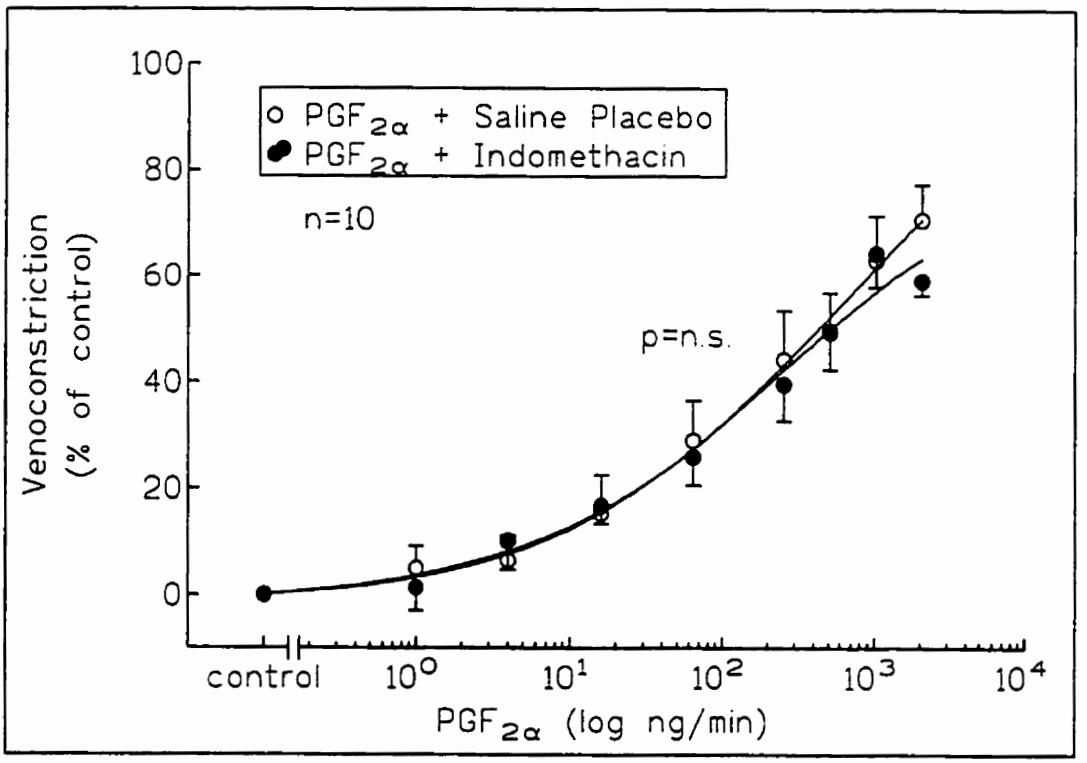


**Figure 7.1** Average response to the non-adrenergic venoconstrictor, $PGF_{2\alpha}$, in the dorsal hand veins of normal subjects in the presence and absence (saline placebo) of indomethacin.

not simply blocking basally released prostaglandins present prior to agonist. Since there was no release of vasodilatory prostaglandins with $PGF_{2\alpha}$ stimulation, this suggests that the release is specific to those receptors being stimulated and not a nonspecific response to vasoconstriction.

In the previous study, using $PGF_{2\alpha}$, we did not achieve full dose response curves, and therefore in this study, we increased the highest dose that we infused to 2048 ng/min from 1024 ng/min. In this study, the dose-response curves for some individuals reached a plateau, however, when all the individual curves were averaged together, a complete sigmoidal curve was not achieved. Thus, it may have been beneficial to have increased the infusion doses even more. Although the dose response curves obtained are not complete ones, we can still conclude that the curves are not significantly different between the indomethacin and the saline infusions. All but one subject achieved $\geq$ 57% constriction.

This study was only performed in normal subjects because we previously showed that the release of vasodilatory prostaglandins is blunted in CHF patients. Therefore, since the purpose of this study was to determine if the release of vasodilatory prostaglandins occurs upon NPY receptor stimulation or whether they are present in the background, it would not be appropriate to study a group of subjects that we already know don't have release of vasodilatory prostaglandins.

## 7.5 Summary

We have shown that the venoconstriction caused by the non-adrenergic, non-NPY

agonist, $PGF_{2\alpha}$ is not influenced during co-infusion of indomethacin in normal subjects. Thus, this provides evidence for no release of vasodilatory prostaglandins with $PGF_{2\alpha}$ stimulation suggesting that the release to NPY and adrenergic stimulation is specific to those receptors being stimulated and not a nonspecific response to vasoconstriction.

# CHAPTER 8

# CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

## 8.1 Introduction

Traditionally, the control of peripheral vascular tone has been attributed to the SNS, resulting in smooth muscle contraction through the release of specific neurotransmitters, one of which more recently recognised is NPY, which act at receptors located in the membrane of the innervated tissue. About 20 years ago, the endothelial cells were found to release many substances that can either relax or contract the smooth muscle. It is now known that these vasoactive factors are released from the endothelial cells in response to a variety of stimuli including hormonal stimulation. Thus, control of vascular smooth muscle tone can now be regarded as a function of at least 2 regulatory systems working together: the SNS and the endothelium. Alterations in the activity of these 2 systems would be expected to result in corresponding changes in the responsiveness of the NPY receptors. Such changes in receptor responsiveness have important implications in disease states in which these modulating systems are chronically altered, such as CHF. There have been several studies which have investigated changes in the arterial side of the circulation, however, despite the importance of the venous system in the regulation of venous return and cardiac output, few studies have focused on alterations in receptor function in these vessels. The studies presented in the previous chapters have attempted to provide important information regarding the changes in venous NPY receptor responsiveness during the increased SNS activity that occurs in CHF, and the possible role

of alterations in endothelial function.

Several conclusions can be made based on the reported results.

1) The first objective was to determine if NPY receptors were present in the dorsal hand veins of normal subjects and if the infusion of NPY produces dose-dependent venoconstriction. The results showed that NPY produced dose-dependent venoconstriction in the dorsal hand veins of all but one subject. This result is consistent with the presence of NPY receptors on vascular smooth muscle cells.

2) Additional observations showed that the responsiveness to NPY was decreased with increasing age. This result is consistent with other findings of decreased responsiveness of receptors that are coupled to the cAMP-adenylate cyclase second messenger cascade system. This result was important as it demonstrated the importance of only using age-similar control subjects in future studies where normals are compared to CHF patients.

3) The second objective was to determine in CHF patients whether NPY receptors are functioning normally or are hypo- or hyper-responsive. These results showed that in patients with mild to moderate LV dysfunction, NPY responsiveness was increased compared to age-similar normal controls. However, in patients with CHF and severe LV dysfunction, venous NPY responsiveness was not different from normal controls but was significantly decreased compared to patients with mild to moderate CHF. The mechanism of this increased NPY responsiveness in

mild to moderate CHF was not known, but it is most likely not due to receptor upregulation since sympathetic activity and NPY release are not decreased in these patients. Involvement of endothelial released hormones was subsequently postulated as contributing to the increased response based on previous research in our lab with $\alpha$ receptors.

4) The third objective was therefore to determine whether NPY stimulation promotes the release of endothelial vasodilator autacoids, mainly $PGI_2$, which may influence NPY receptor responsiveness, and to determine if this is altered in CHF patients. The results consistently showed that NPY responsiveness was significantly increased in the presence of indomethacin in normal subjects, most likely due to the blockade of $PGI_2$ synthesis and release. Since PGI is predominantly synthesized and released by endothelial cells, our results would suggest that there may be a second population of NPY receptors that exist on the vascular endothelium, in addition to those located on the vascular smooth muscle. In addition, our results showed that the NPY responsiveness in CHF patients was unaltered in the presence of indomethacin, thus suggesting endothelial dysfunction, and therefore a lack of vasodilatory autacoids being released upon stimulation. Thus, when the "true" NPY response is seen when the vasodilatory prostaglandins are blocked by indomethacin, the response seen in mild to moderate CHF patients is unaltered from normals, but the response in severe CHF patients is significantly decreased compared to normals and mild to moderate CHF patients.

5) Although these results showed that NPY responsiveness is decreased in severe CHF patients, we could not be sure that this is due to a receptor-related defect and not due to a nonspecific vascular smooth muscle defect. Thus, we used the non-adrenergic, non-NPY venoconstrictor, $PGF_{2\alpha}$ in normals and CHF patients to determine whether the responsiveness is altered with this agonist in CHF patients. The results showed that the responsiveness to $PGF_{2\alpha}$ was not significantly different in CHF patients compared to normal controls, thus demonstrating that the decreased responsiveness to NPY in CHF patients was in fact due to an abnormal response related to the receptor rather than smooth muscle.

6) Although we previously concluded that NPY stimulates endothelial receptors to release vasodilatory prostaglandins to counteract the vasoconstrictor response to NPY in normal subjects, we could not be sure that the release is specific to those receptors being stimulated and not a nonspecific response to vasoconstriction. Thus, to answer this question, we infused $PGF_{2\alpha}$ in the absence and presence of indomethacin and found that the response to $PGF_{2\alpha}$ was unaltered in the presence of indomethacin as compared to saline placebo. Therefore, these results demonstrate that $PGF_{2\alpha}$ and smooth muscle constriction does not stimulate the release of vasodilatory prostaglandins supporting our hypothesis that their release is NPY mediated.

7) Therefore, we conclude that both SNS activity and endothelial function act together to significantly modulate venous NPY receptor mediated responsiveness.

## 8.2 Limitations

In all of the studies, especially those in heart failure patients, medications were withheld for 5 times the plasma half-life of the drug and all subjects were instructed to refrain from taking caffeine or alcohol containing substances for at least 12 hours prior to the study. While patient compliance with these criteria was reported to be complete, the possibility that lingering pharmacological effects of these drugs, while probably minimal, or patients not following instructions properly, cannot be excluded. Because the medications were withheld for 5 times the plasma half-life of the drug, any involvement of the medications is unlikely though local tissue binding might prolong the duration of their effects. In addition, while the alternative *in vivo* effects of indomethacin are unlikely as discussed in Chapter 5, they cannot be definitively excluded.

Although the LVDT method of assessing venous responsiveness has been shown to be representative of the general vasculature with respect to the $\alpha$ receptor, comparisons of superficial vein responsiveness to local concentrations of drugs with deep or splanchnic veins have not, to our knowledge, been investigated. Thus, further experiments are needed in order to determine whether superficial venous responsiveness is representative of these veins. In addition, although some evidence exists to suggest that NPY receptor responsiveness in cutaneous veins is similar to those of the arteries, more research is

necessary to confirm these conclusions. Thus, we cannot exclude the possibility that responses we have observed may be different in other components of the circulation.

In the studies involving the infusion of NPY and $PGF_{2\alpha}$, full dose response curves were not obtained. Thus for NPY, we compared the curves and did not compare $ED_{50}$ values between the groups of subjects. Despite this difficulty, we could still detect a significant difference between the dose response curves obtained demonstrating altered responsiveness. In the future, it would be useful to infuse a greater concentration to obtain full dose-response curves. The doses that we did infuse were greater than those previously used in the literature.

Although the LVDT technique is a useful method to assess the direct effects of drugs by local infusion into the vein being studied, little information is given regarding the mechanisms associated with the responses observed. Thus, although we observed a decreased responsiveness to NPY in severe CHF patients, we did not directly assess the mechanisms involved, although it most likely results from receptor downregulation or an alteration in intracellular coupling mechanisms or a combination of both. Thus, further experiments need to be done in order to clarify the specific mechanism involved.

## 8.3 Suggestions for Future Research

The studies presented in this thesis have attempted to describe the modulation in venous NPY receptor responsiveness that are associated with alterations in the activity of the SNS as well as the interaction of these receptors with endothelial released mediators, as both

have been shown to control cardiovascular function. The original objectives of this thesis have been met, however additional questions have arisen which demonstrate the necessity for future research to be done in this area. Some possible areas that need further investigation are outlined below.

SNS activity and endothelial released mediators have been shown to be important modifiers of venous NPY receptor responsiveness. However, as mentioned previously, these results may possibly be different in deep or splanchnic venous beds and also in arteries. Thus, additional *in vivo* studies need to be performed to confirm the results of this thesis in these alternate vascular beds, although this would be technically much more difficult than in superficial veins.

The importance of the endothelium in NPY receptor-mediated events has been clearly demonstrated in this thesis. Vasodilatory prostanoids have been shown to play an influential role in the responses obtained to NPY in normal subjects. Although research suggests that the vasodilatory prostanoid being released is most likely $PGI_2$, in order to be certain, it would be beneficial to measure $PGI_2$ levels in the blood both before and after NPY receptor stimulation. This would enable us to see if there is indeed an increased release of $PGI_2$ upon NPY receptor stimulation. However, this is not easy to do, as explained previously in Chapter 5.

Furthermore, these studies have shown little about the mechanisms associated with $PGI_2$ release secondary to NPY receptor stimulation. The goal of this thesis was to initially determine if receptor responsiveness was altered, rather than identifying the mechanisms involved. The intracellular mechanisms associated with $PGI_2$ activity have

not yet been determined and therefore it is important to determine what these mechanisms are. It seems likely that endothelial cells contain NPY receptors in their cell membranes, which mediate the release of $PGI_2$ most likely through the same intracellular pathways that these receptors exhibit in the vascular smooth muscle.

While this thesis has provided important results regarding exogenously infused NPY and the release of vasodilatory prostaglandins, it is important to determine whether endogenously released substances also stimulate the release of prostaglandins since these would be at physiological doses, rather than the pharmacological doses that we infused. Thus, upon giving physiological stimuli such as lower body negative pressure, mental arithmetic and the cold pressor test in the presence and absence of indomethacin, we could determine whether the release of endogenous agonists such as NE and NPY are able to release vasodilatory prostaglandins that would be blocked by indomethacin.

Our *in vivo* studies suggest that NPY receptor stimulation promotes the release of vasodilatory prostaglandins, most likely $PGI_2$, which antagonize the NPY receptor-mediated constriction of vascular smooth muscle. Since early studies suggest the endothelium is the major site of production and release of vasodilator prostaglandins, our results may be consistent with the existence of NPY receptors within the vascular endothelium. Alternatively, as recent research suggests that smooth muscle itself is capable of releasing appreciable amounts of vasodilatory prostaglandins, albeit less than the endothelium, the possibility exists that stimulation of smooth muscle NPY receptors also results in the direct release of prostaglandins or an intermediary which promotes prostaglandin release. It is not currently possible *in vivo* to selectively stimulate NPY

receptors which may be located on the endothelium without stimulating those on the vascular smooth muscle. Thus, we could study isolated vessels *in vitro*, where vessels can be examined with and without (denuded) an intact endothelium, in order to identify the predominant source of $PGI_2$ release to NPY stimulation. In addition, such studies could also be performed to assess whether $PGI_2$ release secondary to NPY receptor stimulation occurs in arterial vessels to a similar extent as that seen in veins.

In addition, diseases other than CHF such as hypercholesterolemia, atherosclerosis, and diabetes are known to affect endothelial function. The results in this thesis have demonstrated that CHF affects endothelial function which results in decreased vasodilatory prostaglandins being released from the endothelium. It would be interesting in the future, to investigate the effects that these other diseases have on NPY receptor function.

Therefore, in conclusion, this thesis has provided important information on venous NPY receptor modulation as it is clearly influenced by SNS activity and endothelial released mediators. While these findings regarding NPY responsiveness *in vivo* are important in their own right, they also provide an avenue for important future research.

# APPENDIX

## Study #1

## LETTER OF INFORMATION FOR NORMAL SUBJECTS

### Neuropeptide Y in the Control of Venous Tone in Normals and Patients with Heart Failure.

**J.M.O. Arnold, M.D., Q.P. Feng, M.D., I.D. Callow, B.Sc., M.L. Lambert, B.Sc.**

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A disease such as congestive heart failure influences vascular function. The purpose of the study is to determine the responsiveness in your hand veins to two compounds: neuropeptide Y and noradrenaline. Neuropeptide Y and noradrenaline are natural hormones released at nerve terminals in your body. Both compounds cause short-lived constriction or narrowing of blood vessels. The infusions of very small amounts of these two drugs in your hand vein will result in changes in vein diameter which will be measured by a device resembling a small tripod that can be positioned to rest on the back of the hand while applying a low pressure in a blood pressure cuff. Your results will be compared to those of a patient with heart failure.

A medical history, physical examination and standard ECG will be done prior to the study. It is required that all participants should abstain from drinking any alcohol or caffeine-containing beverages for at least 24 hours prior to the study. On the study morning, you will be allowed to lie down and rest in a quiet room where the remainder of the study will be performed. Throughout the study you will be lying down with your upper arm slightly elevated. Your hand will be placed comfortably on a padded support at the level of your shoulders. The veins in the hand are distended slightly by applying a very mild pressure around the upper arm using a blood pressure cuff. The increases in pressure will not affect the flow of the blood into or out of the arm and may result in a slight sensation of fullness in the limb. A second cuff will be placed around the other arm to allow us to monitor your heart rate and blood pressure throughout the study. A small needle will be inserted into a vein on the back of your hand from which a sample of blood (approximately a tablespoon full) is taken to allow measurement of natural hormones in your blood. This needle will be used for the remainder of the study. There may be a small discomfort associated with the insertion of the needle. Aseptic technique will be used to avoid any risk of infection and it will be inserted when you are lying on the bed to avoid fainting. Neuropeptide Y will be sterilized in the hospital pharmacy prior to use. This process will be done using aseptic technique to avoid any risk of infection and cultures will be done to test the sterility of the drug prior to use. Bruising will be minimised by applying firm pressure over the vein upon removal of the needle.

The study will be carried out on two separate days with at least 3 days but less than 7 days in between. On the first day, neuropeptide Y will be infused at a very low concentration and a slow infusion rate and the amount will be increased at intervals of 5 minutes until the hand vein has constricted. We will then infuse saline for at least 60 minutes to allow the effects of neuropeptide Y to disappear. These data will be used to calculate a

very low-dose (this dose, by itself, will not cause constriction of the vein but may enhance or increase the effects of noradrenaline) of neuropeptide Y to be used on the second study day. In a similar manner, on the second study day, noradrenaline will be infused with increasing doses at 5 minute intervals until the vein has constricted. This will again be followed by a 60 min. period of saline infusion to allow the effects of noradrenaline to disappear. We will then repeat the infusion of increasing doses of noradrenaline but will do so in conjunction with an infusion of the very low-dose of neuropeptide Y calculated on the first study day. In the middle of each five minute interval the cuff pressure will be inflated to allow measurement of any changes in the vein diameter, after which the cuff is immediately deflated. The amounts of neuropeptide Y and noradrenaline infused are extremely small in an effort to produce only local constriction of your hand vein without any effects on other blood vessels in your body. Mild associated changes in blood pressure and heart rate may occur with much larger doses of noradrenaline and neuropeptide Y. Your blood pressure and heart rate will be monitored every 5 minutes by an automated blood pressure recorder on your opposite arm. Should the pulse rate rise above 120 beats/min or fall below 50 beats/min, or if the blood pressure is greater than 160/90 mmHg or less than 80/60 mmHg the infusions will be discontinued immediately. Since the drugs are short acting, any change in blood pressure or heart rate should recover rapidly. Large changes are not likely to occur in the present study since you will be monitored continuously and very closely. By giving drug doses which are effective only locally in your hand vein, it is extremely unlikely we will produce any significant change in your blood pressure and heart rate.

The approximate time of the study each day will be 4 hours (total 8 hours). There will be no direct benefit to you by being involved in this study. You will not be identified by name in any future scientific communications concerning this study and records will be carefully kept in a locked office within the hospital. If you are already participating in another research project at this time, you must inform the investigator promptly to determine whether it is appropriate to begin participation in this study. You may refuse to participate and are free to withdraw at any time with no effect on your standing in school or future medical care. Should you experience any symptoms which you believe are the result of the drug or if you simply wish to leave the study for any other reason at any time your participation will be discontinued. Your travel expenses and parking costs will be reimbursed.

Your questions concerning the nature of the study should be answered to your satisfaction before you sign the consent form. If you have any further questions about the study, please do not hesitate to ask. You may contact Dr. Arnold at his office in Victoria Hospital (667-6650).

The UNIVERSITY of WESTERN ONTARIO
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REVIEW BOARD FOR HEALTH SCIENCES RESEARCH INVOLVING HUMAN SUBJECTS

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THE REVIEW BOARD HAS EXAMINED THE RESEARCH PROJECT ENTITLED:
"Neuropeptide Y in the control of venous tone in normals and patients with heart failure."

REVIEW NO: 4003R

AS SUBMITTED BY: Dr. J.M.O. Arnold, Medicine, Victoria/University Hospital

AND CONSIDERS IT TO BE ACCEPTABLE ON ETHICAL GROUNDS FOR RESEARCH INVOLVING HUMAN SUBJECTS UNDER CONDITIONS OF THE UNIVERSITY'S POLICY ON RESEARCH INVOLVING HUMAN SUBJECTS.

APPROVAL DATE: 22 October 1996 (see correspondence dated Oct.15/96)

AGENCY:

TITLE:

Bessie Borwein

Bessie Borwein, Chairman

c.c. Hospital Administration

## Study #2

## LETTER OF INFORMATION FOR CHF PATIENTS

### Neuropeptide Y in the Control of Venous Tone in Normals and Patients with Heart Failure.

**J.M.O. Arnold, M.D., Q.P. Feng, M.D., I.D. Callow, B.Sc., M.L. Lambert, B.Sc.**

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A disease such as congestive heart failure influences vascular function. The purpose of the study is to determine the responsiveness in your hand veins to two compounds: neuropeptide Y and noradrenaline. Neuropeptide Y and noradrenaline are natural hormones released at nerve terminals in your body. Both compounds cause short-lived constriction or narrowing of blood vessels. The infusions of very small amounts of these two drugs in your hand vein will result in changes in vein diameter which will be measured by a device resembling a small tripod that can be positioned to rest on the back of the hand while applying a low pressure in a blood pressure cuff.

A medical history, physical examination and standard ECG will be done prior to the study. It is required that all participants should abstain from drinking any alcohol or caffeine-containing beverages for at least 24 hours prior to the study. On the study morning, you will be allowed to lie down and rest in a quiet room where the remainder of the study will be performed. Throughout the study you will be lying down with your upper arm slightly elevated. Your hand will be placed comfortably on a padded support at the level of your shoulders. The veins in the hand are distended slightly by applying a very mild pressure around the upper arm using a blood pressure cuff. The increases in pressure will not affect the flow of the blood into or out of the arm and may result in a slight sensation of fullness in the limb. A second cuff will be placed around the other arm to allow us to monitor your heart rate and blood pressure throughout the study. A small needle will be inserted into a vein on the back of your hand from which a sample of blood (approximately a tablespoon full) is taken to allow measurement of natural hormones in your blood. This needle will be used for the remainder of the study. There may be a small discomfort associated with the insertion of the needle. Aseptic technique will be used to avoid any risk of infection and it will be inserted when you are lying on the bed to avoid fainting. Neuropeptide Y will be sterilized in the hospital pharmacy prior to use. This process will be done using aseptic technique to avoid any risk of infection and cultures will be done to test the sterility of the drug prior to use. Bruising will be minimised by applying firm pressure over the vein upon removal of the needle.

The study will be carried out on two separate days with at least 3 days but less than 7 days in between. On the first day, neuropeptide Y will be infused at a very low concentration and a slow infusion rate and the amount will be increased at intervals of 5 minutes until the hand vein has constricted. We will then infuse saline for at least 60 minutes to allow the effects of neuropeptide Y to disappear. These data will be used to calculate a

very low-dose (this dose, by itself, will not cause constriction of the vein but may enhance or increase the effects of noradrenaline) of neuropeptide Y to be used on the second study day. In a similar manner, on the second study day, noradrenaline will be infused with increasing doses at 5 minute intervals until the vein has constricted. This will again be followed by a 60 min. period of saline infusion to allow the effects of noradrenaline to disappear. We will then repeat the infusion of increasing doses of noradrenaline but will do so in conjunction with an infusion of the very low-dose of neuropeptide Y calculated on the first study day. In the middle of each five minute interval the cuff pressure will be inflated to allow measurement of any changes in the vein diameter, after which the cuff is immediately deflated. The amounts of neuropeptide Y and noradrenaline infused are extremely small in an effort to produce only local constriction of your hand vein without any effects on other blood vessels in your body. Mild associated changes in blood pressure and heart rate may occur with much larger doses of noradrenaline and neuropeptide Y. Your blood pressure and heart rate will be monitored every 5 minutes by an automated blood pressure recorder on your opposite arm. Should the pulse rate rise above 120 beats/min or fall below 50 beats/min, or if the blood pressure is greater than 160/90 mmHg or less than 80/60 mmHg the infusions will be discontinued immediately. Since the drugs are short acting, any change in blood pressure or heart rate should recover rapidly. Large changes are not likely to occur in the present study since you will be monitored continuously and very closely. By giving drug doses which are effective only locally in your hand vein, it is extremely unlikely we will produce any significant change in your blood pressure and heart rate.

If you are taking a long acting angiotensin converting enzyme (ACE) inhibitor drug, this will be changed to a short acting drug (captopril) of the same class in an equivalent dose for approximately 1 week before the study. The dose may be adjusted depending on your symptoms though it is unlikely that there would be significant change in your symptoms since captopril is a drug with proven benefit. You should also abstain from taking some of your medications over the 24 hours prior to the study as directed by Dr. Arnold. If you abstain from taking your medications it is possible you may experience some increased symptoms of your heart failure, such as shortness of breath. Because the medication is held for only a short period of time this is unlikely but, if it should occur, you may take nitroglycerine under your tongue and/or restart your previous medication and we will not proceed with the rest of the study. You will be given the telephone number of the Cardiologist on call in Victoria Hospital (685-8500 Beeper # 8965) if you have any concerns during that period. You will be given your normal medications as soon as the study measurements are completed each morning.

The approximate time of the study each day will be 4 hours (total 8 hours). There will be no direct benefit to you by being involved in this study. You will not be identified by name in any future scientific communications concerning this study and records will be carefully kept in a locked office within the hospital. If you are already participating in another research project at this time, you must inform the investigator promptly to determine whether it is appropriate to begin participation in this study. You may refuse to participate and are free to withdraw at any time with no effect on your future medical care. Should you experience any symptoms which you believe are the result of the drug or if you simply wish to leave the study for any other reason at any time your participation will be discontinued. Your travel expenses

and parking costs will be reimbursed.

Your questions concerning the nature of the study should be answered to your satisfaction before you sign the consent form. If you have any further questions about the study, please do not hesitate to ask. You may contact Dr. Arnold at his office in Victoria Hospital (667-6650).

**Study #3 and #4**

## LETTER OF INFORMATION FOR NORMAL SUBJECTS

### Neuropeptide Y in the Control of Venous Tone in Normals and Patients with Heart Failure.

**J.M.O. Arnold, M.D., Q.P. Feng, M.D., I.D. Callow, B.Sc., M.L. Lambert, B.Sc.**

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Many substances naturally released from various cells within the body influence blood vessel function. Prostacyclin is a naturally occurring substance, normally released from cells (called endothelial cells) which line the inside of the blood vessels, and causes a profound relaxation of the veins. The purpose of this study is to determine whether the release of prostacyclin alters the responsiveness in your hand veins to a naturally released hormone neuropeptide Y which causes constriction of the vein. The activity of the endothelial cells will also be studied. The vein will be constricted by a naturally released substance called $PGF_{2\alpha}$. Once the vein is constricted, increasing doses of either acetylcholine or nitroprusside will be given to relax the vein. Acetylcholine is a natural hormone released at nerve terminals in your body which causes short-lived relaxation or widening of blood vessels. Nitroprusside is a short-acting drug which also causes the relaxation of blood vessels and is commonly used for hypertensive patients.

If you agree to participate, a medical history, physical examination and standard ECG will be done prior to the study. It is required that all participants must abstain from drinking any alcohol or caffeine-containing beverages for at least 12 hours prior to the study. On the study morning, you will lie down and rest in a quiet room where the remainder of the study will be performed. Throughout the study you will be lying down with your upper arm slightly elevated. Your hand will be placed comfortably on a padded support at the level of your shoulders. The veins in the hand are distended slightly by applying a very mild pressure around the upper arm using a blood pressure cuff. The increases in pressure will not affect the flow of the blood into or out of the arm and may result in a slight sensation of fullness in the limb. A second cuff will be placed around the other arm to allow us to monitor your heart rate and blood pressure every 5 minutes throughout the study. Two small needles will be inserted into a vein on the back of your hand and this may be associated with a small amount of discomfort. Sterile techniques will be used to avoid any risk of infection. Bruising will be minimised by applying firm pressure over the vein upon removal of the needles. A small amount of blood (60 ml) will be drawn in order to assess natural hormones in your blood (10 ml). The other 50 ml will be used in an alternate study to assess receptor components in your blood.

The study will be carried out on three separate days with at least 3 days but less than 7 days in between each study day. On one day, neuropeptide Y will be infused, through one of the needles, into the hand vein and the amount will be increased at intervals of 5 minutes

until that hand vein has constricted. This infusion of neuropeptide Y will be given together with a second, separate infusion (via a separate syringe and the second i.v. needle in your vein) containing saline. On the other study day, the identical procedure will be repeated but the second infusion will be a drug called indomethacin, which blocks the formation of prostacyclin and is often used in tablet form to relieve some symptoms of arthritis. On the third study day, the hand vein will first be constricted with $PGF_{2\alpha}$ (256-1024 ng/min) and this dose will be infused continuously into this needle to keep the vein constricted. Then acetylcholine (0.01-1.0 nmol/min) will be infused through the other needle into the hand vein and the amount will be increased at intervals of 5 minutes which will cause the vein to dilate. Once this is complete, the acetylcholine will be washed out using saline and the same procedure will be repeated using nitroprusside (0.3-10 nmol/min) instead of acetylcholine. In the middle of each five minute infusion of neuropeptide Y, acetylcholine and nitroprusside, the cuff pressure will be inflated to allow measurement of any changes in the vein diameter, after which the cuff is immediately deflated. Vein diameter will be measured by a lightweight device resembling a small tripod that can be positioned to rest comfortably on the back of the hand.

The amounts of neuropeptide Y, indomethacin, $PGF_{2\alpha}$, acetylcholine, and nitroprusside infused are extremely small and produce only local constriction or dilation of your hand vein without any effects on other blood vessels in your body. Mild associated changes in blood pressure and heart rate may occur with much larger doses of these drugs. Should the pulse rate rise above 120 beats/min or fall below 50 beats/min, or if the blood pressure is greater than 160/90 mmHg or less than 80/60 mmHg the infusions will be discontinued immediately. Since the drugs are short acting, any change in blood pressure or heart rate will recover rapidly. If you have a history of an allergic reaction to aspirin or indomethacin, you should not participate in this study.

The approximate time of the study each day will be 2-2.5 hours (total 6-7.5 hours). There will be no direct benefit to you by being involved in this study. You will not be identified by name in any future scientific communications concerning this study and records will be carefully kept in a locked office within the hospital. If you are already participating in another research project at this time, you must inform the investigator promptly to determine whether it is appropriate to begin participation in this study. You may refuse to participate or to withdraw from the study at any time with no effect on your standing in school, future medical care or employment status. Should you experience any symptoms which you believe are the result of the drugs or if you simply wish to leave the study for any other reason at any time your participation will be discontinued. Your travel expenses and parking costs will be reimbursed.

Your questions concerning the nature of the study should be answered to your satisfaction before you sign the consent form. If you have any further questions about the study, please do not hesitate to ask. You may contact Dr. Arnold at his office in Victoria Hospital (667-6650).

**Study #3 and #4**

## LETTER OF INFORMATION FOR CHF PATIENTS

### Neuropeptide Y in the Control of Venous Tone in Normals and Patients with Heart Failure.

**J.M.O. Arnold, M.D., Q.P. Feng, M.D., I.D. Callow, B.Sc., M.L. Lambert, B.Sc.**

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Many substances naturally released from various cells within the body influence blood vessel function. Prostacyclin is a naturally occurring substance, normally released from cells (called endothelial cells) which line the inside of the blood vessels, and causes a profound relaxation of the veins. The purpose of this study is to determine whether the release of prostacyclin alters the responsiveness in your hand veins to a naturally released hormone neuropeptide Y which causes constriction of the vein. The activity of the endothelial cells will also be studied. The vein will be constricted by a naturally released substance called $PGF_{2\alpha}$. Once the vein is constricted, increasing doses of either acetylcholine or nitroprusside will be given to relax the vein. Acetylcholine is a natural hormone released at nerve terminals in your body which causes short-lived relaxation or widening of blood vessels. Nitroprusside is a short-acting drug which also causes the relaxation of blood vessels and is commonly used for hypertensive patients.

If you agree to participate, a medical history, physical examination and standard ECG will be done prior to the study. It is required that all participants should abstain from drinking any alcohol or caffeine-containing beverages for at least 24 hours prior to the study. On the study morning, you will be allowed to lie down and rest in a quiet room where the remainder of the study will be performed. Throughout the study you will be lying down with your upper arm slightly elevated. Your hand will be placed comfortably on a padded support at the level of your shoulders. The veins in the hand are distended slightly by applying a very mild pressure around the upper arm using a blood pressure cuff. The increases in pressure will not affect the flow of the blood into or out of the arm and may result in a slight sensation of fullness in the limb. A second cuff will be placed around the other arm to allow us to monitor your heart rate and blood pressure throughout the study. A small needle will be inserted into a vein on the back of your hand from which a sample of blood (approximately a tablespoon full) is taken to allow measurement of natural hormones in your blood. This needle will be used for the remainder of the study. There may be a small discomfort associated with the insertion of the needle. Aseptic technique will be used to avoid any risk of infection and it will be inserted when you are lying on the bed to avoid fainting. Neuropeptide Y will be sterilized in the hospital pharmacy prior to use. This process will be done using aseptic technique to avoid any risk of infection and cultures will be done to test the sterility of the drug prior to use. Bruising will be minimised by applying firm pressure over the vein upon removal of the needle. A small amount of blood (60 ml) will be drawn in order to assess natural hormones in your blood (10 ml). The other 50 ml will be used in an alternate study to assess receptor

components in your blood.

The study will be carried out on three separate days with at least 3 days but less than 7 days in between each study day. On one day, neuropeptide Y will be infused, through one of the needles, into the hand vein and the amount will be increased at intervals of 5 minutes until that hand vein has constricted. This infusion of neuropeptide Y will be given together with a second, separate infusion (via a separate syringe and the second i.v. needle in your vein) containing saline. On the other study day, the identical procedure will be repeated but the second infusion will be a drug called indomethacin, which blocks the formation of prostacyclin and is often used in tablet form to relieve some symptoms of arthritis. On the third study day, the hand vein will first be constricted with $PGF_{2\alpha}$ (256-1024 ng/min) and this dose will be infused continuously into this needle to keep the vein constricted. Then acetylcholine (0.1-1.0 nmol/min) will be infused through the other needle into the hand vein and the amount will be increased at intervals of 5 minutes which will cause the vein to dilate. Once this is complete, the acetylcholine will be washed out using saline and the same procedure will be repeated using nitroprusside (0.3-10 nmol/min) instead of acetylcholine. In the middle of each five minute infusion of neuropeptide Y, acetylcholine and nitroprusside, the cuff pressure will be inflated to allow measurement of any changes in the vein diameter, after which the cuff is immediately deflated. Vein diameter will be measured by a lightweight device resembling a small tripod that can be positioned to rest comfortably on the back of the hand.

The amounts of neuropeptide Y, indomethacin, $PGF_{2\alpha}$, acetylcholine, and nitroprusside infused are extremely small and produce only local constriction of your hand vein without any effects on other blood vessels in your body. Mild associated changes in blood pressure and heart rate may occur with much larger doses of neuropeptide Y. Should the pulse rate rise above 120 beats/min or fall below 50 beats/min, or if the blood pressure is greater than 160/90 mmHg or less than 80/60 mmHg the infusions will be discontinued immediately. Since the drugs are short acting, any change in blood pressure or heart rate will recover rapidly. If you have a history of an allergic reaction to aspirin or indomethacin, you should not participate in this study.

If you are taking a long acting angiotensin converting enzyme (ACE) inhibitor drug, this will be changed to a short acting drug (captopril) of the same class in an equivalent dose for approximately 1 week before the study. The dose may be adjusted depending on your symptoms though it is unlikely that there would be significant change in your symptoms since captopril is a drug with proven benefit. You should also abstain from taking some of your medications over the 24 hours prior to the study as directed by Dr. Arnold. If you abstain from taking your medications it is possible you may experience some increased symptoms of your heart failure, such as shortness of breath. Because the medication is held for only a short period of time this is unlikely but, if it should occur, you may take nitroglycerine under your tongue and/or restart your previous medication and we will not proceed with the rest of the study. You will be given the telephone number of the Cardiologist on call in Victoria Hospital (685-8500 Beeper # 8965) if you have any concerns during that period. You will be given your normal medications as soon as the study measurements are completed each morning.

The approximate time of the study each day will be 2-2.5 hours (total 6-7.5 hours). There will be no direct benefit to you by being involved in this study. You will not be identified by name in any future scientific communications concerning this study and records will be carefully kept in a locked office within the hospital. If you are already participating in another research project at this time, you must inform the investigator promptly to determine whether it is appropriate to begin participation in this study. You may refuse to participate and are free to withdraw at any time with no effect on your future medical care. Should you experience any symptoms which you believe are the result of the drug or if you simply wish to leave the study for any other reason at any time your participation will be discontinued. Your travel expenses and parking costs will be reimbursed.

Your questions concerning the nature of the study should be answered to your satisfaction before you sign the consent form. If you have any further questions about the study, please do not hesitate to ask. You may contact Dr. Arnold at his office in Victoria Hospital (667-6650).

## Study #5

## LETTER OF INFORMATION

### Is Prostacyclin liberated by alpha-adrenoceptor stimulation or is it present prior to agonist addition?

**J.M.O. Arnold, MD FRCPC, M.L. Lambert, HBSc**

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Many substances naturally released from various cells within the body influence blood vessel function. Prostacyclin is a naturally occurring substance, normally released from cells (called endothelial cells) which line the inside of the blood vessels, and causes a profound relaxation of the veins. The purpose of this study is to determine whether the release of prostacyclin alters the responsiveness in your hand veins to a drug called norepinephrine and a drug called $PGF_{2\alpha}$ which are both naturally released substance that cause constriction of the vein. This will help in understanding the vascular responsiveness in normal subjects.

If you agree to participate, a medical history, physical examination and standard ECG will be done prior to the study. It is required that all participants must abstain from drinking any alcohol or caffeine-containing beverages for at least 12 hours prior to the study. On the study morning, you will lie down and rest in a quiet room where the remainder of the study will be performed. Throughout the study you will be lying down with your upper arm slightly elevated. Your hand will be placed comfortably on a padded support at the level of your shoulders. The veins in the hand are distended slightly by applying a very mild pressure around the upper arm using a blood pressure cuff. The increases in pressure will not affect the flow of the blood into or out of the arm and may result in a slight sensation of fullness in the limb. A second cuff will be placed around the other arm to allow us to monitor your heart rate and blood pressure every 5 minutes throughout the study. Two small needles will be inserted into a vein on the back of your hand and this may be associated with a small amount of discomfort. Sterile techniques will be used to avoid any risk of infection. Bruising will be minimised by applying firm pressure over the vein upon removal of the needles. A sample of blood (less than 4 tablespoons) will be taken to allow for measurement of naturally occurring hormones in your blood, and also to measure your cholesterol and glucose levels.

The study will be carried out on two separate days with at least 3 days but less than 7 days in between. On one day, norepinephrine (0.5-1024 ng/min) will be infused, through one of the needles, into the hand vein and the amount will be increased at intervals of 5 min until that hand vein has constricted. This infusion of norepinephrine will be given together with a second, separate infusion (via a separate syringe and the second i.v.needle in your vein) containing saline. Once the vein is constricted, the norepinephrine syringe will be replaced with a syringe containing saline to wash out the norepinephrine. Once the

vein has returned to baseline distention, $PGF_{2\alpha}$ (1-2048 ng/min) will be infused, through one of the needles, into the hand vein and the amount will be increased at intervals of 15 min until that hand vein has constricted. This infusion of $PGF_{2\alpha}$ will be given together with a second, separate infusion (via a separate syringe and the second i.v. needle in your vein) containing saline. On the other study day, the identical procedure will be repeated but the second infusion will be a drug called indomethacin, which blocks the formation of prostacyclin and is often used in tablet form to relieve some symptoms of arthritis. In the middle of each infusion of norepinephrine and $PGF_{2\alpha}$, the cuff pressure will be inflated to allow measurement of any changes in the vein diameter, after which the cuff is immediately deflated. Vein diameter will be measured by a lightweight device resembling a small tripod that can be positioned to rest comfortably on the back of the hand.

The amounts of norepinephrine, $PGF_{2\alpha}$ and indomethacin infused are extremely small and produce only local constriction of your hand vein without any effects on other blood vessels in your body. Mild associated changes in blood pressure and heart rate may occur with much larger doses of $PGF_{2\alpha}$. Should the pulse rate rise above 120 beats/min or fall below 50 beats/min, or if the blood pressure is greater than 160/90 mmHg or less than 80/60 mmHg the infusions will be discontinued immediately. Since the drugs are short acting, any change in blood pressure or heart rate will recover rapidly. If you have a history of an allergic reaction to aspirin or indomethacin, you cannot participate in this study.

The approximate time of the study each day will be 4.5-5 hours (total 9-10 hours). There will be no direct benefit to you by being involved in this study.

You will not be identified by name in any future scientific communications concerning this study and records will be carefully kept in a locked office within the hospital. If you are already participating in another research project at this time, you must inform the investigator promptly to determine whether it is appropriate to begin participation in this study. Participation in the study is voluntary. You may refuse to participate or to withdraw from the study at any time with no effect on your standing in school, future medical care or employment status. Should you experience any symptoms which you believe are the result of the drugs or if you simply wish to leave the study for any other reason at any time your participation will be discontinued. Your travel expenses and parking costs will be reimbursed.

Your questions concerning the nature of the study should be answered to your satisfaction before you sign the consent form. If you have any further questions about the study, please do not hesitate to ask. You may contact Dr. Arnold at his office in Victoria Hospital (667-6650).

# *The* UNIVERSITY *of* WESTERN ONTARIO

*Vice-Provost • Health Sciences • Health Sciences Centre*

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1996-97 CERTIFICATION OF APPROVAL OF HUMAN RESEARCH

ALL HEALTH SCIENCES RESEARCH INVOLVING HUMAN SUBJECTS AT THE UNIVERSITY OF WESTERN ONTARIO IS CARRIED OUT IN COMPLIANCE WITH THE MEDICAL RESEARCH COUNCIL OF CANADA "GUIDELINES ON RESEARCH INVOLVING HUMAN SUBJECT."

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THE REVIEW BOARD HAS EXAMINED THE RESEARCH PROJECT ENTITLED:
"Is prostacyclin liberated by alpha-adrenoceptor stimulation or is it present prior to agonist addition?"

REVIEW NO: 5613

AS SUBMITTED BY: Dr. Dr. J.M.O. Arnold, Medicine, London Health Sciences Centre, Victoria Campus

AND CONSIDERS IT TO BE ACCEPTABLE ON ETHICAL GROUNDS FOR RESEARCH INVOLVING HUMAN SUBJECTS UNDER CONDITIONS OF THE UNIVERSITY'S POLICY ON RESEARCH INVOLVING HUMAN SUBJECTS.

APPROVAL DATE: 10 February 1997 (UWO Protocol, Letter of Information & Consent)

AGENCY: MEDICAL RESEARCH COUNCIL

TITLE:

Bessie Borwein, Chairman

c.c. Hospital Administration

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## SAMPLE CONSENT FORM

STUDY: NEUROPEPTIDE Y IN THE CONTROL OF VENOUS TONE IN NORMALS AND PATIENTS WITH HEART FAILURE.

J.M.O. Arnold, M.D., Q.P. Feng, M.D., I.D. Callow, B.Sc., M.L. Lambert, B.Sc.

I _____ agree to participate in the above study and have had the opportunity to read the letter of information regarding this study. All my questions regarding this study have been answered to my satisfaction.

DATE: _____ SIGNATURE: _____

# REFERENCES

Aakerlund, L., Gether, U., Fuhlendorff, J., Schwartz, T.W. & Thastrup, O. (1990). Y1 receptors for neuropeptide Y are coupled to mobilization of intracellular calcium and inhibition of adenylate cyclase. *FEBS Letters* **260 (1)**, 73-78.

Abboud, F.M. (1979). Integration of reflex responses in the control of blood pressure and vascular resistance. *Am.J.Cardiol.* **44**, 903-911.

Abrass, I.B. & Scarpace, P.J. (1981). Human lymphocyte β-adrenergic receptors are unaltered with age. *J.Gerontology* **36**, 298-301.

Adrian, T.E., Allen, J.M., Bloom, S.R. & Ghatei, M.A. (1983). Neuropeptide Y distribution in human brain. *Nature* **306**, 584-586.

Aellig, W.H. (1981). A new technique for recording compliance of human hand veins. *Br.J.Clin.Pharmac.* **11**, 237-243.

Aellig, W.H. (1983). Influence of pizotifen and ergotamine on the venoconstrictor effect of 5-hydroxytryptamine and noradrenaline in man. *Eur.J.Clin.Pharmac.* **25**, 759-762.

Aellig, W.H. (1990). Superficial hand and foot veins show no difference in sensitivity to constrictor agents. *Clin.Pharmacol.Ther.* **48**, 96-101.

Aellig, W.H. (1994a). Clinical pharmacology, physiology and pathophysiology of superficial veins -1. *Br.J.Clin.Pharmac.* **38**, 181-196.

Aellig, W.H. (1994b). Clinical pharmacology, physiology and pathophysiology of superficial veins -2. *Br.J.Clin.Pharmac.* **38**, 289-305.

Aizawa, Y., Murata, M., Hayashi, M., Funazaki, T., Seiki, I. & Shibata, A. (1985). Vasoconstrictor effect of neuropeptide Y (NPY) on canine coronary artery. *Jap.Circ.J.* **49**, 584-588.

Allen, J.M. & Bloom, S.R. (1986a). Neuropeptide Y: A putative neurotransmitter. *Neurochem* **8**, 1-8.

Allen, J.M., Gjorstrup, P., Bjorkman, J.A., Ek, L., Abrahamsson, T. & Bloom, S.R. (1986b). Studies on cardiac distribution and function of neuropeptide Y. *Acta.Physiol.Scand.* **126**, 405-411.

Allen, J.M., Yeats, J.C., Causon, R., Brown, M.J. & Bloom, S.R. (1987). Neuropeptide Y and its flanking peptide in human endocrine tumors and plasma. *J.Clin.Endocrinol.Metab.* **64**, 1199-1204.

Allen, JM., Gibson, SJ., Adrian, TE., Polak, JM. & Bloom, SR. (1984). Neuropeptide Y in human spinal cord. *Brain Res.* **308**, 145-148.

Allen, Y.S., Adrian, T.E., Allen, J.M., Tatemoto, K., Crow, T.J., Bloom, S.R. & Polak, J.M. (1983). Neuropeptide Y distribution in the rat brain. *Science* **221**, 877-879.

Alradi, A.O. & Carruthers, S.G. (1985). Evaluation and application of the linear variable differential transformer technique for the assessment of human dorsal hand vein alpha-receptor activity. *Clin.Pharmacol.Ther.* **38**, 495-502.

Aminu, J.M. & Vere, D.W. (1972). The effects of oral propranolol on the distensibility of resting superficial veins in man. *Clin.Sci.* **42**, 3P.

Anton, A.H. & Sayre, D.F. (1962). A study of the factors affecting the aluminum oxidetrihydrooxyindole procedure for the analysis of catecholamines. *J.Pharmacol.Exp.Ther.* **138**, 360-374.

Appenzeller, O. (1982). *Autonomic Nervous System*. Elsevier, New York.

Arner, M. & Hogestatt, E.D. (1990). Influence of temperature and extracellular pH on contractile responses in isolated human hand veins. *Pharmacol.Toxicol.* **67**, 141-146.

Arner, M., Uski, T. & Hogestatt, E.D. (1994). Endothelium dependence of prostanoid-induced relaxation in human hand veins. *Acta.Physiol.Scand.* **150**, 267-272.

Arnold, J.M.O., Feng, Q.P., MacLeod, A.P., Lui, A.S., Brown, J.E., Kostuk, W.J. & Feldman, R.D. (1997). Venous alpha-1 adrenoceptor responsiveness in patients with heart failure. *Circulation* (submitted).

Arnold, J.M.O. & McDevitt, D.G. (1983). Contribution of the vagus to the hemodynamic responses following intravenous boluses of isoprenaline. *Br.J.Clin.Pharmac.* **15**, 423-429.

Baranowska, B., Gutkowska, J., Lemire, A., Cantin, M. & Genest, J. (1987). Opposite effects of neuropeptide Y (NPY) and polypeptide YY (PYY) on plasma immunoreactive atrial natriuretic factor (IR-ANF) in rats. *Biochem.Biophys.Res.Commun.* **145**, 680-685.

Barr, M.L. & Kiernan, J.A. (1993). *The human nervous system*. J.B. Lippincott Company, Philadelphia.

Bedarida, G., Bushell, E., Blaschke, T.F. & Hoffman, B.B. (1995). H1- and H2-histamine receptor-mediated vasodilation varies with aging in humans. *Clin.Pharmacol.Ther.* **58**, 73-80.

Bell, L. & Rutlen, D.L. (1990). Quantitative radionuclide assessment of total pulmonary vascular volume changes. *Can.J.Physiol.Pharmacol.* **68**, 727-732.

Bergdahl, A., Valdemarsson, S., Pantev, E., Ottosson, A., Feng, Q.P., Sun, X.Y., Hedner, T. & Edvinsson, L. (1995). Modulation of vascular contractile responses to alpha-1 and alpha-2 adrenergic and Neuropeptide Y receptor stimulation in rats with ischemic heart failure. *Acta.Physiol.Scand.* **154**, 429-437.

Bevan, J.A. & Brayden, J.E. (1987). Nonadrenergic neural vasodilator mechanisms. *Circ.Res.* **60 (3)**, 309-326.

Bischoff, A., Erdbrugger, W., Smits, J. & Michel, M.C. (1994). Dissociation of renal vasoconstrictory and diuretic effects of neuropeptide Y (NPY). *Naunyn Schmiedeberg's Arch Pharmacol* **349 (suppl)**, R39.

Blochl-Daum, B., Korn, A., Wolzt, M., Schmidt, E. & Eichler, H-G. (1991). In vivo studies on alpha-adrenergic receptor subtypes in human veins. *Naunyn Schmiedeberg's Arch.Pharmacol.* **344**, 302-307.

Bodelsson, M., Arneklo-Nobin, B., Nobin, A., Owman, CH., Sollerman, C. & Tornebrandt, K. (1990a). Cooling enhances alpha2-adrenoceptor-mediated vasoconstriction in human hand veins. *Acta.Physiol.Scand.* **138**, 283-291.

Bodelsson, M., Arneklo-Nobin, B. & Tornebrandt, K. (1990b). Effect of cooling on smooth muscle response to 5-hydroxytryptamine in human hand veins. *Acta.Physiol.Scand.* **140**, 331-339.

Bouvier, M., Leeb-Lundberg, L.M.F., Benovic, J.C., Caron, M.G. & Lefkowitz, R.J. (1987). Regulation of adrenergic receptor function by phosphorylation. *J.Biol.Chem.* **262**, 3106-3113.

Braunwald, E. (1987). Pathophysiology of heart failure. In *Heart Disease* (Braunwald, E., ed.), Saunders, New York.

Bristow, M.R., Ginsburg, R., Fowler, M., Minobe, W., Rasmussen, R., Zera, P., Menlove, R., Shah, P. & Stinson, E. (1986). β1 and β2-adrenergic receptor subpopulations in normal and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β1-receptor downregulation in heart failure. *Circ.Res.* **59**, 297-309.

Broadley, K.J. (1996). Anatomy and Physiology of the Autonomic Nervous System. In *Autonomic Pharmacology* (Taylor and Francis, London, pp. 1-39.

Burch, G.E. (1978). The role of the central nervous system in chronic congestive heart failure. *Am.Heart.J.* **95 (2)**, 255-261.

Burch, G.E. & Murtadha, M. (1956). A study of the venomotor tone in a short intact venous segment of the forearm of man. *Am.Heart.J.* **51**, 807-828.

Burnstock, G. & Hoyle, C.H.V. (1992). *Autonomic Neuroeffector Mechanisms*. Harwood Academic Publishers,

Busse, R., Fleming, I. & Hecker, M. (1993). Endothelium-derived bradykinin: Iplications for Angiotensin-Converting Enzyme-Inhibitor Therapy. *J.Cardiovasc.Pharmacol.* **22 (Suppl.5)**, S31-S36.

Bylund, J.B. (1988). α2-Adrenergic receptors. A historical perspective. In *The α2-Adrenegic Receptor* (Limbind, L.E., ed.), Humana Press, New Jersey, pp. 1-13.

Callow, I.D. (1995). Modulation of venous alpha adrenoceptor responsiveness. *MSc.Thesis*

Callow, I.D., Campisi, P., Feng, Q.P. & Arnold, J.M.O. (1997). Enhanced in vivo alpha-1 and alpha-2 adrenoceptor venoconstriction with prostaglandin inhibition. *Am.J.Physiol.* **(submitted)**,

Cannon, W.B. (1929). Organisation for physiological homeostasis. *Physiol.Rev.* **9**, 399-431.

Chang, R. & Lotti, V.J. (1988). Specific [3H]proprionyl-neuropeptide Y (NPY) binding in rabbit aortic membranes: comparison with binding in rat brain and biological responses in rat vas deferens. *Biochem.Biophys.Res.Commun.* **151**, 1213-1219.

Chidsey, C.A., Harrison, D.C. & Braunwald, E. (1962). Augmentation of the plasma norepinephrine response to exercise in patients with congestive heart failure. *N.Engl.J.Med.* **267**, 650-654.

Christensen, N.J. (1986). Is plasma NA an index of biological age? In *The Sympathoadrenal System: Physiology and Pathophysiology* (Christensen, N.J., Henriksen, O. and Lassen, N.A., eds.), Munksgaard, Copenhagen, pp. 266-272.

Clarke, J., Benjamin, N., Larkin, S., Webb, D., Maseri, A. & Davies, G. (1991). Interaction of neuropeptide Y and the sympathetic nervous system in vascular control in man. *Circulation* **83**, 774-777.

Clarke, J.G., Davies, G.J., Kerwin, R., Hackett, D., Larkin, S., Dawbarn, D., Lee, Y., Bloom, S.R., Yacoub, M. & Maseri, A. (1987). Coronary artery infusion of neuropeptide Y in patients with angina pectoris. *Lancet* **1**, 1057-1059.

Clements, I.P., Strelow, D.A., Becker, G.P., Vlietstra, R.E. & Brown, M.L. (1981). Radionuclide evaluation of peripheral circulatory dynamics: new clinical application of blood pool scintigraphy for measuring limb venous volume, capacity, and blood flow. *Am.Heart.J.* **102**, 980-983.

Cody, R.J., Covit, A.B., Schaer, G.L., Laragh, J.H., Sealey, J.E. & Feldschuh, H. (1986). Sodium and water balance in chronic congestive heart failure. *J.Clin.Invest.* **77**, 1441-1452.

Cohen, M.L. & Berkowitz, B.A. (1974). Age-related changes in vascular responsiveness to cyclic nucleotides and contractile agonists. *J.Pharmacol.Exp.Ther.* **191**, 147-155.

Cohn, J.N. (1990). Abnormalities of peripheral sympathetic nervous system control in congestive heart failure. *Circulation* **81 (Suppl. I)**, I59-I67.

Cohn, J.N., Johnson, G.R., Shabetai, R., Loeb, H., Tristani, F., Rector, T., Smith, R. & Fletcher, R. (1993). Ejection fraction, peak exercise oxygen consumption, cardiothoracic ratio, ventricular arrhythmias and plasma norepinephrine as determinants of prognosis in heart failure. *Circulation* **87**, VI-5-VI-16.

Cohn, J.N., Levine, T.B., Olivari, M.T., Garberg, V., Tura, D., Francis, G.S., Simon, A.B. & Rector, T. (1984). Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N.Engl.J.Med.* **311**, 819-823.

Collier, J. & Vallance, P. (1990). Biphasic response to acetylcholine in human veins in vivo: the role of the endothelium. *Clin.Sci.* **78**, 101-104.

Colucci, W.S. (1994). The Sympathetic Nervous System in Congestive Heart Failure. In *Congestive Heart Failure: Pathophysiology, Diagnosis, and Comprehensive Approach to Management* (Hosenpud, J.D. and Greenberg, B.H., eds.), Springer-Verlag, New York, pp. 126-135.

Corder, R., Lowry, P.J., Emson, P.C. & Gaillard, R.C. (1985). Chromatographic characterisation of the circulating neuropeptide Y immunoreactivity from patients with phaeochromocytoma. *Regul.Peptides* **10**, 91-97.

Craig, C.R. (1986). *Modern Pharmacology*. Little, Brown and Company, Toronto.

Dahlof, C., Dahlof, P. & Lundberg, JM. (1985a). Neuropeptide Y (NPY) reduces field stimulation-evoked release of noradrenaline and enhances force of contraction in the rat portal vein. *Naunyn Schmiedeberg's Arch.Pharmacol.* **328**, 327-330.

Dahlof, C., Dahlof, P. & Lundberg, JM. (1985b). Neuropeptide Y (NPY) enhancement of blood pressure upon alpha-adrenoceptor activation and direct pressor effects in pithed rat. *Eur.J.Pharmacol.* **109**, 289-292.

Dalsgaard, C.J., Franco-Cereceda, A., Saria, A., Lundberg, J.M., Theodorsson-Norheim, E. & Hokfelt, T. (1986). Distribution and origin of substance P and neuropeptide Y immunoreactive nerves in the guinea pig heart. *Cell Tissue Res.* **243**, 477-485.

Dawbarn, D., Hung, S.P. & Emson, P.C. (1984). Neuropeptide Y: Regional distribution chromatographic characterization and immunohistochemical demonstration in post-mortem human brain. *Brain Res.* **296**, 168-173.

De Quidt, M.E., Richardson, P.J. & Emson, P.C. (1985). Subcellular distribution of neuropeptide Y-like immunoreactivity in guinea pig neocortex. *Brain Res.* **335**, 354-359.

Derchi, G., Dupuis, J., deChamplain, J. & Rouleau, J.L. (1993). Paradoxical decrease in circulating neuropeptide Y-like immunoreactivity during mild orthostatic stress in subjects with and without congestive heart failure. *Euro.Heart J.* **14**, 34-39.

DiBianco, R. (1994). The changing syndrome of heart failure: an annotated review as we approach the 21st century. *J.Hypertension* **12 (Suppl. 4)**, S73-S87.

Docherty, J.R. & Hyland, L. (1985). Vascular α2-adrenoceptors can mediate nerve stimulation-evoked contractions. *Clin.Sci.* **68 (suppl.10)**, 117s-120s.

Donald, D.E. & Shepherd, J.T. (1979). Cardiac receptors: normal and disturbed function. *American Journal of Cardiology* **44 (5)**, 873-878.

Dorn, G.W., Becker, M.W. & Davis, M.G. (1992). Dissociation of the Contractile and Hypertrophic Effects of Vasoconstrictor Prostanoids in Vascular Smooth Muscle. *J.Biol.Chem.* **267(34)**, 24897-24905.

Doughty, R.N., MacMahon, S. & Sharpe, N. (1994). Beta-Blockers in heart failure: Promising or Proved? *J.Am.Coll.Cardiol.* **23**, 814-821.

Drexler, H., Hayoz, D. & Munzel, T. (1992). Endothelial function in chronic congestive heart failure. *Am.J.Cardiol.* **69**, 1596-1601.

Drexler, H., Hayoz, D., Munzel, T., Just, H., Zelis, R. & Brunner, H.R. (1994). Endothelial dysfunction in chronic heart failure. Experimental and clinical studies. *Drug Res.* **44** (I), 455-458.

Dubois-Rande, J.L., Comoy, E., Merlet, P., Benvenuti, C., Carville, C., Hittinger, L., Macquin-Mavier, I., Bohuon, C. & Castaigne, A. (1992). Relationship among neuropeptide Y, catecholamines and haemodynamics in congestive heart failure. *Euro.Heart J.* **13**, 1233-1238.

DuCharme, D.W. & Weeks, J.R. (1966). Cardiovascular pharmacology of prostaglandin F2α, a unique pressor agent. In *Prostaglandins* (Bergstrom, S. and Samuelsson, B., eds.), Almqvist & Wiksell, Stockholm, pp. 173-181.

DuCharme, D.W., Weeks, J.R. & Montgomery, R.G. (1968). Studies on the mechanism of the hypertensive effect of prostaglandin F2α. *J.Pharmacol.Exp.Ther.* **160 No.1**, 1-10.

Dzau, V.J., Colucci, W.A., Hollenberg, N.K. & Williams, G.H. (1981). Relation of the renin-angiotensin-aldosterone system to the clinical state in congestive heart failure. *Circulation* **63**, 645-651.

Echtenkamp, S.F. & Dandridge, P.F. (1989). Renal actions of neuropeptide Y in the primate. *Am.J.Physiol.* **256**, F524-F531.

Edvinsson, L., Adamsson, M. & Jansen, I. (1990). Neuropeptide Y antagonistic properties of D-myo-inositol-1,2,6-trisphosphate in guinea pig basilar arteries. *Neuropeptides* **17**, 99-105.

Edvinsson, L., Ekblad, E., Hakanson, R. & Wahlestedt, C. (1984). Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. *Br.J.Pharmac.* **83**, 519-525.

Edvinsson, L., Emson, P., McCulloch, J., Tatemoto, K. & Uddman, R. (1983). Neuropeptide Y: cerebrovascular innervation and vasomotor responses in the cat. *Neuroscience Letters* **43**, 79-84.

Edvinsson, L., Hakanson, R., Steen, S., Sundler, F., Uddman, R. & Wahlestedt, C. (1985). Innervation of human omental arteries and veins and vasomotor responses to noradrenaline, neuropeptide Y, substance P and vasoactive intestinal polypeptide. *Regul.Peptides* **12**, 67-79.

Edvinsson, L., Hakanson, R., Wahlestedt, C. & Uddman, R. (1987). Effects of neuropeptide Y on the cardiovascular system. *TIPS* **8**, 231-235.

Ekblad, E., Edvinsson, L., Wahlestedt, C., Uddman, R., Hakanson, R. & Sundler, F. (1984). Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. *Regul.Peptides* **8**, 225-235.

El-Din, M.M. & Malik, K.U. (1988). Meuropeptide Y stimulates renal prostaglandin synthesis in the isolated rat kidney: contribution of Ca++ and calmodulin. *J.Pharmacol.Exp.Ther.* **246**, 479-484.

Ellis, E.F., Neis, A.S. & Oates, J.A. (1977). Cerebral arterial smooth muscle contraction by thromboxane A2. *Stroke* **8**, 480.

Erlinge, D., Edvinsson, L., Brunkwall, J., Yee, F. & Wahlestedt, C. (1993). Human neuropeptide Y Y1 receptor antisense oligodeoxynucleotide specifically inhibits neuropeptide Y-evoked vasoconstriction. *Eur.J.Pharmacol.* **240**, 77-80.

Faxon, D.P., Creager, M.A., Haperin, J.L., Bernard, D.B. & Ryan, T.J. (1984). Redistribution of regional blood flow following angiotensin-converting-enzyme inhibition. Comparison of normal subjects and patients with heart failure. *Am.J.Med.* **76**, 104-110.

Feldman, R.D., Limbird, L.E., Nadeau, J., Robertson, D. & Wood, A.J.J. (1984). Alteration in leukocyte β-adrenoceptor affinity with ageing. *N.Engl.J.Med.* **310**, 815-819.

Feng, Q.P. (1993). *Sympathetic regulation of cardiovascular and renal function in rats with experimental congestive heart failure*. University of Gothenburg, Gothenburg, Sweden, pp. 1-86.

Feng, Q.P., Bergdahl, A., Lu, X.R., Sun, X.Y., Edvinsson, L. & Hedner, T. (1996). Vascular alpha-2 adrenoceptor function is decreased in rats with congestive heart failure. *Cardiovasc.Res.* **31**, 577-584.

Feng, Q.P., Carlsson, S., Thoren, P. & Hedner, T. (1992). Effects of clonidine on renal sympathetic nerve activity, natriuresis and diuresis in chronic congestive heart failure rats. *J.Pharmacol.Exp.Ther.* **261**, 1129-1135.

Feng, Q.P., Hedner, T., Andersson, B., Lundberg, J.M. & Waagstein, F. (1994). Cardiac neuropeptide Y and noradrenaline balance in patients with congestive heart failure. *Br.Heart J.* **71**, 261-267.

Ferguson, D.W., Abboud, F.M. & Mark, A.L. (1984). Selective impairment of baroreflex-mediated vasoconstrictor responses in patients with ventricular dysfunction. *Circulation* **69 (3)**, 451-460.

Ford, G.A., Hoffman, B.B., Vestal, R.E. & Blaschke, T.F. (1992). Age-related changes in adenosine and β-adrenoceptor responsiveness of vascular smooth muscle in man. *Br.J.Clin.Pharmac.* **33**, 83-87.

Forfar, J.C. (1991). Neuroendocrine activation in congestive heart failure. *Am.J.Cardiol.* **67**, 3C-5C.

Forster, C. & Armstrong, P.W. (1990). Pacing-induced heart failure in the dog: Evaluation of peripheral vascular α-adrenoceptor subtypes. *J.Cardiovasc.Pharmacol.* **16**, 708-718.

Francis, G.S., Goldsmith, S.R., Levine, T.B., Olivari, M.T. & Cohn, J.N. (1984). The neurohormonal axis in congestive heart failure. *Ann.Intern.Med.* **101**, 370-377.

Franco-Cereceda, A., Lundberg, J.M. & Dahlof, C. (1985). Neuropeptide Y and sympathetic control of heart contractility and coronary vascular tone. *Acta.Physiol.Scand.* **124**, 361-369.

Franco-Cereceda, A., Owall, A., Settergren, G., Sollevi, A. & Lundberg, J.M. (1990). Release of neuropeptide Y and noradrenaline from the human heart after aortic occlusion during coronary artery surgery. *Cardiovasc Res* **24**, 241-246.

Fried, G., Terenius, L., Hokfelt, T. & Goldstein, M. (1985). Evidence for differential localization of noradrenaline and neuropeptide Y (NPY) in neuronal storage vesicles isolated from rat vas deferens. *J.Neurosci.* **5**, 450-458.

Furchgott, R.F. & Zawadzki, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**, 373-376.

Furness, J.B., Costa, M., Emson, P.C., Hakanson, R., Moghimzadeh, E., Sundler, F., Taylor, I.L. & Chance, R.E. (1983). Distribution pathways and reactions to drug treatment of nerves with neuropeptide Y- and pancreatic polypeptide-like immunoreactivity in the guinea pig digestive tract. *Cell Tissue Res.* **234**, 71-92.

Gabella, G. (1976). *Structure of the Autonomic Nervous System.* Chapman & Hall, London.

Glover, W.E. (1985). Increased sensitivity of rabbit ear artery to noradrenaline following perivascular nerve stimulation may be a response to neuropeptide Y release as a cotransmitter. *Clin.Exp.Pharmacol.Physiol.* **12**, 227-231.

Goodman, L.S. & Gilman, A.G. (1987). *The Pharmacological Basis of Therapeutics.* Macmillan Publishing Company, New York.

Graham, R.M. (1990). Adrenergic receptors: structure and function. *Clin.J.Med.* **57**, 481-491.

Greenberg, R.A. & Sparks, H.V. (1969). Prostaglandins and consecutive vascular segments of the canine hindlimb. *Am.J.Physiol.* **216**, 567-571.

Griendling, K.K., Murphy, T.J. & Alexander, R.W. (1993). Molecular biology of the renin-angiotensin system. *Circulation* **87**, 1816.

Grundemar, L. (1991). Actions of neuropeptide Y on peripheral and central targets. *Acta.Physiol.Scand.* **Ph.D. Thesis.**

Grundemar, L., Jonas, S.E., Morner, N., Hogestatt, E.D., Wahlestedt, C. & Hakanson, R. (1992). Characterization of vascular neuropeptide Y receptors. *Br.J.Pharmac.* **105**, 45-50.

Gu, J., Polak, J.M., Allen, J.M., Huang, W.M., Sheppard, M.N., Tatemoto, K. & Bloom, S.R. (1984). High concentrations of a novel peptide, neuropeptide Y, in the innervation of mouse and rat heart. *J.Histochem.Cytochem.* **32**, 467-472.

Haefeli, W.E., Srivastava, N., Kongpatanakul, S., Blaschke, T.F. & Hoffman, B.B. (1993). Lack of role of endothelium-derived relaxing factor in effects of α-adrenergic agonists in cutaneous veins in humans. *Am.J.Physiol.* **264**, H364-H369.

Hall, J.E., Guyton, A.C., Jackson, T.E. & Coleman, T.G. (1979). Chronic blockade of angiotensin II formation during sodium deprivation. *Am.J.Physiol.* **237**, F424-F432.

Hanasaki, K. & Arita, H. (1989). A common binding site for primary prostanoids in vascular smooth muscle: a definite discrimination of the binding for thromboxane A2/prostaglandin H2 receptor agonist from its antagonist. *Biochem.Biophys.Acta.* **1013**, 28-35.

Hasking, G.J., Esler, M.D., Jennings, G.L., Burton, D., Johns, J.A. & Korner, P.I. (1986). Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation* **73 (4)**, 615-621.

Hauser, G.J., Danchak, M.R., Colvin, M.P., Hopkins, R.A., Wocial, B., Myers, A.K. & Zukowska-Grojec, Z. (1996). Circulating neuropeptide Y in humans: relation to changes in catecholamine levels and changes in hemodynamics. *Neuropeptides* **30(2)**, 159-165.

Hayashi, S., Park, M.K. & Kuehl, T.J. (1986). Effects of prostaglandins and arachidonic acid on baboon cerebral and mesenteric arteries. *Prostaglandins* **32**, 587.

Haynes, W.G. & Webb, D.J. (1993). Endothelium-dependent modulation of responses to endothelin-1 in human veins. *Clin.Sci.* **84**, 427-433.

Henrich, W.L., Anderson, R.J. & Bernes, A.S. (1978). The role of renal nerves and prostaglandins in control of renal hemodynamics and plasma renin activity during hypotensive hemorrhage in the dog. *J.Clin.Invest.* **61**, 744-750.

Herzog, H., Baumgartner, M., Vivero, C., Selbie, L.A., Auer, B. & Shine, J. (1993). Genomic organization, localization, and allelic differences in the gene for the human neuropeptide Y Y1 receptor. *J.Biol.Chem.* **268**, 6703-6707.

Herzog, H., Hort, Y.J., Ball, H.J., Hayes, G., Shine, J. & Selbie, L.A. (1992). Cloned human neuropeptide Y receptor couples to two different second messenger systems. *Proc.Natl.Acad.Sci.USA* **89**, 5794-5798.

Hetland, M.L., Eldrup, E., Bratholm, P. & Christensen, N.J. (1991). The relationship between age and venous plasma concentrations of noradrenaline, catecholamine metabolites, DOPA and neuropeptide Y-like immunoreactivity in normal human subjects. *Scand.J.Clin.Lab.Invest.* **51**, 219-224.

Higgins, C.B., Vatner, S.F., Echberg, D.L. & Braunwald, E. (1972). Alterations in the baroreceptor reflex in conscious dogs with heart failure. *J.Clin.Invest.* **51**, 715-724.

Hiremath, A.N., Pershe, R.A., Hoffman, B.B. & Blaschke, T.F. (1989). Comparison of age-related changes in prostaglandin E1 and beta-adrenergic responsiveness of vascular smooth muscle in adult males. *J.Gerontology* **44**, M13-M17.

Hirsch, A.T., Dzau, V.J. & Creager, M.A. (1987). Baroreceptor function in congestive heart failure; effect on neurohumoral activation and regional vascular resistance. *Circulation* **75** (IV), 36-48.

Hjemdahl, P., Daleskog, M. & Kahan, T. (1979). Determination of plasma catecholamines by high performance liquid chromatography with electrochemical detection: Comparison with a radioenzymatic method. *Life Sci.* **25**, 131-138.

Hollenberg, N.K., Williams, G.H., Taub, K.J., Ishikawa, I., Brown, C. & Adams, D.F. (1977). Renal vascular response to interruption of the renin-angiotensin system in normal man. *Kidney Int.* **12**, 285-293.

Howe, P.R., Rogers, P.F., Morris, M.J., Chalmers, J.P. & Smith, R.M. (1986). Plasma catecholamines and neuropeptide Y as indices of sympathetic nerve activity in normotensive and stroke-prone spontaneously hypertensive rats. *J.Cardiovasc.Pharmacol.* **8**, 1113-1121.

Hulting, J., Sollevi, A., Ullman, B., Franco-Cereceda, A. & Lundberg, J.M. (1990). Plasma neuropeptide Y on admission to a coronary care unit: raised levels in patients with left heart failure. *Cardiovasc.Res.* **24**, 102-108.

Hyman, A.L. (1969). The active responses of pulmonary veins in intact dogs to prostaglandins F2α and E1. *J.Pharmacol.Exp.Ther.* **165**, 267-273.

Ignarro, L.J. (1989). Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. *Circ.Res.* **65**, 1-21.

Inui, A., Okita, M., Inoue, T., Sakatani, N., Oya, M., Moriloka, H., Shii, K., Yokono, K., Mizuno, N. & Baba, S. (1988). Characterization of peptide YY receptors in the brain. *Endocrinology* **124**, 402-409.

Ito, M., Soma, M., Izumi, Y. & Kanmatsuse, K. (1995). Effects of Long-Term Treatment with Angiotensin-Converting Enzyme Inhibitor on Angiotensin II and Prostacyclin Release from Mesenteric Arteries in Spontaneously Hypertensive Rats. *Prostaglandins Leukotrienes and Essential Fatty Acids* **53**, 359-363.

Jackson, E.K., Gerkens, J.F., Brash, A.R. & Branch, R.A. (1982). Acute renal artery constriction increases renal prostaglandin I2 biosynthesis and renin release in the conscious dog. *J.Pharmacol.Exp.Ther.* **222**, 410-413.

Johnson, R.A., Morton, D.R., Kinner, J.H., Gorman, R.R., McGuire, J.R., Sun, F.F., Whittaker, N., Bunting, S., Salmon, J.A., Moncada, S. & Vane, J.R. (1976). The chemical characterization of prostaglandin X (prostacyclin). *Prostaglandins* **12**, 915.

Kahan, T., Taddei, S., Pedrinelli, R., Hjemdahl, P. & Salvetti, A. (1992). Nonadrenergic sympathetic vascular control of the human forearm in hypertension: Possible involvement of neuropeptide Y. *J.Cardiovasc.Pharmacol.* **19**, 587-592.

Kaijser, L., Pernow, J., Berglund, B. & Lundberg, J.M. (1990). Neuropeptide Y is released together with noradrenaline from the human heart during exercise and hypoxia. *Clin.Physiol.* **10**, 179-188.

Katz, S.D. (1995). The Role of Endothelium-Derived Vasoactive Substances in the Pathophysiology of Exercise Intolerance in Patients With Congestive Heart Failure. *Prog.Cardiovasc.Dis.* **38(1)**, 23-50.

Katz, S.D., Biasucci, L. & Sabba, C. (1992). Impaired endothelium-mediated vasodilation in the peripheral vasculature of patients with congestive heart failure. *J.Am.Coll.Cardiol.* **19**, 918-925.

Katzung, B.G. (1995). *Basic and Clinical Pharmacology*. Appleton and Lange, Norwalk.

Kawamura, K., Smith, T.L., Zhou, Q. & Kummerow, F.A. (1991). Neuropeptide Y Stimulates Prostacyclin Production in Porcine Vascular Endothelial Cells. *Biochem.Biophys.Res.Commun.* **179(1)**, 309-313.

Keeton, T.K. & Campbell, W.B. (1980). The pharmacologic alteration of renin release. *Pharmacol.Rev.* **32(2)**, 81-227.

Kimmel, J.R., Hayden, L.J. & Pollock, H.G. (1975). Isolation and characterization of a new pancreatic polypeptide hormone. *J.Biol.Chem.* **250**, 9369-9376.

Klein, C., Gerber, J.G., Payne, N.A. & Nies, A.S. (1990). The effect of age on the sensitivity of the α1-adrenoceptor to phenylephrine and prazosin. *Clin.Pharmacol.Ther.* **47**, 535-539.

Komaru, T., Ashikawa, K., Kanatsuka, H., Sekiguchi, N., Suzuki, T. & Takishima, T. (1990). Neuropeptide Y modulates vasoconstriction in the coronary microvessels in the beating canine heart. *Circ.Res.* **67**, 1142-1151.

Korner, P.I. (1979). Central nervous control of autonomic cardiovascular function. In *Handbook of Physiology. The Cardiovascular System* (Berne, R.M., Sperelakis, N. and Geiger, S.R., eds.), American Physiological Society, Bethesda, pp. 691-739.

Kubo, S.H. & Cody, R.J. (1983). Circulatory autoregulation in chronic congestive heart failure: Responses to head-up tilt in 41 patients. *Am.J.Cardiol.* **52**, 512-518.

Kubo, S.H., Rector, T.S., Bank, A.J., Williams, R.E. & Heifetz, S.M. (1991). Endothelium-dependent vasodilation is attenuated in patients with heart failure. *Circulation* **84**, 1589-1596.

Larhammar, D., Ericsson, A. & Persson, H. (1987). Structure and expression of the rat neuropeptide-Y gene. *Proc.Natl.Acad.Sci.USA* **84**, 2068-2072.

Lee, W.H. & Packer, M. (1986). Prognostic importance of serum sodium concentration and its modification by converting-enzyme inhibition in patients with severe chronic heart failure. *Circulation* **73**, 257-267.

Leimbach, W.N., Wallin, G., Victor, R.G., Aylward, P.E., Sundlof, G. & Mark, A.L. (1986). Direct evidence from intraneuronal recordings for increased central sympathetic outflow in patients with heart failure. *Circulation* **73**, 913-919.

Levine, T.B., Francis, G.S., Goldsmith, S.R. & Cohn, J.N. (1983). The neurohumoral and hemodynamic response to orthostatic tilt in patients with congestive heart failure. *Circulation* **67**, 1070-1075.

Limbird, L.E. (1988). Receptors linked to inhibition of adenylate cyclase: Additional signaling mechanisms. *Faseb J.* **2 (11)**, 2686-2695.

Lin, T.M. & Chance, R.E. (1974). Candidate hormones of the gut. Bovine pancreatic polypeptide (BPP) and avian pancreatic polypeptide (APP). *Gastroenterology* **67**, 737-738.

Lind, H., Erlinge, D., Brunkwall, J. & Edvinsson, L. (1995). Transient vasodilation induced by neuropeptide Y and inhibited by L-NMMA in human subcutaneous blood vessels. *Pharmacol.Toxicol.* **76(suppl IV)**, 57.

Lorenz, J.N. & et al., (1991). Renin release from isolated juxtaglomerular apparatus depends on macular densa chloride transport. *Am.J.Physiol.* **260**, F486.

Lui, A.S. & Arnold, J.M.O. (1996). Response of large superficial dorsal hand veins to locally infused nitroglycerine in young, normal subjects. *Can.J.Physiol.& Pharmacol.* **74**, 1034-1038.

Lundberg, J.M., Anggard, A., Theodorsson-Norheim, E. & Pernow, J. (1984a). Guanethidine-sensitive release of neuropeptide Y-like immunoreactivity in the cat spleen by sympathetic nerve stimulation. *Neuroscience Letters* **52**, 175-180.

Lundberg, J.M., Hemsen, A., Larsson, O., Rudehill, A., Saria, A. & Fredholm, B. (1985a). Neuropeptide Y receptor in pig spleen: binding characteristics reduction of cyclic AMP formation and calcium antagonist inhibition of vasoconstriction. *Eur.J.Pharmacol.* **145**, 21-29.

Lundberg, J.M., Hemsen, A., Larsson, O., Rudehill, A., Saria, A. & Fredholm, B. (1988). Neuropeptide Y receptor in pig spleen: binding characteristics, reduction of cyclic AMP formation and calcium antagonist inhibition of vasoconstriction. *Eur.J.Pharmacol.* **145**, 21-29.

Lundberg, J.M., Hua, X.Y. & Franco-Cereceda, A. (1984b). Effects of neuropeptide Y (NPY) on mechanical activity and neurotransmission in the heart, vas deferens and urinary bladder of the guinea-pig. *Acta.Physiol.Scand.* **121**, 325-332.

Lundberg, J.M., Martinsson, A., Hemsen, A., Theodorsson-Norhein, E., Svedenhag, J., Ekblom, B. & Hjemdahl, P. (1985b). Co-release of neuropeptide Y and catecholamines during physical exercise in man. *Biochem.Biophys.Res.Commun.* **133 (1)**, 30-36.

Lundberg, J.M., Rudehill, A., Sollevi, A. & Hamberger, B. (1989). Evidence for co-transmitter role of neuropeptide Y in the pig spleen. *Br.J.Pharmac.* **96**, 675-687.

Lundberg, J.M. & Tatemoto, K. (1982). Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to α-adrenoceptor blockade. *Acta.Physiol.Scand.* **116**, 393-402.

Lundberg, J.M., Terenius, L., Hokfelt, T. & Goldstein, M. (1983). High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. *Neuroscience Letters* **42**, 167-172.

Lundberg, J.M., Terenius, L., Hokfelt, T., Martling, C.R., Tatemoto, K., Mutt, V., Polak, J. & Goldstein, M. (1982). Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta.Physiol.Scand.* **116**, 477-480.

Lundberg, JM., Fried, G., Pernow, J. & Theodorsson-Norheim, E. (1986). Co-release of neuropeptide Y and catecholamines upon adrenal activation in the cat. *Acta.Physiol.Scand.* **126**, 231-238.

Luscher, T.F. & Yang, Z. (1993). Calcium antagonists and ACE inhibitors. Effect on endothelium and vascular smooth muscle. *Drugs* **46 (Suppl. 2)**, 121-132.

Luthra, A., Borkowski, K.R. & Carruthers, S.G. (1989). Comparison of upper and lower limb superficial vein responsiveness to noradrenaline. *Eur.J.Clin.Pharmac.* **36**, A206.

Luthra, A., Borkowski, K.R. & Carruthers, S.G. (1991). Genetic aspects of variability in superficial vein responsiveness to norepinephrine. *Clin.Pharmacol.Ther.* **49**, 355-361.

Mabe, Y., Perez, R., Tatemoto, K. & Huidobro-Toro, J.P. (1987). Chemical sympathectomy reveals pre- and postsynaptic effects of neuropeptide Y (NPY) in the cardiovascular system. *Experientia* **43**, 1018-1020.

MacKerell, A.D., Hemsen, A., Lacroix, J.S. & Lundberg, J.M. (1989). Analysis of structure-function relationships of neuropeptide Y using molecular dynamics simulations and pharmacological activity and binding measurements. *Regul.Peptides* **25**, 295-313.

Maisel, A.S., Scott, N.A., Motulsky, H.J., Michel, M.C., Boublik, J.H., Rivier, J.E., Ziegler, M., Allen, R.S. & Brown, M.R. (1989). Elevation of plasma neuropeptide Y levels in congestive heart failure. *Am.J.Med.* **86**, 43-48.

Mancia, G. (1990). Sympathetic activation in congestive heart failure. *Euro.Heart J.* **11 (Suppl. A)**, 3-11.

Mark, A.L., Schmid, P.G., Eckstein, J.W. & Wendling, M.G. (1971). Venous responses to prostaglandin F2α. *Am.J.Physiol.* **220(1)**, 222-226.

Martin, W., Villani, G.M., Jothianandan, D. & Furchgott, R.F. (1985). Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J.Pharmacol.Exp.Ther.* **232**, 708-716.

McDermott, B.J., Millar, B.C. & Piper, H.M. (1993). Cardiovascular effects of neuropeptide Y: receptor interactions and cellular mechanisms. *Cardiovasc.Res.* **27**, 893-905.

Mellander, S. (1960). Comparative studies on the adrenergic neuro-hormonal control of resistance and capacitance blood vessels in the cat. *Acta.Physiol.Scand.* **50 (suppl 176)**, 1-86.

Millar, B.C., Schluter, K.D., Zhou, X.J., McDermott, B.J. & Piper, H.M. (1994). Neuropeptide Y stimulates hypertrophy of adult ventricular cardiomyocytes. *Am.J.Physiol.* **266**, C1271-C1277.

Miller, V.M. (1991). Interactions between neural and endothelial mechanisms in control of vascular tone. *NIPS* **6**, 60-63.

Milnor, W.R. (1990). *Cardiovascular Physiology*. Oxford University Press, New York.

Minami, M., Yasuda, H. & Yamazaki, N. (1983). Plasma norepinephrine concentration and plasma dopamine-beta-hydroxylase activity in patients with congestive heart failure. *Circulation* **67**, 1324-1329.

Minth, C.D., Bloom, S.R., Polak, J.M. & Dixon, J.E. (1984). Cloning, characterization, and DNA sequence of a human cDNA encoding neuropeptide tyrosine. *Proc.Natl.Acad.Sci.USA* **81**, 4577-4581.

Modin, A., Pernow, J. & Lundberg, J.M. (1991). Evidence for two neuropeptide Y receptors mediating vasoconstriction. *Eur.J.Pharmacol.* **203**, 165-171.

Moffet, S.M. (1993). *Human Physiology*. Mosby, St. Louis.

Moncada, S., Herman, A.G., Higgs, E.A. & Vane, J.R. (1977). Differential formation of prostacyclin (PGX or PGI2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. *Thromb.Res.* **11**, 323-324.

Moncada, S. & Vane, J.R. (1979). Arachidonic acid metabolites and the interaction between platelets and blood vessel walls. *N.Engl.J.Med.* **300**, 1142-1147F.

Morris, MJ., Russel, AE. & Kapoor, V. (1986). Increases in plasma neuropeptide Y concentrations during sympathetic activation in man. *J.Auton.Nerv.Syst.* **17**, 143-149.

Murad, F. (1986). Cyclic guanosine monophosphate as a mediator of vasodilation. *J.Clin.Invest.* **78**, 1-5.

Mycek, M.J., Harvey, R.A. & Champe, P.C. (1997). *Pharmacology*. Lippincott-Raven, New York.

Nachev, C., Collier, J. & Robinson, B. (1971). Simplified method for measuring compliance of superficial veins. *Cardiovasc.Res.* **5**, 147-156.

Nakamura, M., Funakoshi, T., Arakawa, N., Yoshida, H., Makita, S. & Hiramori, K. (1994). Effect of Angiotensin-Converting Enzyme Inhibitors on Endothelium-Dependent Peripheral Vasodilation in Patients With Chronic Heart Failure. *JACC* **24 (5)**, 1321-1327.

Netter, F.H. (1974). *Nervous System*. Ciba Medical Education Division, New Jersey.

Nishimura, H., Kubo, S., Ueyama, M., Kubota, J. & Kawamura, K. (1989). Peripheral hemodynamic effects of captopril in patients with congestive heart failure. *Am.Heart.J.* **117**, 100.

Nossel, H.L. & Vogel, H.J. (1982). *Pathobiology of the endothelial cell*. Academic Press, New York.

Oellerich, W.F. & Malik, K.U. (1993). Neuropeptide Y modulates the vascular response to periarterial nerve stimulation primarily by a postjunctional action in the isolated perfused rat kidney. *J.Pharmacol.Exp.Ther.* **266**, 1321-1329.

Ohtomo, Y., Meister, B., Hokfelt, T. & Aperia, A. (1994). Coexisting NPY and NE synergistically regulate renal tubular Na+,K+-ATPase activity. *Kidney Int.* **45**, 1606-1613.

Olivari, M.T., Levine, T.B. & Cohn, J.N. (1983). Abnormal neurohumoral response to nitroprusside infusion in congestive heart failure. *J.Am.Coll.Cardiol.* **2**, 411-417.

Packer, M. (1988). Neurohormonal interactions and adaptations in congestive heart failure. *Circulation* **77 (4)**, 721-729.

Packer, M., Lee, W.H. & Kessler, P.D. (1986). Preservation of glomerular filtration rate in human heart failure by activation of the renin-angiotensin system. *Circulation* **74**, 766-774.

Pan, H.Y-M., Hoffman, B.B., Pershe, R.A. & Blaschke, T.F. (1986). Decline in beta adrenergic receptor-mediated vascular relaxation with aging in man. *J.Pharmacol.Exp.Ther.* **239**, 802-807.

Pang, C.C.Y. (1994). *The effects of drugs on the venous system*. R.G. Landes Company, Austin.

Parmley, W.W. (1989). Pathophysiology and therapy of congestive heart failure. *J.Am.Coll.Cardiol.* **13**, 771-785.

Peduzzi, P., Simper, D., Linder, L., Strobel, W. & Haefeli, W.E. (1995). Neuropeptide Y in human hand veins: Pharmacologic characterization and interaction with cyclic guanosine monophosphate-dependent venodilators in vivo. *Clin.Pharmacol.Ther.* **58**, 675-683.

Pelletier, G., Desy, L., Kerkerian, L. & Cote, J. (1984). Immunocytochemical localization of neuropeptide Y (NPY) in the human hypothalamus. *Cell Tissue Res.* **238**, 203-205.

Pernow, J. & Lundberg, J.M. (1989). Modulation of noradrenaline and neuropeptide Y (NPY) release in the pig kidney in vivo: involvement of alpha2, NPY and angiotensin II receptors. *Naunyn Schmiedeberg's Arch.Pharmacol.* **340**, 379-385.

Pernow, J., Lundberg, J.M. & Kaijser, L. (1986a). Vasoconstrictor effects in vivo and plasma disappearance rate of neuropeptide Y in man. *Life Sci.* **40**, 47-54.

Pernow, J., Lundberg, J.M. & Kaijser, L. (1988). Alpha-adrenoceptor influence on plasma levels of neuropeptide Y-like immunoreactivity and catecholamines during rest and sympatho-adrenal activation in humans. *J.Cardiovasc.Pharmacol.* **12**, 593-599.

Pernow, J., Lundberg, JM. & Kaijser, L. (1987). Vasoconstrictor effects in vivo and plasma disappearance rate of neuropeptide Y in man. *Life Sci.* **40**, 47-54.

Pernow, J., Saria, A. & Lundberg, J.M. (1986b). Mechanisms underlying pre- and postjunctional effects of neuropeptide Y in the sympathetic control. *Acta.Physiol.Scand.* **126**, 239-249.

Persson, P.B., Ehmke, H., Nafz, B., Lang, R., Hackenthal, E. & Nobiling, R. (1991). Effects of neuropeptide Y on renal function and its interaction with sympathetic stimulation in conscious dogs. *J.Physiol.* **444**, 289-302.

Pohl, U. & Kaas, J. (1994). Interactions of hormones with the vascular endothelium. Effects on the control of vascular tone. *Drug Res.* **44(I)**, 459-461.

Rang, H.P. & Dale, M.M. (1991). *Pharmacology*. Churchill Livingstone, New York.

Rea, R.F. & Berg, W.J. (1990). Abnormal baroreflex mechanisms in congestive heart failure. Recent insights. *Circulation* **81**, 2026-2027.

Robinson, B.F. (1978). Assessment of the effect of drugs on the venous system in man. *Br.J.Clin.Pharmac.* **6**, 381-386.

Robinson, B.F., Collier, J.G., Karim, S.M.M. & Somers, K. (1973). Effect of Prostaglandins A1,A2,B1,E2 and F2alpha on Forearm Arterial Bed and Superficial Hand Veins in Man. *Clin.Sci.* **44**, 367-376.

Ross,M.H., Romrell, L.J. (1989). *Histology. A Text and Atlas*. Williams and Wilkins, Baltimore, Maryland.

Rothe, C.F. (1983). Reflex control of veins and vascular capacitance. *Physiol.Rev.* **63**, 1281-1342.

Salvemini, D., Misko, T.P., Masferrer, J.L., Seibert, K., Currie, M.G. & Needleman, P. (1993). Nitric oxide activates cyclooxygenase enzymes. *Proc.Natl.Acad.Sci.* **90**, 7240-7244.

Sandler, D.A. (1990). The pathophysiology of heart failure. In *Heart Failure* (Sandler, G. and Fry, J., eds.), Clinical Press, London, pp. 1-16.

Saria, A. & Beubler, E. (1985). Neuropeptide Y (NPY) and peptide YY (PYY) inhibit prostaglandin E2-induced intestinal fluid and electolyte secretion in the rat jejunum in vivo. *Eur.J.Pharmacol.* **119**, 47-52.

Schnizer, W., Klatt, R., Baeker, H. & Rieckert, H. (1978). Comparison of scintigraphic and plethysmographic measurements for determination of capillary filtration co-efficient in human limbs. *Basic Res.Cardiol.* **73**, 77-84.

Schror, K. (1985). Prostaglandins, other eicosanoids and endothelial cells. *Basic Res.Cardiol.* **80**, 502-514.

Schwartz, J.C. (1992). Multiple dopamine receptors: functional implications. *Clin.Neuropharmacol.* **15 (Suppl. 1 Pt A)**, 1A-2A.

Schwartz, T.W., Fuhlendorff, J., Kjems, L.L., Kristensen, M.S., Vervelde, M., O'Hare, M., Kretenansky, J.L. & Bjornholm, B. (1990). Signal epitopes in the three-dimensional structure of NPY interaction with Y1, Y2 and PP receptors. *Ann.NY Acad.Sci.* **611**, 35-47.

Shebuski, R.J. & Aiken, J.W. (1980). Angiotensin II Stimulation of Renal Prostaglandin Synthesis Elevates Circulating Prostacyclin in the Dog. *J.Cardiovasc.Pharmacol.* **2**, 667-677.

Sheikh, S.P., Roach, E., Fuhlendorff, J. & Williams, J.A. (1991). Localization of Y1 receptors for NPY and PYY on vascular smooth muscle cells in rat pancreas. *Am.J.Physiol.* **260**, G250-G257.

Shepherd, J.T. & Vanhoutte, P.M. (1975). *Veins and their control.* WB Saunders, Philadelphia, pp. 99-209.

Shigeri, Y., Mihara, S. & Fujimoto, M. (1991). Neuropeptide Y Receptor in Vascular Smooth Muscle. *Journal of Neurochemistry* **56**, 852-859.

Sicuteri, F., DelBianco, P.L., Fanciullacci, M. & Franchi, G. (1964). Il test della venoconstrizione per la misura della sensibilita alla 5-idrossitriptamina e alle catecolamine nell'uomo. *Boll.Soc.Ital.Biol.Sperim.* **40**, 1148-1150.

Silberbauer, K., Stanek, B. & Templ, H. (1982). Acute hypotensive effect of captopril in man modified by prostaglandin synthesis inhibition. *Br.J.Clin.Pharmac.* **14**, 87S-93S.

Smith, W.L., Sonnenburg, W.K., Witanabe, T. & Umagaki, K. (1988). Mechanisms of action of prostaglandin E2 and prostaglandin F2$\alpha$. In *PGE and PGF$\alpha$* (Halushka, P.V. and Mais, D.E., eds.), MTP Press, Lancaster, England, pp. 232-247.

Sternini, C. & Brecha, N. (1985). Distribution and colocalization of neuropeptide Y and tyrosine hydroxylase-like immunoreactivity in the guinea pig heart. *Cell Tissue Res.* **241**, 93-102.

Sundler, F., Bottcher, G., Ekblad, E. & Hakanson, R. (1993). PP, PYY and NPY. Occurrence and distribution in the periphery. In *The Biology of Neuropeptide Y and Related Peptides* (Colmers, W.F. and Wahlestedt, C., eds.), Humana Press, New Jersey, pp. 157-196.

Swedberg, K., Viquerat, C. & Rouleau, J.L. (1984). Comparison of myocardial catecholamine balance in chronic congestive heart failure and in angina pectoris without failure. *Am.J.Cardiol.* **54**, 783-786.

Tatemoto, K., Carlquist, M. & Mutt, V. (1982). Neuropeptide Y - A novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* **296**, 659-660.

Taylor, A.A. (1994). Autonomic control of cardiovascular function: Clinical evaluation inhealth and disease. *J.Clin.Pharmacol.* **34**, 363-374.

Teerlink, J.R., Gray, G.A., Clozel, M. & Clozel, J-P (1994a). Increased vascular responsiveness to norepinephrine in rats with heart failure is endothelium dependent. Dissociation of Basal and Stimulated nitric oxide release. *Circulation* **89**, 393-401.

Teerlink, J.R., Loffler, B.M., Hess, P., Maire, J.P., Clozel, M. & Clozel, J.P. (1994b). Role of Endothelin in the Maintenance of Blood Pressure in Conscious Rats With Chronic Heart Failure. *Circulation* **90(5)**, 2510-2518.

Thomas, J.A. & Marks, B.H. (1978). Plasma norepinephrine in congestive heart failure. *Am.J.Cardiol.* **41**, 233-243.

Tkachenko, B.I. & Chernjavskaja, G.V. (1971). Neurogenic responses of resistance and capacitance vessels. *Experientia* **27**, 782-784.

Toda, N. & Miyazaki, M. (1978). Responses of isolated dog cerebral and peripheral arteries to prostaglandins after application of aspirin and polyphloretin phosphate. *Stroke* **9**, 490.

Tyagi, M.G., Kan, H., Ruan, Y. & Malik, K.U. (1996). Studies on the Characterization of the Subtype(s) of Muscarinic Receptor Involved in Prostacyclin Synthesis in Rabbit Cardiomyocytes. *J.Of Receptor & Signal Transduction Research* **16(5&6)**, 273-296.

Unden, A., Tatemoto, K., Mutt, V. & Bartfai, T. (1984). Neuropeptide Y receptors in the rat brain. *Eur.J.Biochem.* **145**, 525-530.

Valdemarsson, S. & Edvinsson, A.B.L. (1994). Relationships between plasma levels of catecholamines and neuropeptides and the survival time in patients with congestive heart failure. *J.Intern.Med.* **235**, 595-601.

Valdemarsson, S., Edvinsson, L., Ekman, R., Hedner, P. & Sjoholm, A. (1991). Increased plasma level of substance P in patients with severe congestive heart failure treated with ACE inhibitors. *J.Intern.Med.* **230**, 325-331.

Vallance, P., Collier, J. & Moncada, S. (1989a). Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* **1**, 997-1000.

Vallance, P., Collier, J. & Moncada, S. (1989b). Nitric oxide synthesized from L-arginine mediates endothelium dependent dilatation in human veins in vivo. *Cardiovasc.Res.* **23**, 1053-1057.

Van Brummelen, P., Buhler, F.R., Kiowski, W. & Amann, F.W. (1981). Age-related decrease in cardiac and peripheral vascular responsiveness to isoprenaline: studies in normal subjects. *Clin.Sci.* **60**, 571-577.

Vane, J.R. & Botting, R.M. (1995). Pharmacodynamic profile of prostacyclin. *Am.J.Cardiol.* **75**, 3A-10A.

Vanhoutte, P.M. & Miller, V.M. (1989). Alpha2-adrenoceptors and endothelium-derived relaxing factor. *Am.J.Med.* **87 (Suppl.3C)**, 3C-1S-3C-5S.

Vanhoutte, P.M. & Shepherd, J.T. (1970). Effect of temperature on reactivity of isolated cutaneous veins of the dogs. *Am.J.Physiol.* **218**, 187-190.

Vatner, S.F., Hittinger, L. & Shannon, R. (1990). Reduced subendocardial coronary reserve. A potential mechanism for impaired diastolic function in the hypertrophied and failing heart. *Circulation* **81 (2 suppl)**, 1114-1118.

Vestal, R.E., Wood, A.J.J. & Shand, D.G. (1979). Reduced β-adrenoceptor sensitivity in the elderly. *Clin.Pharmacol.Ther.* **26**, 181-186.

Vincent, J., Blaschke, T.F. & Hoffman, B.B. (1992). Vascular reactivity to phenylephrine and angiotensin II: Comparison of direct venous and systemic vascular responses. *Clin.Pharmacol.Ther.* **51**, 68-75.

Vinet, R., Brieva, C., Pinardi, G. & Penna, M. (1991). Modulation of α-Adrenergic-Induced Contractions By Endothelium-Dervied Relaxing Factor in Rat Aorta. *Gen.Pharmac.* **22, No. 1**, 137-142.

Wahlestedt, C., Edvinsson, L., Ekblad, E. & Hakanson, R. (1985). Neuropeptide Y potentiates noradrenaline-evoked vasoconstriction: Mode of action. *J.Pharmacol.Exp.Ther.* **234 (3)**, 735-741.

Wahlestedt, C., Edvinsson, L., Ekblad, E. & Hakanson, R. (1987). Effects of neuropeptide Y at sympathetic neuroeffector junctions: Existence of Y1 and Y2 receptors. In *Neuronal Messengers in Vascular Function* (Nobin, A. and Owman, C., eds.), Fernstrom Symposium, pp. 231-242.

Wahlestedt, C. & Hakanson, R. (1986a). Effects of neuropeptide Y (NPY) on the sympathetic neuroeffector junction. Can pre-, postjunctional receptors be distinguished? *Med. Biol.* **64**, 85-88.

Wahlestedt, C., Hakanson, R., Vaz, C.A. & Zukowska-Grojec, Z. (1990). Norepinephrine and neuropeptide Y: vasoconstrictor cooperation in vivo and in vitro. *Am.J.Physiol.* **258**, R736-R742.

Wahlestedt,C., Reis,D.J. (1993). Neuropeptide Y-Related Peptides and Their Receptors - Are The Receptors Potential Therapeutic Drug Targets? *Annu.Rev.Pharmacol.Toxicol.* **32**, 309-352.

Wahlestedt, C., Yanaihara, N. & Hakanson, R. (1986b). Evidence for different pre- and post-junctional receptors for neuropeptide Y and related peptides. *Regul.Peptides* **13**, 307-318.

Wang, W., Chen, J.S. & Zucker, I.H. (1990). Carotid sinus baroreceptor sensitivity in experimental heart failure. *Circulation* **81**, 1959-1966.

Webb-Peploe, M.M & Shepherd, J.T. (1968). Veins and their control. *N.Engl.J.Med.* **278 (No. 6)**, 317-322.

Westfall, T.C., Carpentier, S., Chen, X., Beinfeld, M.N.C., Naes, L. & Meldrum, M.J. (1987). Prejunctional and postjunctional effects of neuropeptide Y at the noradrenergic neuroeffector junction of the perfused mesenteric arterial bed of the rat. *J.Cardiovasc.Pharmacol.* **10**, 716-722.

Westfall, T.C., Han, S.P., Kneupfer, M., Martin, J., Chen, X., Del Valle, , Ciarleglio, A. & Naes, L. (1990). Neuropeptides in hypertension: role of neuropeptide Y and calcitonin gene related peptide. *Br.J.Clin.Pharmac.* **30**, 755-825.

Wharton, J., Gordon, L., Byrne, J., Hrzog, H., Selbie, L.A., Moore, K., Sullivan, M.H.F., Elder, M.G., Moscoso, G., Taylor, K.M., Shine, J. & Polak, J.M. (1993). Expression of the human neuropeptide tyrosine Y1 receptor. *Proc.Natl.Acad.Sci.USA* **90**, 687-691.

Wheater, P.R., Burkitt, H.G. & Daniels, V.G. (1987). *Functional Histology. A Text and Atlas*. Churchill Livingstone, New York.

White, C. & Udwadia, B.P. (1975). β-adrenoceptors in the human dorsal hand vein, and the effects of propranolol and practolol on venous sensitivity to noradrenaline. *Br.J.Clin.Pharmac.* **2**, 99-105.

Whitney, R.J. (1953). The measurement of volume changes in human limbs. *J.Physiol.* **121**, 1-27.

Wiedeman, M.P. (1963). Dimensions of blood vessels from distributing artery to collecting vein. *Circ.Res.* **12**, 375-378.

Winther, JE., Espersen, K., Kanstrup, IL. & Christensen, N. (1992). Age-related changes of exercise-induced plasma catecholamines and neuropeptide Y responses in normal human subjects. *Acta.Physiol.Scand.* **144**, 129-133.

Zelis, R. & Flaim, S.F. (1982). Alterations in vasomotor tone in congestive heart failure. *Prog.Cardiovasc.Dis.* **24(6)**, 437-459.

Zhu, P., Zaugg, C.E., Simper, D., Hornstein, P., Allegrini, P.R. & Buser, P.T. (1995). Bradykinin improves postischaemic recovery in the rat heart: role of high energy phosphates, nitric oxide, and prostacyclin. *Cardiovasc.Res.* **29**, 658-663.

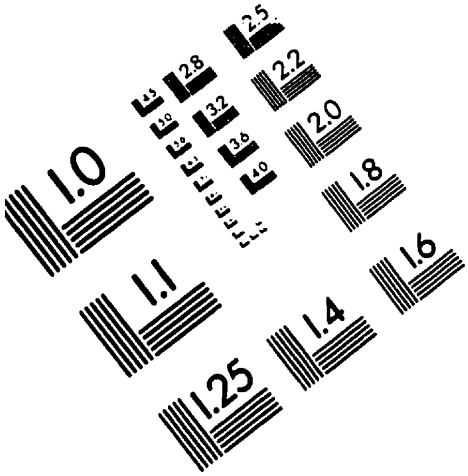
Zucker, I.H., Share, L. & Gilmore, J.P. (1979). Renal effects of left atrial distension in dogs with chronic congestive heart failure. *Am.J.Physiol.* **236**, H554-H560.

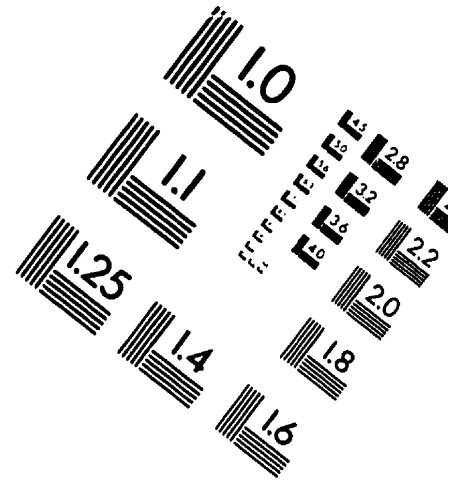
Zukowska-Grojec, Z., Shen, G.H., Deka-Starosta, A., Myers, K.A., Kvetnansky, R. & McCarty, R. (1992). Neuronal, adrenomedullary and platelet-derived neuropeptide Y responses to stress in rats. In *Stress: Neuroendocrine and Molecular Approaches* (Kvetnansky, R., McCarty, R. and Axelrod, J., eds.), Gordon and Breach Science Publishers, New York, pp. 197-209.

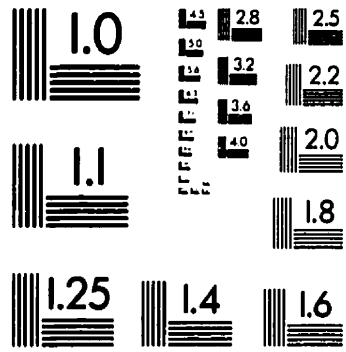
Zukowska-Grojec, Z. & Wahlestedt, C. (1993). Origin and Actions of Neuropeptide Y in the Cardiovascular System. In *The Biology of Neuropeptide Y and Related Peptides* (Colmers, W.F. and Wahlestedt, C., eds.), Humana Press, New Jersey, pp. 315-388.

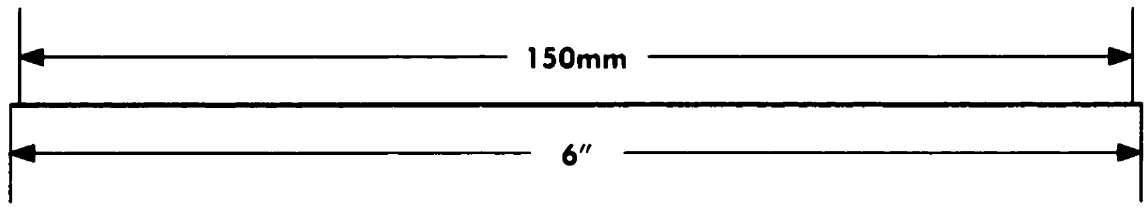
Zusman, R.M. & Keiser, H.R. (1977). Prostaglandin biosynthesis by rabbit renomedullary interstitial cells in tissue culture. Stimulation by angiotensin II, bradykinin, and arginine vasopressin. *J.Clin.Invest.* **60(1)**, 215-223.

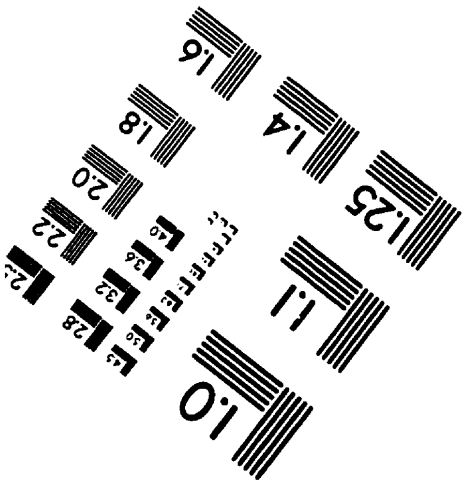
# IMAGE EVALUATION TEST TARGET (QA-3)

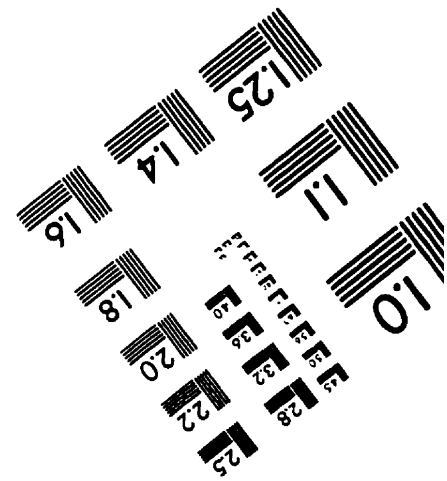
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