Antidepressants, in addition to being effective therapeutic agents for depression, have also proved to be multifaceted drugs useful for treating a number of other psychiatric and neurologic disorders. Despite the widespread use of these drugs, much remains to be understood about their mechanisms of action and other important aspects, such as their metabolism and potential interactions with other drugs. This article reviews research conducted in the authors’ laboratories on various aspects of antidepressants, including trace amines and antidepressants, γ-aminobutyric acid and antidepressants, drug metabolism, development and application of rapid, sensitive assay procedures for antidepressants and their metabolites, and drug development based on analogues of the antidepressants phenelzine and tranylcypromine. The significance of this work to future drug development is also discussed.

Outre qu’ils sont des agents thérapeutiques efficaces contre la dépression, les antidépresseurs ont aussi prouvé leur utilité contre de nombreux autres troubles psychiatriques et neurologiques. Malgré leur utilisation répandue, il reste encore beaucoup à comprendre au sujet du mode d’action et de certains autres aspects importants de ces médicaments, notamment leur métabolisme et leur interaction possible avec d’autres médicaments. Cet article traite des recherches effectuées dans les laboratoires des auteurs sur divers aspects des antidépresseurs, y compris leur effet sur les amines-traces et l’acide γ-aminobutyrique, le métabolisme des médicaments et la mise au point de méthodes sensibles de dosage des antidépresseurs et de leurs métabolites, ainsi que du développement de médicaments basés sur deux analogues des antidépresseurs, la phénélzine et la tranylcypromine. Il est aussi question de l’importance de ces travaux pour la mise au point future de médicaments.

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Antidepressants are obviously of primary interest for their ability to alleviate the suffering of countless millions of people whose lives have been disrupted by depression. However, they are multifaceted drugs that have proven useful for the treatment of a number of other disorders, including various anxiety disorders, eating disorders, premenstrual dysphoric disorder, some personality disorders, migraine and chronic pain syndromes. In many cases, they produce a wide array of side effects due to blockade of receptors for numerous neurotransmitters. Many antidepressants are metabolized extensively by and/or inhibit cytochrome P450 enzymes, opening up the possibility of pharmacokinetic drug–drug interactions in patients taking other drugs concomitantly. In addition, several antidepressants have chiral centres or centres of unsaturation, resulting in stereo-isomers or geometric isomers, which may affect the overall profile of the parent drug. Over the past 20 years, we have been involved in research on several of the aspects of antidepressants mentioned above. We hope that this review will provide a concise overview of some of that work. Although many groups, including ours, have conducted research on possible mechanisms of action of antidepressants, it is beyond the scope of this review to cover such research in detail, and the reader is referred to several recent excellent reviews on this topic. Aspects of antidepressants discussed in the present review include involvement of trace amines and γ-aminobutyric acid (GABA) in antidepressant action; relevance of drug metabolism to the actions of antidepressants; development of assay techniques for analysis of antidepressants or their metabolites; and possible future drug development based on analogues of monoamine oxidase (MAO) inhibitors.

**Trace amines and antidepressants**

The inhibition of monoamine oxidase (MAO) by drugs such as phenelzine and tranylcypromine results in an often-dramatic elevation of a number of brain amines (2-phenylethylamine [PEA], m- and p-tyramine, octopamine, tryptamine), which are termed “trace amines” because of their low absolute concentrations in the brain relative to the classical neurotransmitter amines such as the catecholamines dopamine (DA) and norepinephrine (NE) and the indolealkylamine 5-hydroxytryptamine (5-HT, serotonin). These trace amines can have marked effects on uptake or release of the catecholamines or 5-HT at nerve endings or may act as neuromodulators through direct actions on receptors for the catecholamines or 5-HT. Our interest in the trace amines was further stimulated by findings in our laboratories and those of others that PEA is actually a metabolite of phenelzine. Since phenelzine has been shown in receptor binding studies and in behavioural tests to produce down-regulation of β-adrenergic receptors, we conducted further experiments, using a behavioural response (change in locomotor activity) to salbutamol, to examine the effects on β-adrenergic activity of conditions that resulted in elevations of PEA. These studies provided evidence that PEA may contribute to the effects of phenelzine on β-adrenergic receptor function.

The possible involvement of tryptamine in depression had been postulated some time ago, but it was not until the 1980s that a saturable and specific high-affinity binding site for tryptamine in brain was demonstrated. Using radioligand binding studies, we demonstrated that the MAO inhibitors tranylcypromine, phenelzine and clorgyline, administered for long periods and at doses at which they produce a down-regulation of β-adrenergic receptors, produce a decrease in β-tryptamine binding site density in rat brain, without any effects on the affinity of this site. Further studies in rats receiving low and high doses of tranylcypromine comparable, on a dose-per-body-weight basis, to usual therapeutic doses and doses sometimes used to treat refractory depression in humans, indicated that the higher doses of tranylcypromine produce a more rapid down-regulation of tryptamine receptors in the hippocampus and a greater reduction of such receptors in striatum than do low doses.

**Antidepressants and GABA**

Since the introduction of the original theories suggesting that depression is the result of a functional deficiency of biogenic amines at central synapses, research on depressive disorders has concentrated on searching for biochemical lesions involving biogenic amines, particularly NE and 5-HT, and on investigating the effects of antidepressant drugs on the reuptake, metabolism and receptor activity of these amines. However, the biogenic amine hypothesis as originally stated has not been entirely satisfactory in explaining the delay in therapeutic actions of antidepressants. The possible role of other neurotransmitters and neuromodulators in the etiology and pharmacotherapy of depression has
Neurochemical and metabolic aspects of antidepressants

received considerable attention in recent years. One such neurotransmitter is GABA. This amino acid has long been thought to be important in generalized anxiety disorder, since the benzodiazepine anxiolytics are known to act through the GABA<sub>A</sub> receptor. GABA has also been proposed as being important in the actions of antipanic drugs, which is of particular interest since there is considerable comorbidity of depression and anxiety disorders, including panic disorder.

There is now a voluminous literature on the possible involvement of GABA in the pathophysiology of mood disorders. This literature, which includes studies of animal models of depression, radioligand binding and functional studies in rodent brain, measurements of GABA in cerebrospinal fluid (CSF), plasma and post mortem brain tissue and neuroendocrine challenge studies is not without conflicting findings, but does implicate GABA in the pathophysiology of depression.

Antidepressant actions of the GABA agonists progabide, baclofen, muscimol and fengabine have been reported in animal models and in human subjects, although there is controversy in this area. The anticonvulsants vigabatrin and valproic acid, both GABAergic drugs, have also been reported to have anxiolytic and antipanic effects in humans and animal models. Valproic acid has also recently been proposed as a possible treatment for major depressive disorder. The triazolobenzodiazepines alprazolam and adinazolam (benzodiazepines are agonists at the benzodiazepine site of the GABA<sub>A</sub> receptor) have been reported to be effective antidepressants.

Long-term administration of several types of antidepressants as well as repeated electroshocks have been reported by some workers to result in an upregulation of GABA<sub>A</sub> receptors in rat cortex, but these findings have been disputed by others, including researchers in our laboratories.

Suranyi-Cadotte et al reported that long-term administration of several types of antidepressants to rats resulted in a decreased number of H-flunitrazepam binding sites in rat brain. In contrast, Kimber et al found no such decrease after long-term administration of desipramine, tranylcypromine or zimelidine. Barbaccia et al found that maprotiline, when administered to rats for 21 days, produced a significant decrease in the number of H-flunitrazepam binding sites.

We found no change in H-flunitrazepam binding in rat cerebral cortex after long-term administration of the antidepressants phenelzine, clomipramine, desipramine or maprotiline. We also found that long-term administration of phenelzine resulted in no changes in binding of the GABA<sub>A</sub> receptor agonist H-muscimol in rat cortical homogenates or in GABA-stimulated Cl<sup>-</sup> flux in synaptoneurosomes, but did result in changes in steady-state levels of some isoforms of GABA<sub>A</sub> receptor subunits (see the discussion of this aspect later in this section).

Our interest in GABA and antidepressants was stimulated initially by early reports in the literature indicating that phenelzine, an MAO-inhibiting antidepressant and antipanic drug, produces an elevation of rat brain GABA. Subsequent experiments in our laboratories indicated that this is a rather dramatic effect, with GABA levels remaining elevated for lengthy periods after a single dose of phenelzine (Fig. 1).

Similar increases of brain alanine (ALA) occurred, while under the same conditions levels of glutamine (GLN) declined markedly (Fig. 2), although this decrease was briefer that the increases seen with GABA and ALA. Experiments in our laboratories with long-term administration of phenelzine (14 and 28 days) have also demonstrated significant elevations of GABA and ALA and decreases in GLN compared with vehicle-treated control values in rat brains (unpublished data). Plasma GABA levels in humans are also significantly elevated after long-term administration of phenelzine. We have also demonstrated recently, using in vivo microdialysis, that phenelzine increases extracellular GABA and ALA in various brain areas.

![Fig. 1: Rat brain levels of GABA (% of controls treated with vehicle at the same time intervals) at various times after injection of a single dose of phenelzine (15 mg/kg intraperitoneally). All values are significantly higher than controls. Control levels were mean 234 µg/g, standard deviation (SD) 8 µg/g (n = 30). Adapted from Baker et al.](image-url)
GABA is formed in the metabolic pathway referred to as the GABA shunt, and formation of GLN is closely associated with the GABA shunt through another loop in which GABA is taken up by glial cells and metabolized by GABA-T. The glial cells lack glutamic acid decarboxylase (GAD), so the glutamate (GLU) formed in the transamination reaction is transformed by GLN synthetase (present only in glia) into GLN, which can be returned to the nerve ending to be converted back to GLU by the enzyme glutaminase. It is thus feasible that phenelzine may also be altering levels of GLU and GLN through actions on GLN synthetase or glutaminase, but such actions have not, to our knowledge, been investigated. It is interesting in this regard that Collins et al reported that the anticonvulsant valproic acid, which increases brain levels of GABA, also increases glutaminase activity and decreases GLN synthetase activity in cultures of rat brain astrocytes.

There is relatively little known about the function of ALA in the central nervous system, but levels are approximately 25% to 40% of those for GABA. Like glycine, ALA is a co-agonist of N-methyl-D-aspartate (NMDA) excitatory amino acid receptors, although it is weaker than glycine in this regard. ALA is also related metabolically to lactate in the brain. Since sodium lactate is a panicogenic agent, and since recent magnetic resonance spectroscopy studies have shown that higher levels of lactate are attained in brains of patients with panic disorder than in controls after a lactate challenge, it will be interesting to see if the increase in brain ALA produced by phenelzine is accompanied by a decrease in plasma, CSF or brain lactate. ALA has also been reported to be an inhibitor of GLN synthetase, which may contribute to the GLN reduction we have observed in rat brain after phenelzine administration. The study of the effects of phenelzine on the various amino acids and their metabolism is made even more intriguing by reports that GLN serves as a precursor for GABA, GLU and ALA.

Phenelzine is an inhibitor of the catabolic enzymes GABA transaminase (GABA-T) and ALA transaminase (ALA-T), presumably accounting for the increase in brain levels of GABA and ALA mentioned above, although other factors may be important, since marked increases in levels of these amino acids (e.g., 2–4 fold increases) are observed even when inhibition of the enzymes is 50% or less. Phenelzine is apparently not just a nonspecific inhibitor of transaminases, since we found no effect of the drug on brain levels of leucine, isoleucine and valine, which are also metabolized by pyridoxal phosphate-dependent transaminases.

It is of interest that phenelzine is a much more potent inhibitor, in vitro and in vivo, of GABA-T than is vigabatrin, a GABA-T inhibitor marketed as anticonvulsant. Like phenelzine, vigabatrin has been reported to have anxiolytic properties. Shuaib et al also found vigabatrin to be a potential anti-ischemic agent, prompting preliminary studies of phenelzine in this regard. Although neuroprotective effects were evident at relatively high doses of phenelzine, further dose–response and time–response studies are required. It would also be of interest to investigate in some detail the anticonvulsant properties of phenelzine, particularly since several antidepressants actually increase susceptibility to seizures. If anticonvulsant effects are evident, they could be due to the GABAergic actions of phenelzine or to inhibition of MAO-A, as has been demonstrated with other MAO inhibitors. Because we have an analogue of phenelzine (N-acetylphenelzine — see Future Directions section of this paper) that retains

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**Fig. 2:** Effects of administration of phenelzine (P) at 15 and 30 mg/kg intraperitoneally 3 hours previously on glutamate levels in hypothalamus (white bars). The decreases in glutamate levels were blocked by pretreatment with another MAO inhibitor, (tranylcypromine [TP], 5 mg/kg) 1 hour before phenelzine administration (hatched bars). Results are expressed as mean percentages (and standard errors of the mean) of values in vehicle-treated controls. Values at all time intervals were significantly higher (p < 0.05) in the phenelzine-treated rats than in the corresponding vehicle-treated rats. Reproduced from Progress in Brain Research, vol. 106, Paslawski TM, Sloley DB, Baker GB, “Effects of the MAO inhibitor phenelzine on glutamate and GABA concentrations in rat brain,” p. 181-6, © 1995, with permission from Elsevier Science.
the MAO-inhibiting properties of phenelzine but has no effect on GABA, it should be possible to ascertain which neurochemical actions of phenelzine are most relevant to any anticonvulsant effects which may be observed.

Popov and Matthies\textsuperscript{64} showed many years ago that the GABA-elevating effect of phenelzine (apparently as a result of inhibition of GABA-T) in rats could be reduced dramatically by pretreating rats with other MAO inhibitors. They said that this result implied that a metabolite of phenelzine, produced by the action of MAO on phenelzine, was responsible for the effect on GABA; this seemed likely since Clineschmidt and Horita\textsuperscript{92,93} had demonstrated that not only was phenelzine an inhibitor of MAO, but it was also a substrate for the enzyme, with phenylacetic acid apparently one of the metabolites. Despite these exciting early reports, the metabolite of phenelzine responsible for the GABA-elevating action still remains unknown. 1-(2-phenethyl)diazine (PhCH$_2$CH$_2$N=NH) and phenylethylidenedrazine (PhCH$_2$CH=N-NH$_2$) have been suggested as possible metabolites formed by the action of MAO on phenelzine.\textsuperscript{92,93} It is doubtful that the former would exist because of the ease with which it would presumably expel a molecule of nitrogen. The latter proposed metabolite, phenylethylidenedehydrazine (PEH), can be prepared by the interaction of phenylacetaldehyde and excess hydrazine. We have now synthesized this compound, and preliminary studies have shown the following: like phenelzine, PEH elevates brain GABA and ALA and depletes brain GLN; unlike phenelzine, it is not a potent inhibitor of MAO.\textsuperscript{94}

As can be seen from this literature overview, there is controversy about the effects of antidepressant drugs on GABA receptors at the molecular level. However, it may be that many of the procedures used to date are not sufficiently precise to pick up subtle changes in GABA receptors; recent studies with benzodiazepines show that these drugs, which often fail to cause perceptible changes in benzodiazepine receptor number in radioligand binding studies, do cause changes in expression of some of the messenger RNAs for isoforms of GABA$_\alpha$ receptor subunits. The GABA$_\alpha$ receptor is an oligomeric structure comprised of 5 binding domains.\textsuperscript{25} The GABA$_\alpha$ receptor subunits, within the functional receptor oligomer, are from at least 7 distinct classes: $\alpha$, $\beta$, $\gamma$, $\delta$, $\epsilon$, $\pi$ and $\theta$.\textsuperscript{95,96} Some of the classes have a number of different isoforms, e.g., $6\alpha$, $3\beta$ and $3\gamma$. The precise subunit isoform composition of any GABA$_\alpha$ receptor in vivo is currently unknown, but transient expression studies have demonstrated the importance of the subunit isoform composition to the recognition properties and functional characteristics of the resulting oligomer.\textsuperscript{97–99} The effects of anxiolytic and antidepressant drugs may be due, at least in part, to the substitution of one subunit isoform in a given functional receptor with another. This would change the characteristics of the drug response without necessarily changing binding capacity.

Our experiments with imipramine and phenelzine indicate that they do modulate GABA$_\alpha$ receptor subunit gene expression. The major GABA$_\alpha$ receptor subtype in mammalian brain is believed to be comprised of $\alpha 1$, $\beta 2$ and $\gamma 2$ subunits.\textsuperscript{97–100} In rats, 21-day treatment with either phenelzine or imipramine caused significant increases in $\beta 2$- and $\gamma 2$-subunit gene expression in brain stem compared with vehicle controls; in contrast, $\alpha 1$-subunit mRNA levels were decreased by phenelzine.\textsuperscript{101} We are currently examining other GABA$_\alpha$ receptor subunit transcripts to determine the profile of changes in gene expression produced by these therapeutic agents. We have preliminary data to suggest that, in other brain regions, phenelzine causes differential changes in gene expression to those produced in brain stem. Thus, we have demonstrated the capacity of phenelzine to induce changes in GABA$_\alpha$ receptor gene expression and have revealed the need to conduct such experiments on a brain-regional basis. We have recently also shown that long-term administration of phenelzine causes an increase in steady-state levels of mRNA for the GABA transporter GAT-1 in rat cerebral cortex.\textsuperscript{102} Thus, long-term phenelzine treatment modulates the expression of genes that encode components of GABAergic transmission. Further, the long-term changes in gene expression (in contrast to acute pharmacodynamic effects), correlate temporally with the known therapeutic latency of antidepressant/antipanic agents.

**Development of assay procedures for antidepressant drugs**

A major research emphasis in the Neurochemical Research Unit is the development of sensitive procedures for analysis of biogenic amines, amino acids and psychotropic drugs, including antidepressants. Such assays for antidepressants permit us to monitor tissue and body-fluid levels of the drugs of interest and their major metabolites, and to examine possible pharmacokinetic drug–drug interactions. Although high-pressure
liquid chromatography (HPLC) and combined gas chromatography-mass spectrometry (GC-MS) have been used for some of this work in our laboratories, the emphasis has been on developing gas chromatographic procedures with electron-capture or nitrogen-phosphorus detection, which provide sensitive, economical assays for routine analyses not only of the antidepressants, but also of various other drugs, biogenic amines and amino acids.\textsuperscript{103-113} Several of the assay procedures developed, e.g., those using reactions with acetic anhydride, pentafluorobenzoyl chloride or pentafluorobenzensulfonyl chloride, have permitted the extractive derivatization of such drugs under aqueous conditions, providing relatively rapid assays.\textsuperscript{104-107,112,113}

The availability of such analytical techniques facilitates clinical investigations of the metabolism of antidepressants. However, we also routinely measure brain levels of antidepressants under investigation in laboratory animals, thus ensuring that adequate levels have been attained in brain.\textsuperscript{43,101,114} such measurements are particularly important when using osmotic minipumps, to ensure that the drugs are being delivered adequately from the pumps. These routine assay procedures are also very useful for measuring metabolites of antidepressants and for studying pharmacokinetic drug–drug interactions in laboratory animals. The formation of metabolites is often neglected in such studies, the assumption being that any behavioural or neurochemical effects are the result of the parent drug; however, many drugs used to treat psychiatric disorders are biotransformed extensively in the body into active metabolites, and the investigator should be aware of the brain levels of those metabolites. In addition, some drugs may be metabolized to a much different extent in the laboratory animals than in humans. It is important to be aware of such differences, in case behavioural or neurochemical studies conducted in particular animal species have no relevance to the situation in humans. In addition, many behavioural studies in animals involve the use of multiple drugs, with the assumption that the drugs are interacting pharmacodynamically, when in fact pharmacokinetic interactions are occurring and possibly giving misleading results. By applying our analytical assay procedures to rat brain, we have observed in recent years several such pharmacokinetic interactions involving selective serotonin reuptake inhibitors (SSRIs) in rat brain.\textsuperscript{114-116}

Another factor to consider in analyzing antidepressants is that, like numerous other drugs commercially available,\textsuperscript{117} several antidepressants have chiral centres or centres of unsaturation, and thus enantiomers or geometric isomers of the same drug may exist. The individual enantiomers or geometric isomers may differ in their pharmacological or pharmacokinetic profiles,\textsuperscript{118,119} including metabolic interactions with other drugs. While some antidepressants (e.g., sertraline and paroxetine) are marketed as the individual enantiomers, others (e.g., fluoxetine, tranylcypromine, trimipramine, citalopram) are marketed as racemic mixtures of the enantiomers. In the latter case, it may be important to be able to monitor levels of the individual enantiomers. We have used reactions with individual enantiomers of halogenated derivatizing reagents followed by GC with electron-capture detection to investigate the levels of enantiomers of antidepressants such as tranylcypromine and fluoxetine (and of its metabolite, norfluoxetine) in tissues and body fluids (Fig. 3).\textsuperscript{110,120,121}

![Fig. 3: Gas chromatogram of derivatized extract of rat brain from a fluoxetine-treated (10 mg/kg) animal killed 3 hours after administration. The gas chromatography peaks of the stereoisomers are identified as follows: 1 = (S)-norfluoxetine; 2 = (R)-norfluoxetine; IS = internal standard (alprenolol); 3 = (R)-fluoxetine; 4 = (S)-fluoxetine.](image)

Metabolism of antidepressants

Our interest in the metabolism of antidepressants stemmed from 3 primary observations or developments: (1) very little was known about the metabolism of phenelzine and tranylcypromine, despite the fact that they had been on the market for considerable lengths of time; (2) although it had been recognized for many years that there could be metabolic (pharmacokinetic) drug–drug interactions between antidepressants and other drugs that psychiatric patients were taking concomitantly, such potential interactions gained increased attention with the introduction of SSRIs, several of which are relatively potent inhibitors of enzymes involved in drug metabolism; and (3) the recent ready access to human liver microsomes and individual complementary DNA-expressed cytochrome P450 enzymes has facilitated in vitro studies of drug metabolism.

Much is still unknown about the metabolism of phenelzine and tranylcypromine and the contribution of metabolites to their overall pharmacological profiles.122-127 Numerous studies have been carried out on the acetylator status of patients and their subsequent response to treatment with phenelzine. These investigations were conducted based on the assumption that phenelzine is acetylated because it is similar in structure to drugs such as isoniazid, which are known to be acetylated. In fact, the existence of N-acetylphenelzine as a metabolite of phenelzine had not been adequately demonstrated until the 1980s, and indications are that it is only a minor metabolite.128-130 Phenelzine is an unusual drug that it not only inhibits MAO, but also apparently constitutes a substrate for MAO. Clineschmidt and Horita,90,91 using radiolabelled phenelzine, suggested that phenylacetic acid (PAA) is a major metabolite of phenelzine. In a later study using mass spectrometry to identify metabolites,124 PAA and 4-hydroxyphenylacetic acid (4-OH-PAA) were identified in human urine samples as major metabolites of phenelzine. 4-OH-PAA is also of interest because it is a metabolite of the endogenous amine p-tyramine (4-OH-PEA). PEA is also a known metabolite of phenelzine,129,132 and there is now indirect evidence for the formation of 4-hydroxyphenelzine (4-OH-phenelzine) from phenelzine.131 These observations raise the question of the routes of formation of 4-OH-PAA. Possible routes are as follows: phenelzine → PAA → 4-OH-PAA; phenelzine → PEA → p-tyramine (and/or PAA) → 4-OH-PAA; and phenelzine → 4-OH-phenelzine → 4-OH-PAA (with or without a p-tyramine intermediate) (Fig. 4). These routes have not been studied thoroughly, nor is there detailed information on the pharmacological activity of metabolites such as 4-OH-phenelzine. Another possible route of metabolism of phenelzine is N-methylation, which was demonstrated by Yu et al125 using enzymes obtained from bovine and adrenal glands.

Alleva126 reported that hippuric acid is a metabolite of tranylcypromine, but concluded that amphetamine was not involved as an intermediate in this metabolism. The metabolic formation of amphetamine from tranylcypromine continues to be debated. Youdim et al134 reported the presence of amphetamine in the plasma of a patient who had overdosed on tranylcypromine, but studies conducted by others did not find amphetamine in tissues or body fluids from subjects taking tranylcypromine.135-137 Comprehensive studies in our laboratories146 on humans and rats have not revealed amphetamine in human urine or in rat brain, liver or plasma after the administration of pharmacologically relevant doses of tranylcypromine. The presence of the N-acetyl104 and ring hydroxylated144,145 metabolites of tranylcypromine have been demonstrated in rat brain after intraperitoneal administration of tranylcypromine. Kang and Chung146 confirmed the formation of N-acetyltranylcypromine and also identified N-acetyl-4-hydroxytranylcypromine as a tranylcypromine metabolite in rat urine.

A better understanding of the metabolism of drugs such as phenelzine and tranylcypromine may be useful in future drug design studies. Our investigations of phenelzine metabolism led us to synthesize and characterize N-acetylphenelzine and PEH. Although the former seems to be only a very minor metabolite of phenelzine and we have not yet demonstrated that the latter is a metabolite of phenelzine, both drugs have pharmacological profiles quite different from phenelzine and from each other and may be potential therapeutic agents in their own right (see the section on Future directions: drug development later in this review). Several analogues of tranylcypromine in which the 4 position of the phenyl ring is protected from hydroxylation have been synthesized and investigated in our laboratories. Two of these analogues, namely 4-fluorotranycypromine and 4-methoxytranylcypromine, have proved to be potent MAO inhibitors.148-150 Further studies of 4-fluorotranycypromine have revealed that it is a stronger inhibitor of MAO than tranylcypromine, it has a better pharmacokinetic profile
than tranylcypromine, it has a different pattern of activity than the parent drug with regard to inhibition of reuptake of biogenic amines, and it is less likely than tranylcypromine to interact pharmacokinetically with inhibitors of cytochrome P450 enzymes.149

**Cytochrome P450 and metabolism of antidepressants**

Formation of metabolites or drugs is often ignored, the assumption being made that the drug itself is the active factor. However, metabolites may contribute significantly to therapeutic effects and adverse side effects, and many drugs used to treat psychiatric disorders undergo extensive metabolism.150–156 While many enzymes may be involved in drug metabolism reactions, cytochrome P450 (CYP) enzymes are of particular importance in oxidative metabolism. There are at least 14 different gene families (1–5, 7, 8, 11, 17, 19, 21, 24, 27, 51) of CYP enzymes in humans, and families 1–3 are particularly implicated in the metabolism of numerous drugs.157

Knowledge of which CYP enzyme is involved in a metabolic process is important. If 2 or more drugs significantly metabolized by or inhibiting the same CYP enzyme are administered concomitantly to a patient, there may be competition for the enzyme, and the phar-
macokinetic properties of each drug may differ from those observed when each drug is administered individually. If the pharmacological activity of a drug resides primarily in the parent compound rather than its metabolites, the active drug may accumulate in this situation and could demonstrate exaggerated effects. However, the parent compound might be a prodrug, and the metabolic bioactivation necessary for drug effects may proceed via the CYP enzyme inhibited by the concomitantly administered drug. In this case, smaller amounts of the active metabolite will be formed, and there will be diminished therapeutic effects.

For many years, researchers have been aware of the possibility of metabolic (pharmacokinetic) drug–drug interactions involving psychiatric drugs (e.g., phenothiazone antipsychotic drugs and tricyclic antidepressants), but there has been a marked increase in research in this area and increased awareness by physicians since the introduction of the SSRI antidepressants. Several developments have enhanced research on drug metabolism involving antidepressants and increased awareness of the potential for pharmacokinetic drug–drug interactions:113–116 the popularity of the SSRLs, the knowledge that several are quite potent inhibitors of CYP enzymes, and advances in CYP research (e.g., the increased availability of human liver microsomes and the use of molecular biological techniques to express CYP enzymes from human sources in various cell lines easily accessible to researchers). SSRLs (which are substrates for or inhibitors of several CYP enzymes) are often administered in combination with other neuropsychiatric drugs, such as benzodiazepines, antipsychotics, anticonvulsants and even other antidepressants, which are substrates for one or more CYP enzymes. Thus, the possibility of drug–drug interactions is a serious consideration.

For the past several years, we have used gas chromatographic techniques developed in our laboratories108–113 and HPLC techniques to investigate metabolism and metabolic drug–drug interactions involving the following antidepressants: imipramine, amitriptyline, trimipramine, iprindole, fluoxetine, tranylcypromine, phenelzine, trazodone and nefazodone. In studies of urine samples obtained from rats treated with antidepressants, the existence of several hitherto unidentified hydroxylated metabolites of imipramine, trimipramine and iprindole were demonstrated.108–113 In studies with cDNA-expressed CYP enzymes from human sources, CYP2D6 was demonstrated to be involved in a wider range of metabolic reactions than had been previously thought.114–117 Levels of fluoxetine and norfluoxetine in rat brain were shown to be increased by coadministration of desipramine or iprindole,118,119 known inhibitors of CYP2D6 in humans. An assay procedure for p-trifluoromethylphenol was developed, and this compound was shown to be present in substantial amounts in rat brain, urine and liver and in human plasma and urine from subjects treated with fluoxetine.120 More recent experiments, using human liver microsomes and individual cDNA-expressed CYP enzymes, have demonstrated that (1) formation of m-chlorophenylpiperazine (mCPP) from nefazodone and trazodone (Fig. 5) and of hydroxynefazodone and triazolodine (TD) from nefazodone are mediated principally by CYP3A4,168,170 and (2) hydroxylation of mCPP is largely under the control of CYP2D6.171 These experiments were also conducted in the presence and absence of inhibitors of CYPs 3A4 and 2D6 (ketonazole and quinidine, respectively).

Space does not permit further detailed discussion of these metabolism studies, but similar techniques were also extended in our laboratories to investigations of the involvement of various CYP enzymes in the metabolism of the antipsychotic drugs haloperidol,122 clozapine172 and risperidone.123

The availability of information on the effect of CYP enzymes from human sources on in vitro metabolism of antidepressants is important not only for a better understanding of the therapeutic and side effects of these drugs but as a warning for potential pharmacokinetic drug–drug interactions involving these agents. Ultimately, such interactions must be investigated in vivo to assess their practical clinical relevance, but the in vitro studies represent a relatively economical and rapid method of determining potential hazards and aiding in more effectively conducting future clinical investigations.

Future directions: drug development

Although much of the research described above began as basic studies on the metabolism or mechanisms of action of antidepressants, the resulting findings, primarily with phenelzine, have led us into other areas of research into neuropsychiatric disorders and have provided important clues for future drug development. As part of our investigations of phenelzine metabolism, we synthesized N2-acetylphenelzine and PEH (Fig. 6), each
of which differs importantly from the parent drug with regard to pharmacological properties. While phenelzine inhibits both MAO and GABA-T, N\textsuperscript{\textregistered}-acetylphenelzine inhibits MAO but not GABA-T (175) and PEH inhibits GABA-T but has minimal effects on MAO. The use of all 3 drugs as pharmacological tools should allow us to tease out the involvement of GABA and biogenic amines in individual neuropsychiatric disorders such as depression, anxiety, epilepsy and stroke. For example, N\textsuperscript{\textregistered}-acetylphenelzine has demonstrated antidepressant activity in the forced swimming test,\textsuperscript{175} but not anxiolytic properties in the elevated plus maze test.\textsuperscript{176} In the same tests, phenelzine demonstrated both antidepressant and anxiolytic actions.\textsuperscript{175,176} PEH has now been tested, using a global ischemia model in gerbils, for potential neuroprotective and anti-ischemic (antistroke) activity, since several other GABAergic agents have previously been shown to be protective in such models of ischemia (stroke).\textsuperscript{86,177,178} When given just before induction of ischemia, PEH provides extensive neuroprotection.\textsuperscript{179} Studies are now under way to determine effective “windows” (times after induction of ischemia) when PEH is effective in providing neuroprotection; such experiments will also include comparisons with phenelzine itself. Since GABAergic agents have also been shown to be effective mood stabilizers, anticonvulsants and anxiolytics, comprehensive comparisons of phenelzine and PEH in animal models for screening for such drugs are planned.

As mentioned in the introduction to this review, many antidepressants are multifaceted drugs with a wide array of uses. Although phenelzine is known to be effective in treatment of depression, panic disorder and social phobia, it may also be useful in epilepsy and stroke or, through its analogues, give important clues to development of future drugs effective in these conditions.

**Conclusions**

For many years, research in our laboratories has focused on antidepressants. This research included extensive studies on the involvement of trace amines...
and GABA in the action of antidepressants. The work on trace amines necessitated the development of sensitive gas chromatographic assay procedures for these substances in the brain. These methods were subsequently modified to provide sensitive, relatively rapid assays for numerous antidepressants and their metabolites, which were applied to measurements in tissues and body fluids and to studies on drug metabolism using CYP enzymes. The studies of GABA have resulted in a greater understanding of the possible role of this neurotransmitter amino acid in the actions of antidepressants, particularly phenelzine. Extensions of the GABA research and of the work on metabolism of MAO inhibitors have resulted in the development of several new drugs with considerable potential as useful pharmacological tools and therapeutic agents, not only for depression but also for other neuropsychiatric disorders. The findings on the effects of phenelzine on GABA have suggested that this drug, which is already useful in treatment of a number of depressive and anxiety disorders, should also be investigated for its usefulness as a neuroprotective agent. Thus, studies on the mechanisms of action and metabolism of antidepressants, as multifaceted drugs, have provided not only useful information about the etiology of depression and about possible pharmacokinetic interactions involving these drugs, but also important clues about the treatment of other neuropsychiatric disorders and the future development of potentially useful drugs for these disorders.

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**2000 CCNP Award Winners**

**Heinz Lehmann Award**

**Dr. Meir Steiner**

Dr. Steiner is currently a Professor in Psychiatry and Behavioural Neurosciences at McMaster University and Director of Research in the Department of Psychiatry at St. Joseph’s Hospital in Hamilton. This award is designed to recognize outstanding research achievements by Canadian scientists in the field of neuropsychopharmacology. The award, donated by Hoffmann-La Roche Limited, consists of $5000 and an engraved plaque.

**Innovations in Neuropsychopharmacology Award**

**Professor Mirko Diksic**

Dr. Diksic is currently a Professor in the Department of Neurology and Neurosurgery at McGill University, Montreal, and Director of Radiochemistry at the Cyclotron Unit, McConnell Brain Imaging Centre, Montreal Neurological Institute and Hospital, McGill University. This award is designed to recognize outstanding research innovations in the basic or clinical fields of neuropsychopharmacology. The award, donated by Janssen Research Foundation – Janssen Pharmaceutica, consists of $5000 and an engraved plaque.

**CCNP Medal**

**Dr. A. George Awad**

Dr. Awad is currently Psychiatrist-in-Chief at the Humber River Regional Hospital in Toronto and Professor Emeritus at the University of Toronto. This award is designed to honour individuals for a meritorious career in, and outstanding contribution to, neuropsychopharmacology. The award consists of a bronze medal engraved with the name of the recipient.

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Dr. Semeniuk is currently a resident in the Psychiatry Program at the University of Alberta, Edmonton, Alberta. This award is designed to recognize the best poster presentation by a research trainee at the Annual Meeting of the CCNP. The award, donated by the CCNP, consists of $500.