UNIVERSITY OF ALBERTA

NEUROCHEMICAL STUDIES OF TRANYLCYPROMINE AND
RING-SUBSTITUTED ANALOGUES

by

RHONDA LYNN SHERRY-McKENNA

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
PHARMACEUTICAL SCIENCES (NEUROCHEMISTRY)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES AND
FACULTY OF MEDICINE (PSYCHIATRY)
EDMONTON, ALBERTA
FALL, 1996

ISBN 0-612-18080-8

UNIVERSITY OF ALBERTA

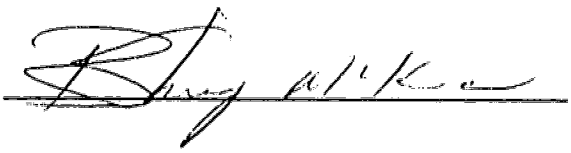
RELEASE FORM

NAME OF AUTHOR: Rhonda Lynn Sherry-McKenna

TITLE OF THESIS: Neurochemical studies of tranylcypromine and ring-substituted analogues

DEGREE FOR WHICH THESIS WAS PRESENTED: Doctor of Philosophy

YEAR THIS DEGREE GRANTED: 1996



#1212 - 8210 - 111 Street

Edmonton, Alberta

T6J 2C6

DATED: October 1, 1996

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled NEUROCHEMICAL STUDIES OF TRANYLCYPROMINE AND RING-SUBSTITUTED ANALOGUES by RHONDA LYNN SHERRY-McKENNA in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Pharmaceutical Sciences (Neurochemistry).

J.R. Mercer (Chair)

G.B. Baker (Supervisor)

R.T. Coutts (Cosupervisor)

I.L. Martin

R.C. Bland

R.F Butterworth (External Examiner)

DATED: September 30, 1996

ACKNOWLEDGEMENTS

I would like to thank the members of my supervisory committee, namely Drs. Baker, Coutts, Martin and Martin-Iverson for their advice and encouragement over the years. I am grateful to the Alberta Mental Health Research Fund for providing me with a Scholarship, to Gail Rauw for technical assistance and to Sally Omura and Jordyce van Muyden for assisting me with the typing and preparation of figures for this thesis.

ABSTRACT

Experiments designed to investigate some neurochemical and neuropharmacological properties of phenyl ring-substituted analogues of the monoamine oxidase (MAO)-inhibiting antidepressant tranylcypromine (TCP) are described. Tranylcypromine is similar structurally to amphetamine, and it has been proposed that its amphetamine-like actions, particularly on noradrenaline, contribute to side effects of this drug. Phenyl ring-substituted analogues had been prepared by other researchers in the hope that by inhibiting metabolism at the 4-position of the ring, antidepressant drugs with improved pharmacokinetic and side effect profiles would result. After an initial screen, two analogues, 4-fluoro-TCP (FTCP) and 4-methoxy-TCP (MeOTCP), were selected for further investigation. Both analogues were ≥ TCP with regard to inhibition of rat brain MAO after acute and chronic administration. Unlike some other phenyl-ring substituted amphetamine analogues, FTCP and MeOTCP cause an elevation (>TCP) rather than a depletion of rat brain 5-HT.

At a dose equivalent to the usual clinical dose of TCP, the two analogues, like TCP, produced a down-regulation of β-adrenergic and tryptamine receptors after chronic administration, further suggesting that they might have antidepressant properties.

After acute administration of equimolar doses of TCP and MeOTCP to rats, levels of MeOTCP were lower than those of TCP in brain, liver and heart (previous experiments had shown that FTCP attained higher levels than TCP). A pretreatment study with iprindole and trifluoroperazine, which caused marked elevations of TCP in rat brain, showed no such elevations with FTCP and MeOTCP, suggesting that these drugs are not as susceptible as TCP to metabolic drug-drug interactions.

Uptake and release experiments in vitro with brain prisms revealed that the analogues had quite different effects from TCP on uptake and/or release of noradrenaline, dopamine and/or 5-HT, a factor which may have important implications for the overall pharmacological profile of these drugs.

Like TCP, the analogues did not elevate rat brain tryptophan levels after chronic administration. An electron-capture gas chromatography study indicated that amphetamine and 3-phenylpropylamine, two potential products of side chain cyclopropyl ring opening, are not metabolites of TCP in rats or humans.

TABLE OF CONTENTS

FIGURE LEGENDS

TABLE LEGENDS

LIST OF ABBREVIATIONS

AC	adenylyl cyclase
AMP	amphetamine
cAMP	cyclic adenosine monophosphate
bid	twice daily
CAM kinase II	calcium/calmodulin-dependent kinase
cDNA	complementary DNA
CNS	central nervous system
CYP	cytochrome P-450
DA	dopamine
DAG	diacylglycerol
DHA	dihydroalprenolol
DOPAC	3,4-dihydroxyphenylacetic acid
dpm	disintegrations per minute
DSM-IV	*Diagnostic & Statistical Manual of the American Psychiatric Association, Fourth Edition*
EDTA	ethylenediaminetetraacetic acid
ECD	electron capture detection
ECT	electroconvulsive shock therapy
FLU	fluoxetine
FTCP	4-fluorotranylcypromine
GABA	γ-aminobutyric acid
GC	gas chromatography
h	hour(s)
H_2O	water
HPLC	high performance liquid chromatography
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
HVA	homovanillic acid
5-HIAA	5-hydroxyindole-3-acetic acid

ip	intraperitoneally
Kd	kilodatton
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MDMA	3,4-methylenedioxymethamphetamine
MDA	3,4-methylenedioxyamphetamine
MeOTCP	4-methoxytranylcypromine
MeO_2TCP	methylenedioxytranylcypromine
NA	noradrenaline
NCP	naphthylcyclopropylamine
n.d.	not detectable
NFLU	norfluoxetine
OHTCP	4-hydroxytranylcypromine
pCA	p-chloroamphetamine
PEA	β-phenylethylamine
PI	phosphoinositide
PFBC	pentafluorobenzoyl chloride
PKA	protein kinase A
PKC	protein kinase C
PLZ	phenelzine
K_2CO_3	potassium carbonate
B_{max}	receptor density
SAD	seasonal affective disorder
SEM	standard error of the mean
TA	tyramine
TCA	tricyclic antidepressant
TCP	tranylcypromine
Trp	tryptophan
T	tryptamine

INTRODUCTION

A. The Evolution of Monoamine (MAO) Oxidase Inhibitors in the Treatment of Depression

Monoamine oxidase inhibitors (MAOIs) were discovered serendipitously in the 1950s to be efficacious in the treatment of affective disorders. Following the administration of iproniazid (Marsilid®), it was observed that this compound induced mood-elevating properties in patients suffering from pulmonary tuberculosis (Selikoff et al., 1952; Bloch et al., 1954). At about the same time, Zeller et al. (1952) demonstrated that iproniazid was a potent inhibitor of MAO both in vitro and ex vivo and that this metabolic enzyme affected levels of brain amines. The proposed antidepressant mechanism of action of iproniazid was thought to be related to the inhibition of MAO and the resultant increase in brain levels of catecholamines and 5-hydroxytryptamine (5-HT).

Reserpine was used in the 1950s to lower high blood pressure as well as tranquillize schizophrenic patients. However, reserpine is responsible for numerous side effects, including depression and attenuation of motor activity. It was known that reserpine depleted central stores of 5-HT as well as of the catecholamines. (Brodie et al., 1957), and Kline (1958) made the connection that reserpine-induced depression was related to decreases in brain catecholamine and 5-HT levels.

Clinical trials were launched by Crane (1957) and Kline (1958) in which populations of depressed patients institutionalized for an average of 20 years showed remarkable improvement following administration of iproniazid. In fact,

Kline titled MAOIs as "psychic energizers". This initial evidence was supported subsequently by other successful drug trials. As a result, iproniazid was the first MAO inhibitor to be employed successfully in the treatment of depression. It was not long before several hydrazine analogues of this compound were synthesized and a variety of non-hydrazine compounds were also developed in order to eliminate liver toxicity. Tranylcypromine (TCP) was the first of these non-hydrazine drugs to be used clinically. With the revelation that these newer compounds also elevated brain concentrations of catecholamines and 5-HT, the monoaminergic hypothesis of depression emerged. In its original form, the theory simply attributed depression to a decline in neuronal noradrenergic and/or serotoninergic function in brain (Everett and Tomon, 1959; Pare and Sandler, 1959; Schildkraut, 1965; Coppen, 1967; Lapin and Oxenkrug, 1969).

B. Depression

B.1 Clinical Features of Affective Disorders

Affective disorders, or more commonly depression together with mania (bipolar disorder), are categorized separately under the broad heading of mood disorders. The major criteria for depression as described in the Diagnostic and Statistical Manual of the APA, Fourth Edition (DSM-IV) are summarized in Table 1. This categorization is related to the patient's past history of mental illness. For example, if the depression is accompanied by one or more episodes of mania

At least five of the following symptoms present for a 2-week period and represent a change from previous functioning. At least one of the symptoms is either 1 or 2 from this list:

1. depressed/irritable mood experienced daily
2. loss of interest/pleasure in daily activities (anhedonia)
3. weight loss/gain; appetite increase/decrease
4. insomnia/hypersomnia
5. psychomotor agitation/decrease
6. significant fatigue
7. feelings of worthlessness/excessive guilt
8. decreased ability to think or concentrate
9. recurrent thought of death, suicidal ideation with no specific plan, or a suicide attempt or a specific plan for suicide.

Table 1: Summary of symptoms involved in depression [adapted from the *Diagnostic and Statistical Manual of the American Psychiatric Association, 4th Edition (DSM-IV)*].

(Table 2), the patient is diagnosed as having bipolar disorder. However, should the patient be experiencing a constant mood disturbance such that a depressive episode is rapidly obscured by a bout of mania only to evolve into depression again, the patient is said to be cyclothymic. This mood instability must be present for a two year period (Talbott et al., 1988). Although it has been somewhat contentious, manic or hypomanic episodes are absent in individuals suffering from major depressive disorder. Dysthymia is differentiated from major depression by means of chronicity and is defined as an ongoing illness (at least two years) with the absence of a diagnosis of major depression (DSM-IV).

The current DSM-IV classification is not without its problems, as there are gray areas in the design of its classification and, because of its failings, certain mood disorders will be undiagnosed or misdiagnosed.

B.2 Treatment Strategies in Depression

The current philosophy in psychiatry supports the heterogeneous nature of affective disorders. It follows that the pharmacotherapy is also as diverse as is the prevalent disorder. Although antidepressants were discovered serendipitously, the monoamine deficiency hypothesis of depression (see Section A of Introduction) soon emerged and it was on the basis of this theory that subsequent antidepressant agents were designed and targeted. For many years, tricyclic antidepressants (TCAs) [see Figure 1] have generally been the preferred choice for the treatment of major depression despite the fact that 10-30% of patients do not respond favour-

1.	Abnormally elated expansive mood or extreme irritability
2.	Three of the following symptoms present (four if patient experiences only irritability in #1):
(a)	increased self esteem/grandiosity
(b)	significant decrease in sleep
(c)	excessively talkative
(d)	flight of ideas/racing thoughts
(e)	easily distracted
(f)	increased goal-directed activity/psychomotor agitation
(g)	extreme drive for pleasure at the risk of negative consequences
3.	Impaired occupational/societal functioning or disturbance in inter-personal relationships
4.	Delusions/hallucinations not present
5.	Psychotic disorder not present
6.	No established organic factor detected

Table 2: Symptoms of mania (adapted from the *DSM-IV*).

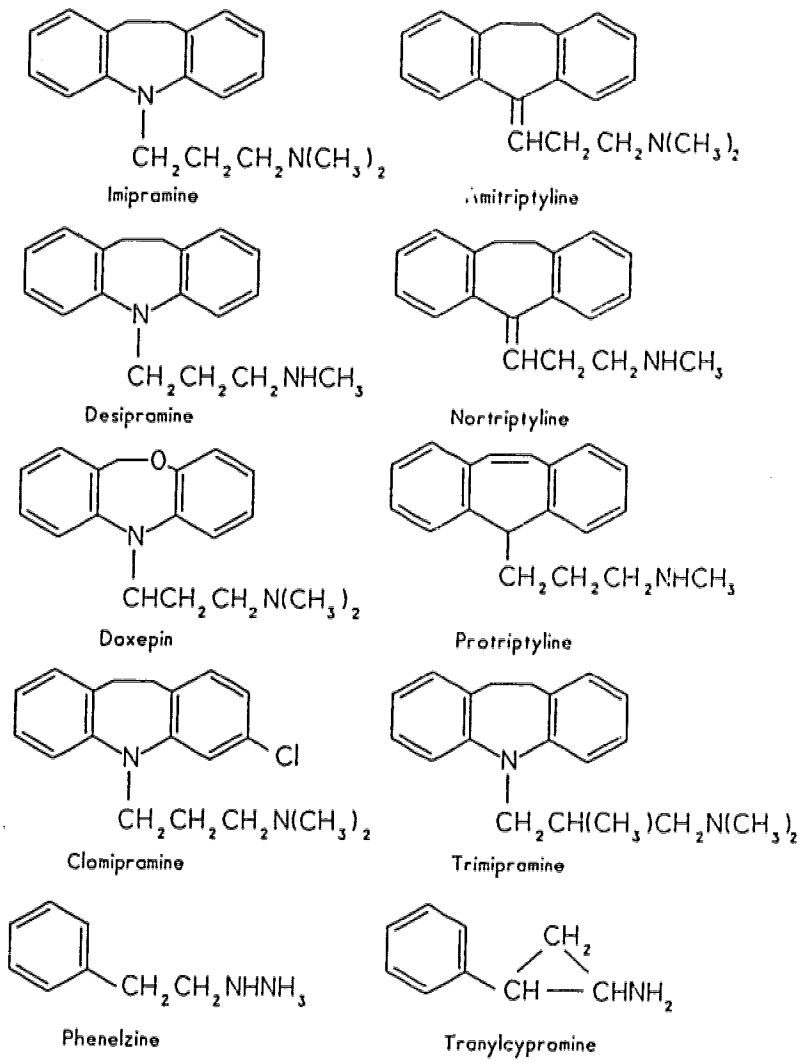


Figure 1: Structures of some commonly used tricyclic antidepressants and monoamine oxidase (MAO)-inhibiting antidepressants.

ably to treatment with these agents (Kielholz et al., 1979; Perez de Francisco, 1979). Even though MAOIs have been available for a longer period of time than the TCAs, they have not been used as extensively. However, there is now general agreement that the side effects associated with MAOIs have been overemphasized in the literature and by the medical profession in general, and there has been a resurgence in the use of MAOIs in recent years (Martin et al., 1994; Murphy et al., 1987; Schmauss et al., 1988).

One study evaluating the clinical efficacy of MAOIs reported that these compounds were ineffective in the treatment of endogenous and melancholic depression (Paykel, 1979), and common practice held that MAOIs were of clinical use only after the patient had failed to respond to a variety of TCAs or were useful only in the treatment of atypical depression. Attempts to characterize and define a standard set of symptoms for atypical depression have proven difficult (Paykel et al., 1983; Davidson and Pelton, 1986), but atypical depression was initially thought to consist of a cluster of symptoms that were of a nonendogenous nature in which reversed vegetative signs (hypersomnia, hyperphagia and a general feeling of well being only in the morning) predominated (Sovner, 1981; Murphy et al., 1987). High anxiety, panic, anergia, and hysteroid dysphoria were also features typically associated with atypical depression (Sargant, 1962; Klein and Davis, 1969; Kelly, 1973; Himmelhoch et al., 1982). Other studies have not agreed that MAOIs are really only useful in treating atypical depression (Robinson et al., 1985; McGrath et al., 1986; Nolen et al., 1988; Martin et al., 1994; Volz et al., 1994), and the following is a statement taken from Murphy et al. (1987):

> "A survey of recent, major double-blind, random assignment studies of MAO inhibitors available for prescription in the United States accomplished during the 1980s reveals that phenelzine, tranylcypromine, and isocarboxazid continue to be found more effective than placebo and, in general, equally as effective as the standard tricyclic antidepressants, confirming the earlier conclusions of a 1979 review. Moreover, these MAO inhibitors were demonstrated to be as effective as tricyclics in all recent controlled studies of typical populations of depressed patients meeting current diagnostic criteria as in those patients with so-called atypical depression. This is a major finding, which would appear to contradict quite widely held clinical lore that 'atypical' patients are preferentially responsive to MAO inhibitors, whereas typical depressed patients respond best to tricyclics and related heterocyclic agents."

The two most commonly prescribed MAOIs are phenelzine (PLZ) and TCP (see Figure 1 for structures).

Electroconvulsive shock therapy (ECT) was used prior to 1950 for severe endogenous depression and still remains the preferred therapy for patients suffering from debilitating agitated or retarded depression, especially if the risk of suicide is

imminent (Baldessarini, 1985; Sackeim et al., 1995).

Although its role in the management of major depression is somewhat debatable, lithium carbonate originated in psychiatry as a mood stabilizing compound. Lithium has been indicated for the management of mania in bipolar disorder. However, there is a growing body of evidence indicating that lithium is potentially efficacious in the treatment of unipolar depression, especially in depressives refractory to the usual antidepressants at the usual doses (de Montigny et al., 1981; Ramsey and Mendels, 1982; Coppen and Abou-Saleh, 1988). The addition of lithium to other antidepressant medication has been the subject of considerable interest in recent years, and improvement in depressives otherwise refractory to tricyclics and mianserin by adding lithium has been observed (Lingjaard, 1973; de Montigny et al., 1983; Heninger et al., 1983; Joyce et al., 1983; Garbutt et al., 1986; Joffe, 1988; review: Kramlinger and Post, 1989; Katona, 1995; Thase and Rush, 1995), although the speed at which this improvement occurs and the sustainment of that response continue to be matters of debate (Browne et al., 1990; Nierenberg et al., 1990). Concomitant lithium-MAOI administration has also been reported to be effective in treatment of refractory depressives (Zall, 1971; Himmelhoch et al., 1972; Nelson and Byck, 1982; Price et al., 1985).

A number of other drugs which are neither TCAs nor MAOIs are now available for treatment of depression and have been grouped under the general heading "novel antidepressants". Some of these drugs are listed in Figure 2. Maprotiline, mianserin, iprindole, viloxazine, trazodone and alprazolam are

sometimes termed "second generation antidepressants". Maprotiline, like the secondary amine TCAs, is a relatively potent inhibitor of reuptake of noradrenaline (NA) into nerve terminals, but is a tetracyclic compound. The other five drugs mentioned above do not inhibit reuptake of NA or 5-HT, possess a tricyclic structure or inhibit MAO. Fluoxetine, fluvoxamine, sertraline and paroxetine are selective serotonin reuptake inhibitors (SSRIs) with very little effect on NA reuptake (Hyttel, 1994) and are sometimes referred to as "third generation antidepressants". Two recently introduced "fourth generation antidepressants" are nefazodone and venlafaxine. The former drug inhibits 5-HT reuptake and is an antagonist at 5-HT_2 receptors while the latter inhibits uptake of NA and 5-HT, but has a much different side effect profile from the tertiary amine TCAs which also inhibit both of these neurotransmitters (Preskorn et al., 1995).

B.3 Proposed Mechanisms of Antidepressant Action

For years scientists and clinicians have attempted to reconcile the lag time between commencement of antidepressant administration and onset of clinical improvement (Osward et al., 1972; Klein et al., 1980; Baldessarini, 1985). In an effort to better understand the ongoing processes, long-term neurochemical effects and neurophysiological changes have been investigated. Early work demonstrated that chronic administration of antidepressants to rats prompted a reduction in adenylate cyclase activity in the limbic forebrain (Vetulani and Sulser, 1975). These findings generated interest in the β-adrenergic receptor as it is coupled to the adenylate cyclase system (Sulser et al., 1978). Not only does long-term administration of TCAs and MAOIs reduce the number of forebrain β-receptors (Hauger and Paul, 1983; Goodman and Charney, 1985; Paul et al., 1985; Baker and Green-

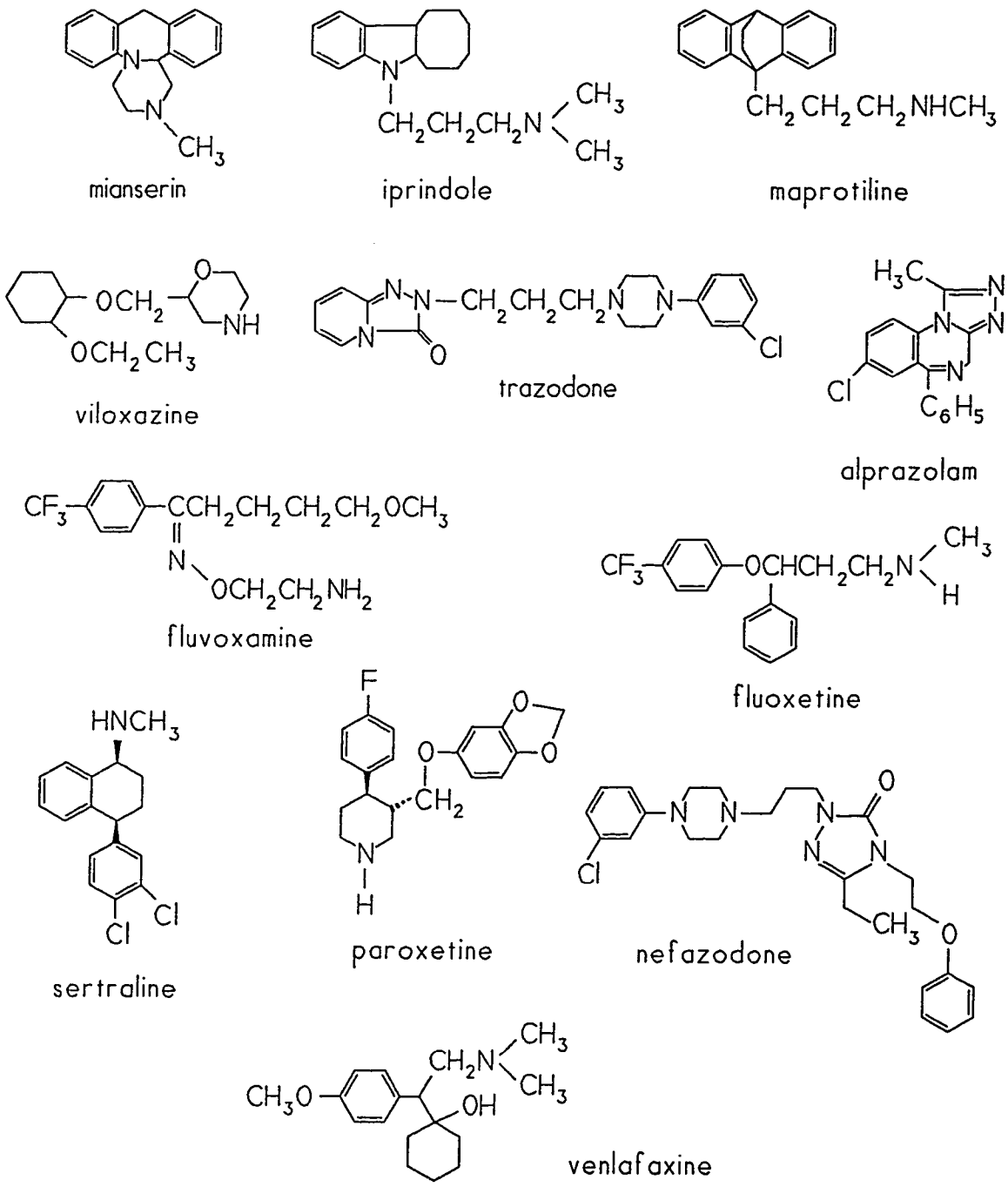


Figure 2: Structures of some 'novel' antidepressants.

shaw, 1989), but many of the so-called "novel" antidepressants (e.g. mianserin, iprindole and trazodone) decrease the number of β-receptors as well (Sulser et al., 1978; Baker and Greenshaw, 1989). The time course required for β-receptor down-regulation may thus help explain the delayed onset of antidepressant response. This early research prompted studies pertaining to the effects of chronic administration of antidepressants on receptors for other putative neurotransmitters. Although the results with many of these receptor studies have not been entirely consistent among laboratories (Heninger and Charney, 1987; Baker and Greenshaw, 1989), strong evidence has emerged revealing a reduction in 5-HT_2 receptors in brain produced by chronic administration of several antidepressants (Peroutka and Snyder, 1980; Fuxe et al., 1983; Eison et al., 1991; Lafaille et al., 1991). It is of interest that most literature reports have not found a decrease in numbers of β-adrenergic or 5-HT_2 receptors after chronic administration of the SSRIs (review: Bourin and Baker, 1996), supporting a suggestion that these drugs may exert their primary action through effects on 5-HT autoreceptors which regulate 5-HT release (Blier and de Montigny, 1994).

Dopamine (DA) has not received as much attention as NA and 5-HT with regard to possible importance in the mechanisms of action of antidepressants. However, there are numerous reports in the literature indicating that behavioural responses to direct and indirect DA agonists in rats are generally enhanced after long-term treatment with some antidepressants (Spyraki and Fibiger, 1981; Martin-Iverson et al., 1983; Campbell et al., 1985; De Ceballos et al., 1985; Plaznik and Kostowski, 1987; Maj et al., 1989; Przegalinski and Jurkowski, 1990). Several

researchers have found that behavioral responses to low doses of the agonist apomorphine (believed to be selective for DA D2 autoreceptors), are usually inhibited following chronic treatment of rats with antidepressants (Antelman and Chiodo, 1981; Serra et al., 1981; Arnt et al., 1984; Volosin et al., 1991). It is well known that DA has a role in maintaining rewarded behaviour (Fibiger and Phillips, 1987), and anhedonia (lack of pleasure) is a common feature of depression. Recent radioligand binding studies have reported decreases in the density of D1 and D2 receptors in rat brain produced by chronic administration of TCP (Paetsch and Greenshaw, 1992; Martin et al., 1995).

There have been numerous reports over the years implicating so-called "trace" amines such as β-phenylethylamine (PEA), tryptamine and tyramine (TA) in the etiology and pharmacotherapy of depression (reviews: Dewhurst, 1968a,b; Boulton, 1984). In recent years, there has been increased interest in tryptamine in this regard, particularly following characterization of a saturable and specific high-affinity binding site for this amine in brain (Cascio and Kellar, 1983; Wood et al., 1984, 1985; Altar et al., 1986; Greenshaw and Dewhurst, 1987; van Nguyen and Juorio, 1989a,b; Mousseau, 1993). Long-term treatment with the MAOI clorgyline was reported several years ago to down-regulate tryptamine binding sites in rat cortex (Wood et al., 1985). A more recent study indicates that a decrease in the number of the receptors for tryptamine in rat cortex following chronic drug administration may be a property shared by several MAOIs (Mousseau et al., 1993).

Research has sparked interest in the possible role of γ-aminobutyric acid (GABA) receptors in the action of antidepressants. The triazolobenzodiazepines

alprazolam and adinazolam which, like other benzodiazepines act on the $GABA_A$ receptor-benzodiazepine receptor-chloride ionophore complex (Enna and Mohler, 1987; Martin, 1987; Richards et al., 1991), have been reported to be efficacious antidepressants (Rickels et al., 1985; Amsterdam et al., 1986). Barbaccia et al. (1986) found that the novel antidepressant maprotiline, when administered to rats for 21 days, failed to affect NA-stimulated adenylate cyclase and β-adrenoceptor number, but did down-regulate ^{3}H-flunitrazepam binding sites. Utilizing microiontophoretic techniques, Bouthillier and de Montigny (1987) found that 3-week treatment of rats with desipramine, trimipramine or citralopram reduced the effect of flunitrazepam application on cholecystokinin-induced activation of hippocampal pyramidal neurons.

Findings by Suranyi-Cadotte et al. (1985, 1990) have indicated that chronic administration of the antidepressants desipramine, zimelidine, bupropion and adinozolam results in down-regulation of ^{3}H-flunitrazepam binding sites in rat brain. However, Kimber et al. (1987) failed to observe a reduction in the number of these receptors following long-term treatment with TCP, desipramine or zimelidine. Similarly, McManus (personal communication) and Todd et al. (1992) from our laboratories were unable to find a change in ^{3}H-flunitrazepam binding site density or affinity following chronic administration of TCP or 4-fluoro-TCP. Suzdak and Gianutsos (1986) found a reduction in density of $GABA_A$ binding sites in rat cortex produced by chronic administration of antidepressants, while Pilc and Lloyd (1984) were unable to find any such change. McKenna et al. (1994) recently found that chronic administration of the MAO-inhibiting antidepressant phenelzine caused no

effects on ^{3}H-muscimol (a $GABA_A$ receptor ligand) binding in rat cortex relative to those observed in vehicle-treated rats.

The picture with regard to the role of $GABA_B$ receptors in the actions of antidepressants is also rather unclear. Chronic administration of antidepressants of every class (tricyclics, MAO inhibitors, novel antidepressants) as well as repeated electroshocks have been reported to result in an up-regulation of $GABA_B$ receptors in rat cortex (Lloyd et al., 1985, 1989; Gray and Green, 1987). This effect of antidepressant drugs has also been observed by Suzdak and Gianutsos (1986), but has been disputed by others (Cross and Horton, 1987; Szekely et al., 1987), including workers in the Neurochemical Research Unit, University of Alberta (McManus and Greenshaw, 1991a).

A recent area of interest with regard to the MAO inhibitors is their possible interaction with imidazoline receptors (Alemany et al., 1995; Carpéné et al., 1995; Holt and Baker, 1995). These receptors seem to be intimately associated with MAO (Alemany et al., 1995). In addition, it has been reported that there is an increased density of imidazoline receptors in frontal cortex of depressed suicide victims (Meana et al., 1993) and that chronic administration of the MAO inhibitors clorgyline, pargyline, phenelzine or TCP results in down-regulation of imidazoline receptors in rat brain (Olmos et al., 1993; Alemany et al., 1995).

B.4 Involvement of Tryptophan in Antidepressant Therapy

The amino acid tryptophan (Trp) is a precursor of 5-HT and tryptamine, two amines which have been implicated in the etiology and pharmacotherapy of

depressive disorders (Baker and Dewhurst, 1985). There have been numerous studies conducted in which Trp levels have been compared in body fluid samples from depressed patients and normal subjects, but conflicting results have been reported (Young, 1991). However, some reports (Moller et al., 1980; DeMyer et al., 1981; Maes et al., 1987) suggest that the plasma ratio of Trp to the amino acids which compete with it for transport through the blood-brain barrier may be a useful marker in depressed patients.

Trp has also been tested as an antidepressant drug but there have been varying results reported (Chouinard et al., 1979; Lundberg et al., 1979; Thomson et al., 1982; van Praag, 1984a,b; Baldessarini, 1985; Young, 1984). van Praag (1984a) has discussed some of the studies that have been carried out with Trp and 5-hydroxytryptophan (5-HTP) and suggested that further research should be conducted using larger doses and longer periods of administration, and that a therapeutic "window" effect should be considered. Dietary investigations have reported that depletion of Trp causes a rapid lowering of mood in normal males (Young et al., 1984) and that such depletion can reverse antidepressant-induced remission (Delgado et al., 1990; Salomon et al., 1993). The latter workers reported a gradual (24-48 h) return to the remitted state on return to regular food intake and that free plasma Trp levels were negatively correlated with depression scores during acute Trp depletion. Although comprehensive studies on the effects of chronic administration of Trp or 5-HTP on 5-HT receptors have not been conducted, Blier et al. (1990) have speculated that these two amino acids would, like the MAOIs, produce decreased sensitivity of the somatodendritic 5-HT autoreceptor.

The seemingly effective, yet controversial, practice of combining MAOIs with Trp in order to enhance the clinical effectiveness of the MAOIs in treating refractory depression has been documented as far back as 1958 (Lauer et al., 1958). Others (Coppen et al., 1963; Pare, 1963; Glassman and Platman, 1969; Lopez-Ibor et al., 1973) also verified the efficacy of combining Trp with an MAOI. Despite the overwhelming success of these five studies, there has been an absence of further research in this area. Perhaps clinicians are somewhat apprehensive due to the potential for serious side effects. Because MAOIs increase 5-HT levels as does oral administration of Trp, many psychiatrists fear that the cumulative increase in 5-HT may precipitate a crisis commonly known as the 5-HT (serotonin) syndrome. Symptoms most commonly associated with this syndrome include alterations in mental status, restlessness, hyperreflexia, diaphoresis, shivering and tremor (Sternbach, 1991; Hyman et al., 1995); in certain cases, death ensues. Treatment is primarily supportive, although in certain instances 5-HT receptor antagonists have also been employed (Hyman et al., 1995).

Acute administration of different types of antidepressants (tricyclics, MAOIs, novel antidepressants) has been reported to produce elevated levels of brain Trp in laboratory animals (Grahame-Smith, 1971; Tabakoff and Moses, 1976; Valzelli et al., 1980; Badawy and Evans, 1982; Edwards and Sorisio, 1988; Badawy and Morgan, 1992). Badawy and Evans (1981, 1982) investigated the acute effects (2 h) of 19 antidepressants of different types at two doses (10 mg/kg and 0.5 mg/kg) on liver Trp pyrrolase activity and on brain Trp concentration; they found significant inhibition of the pyrrolase activity and, presumably secondary to enzyme inhibition,

elevation of brain levels of Trp. Tabakoff and Moses (1976) reported that the MAOI TCP increased brain Trp levels in a dose-dependent manner 2 h post injection; doses ranged from 5 to 50 mg/kg. In a study in which various psychoactive drugs were examined for their effects on both serum and brain Trp levels, Valzelli and coworkers (1980) found that 1 h after injecting the TCAs desipramine, clomipramine, or amitriptyline (10 mg/kg i.p.), brain concentrations of Trp were increased. Grahame-Smith (1971) reported that 90 min after injecting TCP (20 mg/kg i.p.), levels of rat brain Trp were significantly elevated, while plasma Trp was significantly decreased. However, following a time-response and a dose-response study utilizing phenelzine and TCP, Wong (1990) observed increases in rat whole brain Trp only at the highest doses of both compounds and reported that this effect was short lived. At the time of beginning my experiments in this area, there had been no publications reporting on the effects of chronic administration of TCP on brain levels of Trp.

C. MAO

Monoamine oxidase (MAO; EC1.4.3.4.; monoamine:oxygen oxidoreductase) is a ubiquitous flavin-containing mitochondrial enzyme present in both vertebrate and invertebrate species (Blaschko, 1952). Hare discovered MAO in 1928, but she only detected tyramine oxidation in the rabbit liver. Hence, this enzyme was mistakenly titled tyramine oxidase. It was not until 1952, when Zeller correctly identified and categorized amine oxidases according to the substrates oxidized, that we had a better understanding of MAO. This enzyme is distributed in numerous

organ systems, most notably the heart, lung, liver, kidney and brain where it catalyses the oxidative deamination of biogenic and xenobiotic amines in the following manner.

$$R.CH_2NHR^1 + H_2O + O_2 \Rightarrow R.CHO + R^1NH_2 + H_2O_2$$

The R group can be an alkyl, aryl, or arylalkyl moiety, while R^1 can be either a hydrogen atom or a methyl group. MAO oxidizes primary amines very effectively, while secondary amines are catabolized at a slower rate. Amine groups with substituents larger than methyl are not generally favourable substrates for MAO (Blaschko, 1952: Tipton, 1975; Youdim, 1975).

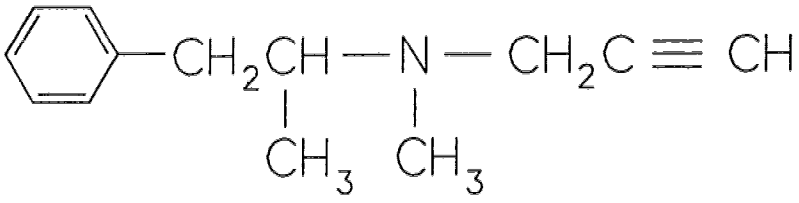
Some amines of interest in the central nervous system (CNS) and periphery that are enzymatically catabolized by MAO are PEA, TA, DA, NA, 5-HT and tryptamine. Not only does MAO apparently maintain homeostatic levels of these putative neurotransmitters and neuromodulators intraneuronally, but it also regulates circulating amine levels following ingestion of foods containing such substances.

C.1 Structure, Function and Distribution of MAO-A and -B

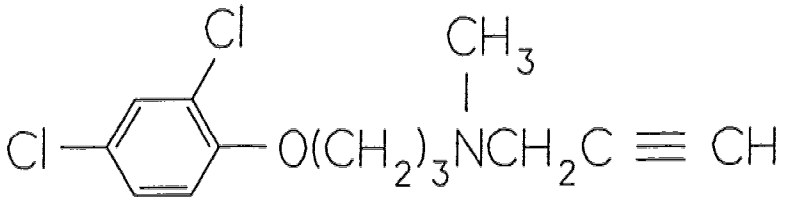
Two isozymes, designated MAO-A and MAO-B, are known to exist. MAO-A is characteristically inhibited by nanomolar concentrations of clorgyline [N-3-(2,4-di-chlorophenoxy)propyl-N-methylpropargylamine] (Johnston, 1968). Noradrenaline and 5-HT are the specific substrates for oxidative deamination by this isozyme

(Gordis and Neff, 1971a,b), whereas MAO-B selectively metabolizes PEA and benzylamine (Knoll and Magyar, 1972). MAO-B is sensitive to inhibition by nanomolar concentrations of deprenyl (N-methyl,N-propargyl-α-methylphenylethyl-amine). The structures of clorgyline and deprenyl are shown in Figure 3. The relative specificities for PEA and 5-HT vary. At concentrations greater than its physiological concentrations, PEA can also be metabolized by the A form of the enzyme (Kinemuchi et al., 1982). and 5-HT can be oxidized by MAO-B when MAO-A is inhibited (Wolf et al., 1985). Dopamine and TA are mixed substrates, i.e. both amines are oxidized by the A and B forms of the enzyme.

SDS-gel electrophoresis (Weyler and Salach, 1985), immunological research (Denny et al., 1982a,b), peptide mapping from enzyme digestion (Cawthon and Breakefield, 1979) and combined quantitative enzyme radioautography and in situ hybridization histochemistry (Saura et al., 1996) have added further support to the hypothesis that there are two MAO isozymes. Most tissues, including the brain (Murphy and Donnelly, 1974) and the blood-brain barrier (de la Tore, 1972; Yu, 1984) contain a mixture of both isozymes. However, only MAO-A is present in placental tissue (Salach and Detmer, 1979), while lymphocytes (Bond and Dundall, 1977) and platelets (Donnelly and Murphy, 1977) contain solely MAO-B. Regional brain distribution of MAO-A and MAO-B also varies. Immunocytochemical studies using monoclonal antibodies demonstrated that MAO-A reactivity is concentrated in certain catecholaminergic areas, i.e. the locus coeruleus, subcoeruleus, and substantia nigra (Westlund et al., 1985; Thorpe et al., 1987). Saura et al. (1990), using autoradiographic procedures, found an abundance of MAO-A not only in the



Deprenyl



Clorgyline

Figure 3: Structures of deprenyl (an irreversible MAO-B-selective inhibitor) and clorgyline (an irreversible MAO-A-selective inhibitor).

locus coeruleus, but also in the paraventricular thalamus, raphé nuclei, solitary tract nucleus, inferior olives, interpeduncular nucleus, claustrum and various peripheral tissues such as the liver, vas deferens, heart, superior cervical ganglia and the pancreas. MAO-B is differentially distributed in the ependyma, all circumventricular organs, raphé nuclei, paraventricular thalamus, posterior pituitary, liver and endocrine nucleus. Using in situ hybridization histochemistry, Saura et al. (1990) reported high activity of MAO-A in the locus coeruleus and of MAO-B in the raphé nuclei. Thorpe et al. (1987) and Westlund et al. (1985) determined independently that the B isozyme shows immunological reactivity in the superior central nucleus, an area strongly innervated by 5-HT, and in astrocytes and radial glia cells. Similar results were found in platelets, cells which have a high concentration of 5-HT. Saura et al. (1996) recently combined quantitative enzyme radioautography and in situ hybridization experiments to demonstrate markedly different regional distributions of the two isozymes in human brain.

A comparison of the two MAO forms suggested that the isozymes possess unique immunological determinants, individual peptide maps, different cleavage sites and molecular weights (Berry et al., 1994; Shih et al., 1994). The subunits of MAO-A have a mass of 63 kd, while MAO-B subunits are of mass 60 kd. Differences in subunit mass are further supported by analysis of purified human MAO-A and B (Weyler and Salach, 1985). Peptide mapping studies combined with cDNA sequencing data strongly infer that MAO-A and MAO-B are coded by different genes (Hsu et al., 1988; Powell et al., 1988; Grimsby et al., 1990; Kanazawa, 1994) situated near each other in the Xp chromosomal region (Sims et al., 1989). In fact,

Ozelius (1988) has located MAO-A and has mapped it to the Xp21-11 region of the human gene. Thus, information to date suggests that MAO-A and MAO-B are structurally highly related, yet distinct enzymes.

C.2 Biological and Behavioral Effects of MAO Inhibitors

Following inhibition of MAO, both intra- and extra-neuronal fluctuations in the levels of various brain amines are observed. As a result, neuronal homeostasis is altered. Most notably, there is a dramatic increase in the concentration of intracellular monoamines. Amines affected include the putative neurotransmitters (catecholamines and more markedly 5-HT) and, to a greater degree, the trace amines [e.g. PEA, octopamine, tryptamine, and N-methylhistamine] (Philips and Boulton, 1979; Murphy and Kalin, 1980; Boulton, 1984). As a consequence of increased neuronal monoamine availability, electrophysiological activity, amine feedback inhibition mechanisms and/or the number of receptors related to these amines may be altered (Campbell et al., 1985; Murphy et al., 1979, 1981, 1984; Baker and Greenshaw, 1989; Glue et al., 1994; Bel and Artigas, 1996). Specifically, there are alterations in the neuronal firing rate of the locus coeruleus and the raphé nucleus, and in monoamine synthesis, and several neuroreceptors are down-regulated (Blier and de Montigny, 1994). Specific studies have shown TCP and PLZ, after chronic administration, to down-regulate β- and α_2-adrenergic receptors (Frazer and Lucki, 1982; Greenshaw et al., 1988; Paetsch and Greenshaw, 1993) as well as tryptamine (Goodnough et al., 1992; Mousseau, 1993) and 5-HT_2 receptors (Lee et al., 1983; Goodnough and Baker, 1992). As mentioned in a

previous section of this thesis, these compensatory effects on neuroreceptor binding might account for the delayed onset of clinical improvement observed with MAOIs.

Numerous studies monitoring the behavioral sequelae of animals treated with high doses of MAOIs have been undertaken. Following administration of nonselective MAOIs such as iproniazid, nialamide or TCP, rodents commonly exhibit increased locomotor activity, twitching, stereotypy, and rectal temperature (Gupta et al., 1971; Modigh and Svensson, 1972; Braestrap et al., 1975; Foldes and Costa, 1975). However, Murphy and Kalin (1980) are extremely critical of these experiments and maintain that it is difficult, if not impossible, to differentiate a specific drug effect from general drug toxicities when doses are in excess of those reported clinically by a factor of 15-50.

The neuroendocrine consequences of MAO inhibition have been reported to include growth and ovulation suppression, miscarriage, and increase in plasma prolactin (Murphy and Kalin, 1980). Both PLZ and TCP were found to effectively reduce prostaglandin release in the rat (Nasser et al., 1988). These investigators found a dose-related decrease of prostacyclin, thromboxane A2, as well as prostaglandin E1 and E2. Interestingly, synthesis of the arachadonic acid-derived prostaglandin E1 is also inhibited by both MAOIs. In addition, TCP has been reported to induce a number of biochemical and physiological changes in rabbits that are thought to be the result of a thiamine deficiency (Ali, 1985).

There is also evidence that TCP and PLZ interact with enzymes of the cytochrome P450 system involved with drug metabolism (Gaultieri and Powell,

1978; Tollefson, 1983; McDaniel, 1986). These MAO inhibitors have been reported to inhibit the metabolic degradation of hexobarbital, ethylmorphine, aminopyrine, meperidine and antipyrine (Eade and Reton, 1970; Clark et al., 1972; Smith, 1980; McDaniel, 1986). Bélanger and Atitsé-Gbeasson (1982a,b) reported that PLZ and TCP inhibit the demethylation of p-nitroanisole and N,N-dimethylalanine and the hydroxylation of aniline in rat liver microsomes. These investigators concluded that both drugs were inhibitors of oxidative microsomal reactions via an interaction with cytochrome P450. In a later series of investigations, Dupont et al. (1987) monitored the effects of MAO inhibitors on cytochrome P450-dependent hydroxylation of bufuralol and antipyrine, as well as the 0-deethylation of 7-ethoxycoumarin, in rat liver microsomes. Although both PLZ and TCP were able to inhibit hydroxylation of antipyrine, PLZ caused a much more potent inhibition of bufuralol hydroxylation and 7-ethoxycoumarin 0-deethylation than did TCP.

C.3 Adverse Effects Associated with MAO Inhibitors

There are two main chemical classes of MAOIs, the hydrazine derivatives [e.g. iproniazid and PLZ] and the non-hydrazine compounds. In the latter group, the phenylcyclopropylamine TCP is an example.

As with most psychiatric drugs, MAOIs are associated with side effects. For instance, the hydrazine compounds are associated with potentially fatal hepatic necrosis. The incidence and severity vary according to the hydrazine compound in question, but estimates of hepatotoxicity have ranged from 1 in 3,000 to 1 in 10,000 of patients treated with the hydrazine MAOIs (Klein et al., 1980). Because the nonhydrazine drugs are not associated with necrosis, it is thought that the hydrazine

moiety is responsible for hepatocellular damage. As with the TCAs, autonomic side effects are frequently encountered with MAOIs. These typically include dizziness, orthostatic hypotension, dry mouth, constipation, gastrointestinal discomfort, delayed micturition and ejaculation, impotence and anorgasmia (Klein et al., 1980). Orthostatic hypotension is characterized by a feeling of dizziness when the patient attempts to stand or sit quickly. Rabkin et al. (1985) found that severe orthostatic hypotension may occur in as many as 10% of patients treated with MAOIs. Severe orthostatic hypotension was found in 70% of cases to occur within the first 2 months of treatment (Rabkin et al., 1985); interestingly, this was the only adverse reaction in which a greater proportion of patients on TCP were affected as compared to PLZ. There has been some speculation that the drop in blood pressure with TCP may be better managed by dosing regimes aimed at administering a lower dose of the MAOI more frequently during the day, thus reducing mean peak plasma levels of TCP (Mallinger et al., 1990). Alternative means of managing this adverse effect include efforts at expanding the intravascular volume by coadministering NaCl tablets or fludrocortisone (Talbott et al., 1988).

Much of the concern associated with prescribing MAOIs is attributed to side effects produced by concomitant ingestion of certain foods; headaches and hypertensive crisis, leading in some cases to patient death may occur. Ingested TA, a monoamine found in high concentrations in foods such as aged cheese, is one of the causative agents and thus this condition has been heralded the TA or "cheese" reaction. The headaches occur precipitously and may be accompanied by increased blood pressure, sweating, pallor, chills, nausea, vomiting, fright,

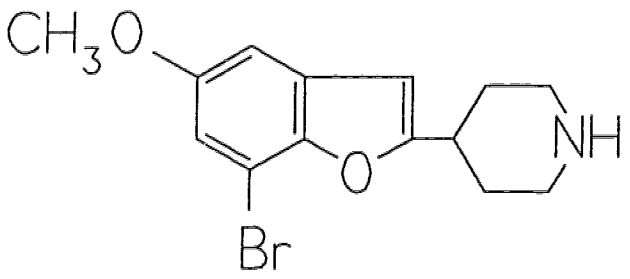
restlessness, muscular fasciculation and a painful, stiff neck (Klein et al., 1980). The locus of these painful headaches is usually in the occipital region of the head, although they may also originate in the temporal region. Other more serious complications may ensue. Hypertension, cardiac palpitation, profuse sweating and pallor followed by eventual collapse may occur, requiring immediate hospitalization of the patient. Finally, the most severe reaction consists of chest pain, palpitation, and intracranial bleeding. The final stages of this reaction can prove to be fatal. In the period 1970-1984, 15 patients taking TCP (out of an estimated 3.5 million) died as a result of cerebrovascular bleeding (Rabkin et al., 1984, 1985). Baldessarini (1985) maintains that even this statistic is meaningless without documenting patient traits such as age, sex, ethnic origin, weight, family history, and tobacco use that might influence the incidence of hypertensive crisis.

Following the ingestion of an MAOI, the enzyme MAO is affected not only centrally, but also peripherally. Importantly, the gut mucosal enzyme is inactivated and, as a consequence, pressor amines such as TA present in ingested foods are not metabolized in the gut. When large proportions of TA are absorbed, the free circulating amine can then displace NA from presynaptic storage granules and invoke hypertensive symptoms. Tyramine is a by-product of aging, ripening and decay and is formed from the decarboxylation of tyrosine during the fermentation process. Foods containing large quantities include red wines, beer, aged cheese, chocolate, beef or chicken livers, pickled herring, caviar, cream, yeast products, broad beans, and stewed bananas (Klein et al., 1980; Rizack, 1995; Folks, 1983; Coutts et al., 1986).

Treatment of a hypertensive crisis is primarily supportive in the most severe of cases. Often an adrenergic antagonist such as phentolamine or phenoxybenzamine is administered intravenously. Chlorpromazine may also be administered intramuscularly because it too has adrenergic antagonist properties (Baldessarini, 1985).

It is of interest that the irreversible MAO-B-selective inhibitor (-)-deprenyl does not exhibit the TA effect, except at very high doses where it starts to inhibit MAO-A as well, but this drug is not a particularly effective antidepressant. Clorgyline, an irreversible selective MAO-A inhibitor, has been demonstrated to have good antidepressant effects (Murphy et al., 1987), but does not appear to have any particular advantage over the nonselective MAOIs because it produces the TA effect. In recent years, reversible MAO-A inhibitors such as brofaromine and moclobemide (see Figure 4) have been developed, and research to date indicates that these drugs are effective antidepressants with a reduced incidence of side effects (including the TA effect) relative to the MAOIs such as PLZ and TCP (Da Prada et al., 1989; Rudorfer and Potter, 1989; Bieck et al., 1993; Baldwin and Rudge, 1993; Nolen et al., 1993; Chouinard et al., 1994; Angst et al., 1995; Paykel, 1995). Moclobemide was introduced on the Canadian market in 1992.

Interestingly, a number of case reports and uncontrolled studies have suggested that an auto-induction of a hypertensive crisis by TCP may occur rarely (Fallon et al., 1988; Kahn, 1988; Keck et al., 1989; Kraus, 1989; Motta and Cordas, 1990; Lavin et at., 1993). There are several reports (Keck et al. 1991, and references therein) that a marked increase in blood pressure may occur 1 to 2 h



Brofaromine

Moclobemide

Figure 4: Structures of brofaromine and moclobemide, reversible inhibitors of MAO-A.

following treatment with TCP and is unrelated to ingestion of TA-containing foods and beverages or indirect sympathomimetics. This blood pressure elevation by TCP appears to be dose-related (Keck et al., 1991). Metabolism of TCP to amphetamine (Youdim et al., 1979; Nies, 1984; Dilsaver, 1988) or intrinsic sympathomimetic activity based on TCP's structural similarity to amphetamine (Hendley and Snyder, 1968; Marley and Blackwell, 1970; Baker et al., 1980; Murphy et al., 1984; Baker and Coutts, 1989) could account for this hypertensive effect. However, because several systematic attempts to positively identify amphetamine have failed to reveal its presence in human body fluid samples taken from patients on TCP, Keck et al. (1991) have discounted the amphetamine theory and prefer to emphasize the possibility that TCP acts as an indirect sympathomimetic amine and/or else directly stimulates β-adrenergic receptors, thus elevating blood pressure.

Other MAOI-associated side effects apparently unrelated to autonomic or cardiovascular symptoms include occasional disorientation, hypomania, insomnia, anorexia, parathesias, pyridoxine deficiencies, rashes, a lupus-like syndrome, and daytime somnolence (Lewis and Winokur, 1982; Rabkin et al., 1984; Teicher et al., 1988; Joffe, 1990; Krishnan, 1995). However, as Rabkin et al. (1984) point out, it is often difficult to differentiate a reported drug-induced side effect from a symptom characteristic of the depressive state itself. A few reports suggest the possibility of TCP addiction and consequent symptomatic withdrawal symptoms (Le Gassicke et al., 1965; Halle et al., 1991; Brady et al., 1991; Dilsaver, 1988; Briggs et al., 1990). In fact, one reported case (Le Gassicke et al., 1965) suggests that one

addict was self administering 200 to 700 mg of TCP per day (the usual recommended daily dose of TCP is 30-60 mg/day, although doses 4-5 times that amount have been used to effectively treat some refractory depressives (Amsterdam and Berwish, 1989).

D. Tranylcypromine

D.1 Therapeutic Efficacy of Tranylcypromine

Despite the fact that use of MAOIs had become relatively unpopular during the 1960s, there has been a recent resurgence in their utility for the treatment of numerous psychiatric disorders (Murphy et al., 1984, 1987; Kennedy and Glue, 1994). TCP received a great deal of bad publicity and was removed from the commercial market in 1964 due to *British Medical Journal* reports citing risk factors attributed to the TA reaction. TCP was later reintroduced for clinical use, albeit under strict dietary precautions, and is presently one of the few MAOIs that has maintained a foothold in the clinical armamentarium for the treatment of depression.

TCP is often the treatment of choice in atypical depressions characterized by anergia (motor retardation, somnolence and volitional inhibition) and reversed vegetative features (Himmelhoch et al., 1982, 1991; Thase et al., 1989). As discussed earlier in this thesis (section B.2), several workers have now indicated that MAOIs such as TCP may also be just as useful in treating typical depression (reviews: Murphy et al., 1987; Martin et al., 1994). The combination of lithium and TCP appears to be an effective treatment for bipolar depression characterized by anergia (Himmelhoch et al., 1972).

For several years, MAOI-TCA combinations were considered highly dangerous and were contraindicated. However, a survey of the literature indicates that the incidence of side effects other than orthostatic hypotension and weight gain is comparable to those associated with each drug alone, provided that the drugs are administered appropriately (Schuckit et al., 1971; Sethna, 1974; Ananth and Luchins, 1977; White and Simpson, 1981; Schmauss et al., 1988; O'Brien et al., 1993). Pare et al. (1982) reported that administration of the TCA amitriptyline actually decreased the pressor response to intravenous TA in patients who were also taking TCP. These workers proposed that a combination of TCP with amitriptyline may, in fact, result in protection of patients from the potential dangers of ingesting TA-containing foods; this protective effect was thought to occur because the TCA inhibits neuronal uptake of both TA and NA. Similarly, Kline et al. (1981) have shown that administration of desipramine in combination with TCP in rats will significantly decrease the hypertensive effect of intravenously administered TA. The findings of Razani et al. (1983) lend further support to the safety and efficacy of the combination of TCP and amitriptyline. A recent noteworthy report indicates that TCP in combination with amphetamine may also be effective in alleviating refractory depression (Sovner, 1990). Ketter et al. (1995) have recently reported that TCP in combination with carbamazepine may also be useful in treating otherwise refractory depressives.

Of recent interest is the potential impact of TCP on the treatment of seasonal affective disorder (SAD). This is a clinically relevant illness characterized by a depressive episode occurring in fall to midwinter and ending with the onset of spring

[mid-February to mid-April] (Rosenthal et al., 1984, 1987). Patients suffer from sadness, increased anxiety, lack of interest in work-related activities, hypersomnia, hyperphagia and carbohydrate craving (Dilsaver and Jaeckle, 1990). Currently, early morning bright light therapy is the treatment of choice in order to phase advance the circadian pacemaker (Avery, 1987). Only a limited number of drug trials has been undertaken, and more studies are required to establish the efficacy of TCP pharmacotherapy for the successful management of SAD (Dilsaver and Jaeckle, 1990).

Other disorders amenable to intervention with MAOIs include bulimia (Walsh et al., 1984; Kennedy and Goldbloom, 1994), chronic pain syndrome (Hyman et al., 1995), panic disorder (Sheehan et al., 1980; Johnson et al., 1994), and obsessive compulsive disorder (Jenike et al., 1983). An increasing number of studies are providing substantial evidence and support for the use of MAOIs over TCAs in the treatment of anxiety disorders characterized by phobias, particularly agoraphobia and social phobia (Tyrer, 1979; Nutt and Glue, 1989; Johnson et al., 1994). Recently, TCP has been reported to be an anticonvulsant and is thought to be a viable alternative antidepressant for patients predisposed to epilepsy (Edwards et al., 1986; Fischer, 1991).

D.2 Differential Properties of Tranylcypromine Enantiomers

Numerous drugs of natural and synthetic origin possess a chiral centre, and thus, enantiomers or nonsuperimposable mirror images of the drugs are possible

(Testa, 1979, 1982; Srinivas et al., 1995; Caldwell, 1996). The individual enantiomers can potentially be differentially metabolized and/or cleared from the body and/or produce varying effects on the biological systems with which they interact (Coutts and Baker, 1989; Jamali et al., 1989).

Because of the aforementioned considerations, racemic mixtures are coming under closer scrutiny from government regulatory bodies and members of the pharmaceutical industry. Recent reports have indicated that as many as 700 of the most commonly prescribed drugs exist as racemates (Coutts and Baker, 1989). TCP is no exception as it exists as a mixture of (+)- and (-)-trans-2-phenylcyclopropylamine (Coutts and Baker, 1989). The geometric cis isomer is not marketed as a drug. The two isomers of TCP differentially inhibit MAO, with the (+)-isomer being a significantly more potent MAOI than the (-)-isomer (Zirkel et al., 1962; Fuentes et al., 1976; Hampson et al., 1986; Smith, 1989). The two TCP isomers also have differential effects on the monoamine reuptake systems. Despite (+)-TCP's superior MAO-inhibiting property, (-)-TCP is a stronger catecholaminergic (Horn and Snyder, 1972) reuptake blocker. There is, however, some controversy as to which isomer more effectively blocks 5-HT reuptake. Tuomisto and Smith (1986) have reported that (+)-TCP is more potent than (-)-TCP at inhibiting binding of ^{3}H-imipramine to its binding sites in brain (these binding sites are thought to be intimately associated with 5-HT uptake sites). However, work in our laboratories (Hampson et al., 1986; Baker, Sherry-McKenna and Rauw, unpublished) indicates that the (-) enantiomer is more potent than the (+) enantiomer at inhibiting 5-HT uptake in rat striatal prisms. Pharmacokinetic differences between the two

enantiomers of TCP have also been reported in human plasma and rat brain (Fuentes et al., 1976; Reynolds et al., 1980; Hampson et al., 1986; Mutschler et al., 1990; Aspeslet et al., 1992).

D.3 Tranylcypromine Metabolism

Despite the fact that TCP has been prescribed for over 30 years, and used extensively in research, very little is known about its metabolism. Alleva (1965) reported that following subcutaneous injection or oral administration of ^{14}C-TCP, 4% was excreted unchanged, and 12% was excreted as ^{14}C-hippuric acid. An estimated 71% of TCP was excreted in urine. The formation of hippuric acid was the first evidence supporting the hypothesis that the cyclopropyl ring undergoes metabolic cleavage. Theoretically, opening the cyclopropyl ring could yield three potential metabolites; 1-methyl-2-phenylethylamine (amphetamine), 2-phenyl-propylamine, and 3-phenylpropylamine (Figure 5). Alleva discounted the presence of amphetamine as a metabolite based on his finding that the chromatograms of rats administered TCP or amphetamine were dissimilar. However, amphetamine, N-methylamphetamine and 2-phenylethylamine were detected in plasma samples of a patient reported to have ingested 250 mg TCP (Youdim et al., 1979). Although many authors cite this one report, and others assume that TCP is metabolized to AMP (Robinson, 1983; Dilsaver, 1988; Kaplan et al., 1994; Silverstone and Turner, 1995), other researchers have been unable to demonstrate such conversion (Reynolds et al., 1980; Mutschler and Mohrke, 1983; Mallinger et al., 1990; Keck et al., 1991; brief review: Jefferson, 1992). As yet, 2-phenylpropylamine and 3-phenyl-

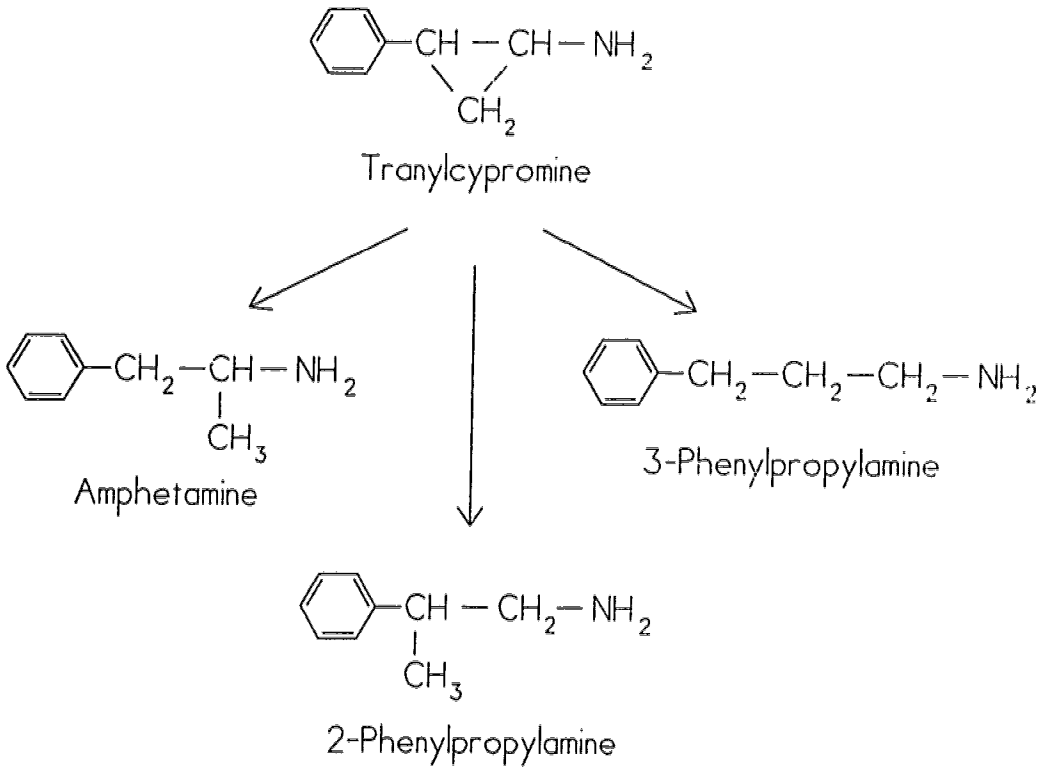


Figure 5: Possible metabolites formed by metabolic cleavage of the cyclopropyl ring of tranylcypromine.

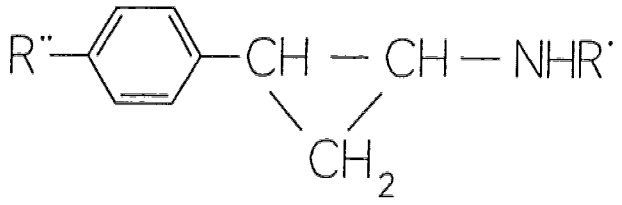
propylamine have not been identified as metabolites of TCP.

Other aspects of the biotransformation of TCP are better understood. The presence of N-acetyl-TCP has been shown to be significant in rat brain and urine samples in rats treated with TCP (Calverley et al., 1981; Kang and Chung, 1984). 4-Hydroxy-TCP has also been detected in rat brain and urine (Baker et al., 1986). In addition, the combined N-acetyl and ring hydroxylated metabolite has been detected and shown to be conjugated with glucuronic acid at the hydroxy position (Kang and Chung, 1984. These metabolites are shown in Figure 6. In a study on the biotransformaion of TCP by the fungus *Cunninghamella echinulata*, Foster et al. (1991) found that the major metabolites were N-acetyl-TCP and N,O-diacetylated 4-OH-TCP.

E. Gas Chromatography

To be able to measure levels of 4-MeOTCP in brain, as required in one of the studies described in this thesis, a gas chromatographic procedure was developed. For this reason, a brief description of gas chromatography is included here.

Gas chromatography (GC) is a method in which chemicals in a mixture are separated on the basis of differential equilibration between a mobile gas phase and a liquid stationary phase. Hence this process is occasionally referred to as gas-liquid chromatography. The stationary phase is a liquid with a characteristically low vapour pressure (high boiling point) that is adsorbed onto support material in the case of packed columns or onto the wall (directly or onto support material) of capillary columns. It is of importance to note that this liquid should have a negligible



	R'	R''
Tranylcypromine (TCP)	H	H
N-Acetyl-TCP	$COCH_3$	H
4-Hydroxy-TCP	H	OH
N-Acetyl-4-Hydroxy-TCP	$COCH_3$	OH

Figure 6: Structure of tranylcypromine and three of its identified metabolites.

vapour pressure at the operating temperature of the column. Numerous stationary phases of varying polarity exist, and polar, non-polar, intermediate and polar acidic phases have been used in GC work. It is the stationary phase that provides a site for different components to adsorb to. Adsorption depends on each chemical's relative partition coefficient or equilibrated distribution between the stationary phase and the mobile gas phase (Baker and Coutts, 1982). The carrier gas, or the mobile phase, must be inert so as not to interact with the samples or the stationary phase. Helium and nitrogen are commonly employed. Fused silica is usually the best choice for column support of the stationary phase as it is inert and efficient, and columns made of this material are flexible and easily installed in gas chromatographs. Borosilicate glass capillary columns were commonly used in the past, but because of the superiority (i.e. increased durability, flexibility) of the fused silica, glass columns (Baker and Coutts, 1982) have become virtually obsolete. The inside diameter of the columns used for high resolution GC is commonly 0.25 mm.

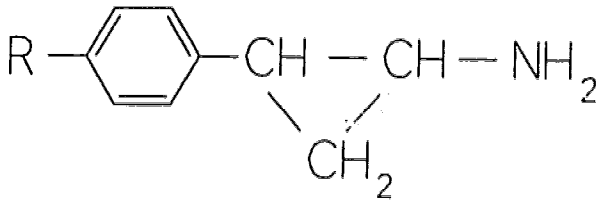
Several GC detectors are available, but for the studies discussed in this thesis, the electron capture detector (ECD) was used. This detector contains a source of radiation (most commonly ^{63}Ni), that sends out a stream of β-particles. Once these high energy β-particles collide with the carrier gas, the carrier gas molecules become ionized, thus forming mobile secondary electrons (Lovecock and Lipsky, 1960). The formation of these lower energy electrons constitutes the standing current. In the presence of electrophoric sample molecules (e.g. fluorinated derivatives of TCP and its analogues), electrons are captured from the standing current; therefore the detector in fact measures and amplifies the ensuing decrease in standing current (Baker et al., 1981).

F. Research Objectives

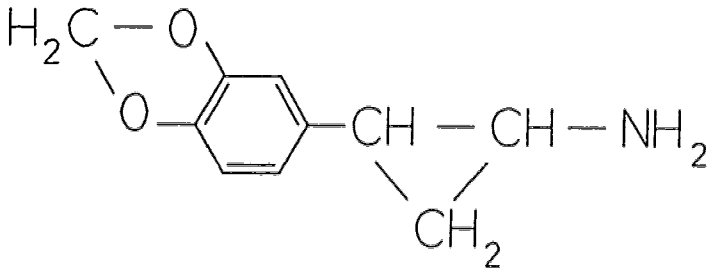
It has been demonstrated in both animals (Fuentes et al., 1976; Calverley et al., 1981; Hampson et al., 1986) and humans (Baselt et al., 1977; Lang et al., 1979; Weber et al., 1984; Edwards et al., 1985; Mallinger et al., 1986) that the biological half-life of TCP is short (2.5 hours in humans). TCP reaches high levels in plasma, brain and heart at short time intervals after administration and is then rapidly eliminated from the body (Fuentes et al., 1976; Coutts et al., 1987; Mallinger et al., 1990). Mallinger et al. (1986) found that in human subjects mean plasma TCP concentrations were correlated with mean orthostatic drop in systolic blood pressure and rise in pulse rate observed 2-7 hours after drug administration. The authors suggested that patients who have hypotensive reactions to this drug may benefit from dose regimens aimed at minimizing peak TCP levels. However, such adjustments could be problematic considering the relatively short half-life of TCP (Edwards et al., 1985; Mallinger et al., 1986). In a later study, the same group (Mallinger et al., 1990) measured TCP levels in plasma 5 h after drug administration and found that TCP levels were significantly higher in nonresponders than in responders. Keck et al. (1991) observed an elevation in patient supine blood pressure shortly after administration of TCP and found that this elevation correlated with TCP dose. These latter authors also observed a drop in the patient's standing systolic blood pressure and an increase in standing heart rate. Based on such evidence, they concluded that the initial hypertensive response might be mediated by effects of TCP on NA uptake and/or release and that the orthostatic hypotension might be a consequence of a direct TCP-α-adrenergic receptor interaction.

The 4-hydroxy metabolite of TCP has been unequivocally identified in rat brain and heart (Baker et al., 1986; Nazarali et al., 1987). Although it is not presently known whether the 4-hydroxy metabolite contributes to the side effects associated with TCP, it has been suggested that ring hydroxylated metabolites of TCAs contribute to cardiotoxic side effects (Jandhyala et al., 1977; Kutcher et al., 1985; Young et al., 1991); in certain instances, these ring hydroxylated metabolites may be associated with poor antidepressant response (review: Potter and Manji, 1990). Another concern pertaining to metabolites is that there is considerable inter-individual variation in metabolism for most antidepressants (review: Rudorfer and Potter, 1987). In an attempt to produce MAOIs with an improved pharmacokinetic profile (i.e. more consistent levels in the brain) and with a potentially reduced incidence of adverse effects, workers in the Neurochemical Research Unit at the University of Alberta synthesized several analogues of TCP in which the 4-position of the phenyl ring was substituted with a chemical constituent in order to protect these compounds from ring hydroxylation at this position. Two of these drugs, namely 4-fluoro-TCP (FTCP) and 4-methoxyTCP (MeOTCP) [see Figure 7] were found to be more potent than TCP at inhibiting rat brain MAO in vitro (Rao et al., 1986). FTCP was subsequently shown to produce longer-lasting drug levels than TCP in rat brain after equimolar intraperitoneal injection (Coutts et al., 1987), and, like TCP, to functionally down-regulate α_2-adrenergic receptors (Greenshaw et al., 1988).

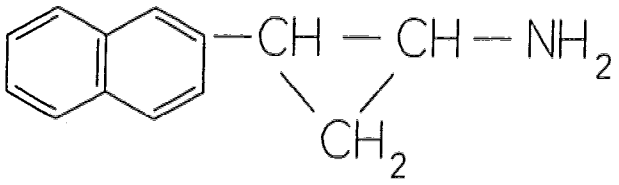
These promising preliminary findings with the novel TCP analogues suggested that further studies were warranted to see if they possessed other



	R
Tranylcypromine (TCP)	H
4-Fluoro-TCP (FTCP)	F
4-Methoxy-TCP (MeOTCP)	CH_3O



3.4-Methylenedioxy-TCP (MeO_2TCP)



Naphthylcyclopropylamine (NCP)

Figure 7: Structures of tranylcypromine and four analogues of interest to this thesis.

neurochemical and neuropharmacological properties characteristic of MAO-inhibiting antidepressants. As a consequence, a series of acute and chronic experiments with FTCP and MeOTCP in rat brain has been conducted in order to study their effects on: MAO activity and levels of biogenic amines and their acid metabolites ex vivo; radioligand binding to β-adrenergic and tryptamine receptors ex vivo; and uptake and release of neurotransmitter amines in vitro. In addition, a short study was conducted to determine tissue concentrations of TCP and MeOTCP after injection of equimolar doses of the drugs at a clinically relevant dose [previous studies by Coutts et al. (1987) had already shown that FTCP reached higher concentrations in brain and liver than did TCP after injection of equimolar doses]. The effects of drugs known to alter brain levels of TCP were also studied for their effects on brain levels of FTCP and MeOTCP. It is important to know if levels of the ring substituted analogues of TCP in tissues are unaffected by such drugs (i.e. whether they are less prone than TCP to metabolic drug-drug interactions). To conduct these last two experiments it was necessary to develop an assay procedure for 4-MeOTCP. Two other experiments were performed to evaluate the possibility of metabolism of TCP to amphetamine and the effects of TCP and its analogues on levels of Trp in brain; as mentioned in this Introduction, there is still considerable controversy about these two aspects of TCP, and it was felt that it was important to investigate them in a comprehensive manner.

MATERIALS AND METHODS

A. Chemicals Used

Table 3: Chemicals used in the studies described in this thesis.

Chemicals	Suppliers
Acetonitrile, HPLC grade, distilled in glass	BDH Chemicals Inc. (Toronto, ON)
Analogues of tranylcypromine 4-Fluorotranylcypromine 4-Hydroxytranylcypromine 4-Methoxytranylcypromine 3,4-Methylenedioxytranylcypromine Naphthylcyclopropylamine	Synthesized by Dr. R.T. Coutts, University of Alberta, Faculty of Pharmacy and Pharmaceutical Sciences
Ascorbic acid	Fisher Scientific (Fairlawn, NJ)
Calcium chloride	Baker Canada (Mississauga, ON)
Chloroform, reagent grade	Fisher Scientific
3,4-Dihydroxyphenylacetic acid	Sigma Chemical Co. (St. Louis, MO)
Dopamine HCl	Sigma Chemical Co.
Ethylenediamine tetraacetate, disodium salt	Fisher Scientific
D-Glucose, anhydrous	Fisher Scientific
Homovanillic acid	Sigma Chemical Co.
Hydrochloric acid, 37-38%	Fisher Scientific
5-Hydroxyindole-3-acetic acid	Sigma Chemical Co.
Hydroxytryptamine binoxalate 5[2-^{14}C]	Dupont, NEN Products (Mississauga, ON)
5-Hydroxytryptamine creatinine sulfate	Sigma Chemical Co.
Isopentane (2-methylbutane)	BDH Chemicals Inc.
Nialamide	Sigma Chemical Co.
(-)-Noradrenaline HCl	Sigma Chemical Co.

Octyl sodium sulfate	Eastman Kodak Co. (Rochester, NY)
Pentafluorobenzoyl chloride	Aldrich
Perchloric acid, 60%	Fisher Scientific
Phentermine HCl	Sigma Chemical Co.
β-Phenylethylamine HCl	Sigma Chemical Co.
Phenylethylamine HCl, 2-[ethyl-1-^{14}C]	Dupont, NEN Products
Phosphoric acid, 85%	Fisher Scientific
Potassium carbonate, anhydrous	Fisher Scientific
Potassium chloride	Fisher Scientific
Saline solution (0.85%) (particle free)	Fisher Scientific
Scintillation fluid (Ready Safe®)	Beckman Canada (Edmonton, AB)
Sodium chloride	Fisher Scientific
Sodium phosphate, dibasic, anhydrous	Fisher Scientific
Sodium phosphate, monobasic	Fisher Scientific
Toluene, glass-distilled	BDH Chemicals Inc.
Toluene, reagent grade	BDH Chemicals Inc.
(±)-Tranylcypromine HCl	Sigma Chemical Co.
(+) and (-) Tranylcypromine HCl	SmithKline & French (Indianapolis, IN)
Tris	Fisher Scientific
Tryptophan	Raylo Chemicals (Edmonton, AB)

A Corning AG-3 or Corning Mega-Pure (3 Litre Automatic) still was used to generate and collect double-distilled water for use in analyses. Stock solutions of Trp were prepared in 0.5M HCl at a concentration of 1 mg/ml; they were stored at -10°C and freshly prepared every week. Stock solutions of neurotransmitter amines and their metabolites were made up in double-distilled water at a concentration of

1 mg/ml, stored at -80°C and freshly prepared every month. Stock solutions of TCP, OHTCP, FTCP, MeOTCP and TA required for uptake and release studies were prepared in double-distilled water at a concentration of 5.0 mM and kept frozen at -10°C for no longer than two weeks.

B. INSTRUMENTATION

B.1 High Performance Liquid Chromatography (HPLC)

Levels of neurotransmitters and their corresponding acid metabolites as well as the amino acid Trp were measured using a modification of the procedure of Baker et al. (1987). A Waters solvent delivery HPLC system in conjunction with an automated WISP710B sample injector was utilized. Analytes were detected with a Bioanalytical Systems (BAS, West Lafayette, IN, U.S.A.) Model LC-4B electrochemical detector. The applied potential was fixed at 0.85 volt. Peak heights were recorded with a Hewlett Packard 3392A integrator. An Econosphere-C18 column [4.6 mm x 250 mm, 5 μm particle size] (Applied Science Labs, Avondale, PA, U.S.A.) coupled to an Econosphere C18 precolumn was used to separate amines, the acid metabolites and Trp. The mobile phase flow rate was adjusted to 1ml/min. The mobile phase consisted of 55 mM $H_2PO_4.H_2O$, 0.85 mM octane sulfonic acid (sodium salt), 0.37 mM disodium EDTA and 9% v/v acetonitrile. Newly prepared mobile phase was filtered through a type HA filter (0.45 μm, Millipore) and then degassed. Phosphoric acid was used to adjust the pH value to 3.0.

B.2 Gas Chromatography

Analyses of compounds of interest discussed in this thesis were conducted on one of two gas chromatographs: an HP 5880A GC equipped with a 25 m long fused silica capillary column [0.31 mm I.D., 0.5 μm film of 5% phenylmethylsilicone as stationary phase] (Hewlett Packard Co. Palo Alto, USA) and interfaced with a 5880A (level 4) integrator or an HP 5890 GC equipped with a 15 m long SP2100 column (0.31 mm I.D., 0.5 μM file of SP2100) and interfaced with an HP 3392A integrator.

B.3 Liquid Scintillation Spectrometry

Two scintillation counters were utilized. A 300 sample capacity Beckman LS7500 liquid scintillation counter (Beckman Co., Fullerton, CA, U.S.A.) was interfaced with an Epson LX 810 dot matrix printer. This instrument has automatic quench compensation, and 10 library programs. A newer model Beckman LS 6000 liquid scintillation spectrophotometer was also employed; it was interfaced with a Beckman dot matrix printer. The sample capacity varied from 336 standard size (20 ml) vials to 648 mini (7 ml) scintillation vials. It also featured automatic quench compensation, and 20 library programs.

Either of two liquid scintillation cocktails was used. The first consisted of 0.4% w/v of butyl-PBD in a mixture of Triton X-100 and toluene (33:66 v/v), and the second cocktail was premade Beckman Ready Safe®.

B.4 Glassware Cleaning

All non-radioactive test tubes were treated with 50 ml Sparkleen industrial detergent (Fisher Scientific Co., Fairlawn, N.J., U.S.A.) and then allowed to soak overnight before sonication in the Baxter Canlab (Mississauga ON) sonicator. Glassware was sonicated three times for a total period of 45 min in a solution of 2% Decon® (BDH Chemicals Inc., Toronto, ON) in distilled water. The tubes and all other laboratory glassware were then transferred to a Miele dishwasher (Miele Electronics, Gütersloh, Germany) for cleaning in a cold water-hot water-hot water wash and rinse cycle. All glassware was then transferred to a Model 18EM Precision Scientific Group convection oven regulated at a temperature of 250-300°C for a period of 1 h. Radioactive glassware was first allowed to soak for a period of 48 h in a solution of Contrad-70 (Mississauga, ON) and then rinsed in tap water before undergoing the cleaning procedure described above.

B.5 Weighing Balances

All compounds were weighed on a Metler AE160 electronic weigh balance (sensitivity: ± 0.1 mg). Rats were weighed in a 700 series triple beam balance (Ohaus, Florham Park, NJ, U.S.A.).

B.6 Tissue Homogenizer

Either of two tissue homogenizers was used. A TRI-Stir-S63C® homogenizer with a variable speed laboratory motor and a Teflon® or glass pestle was sufficient to process brain and liver tissue. Heart tissue was homogenized with a Turax

polytron homogenizer. Samples were homogenized in either a glass homogenization tube with a clearance of 0.10-0.15 mm or in microfuge tubes (1.5 ml capacity). The rotor shaft maintained a maximal speed of 12,000 rpm with a 10 speed setting. Samples were usually homogenized at a setting between 5 and 7.

B.7 Centrifuges

Low speed centrifugation (up to 1,500 rpm) was performed in a Sorval GLC-2B General Laboratory centrifuge (DuPont Instruments). High speed (up to 20,000 rpm) centrifugation was accomplished with a refrigerated Damon-1EC B-20 centrifuge (Damon/IEC, Needham Hts., MA, U.S.A.). High speed centrifugation of small volumes (less than 1.5 ml) was performed on an MSE Macro-Centaur centrifuge (Baxter Canlab, Mississauga, ON).

B.8 Shaker/Mixers

An IKA-Vibrax-VCVR(R) Shaker (Janke and Kunkle Instruments, Staufen, Germany) was employed as well as a Thermolyne Maxi Mix® vortex mixer (Sybron/Thermolyne Instruments).

B.9 Block Heater

A Canlab Temp-Blok Module Heater (Lab Line Instruments, Melrose Park, Il, U.S.A.) was utilized to concentrate solvents or carry out derivatizations for GC analysis.

B.10 pH Meter

Accumet models 610 and 915 pH meters (Fisher Scientific, Fairlawn, NJ, U.S.A.) were used and routinely standardized with certified buffer solutions (Fisher Scientific).

B.11 Uptake Equipment

Uptake of tritiated neurotransmitters into brain prisms was studied with a Millipore filtration device consisting of a 20 cm tank in which 12 Whatman GF/B glass filters could be placed. The system was connected to a vacuum line.

B.12 Release Equipment

A Brandel Superfusion 600 release apparatus designed to simultaneously perfuse each of six tissue samples was employed. Superfusion medium is pumped via a special MultiChannel, Peristaltic Pump (Brandel Research and Development Laboratories, Inc.). The entire system was purchased from Xymotech Biosystems (Montréal, P.Q.).

B.13 Tissue Chopper

A McIlwain (Brinkman Corporation, Rexdale, ON.) tissue chopper was used to prepare hypothalamic and striatal prisms (0.1 x 0.1 x approx. 2 mm) for neurotransmitter uptake and release studies.

B.14 Sample Filter for Radioligand Binding Studies

Tissue was isolated with Whatman GF/B filters contained in a M-24R cell harvester (Brandel, Gaithersburg, MD, U.S.A.). The ideal filtration rate was maintained with a vacuum system attached to a G8CX pump (Emerson, St. Louis MO, U.S.A.).

C. METHODS

C.1 Drug Administration to Animals

Male Sprague-Dawley rats obtained from the Bio Science Animal Service Division (Ellerslie, Alberta) were used. Animals were housed two per cage with food and water provided ad libitum. All rats were fed Lab-Blox Feed (Wayne Feed Division, Continental Grain Company, Chicago, Ill. U.S.A.) consisting of 4.0% crude fat (min), 4.5% crude fiber (max), and 24% crude protein (min). The rats were maintained on a constant 12 h light-dark cycle, and the ambient temperature was 21°C. All animals weighed between 200 and 250 g at the beginning of experiments.

All procedures with rats described subsequently in this thesis were approved by the Health Sciences Animal Welfare Committee, University of Alberta.

C.1.1 Acute Studies

Rats were randomly allocated to vehicle or drug treatment conditions. All drugs administered on an acute basis (1-8 h between administration and sacrifice of rats) were given intraperitoneally. The drugs were dissolved in physiological saline prior to administration at a concentration such that the rats received 2 ml/kg.

Control animals were given physiological saline vehicle. At predetermined time intervals, the rats were killed rapidly by cervical dislocation and decapitation and whole brain was quickly removed and frozen solid in isopentane on solid carbon dioxide. Samples were stored at -80°C until time of analysis, during which half the brain was used to determine ex vivo MAO inhibition and the other half was used to quantitate NA, DA, 5-HT, the acid metabolites HVA, DOPAC and 5-HIAA and the amino acid Trp.

C.1.2 Chronic Studies

Rats were randomly allocated to drug or vehicle treatment conditions and implanted subcutaneously in the dorsal thoracic region with Alzet osmotic minipumps (Alza Corp., Palo Alto, CA). Each pump was filled with a drug solution individually adjusted in concentration (Greenshaw, 1986) or with distilled water (vehicle treatment) according to the group allocation of each animal. Ether or methoxyflurane were the inhalation anaesthetics administered. Incisions were either sutured or stapled. After recovery from surgery, the rats were returned to normal housing conditions. The period of drug administration for drug studies was 28 days, after which animals were killed by rapid guillotine decapitation. Brain, liver and heart were rapidly removed and the liver and heart placed on solid carbon dioxide. Each brain was dissected to yield the following anatomical structures for HPLC analysis: pons medulla, hypothalamus, and hippocampus. After removal of these three brain regions, the cerebral cortex was dissected out for future radioligand binding studies in which ^{3}H-tryptamine or ^{3}H-DHA (radioligand for β-

adrenergic receptors) were the radioligands used (see Section C.4). The remainder of the brain was retained for analysis of MAO activity. All samples were stored at -80°C until time of analysis.

C.2 Assay for MAO

Analysis of MAO activity was based on a radiochemical method developed by Wurtman and Axelrod (1963). ^{14}C-5-HT and ^{14}C-β-PEA were employed as substrates for MAO-A and MAO-B respectively. Whole rat brain was used for the acute 1 to 8 h drug screening procedure. However, in the chronic studies, hippocampus, hypothalamus, pons medulla, and cerebral cortex were removed for other analyses and the remainder of the brain was then used for analysis of MAO activity. Tissues (including heart and liver) were homogenized in 5 volumes of distilled H_2O after which 200 μl aliquots were removed and added to 1000 μl of chilled isotonic KCl. In the procedure leading up to incubation, samples were kept refrigerated on ice. Sodium phosphate buffer (250 μl, 0.5M) was added to each tube, after which 25 μl aliquots of diluted tissue homogenate were added. In the case of blank controls, 25 μl of isotonic KCl was substituted. Solutions of radioactive ^{14}C-5-HT and ^{14}C-PEA (25 μl) were diluted with cold 5-HT or PEA to give final substrate concentrations of 100 μM and 10 μM, respectively. Samples were incubated for 20 min in a 37°C water bath. At the end of this time, HCl (200 μl, 2M) was added to terminate the reaction. The metabolites were extracted into 6 ml of toluene by vortexing for a 5 min period. Tubes were centrifuged for a 5 min period at 1,000 rpm and then placed in a -80°C freezer for at least 1 h in order to freeze

the aqueous layer. In the final step, the toluene layer was decanted into a scintillation vial containing 9 ml scintillation fluid. The vials were placed in a liquid scintillation counter. The amount of radioactivity in the blank vials was averaged and subtracted from all vials. All control (vehicle treatment) values were pooled together and then averaged. The radioactivity of each sample (dpm) was divided by that of the averaged control values and then multiplied by 100 to provide the percentage MAO activity. Percent inhibition was then determined by subtracting percent activity from 100.

C.3 High Performance Liquid Chromatography

The procedure used was essentially that described by Baker et al. (1987). Brain tissues were weighed and homogenized in 5 volumes of ice-cold 0.1 M perchloric acid containing 10 mg% disodium EDTA and 0.05 mM ascorbic acid. The homogenates were centrifuged at 10,000 x g for 15 min at 4°C. A 200 μl portion of the supernatant was retained for analysis and a 15 μl aliquot was injected into the HPLC system by the WISP injector described in section B.1. A set of standards of the neurochemicals of interest (NA, DA, 5-HT, 5-HIAA, HVA and Trp) was prepared and carried through the procedure in parallel in order to prepare a calibration curve.

C.4 Receptor Binding

C.4.1 ^{3}H-Dihydroalprenolol Binding

^{3}H-Dihydroalprenolol (DHA) binding was conducted using a method originally developed by Bylund and Snyder (1976). Cortical tissue was weighed and

homogenized in a glass homogenization tube with a Teflon pestle in 10 vol ice-cold Tris buffer (pH 7.4) and centrifuged at 40,000 g for 10 min (4°C). After the supernatant was discarded, tissue pellets were resuspended in 100 vol 50 mM Tris buffer (pH 7.4). These binding experiments (as well as those on ^{3}H-tryptamine) were conducted in conjunction with Dr. D.D. Mousseau. Dr. Mousseau had already completed a series of saturation experiments for binding of all these radioligands and shown that down-regulation produced by TCP was the result of changes in B_{max}, not of K_d values, in the radioligand (^{3}H-DHA and ^{3}H-tryptamine) binding studies. Because of this, single point analysis was conducted at a concentration of radioligand related to the theoretical K_d determined by him. Aliquots of tissue as prepared above and diluted as described by McManus and Greenshaw (1991b) were added to a series of test tubes containing ^{3}H-DHA at a concentration of 1.5 nM and incubated at 23°C for 1 h. Nonspecific binding was determined by adding l-alprenolol (10 μM) to a parallel set of tubes. The incubation step was terminated by addition of 3 ml buffer and rapid filtration through Whatman GF/B filters. The filters were washed rapidly 3 times with 5 ml of ice-cold buffer, and were place in counting vials to which 5 ml of scintillation cocktail were added. The vials were left overnight before being placed in the scintillation counter for determination of radioactivity. Another set of scintillation vials, containing the prefilter amount of radioligand solution, was run for each concentration to measure total radioactivity added to the corresponding total and non-specific sets of tubes.

C.4.2 ^{3}H-Tryptamine Binding

Analysis of ^{3}H-tryptamine binding in the cortical tissue was based on a method developed by Kellar and Cascio (1982). Tissue was weighed and homogenized in 10 vol ice-cold 50 mM Tris buffer. The homogenates were centrifuged for 10 min at 40,000 x g (4°C). The supernatant was discarded and the tissue pellets were resuspended and incubated for 30 min at 37°C in 10 vol 50 mM Tris buffer to which 10 μM pargyline and 5.6 mM ascorbic acid was added (Mousseau et al., 1993).

Single point determination of tryptamine binding was determined using the method described above. The K_d for tryptamine had been previously estimated by Dr. Mousseau to be 2 nM and this concentration of tryptamine was used in this study. In these binding experiments, nonspecific binding was determined by the addition of 10 μM unlabeled tryptamine to a parallel set of tubes. Incubation was done in a final volume of 1 ml, and tubes were kept on ice for 1 h. The incubation was terminated by addition of 3 ml of buffer followed by rapid filtration through Whatman GF/B filters. The filters were then rapidly washed 3 times with 5 ml of ice-cold buffer, and placed into counting vials. Scintillation cocktail (5 ml) was added to each vial and the vials were left overnight before placing in the scintillation counter for measurement of radioactivity. A triplicate set of scintillation vials, containing the prefilter amount of radioligand solution, was run with each assay to permit measurement of total radioactivity added to the corresponding total and non-specific sets of tubes.

C.5 Protein Determination

The amount of protein in the tissue samples was determined using the procedure developed by Lowry et al. (1951). A set of standards containing known concentrations of bovine serum albumin was included with the samples in each protein determination assay and carried through the entire procedure. Sample tubes were run in duplicate. A total of 250 μl membrane digestor (1:1 NaOH:Na deoxycholate) was added to the test tubes for 10 min, after which 5 ml of Reagent A (1:1:100 1% $CuSO_4$:2% Na-K-tartrate:2% Na_2CO_3) was added. Samples were allowed to sit for 10 min. Folin reagent (500 μl; 1:1 2N Folin-Phenol:H_2O) was added and samples were left standing for 30 min. The absorbance of each sample was read on the spectrophotometer at a wavelength of 660 nm. Protein content per tube was assessed using the Lowry program (McPherson, 1987).

C.6 Uptake and Release Experiments

C.6.1 Preparation of Rat Brain Prisms for Uptake and Release Experiments

The procedures used are those described by Martin et al. (1978). Rats were killed by cervical dislocation and the brains were dissected out and placed in ice-cold saline in order to remove excess blood. The brain was then placed on an ice-cold Petri dish on ice and the hypothalamus and the striatum were dissected out. The striatum was further dissected to exclude the globus pallidus and the nucleus accumbens. The striatal tissue used in these series of experiments extended from the frontal plane of the anterior commissure to the mid-portion of the body of striatal complex. Brain regions were weighed and chopped on a McIlwain tissue chopper to give prisms of dimensions 0.1 x 0.1 x approx. 0.2 mm. The tissue

was then put in an ice-cold incubation mixture containing 123 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 20 mM Tris-HCl buffer (pH 7.4), 10 mM glucose, 12.5 μM nialamide (to inhibit MAO), and 1 mM ascorbic acid. The tissue was resuspended several times using a pipet until the brain suspension was evenly dispersed; the tissue concentration at this point was 5 mg/ml.

C.6.2 Release Protocol

Rat brain tissue was prepared according to section C.6.1 (striatal prisms for ^{3}H-DA and ^{3}H-5-HT uptake and hypothalamic prisms for ^{3}H-NA uptake) and then incubated in 25 ml Erlenmeyer flasks at 37°C for 20 min. ^{3}H-Labelled NA, DA, or 5-HT (0.012 μM final concentration) was added and the tissue was incubated for 10 more minutes. The tissue was then separated from the incubation mixture by rapid filtration through GFB paper filters contained in a Brandel 600 Superfusion release apparatus and washed twice with 1 ml incubation mixture by connecting the stem of the superfusion chamber to a vacuum line. The equipment was adjusted to superfuse and the pump was turned on so that the desired flow of incubation mix was maintained at 0.5 ml/min. After the first two superfusate fractions had been collected, the perfusing solution was changed so that some channels contained solutions of the drugs of interest. One tube was randomly assigned to contain control (drug-free) superfusion medium and another TA (10^{-5}M), a known releaser of catecholamines and 5-HT from nerve terminals (Raiteri et al., 1977). Drug solutions of TCP, OHTCP, FTCP or MeOTCP were contained in the remaining four tubes, and six fractions were collected following superfusion with the drugs.

Scintillation fluid (5ml Ready Safe) was then added to the collection vials and the vials were placed in a scintillation counter.

C.6.3 Uptake Protocol

Rat brain was prepared according to the method described in section C.6.1. The uptake procedure was the one developed by Martin et al. (1978). A total of twelve 25 ml flasks were used for each run. Two flasks were designated as filter blanks (no tissue was added) and the remaining 10 flasks were assigned to control or drug groups. Prisms were added to flasks containing 4 ml of the cold incubation mixture to give a final tissue concentration of 1 mg/5ml. Samples were then incubated in a 37°C water bath for 15 min. Drugs (at varying concentrations) and ^{3}H-labelled NA, DA or 5-HT (final concentration 0.012 μM) were added to the flasks which were incubated for a further 5 min. The tissue was then subsequently separated from the incubation medium by rapid filtration using a 12-channel Millipore filtration device and washed twice rapidly with warm (37°C) incubation medium. All filters were then removed, placed in scintillation vials and liquid scintillation fluid was added. The amount of radioactivity in the samples was measured in a liquid scintillation counter.

C.7 Collection of Samples for Studying Possible Metabolism of TCP to Amphetamine

Urine samples (24 h) were collected by patients who were taking TCP.HCl (Parnate®, 10-20 mg bid). The sample volumes were recorded and aliquots

removed and stored at -60°C until the time of analysis. In the studies on rats, the animals were administered TCP (20 mg/kg i.p.) or vehicle and killed 2 h later by decapitation. Brain, liver and heart were removed, frozen rapidly on solid carbon dioxide, and stored at -60°C until the time of analysis. At the time of decapitation of the rats, blood was collected from the neck region and centrifuged to obtain plasma. The plasma samples were stored at -60°C until the time of analysis.

C.8 GC Analysis of TCP, FTCP and MeOTCP

C.8.1 GC Analysis of Amphetamine

GC analysis of amphetamine was undertaken based on a protocol developed by Paetsch et al. (1992). The procedure consisted of extractive derivatization of amphetamine using pentafluorobenzenesulfonyl chloride followed by analysis on a gas chromatograph equipped with a fused silica capillary column, an electron capture detector and an integrator/printer.

C.8.2 Analysis of TCP, FTCP and MeOTCP

A novel GC method was developed to detect and quantitate MeOTCP and TCP in rat brain, liver and heart. This procedure was subsequently also utilized for analysis of FTCP. Tissue samples were homogenized in 5 volumes of ice-cold 0.1 M perchloric acid. Homogenates were then centrifuged at 12,000 x g for 12 min at 4°C. Portions (2 ml for brain and heart, 1 ml for liver) of the resultant supernatants were used for analysis. p-Chlorophentermine (100 ng) was added as internal standard to each tube. A set of authentic standards of the drugs was also included

in each assay and carried in parallel through the entire procedure to establish a calibration curve. The supernatants were basified with the addition of 1/10th the volume of 25% K_2CO_3 to each tube. Samples were extracted by shaking with 4 ml ethyl acetate for 5 min, after which they were centrifuged at 1,000 x g for 5 min. The top organic layer was transferred to clean tubes and taken to dryness under a stream of nitrogen. To the residue in each tube, 300 μl toluene and 2 μl pentafluorobenzoyl chloride (PFBC) were added. Samples were left to derivatize for 60 min at 60°C and the tubes and contents were allowed to cool at room temperature for 5 min. The organic layer was washed with 3 ml sodium borate by shaking for 1 min and then centrifuged for 1 min. The top layer was transferred to a 400 μl microfuge tube, and an aliquot was used for GC-ECD analysis.

Quantitation was carried out by determining peak area ratios of TCP, FTCP or MeOTCP to internal standard for each sample and comparing the ratios to the values on the calibration curve obtained from the standards run for that particular assay. The calibration curve consisted of a series of tubes of drug-naive brain supernatants containing the same amount of internal standard as in the brain supernatants and varying known concentrations of TCP, FTCP and MeOTCP.

C.9 Statistical Analysis

Results were compared using analysis of variance and, where necessary, the Newman-Keuls test. The conventional $p<0.05$ was utilized to establish statistical significance.

RESULTS

A. Preliminary Comparison of Several TCP Analogues

A.1 Short-Term Study of Ex Vivo Effects on MAO

The finding that TCP underwent metabolic ring hydroxylation (Hampson et al., 1986) and the fact that researchers had reported that the pharmacokinetic profile of TCP (high levels attained in plasma and tissues shortly after administration, but a relatively short elimination half-life) might be responsible for some of the cardiac side effects observed with this drug (Edwards et al., 1985; Mallinger et al., 1986) led researchers in the Neurochemical Research Unit to synthesize some analogues of TCP which were substituted in the 4-position of the phenyl ring (Rao et al., 1986; Coutts et al., 1987). A number of such analogues were synthesized and compared for their ability to inhibit MAO-A and MAO-B *in vitro* (Rao et al., 1986). Four analogues were found to be equipotent to or more potent than TCP *in vitro* (Rao et al., 1986), and these analogues were selected to undergo further *ex vivo* screening for their potential efficacy as MAO inhibitors. In an initial study 4-fluorotranylcypromine (FTCP), 4-methoxytranylcypromine (MeOTCP), 3,4,-methylenedioxytranylcypromine (MeO_2TCP) and 2-napthylcyclopropylamine (NCP) were analyzed for their effects *ex vivo* on MAO inhibition 1 h after administration of equimolar doses of the drug to rats by intraperitoneal injection. Three doses were chosen for comparison to equimolar doses of TCP: 37 μmol/kg, 3.7 μmol/kg (equivalent on a mg/kg basis to the dose of TCP normally used in human subjects) and 1.2 μmol/kg. At the highest dose (37 μmol/kg), each compound strongly

inhibited MAC-A and MAO-B, although NCP was considerably less effective than the other drugs at inhibiting MAO-B (Tables 4 and 5). At the next lowest dose (3.7 μmol/kg), the weaker MAO-inhibiting effects of NCP became more obvious. Differences between the analogues were very apparent at the next dose (1.2 μmol/kg). FTCP and MeOTCP were more potent than TCP at inhibiting both MAO-A and MAO-B at this last dose while MeO_2TCP and NCP were weaker. At this lowest dose, NCP had no significant effect on MAO-A and a very weak effect on MAO-B. Because of the relatively weak effects of MeO_2TCP and NCP relative to FTCP and MeOTCP in this initial comparison, these two drugs were dropped from further investigation. In addition, MeO_2TCP is structurally very similar to MDMA and MDA, two drugs of abuse which have been reported in recent years to be associated with production of neurotoxicity and even death (Dowling, 1990; Pallanti and Mazzi, 1992; Green et al., 1995).

B. A Short-Term Study on the *Ex Vivo* Effects of TCP and its Novel Analogues FTCP and MeOTCP on MAO and Brain Levels of Neurotransmitter Amines and Their Acid Metabolites

B.1 MAO

A time course study was undertaken in order to compare the abilities of TCP, FTCP and MeOTCP to inhibit MAO at 1, 2, 4, and 8 h. For this study, the dose chosen was 1.2 μmol/kg. Both FTCP and MeOTCP were superior inhibitors of MAO-A compared to TCP at each time interval. At 2 and 4 h, FTCP inhibited

Dose (μmol/kg i.p.)	TCP	FTCP	MeOTCP	MeO_2TCP	NCP
37	86.7 ± 2.8 (6)	97.9 ± 0.5 (8)	88.4 ± 3.7 (6)	94.2 ± 1.5 (8)	84.0 ± 3.1 (6)
3.7	80.1 ± 5.1 (5)	86.5 ± 4.5 (6)	89.9 ± 1.6 (6)	86.1 ± 2.2 (6)	14.4 ± 6.3 (6)
1.2	33.7 ± 2.7 (13)	59.0 ± 3.0 (12)	92.3 ± 3.2 (14)	26.0 ± 5.4 (5)	9.3 ± 4.4 (6)

Table 4: Dose-related effects of TCP and its novel analogues on inhibition of MAO-A in rat whole brain 1 h after injection of the drugs. Results are expressed as % mean inhibition ± SEM. Numbers of experiments performed are indicated in parentheses. All values except those at 1.2 μmol/kg of NCP were significantly different ($p<0.05$) from control values.

Dose (μmol/kg i.p.)	TCP	FTCP	MeOTCP	MeO_2TCP	NCP
37	92.6 ± 2.0 (6)	97.9 ± 0.5 (8)	91.3 ± 2.3 (6)	95.6 ± 1.1 (8)	78.6 ±0.19 (6)
3.7	77.8 ± 4.9 (5)	86.6 ± 9.2 (6)	89.8 ± 2.0 (6)	76.1 ± 2.4 (6)	29.8 ±10.9 (6)
1.2	43.0 ± 11.5 (13)	75.7 ± 20.2 (12)	59.0±15.8 (14)	23.9 ± 5.3 (5)	15.4 ± 6.3 (6)

Table 5: Dose-related effects of TCP and its novel analogues on inhibition of MAO-B in rat whole brain 1 h after i.p. injection of the drugs. Results are expressed as % mean inhibition ± SEM. Numbers of experiments performed are indicated in parentheses. All values were significantly different from control values.

MAO-A to a greater extent than did MeOTCP (Figure 8). This effect is most pronounced at 2 h where the mean % inhibition values (± SEM; n=6-13) with TCP, FTCP and MeOTCP, respectively were as follows: MAO-A: 27.9 ± 3.9, 76.7 ± 12.8, 43.3 ± 4.7. A similar trend was apparent with respect to MAO-B inhibition; at each time interval studied, FTCP significantly inhibited the enzyme to a greater degree than either TCP and MeOTCP (Figure 9).

B.2 Neurotransmitter Amines and Their Metabolites

A dose-response study was conducted in order to determine the effects of the novel TCP analogues on levels of the neurotransmitter amines NA, DA and 5-HT and the acid metabolites DOPAC, HVA and 5-HIAA at 1 h after injection of TCP, FTCP or MeOTCP at doses of 1.2 or 3.7 μmol/kg i.p. Following i.p. injection at the dose of 3.7 μmol/kg, FTCP and MeOTCP significantly increased levels of NA, DA and 5-HT and decreased levels of DOPAC, HVA and 5-HIAA (Table 6), while TCP had no effect on levels of any of the amines or 5-HIAA but did cause significant decreases in brain levels of DOPAC and HVA. The decrease in DOPAC observed with TCP at this dose was considerably less than that observed with FTCP and MeOTCP at the same dose. At the lower dose studied (1.2 μmol/kg), TCP did not affect levels of any of the amines or acid metabolites while FTCP and MeOTCP caused increases in brain levels of NA and 5-HT and a decrease in levels of DOPAC. MeOTCP also produced a decrease in brain levels of 5-HIAA. The only significant difference observed between FTCP and MeOTCP at both doses was a

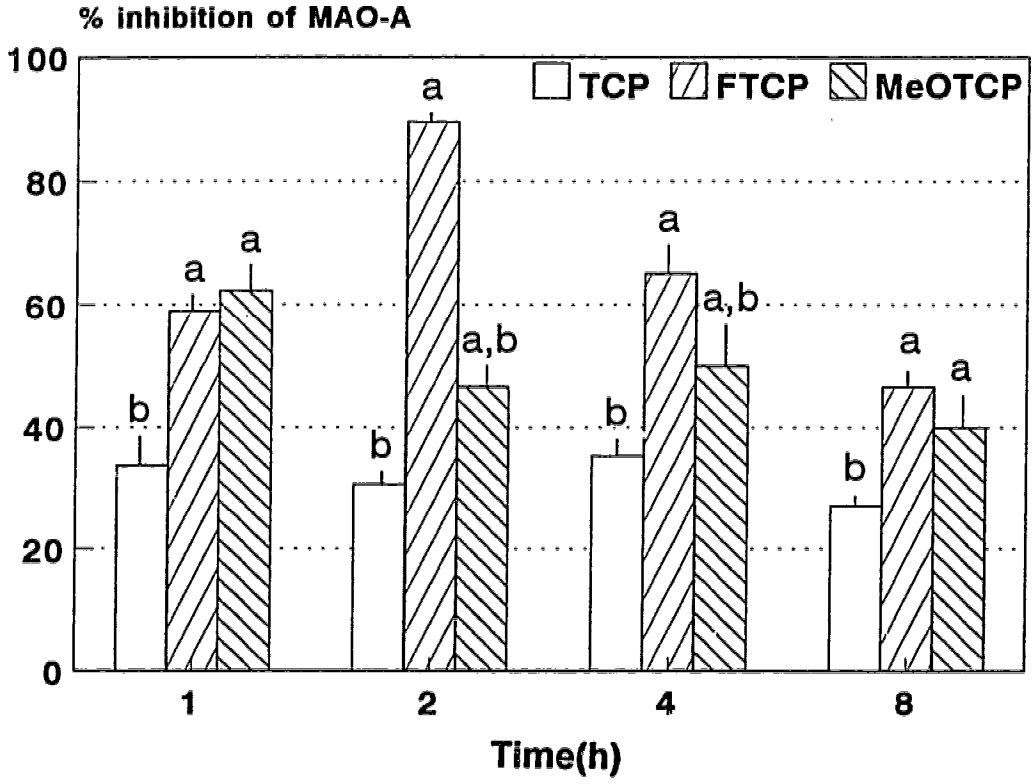


Figure 8: Effects of TCP and analogues (dose = 1.2 μmol/kg i.p.) on MAO-A activity in rat whole brain at 1, 2, 4 and 8 h post-administration. Results expressed as % inhibition of vehicle MAO-A (N=4-13). a = significantly different from TCP-treated group; b = signficantly different from value in FTCP-treated group.

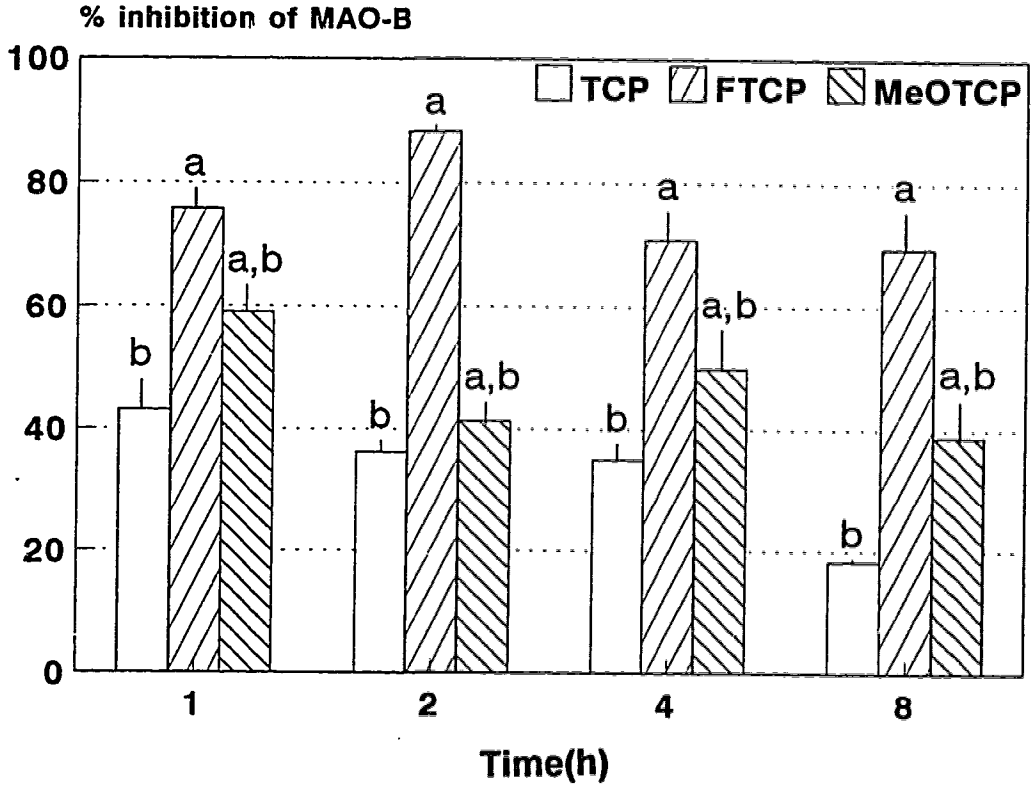


Figure 9: Effects of TCP and analogues (dose = 1.2 μmol/kg i.p.) on MAO-B activity in rat whole brain at 1, 2, 4 and 8 h post-administration. Results expressed as % inhibition of vehicle MAO-B (N=4-13). a = significantly different from TCP-treated group; b = significantly different from FTCP-treated group.

Drug	Dose	NA	DA	5-HT	DOPAC	HVA	5-HIAA
TCP	1.2	112.3 ± 4.0 (12)	98.0 ± 2.0 (12)	105.6 ± 4.1[c] (12)	91.6 ± 2.2[c] (12)	108.1 ± 5.1[c] (9)	101.3 ± 2.7 (9)
	3.7	113.0 ± 15.4[c] (6)	105.6 ± 5.6 (6)	101.3 ± 10.7[c] (6)	58.2 ± 7.1[a,c] (6)	64.0 ± 4.6[a,c] (6)	95.8 ± 8.8[c] (6)
FTCP	1.2	117.6 ± 4.2[a] (14)	101.1 ± 2.6 (14)	127.5 ± 4.7[a,b] (14)	75.1 ± 4.2[a,b] (14)	94.7 ± 5.6 (3)	95.5 ± 8.1 (11)
	3.7	140.5 ± 7.7[a,b] (5)	113.8 ± 5.0[a] (5)	187.6 ± 4.6[a,b] (5)	8.8 ± 0.8[a,b] (5)	43.1 ± 8.6[a,b] (5)	56.8 ± 2.4[a,b] (5)
MeOTCP	1.2	113.9 ± 3.5[a] (14)	102.8 ± 3.0 (13)	127.1 ± 8.0[a] (13)	69.8 ± 3.1[a] (13)	93.5 ± 6.0 (11)	83.1 ± 3.0[a,c] (11)
	3.7	136.4 ± 7.9[a,b] (5)	115.5 ± 5.9[a] (5)	187.3 ± 13.0[a,b] (5)	7.3 ± 1.6[a,b] (5)	49.8 ± 11.4[a] (5)	46.3 ± 3.9[a,b,c] (5)

Table 6: Dose-related effects of TCP, FTCP and MeOTCP on rat whole brain levels of neurotransmitter amines and acid metabolites. Abbreviations: NA (noradrenaline); DA (dopamine); 5-HT (5-hydroxytryptamine); DOPAC (3,4-dihydroxyphenylacetic acid); HVA (homovanillic acid); 5-HIAA (5-hydroxyindole-3-acetic acid). Results are expressed as mean % of control ± SEM. Dose = μmol/kg i.p, 1 h. Numbers of experiments performed are indicated in parentheses. a = significantly different from control values; b = significantly different from values in TCP-treated rats at the same dose; c = significantly different from values in FTCP-treated rats at the same dose. Control values in ng/g (N=26-30) were 274.6 ± 6.6 (NA), 666.7 ± 18.0 (DA), 310.8 ± 13.0 (5-HT), 94.1 ± 2.0 (DOPAC), 77.9 ± 3.3 HVA and 386.6 ± 22.7 (5-HIAA).

greater decrease in 5-HIAA levels in the MeOTCP-treated rats.

The study at a dose of 1.2 μmol/kg was extended to time intervals of 2, 4 and 8 h (see Table 7). At this dose, TCP caused small, but short-lived increases in levels of NA and 5-HT and small decreases in brain levels of DOPAC, HVA and 5-HIAA. FTCP caused a significant increase in levels of NA and 5-HT at all four time intervals. Although only small increases in DA levels were noted at 2 and 4 h, relatively great decreases were observed at all four time intervals in the case of DOPAC and at the last three time intervals in the case of HVA. Significant decreases in levels of 5-HIAA were noted at 2 and 4 h. MeOTCP caused a small, short-lived increase in levels of NA, and increases in 5-HT levels at 1 and 2 h. DOPAC levels were decreased at all four time intervals, while a decrease in HVA levels was noted at 2 h only. Levels of 5-HIAA were decreased below control levels at 1, 2 and 4 h. The patterns of increases of amine levels and decreases in levels of acid metabolites were similar in the FTCP- and MeOTCP-treated rats.

C. Effects of Chronic Administration of TCP, FTCP and MeOTCP in the Rat

Since antidepressants are typically given chronically, with at least 2-3 weeks being required before clinical improvement becomes obvious, it was considered important to compare the TCP analogues to the parent drug chronically with regard to several aspects which could play a role in antidepressant action. These aspects included effects on: MAO activity; brain levels of the neurotransmitter amines and

Drug	Time	NA	DA	5-HT	DOPAC	HVA	5-HIAA
TCP	1	112.3 ± 4.0(12)	98.0 ± 2.0(12)	105.6 ± 4.1(12)[c]	91.6 ± 2.2(9)[a,c]	108.1 ± 5.2(9)	101.3 ± 2.7(9)
	2	111.1 ± 4.7(17)	97.7 ± 2.4(17)[c]	112.5 ± 6.4(17)[c]	83.3 ± 3.1(12)[a,c]	93.7 ± 2.7(17)[c]	95.3 ± 6.4(17)
	4	123.2 ± 5.9(8)[a]	113.2 ± 5.8(8)	131.3 ± 12.7(8)[a]	94.7 ± 5.4(8)[c]	78.7 ± 5.3(8)[a]	76.8 ± 16.0(8)[a]
	8	118.5 ± 15.2(7)	102.8 ± 6.5(7)	112.8 ± 5.7(7)[c]	94.3 ± 12.0(7)[c]	76.0 ± 7.7(7)[a]	110.7 ± 4.9(7)
FTCP	1	117.6±4.2(14)[a]	101.1±2.6(14)	127.5±4.7(14)[a,b]	75.1 ± 4.2(14)[a,b]	94.7 ± 5.6(11)	95.5 ± 8.1(11)
	2	121.2±4.2(22)[a]	117.9±5.0(22)[a,b]	154.5±9.8(22)[a,b]	51.4 ± 5.5(15)[a,b]	71.0±6.3(22)[a,b]	71.8±7.7(22)[a,b]
	4	116.0 ± 8.0(8)[a]	118.9 ± 6.4(8)[a]	142.0 ± 9.0(8)[a]	64.0 ± 13.5(8)[a,b]	62.3 ± 13.6(6)[a]	65.8 ± 10.5(8)[a]
	8	131.9±10.9(6)[a]	118.5 ± 11.1(6)[a]	130.8±6.3(6)[a,b]	64.5 ± 9.2(6)[a,b]	72.1 ± 3.6(6)[a]	103.4 ± 4.9(6)
MeO-TCP	1	113.9 ± 3.5(14)	103.8 ± 3.0(13)	127.1±8.0(14)[a,b]	69.8 ± 3.1(13)[a,b]	93.5 ± 6.0(11)	83.0±3.0(10)[a,b]
	2	118.5±4.5(16)[a]	102.5 ± 2.9(16)[c]	130.4 ± 5.4(16)[c]	73.4±6.2(11)[a,b,c]	82.8 ± 5.6(16)[a]	84.7 ± 8.7(16)[a]
	4	114.3±13.4(8)	96.8 ± 14.0(8)	116.0 ± 19.0(7)	78.2±9.5(8)[a,b]	95.1±24.6(8)[b,c]	77.8 ± 10.8(8)[a]
	8	125.2 ± 12.6(6)	122.7 ± 10.4(6)	114.8 ± 8.7(6)	74.2 ± 12.2(6)[b]	86.4 ± 16.2(6)	103.5 ± 3.2(6)

Table 7: Effects of TCP, FTCP and MeOTCP on rat whole brain levels of neurotransmitter amines and acid metabolites at 1, 2, 4 and 8 h. The dose used was 1.2 μmol/kg i.p. for all three drugs. Results are expressed as mean % of values (± SEM) in rats treated with vehicle for the same periods of time. Numbers of experiments performed are indicated in parentheses. a = significantly different from vehicle-treated rats at the same time interval; b = significantly different from TCP-treated rats at the same time interval, c = significantly different from FTCP-treated rats at the same time interval.

their metabolites; brain tryptophan levels; and binding of radioligands to β-adrenergic and tryptamine receptors.

C.1 Effects on Inhibition of MAO

The effects of chronic administration (28 d) of TCP and its two analogues on brain, heart and liver MAO *ex vivo* are shown in Figures 10-13. A dose of 3.7 μmol/kg was chosen for the initial studies since it corresponds to the usual dose, on a mg/kg basis, of TCP used clinically. At this dose, all three drugs were strong inhibitors of MAO-A and MAO-B, with the two analogues equipotent to or slightly stronger than TCP (Figures 10 and 11).

Ex vivo inhibition of brain and hepatic MAO-A and MAO-B following 28 day administration of TCP and its analogues was also assessed at a dose of 1.2 μmol/kg (Figures 12 and 13). At this dose, both analogues were stronger inhibitors of MAO-A and -B in brain than was TCP. MeOTCP was more potent than FTCP at inhibiting MAO-A. A similar pattern was evident in liver, although in this case FTCP was more potent than MeOTCP at inhibiting MAO-B.

C.2 Effects on Levels of Neurotransmitter Amines and their Acid Metabolites

Because numerous treatment strategies for depression have been influenced by the monoamine theory of depression, it was of interest to measure the brain levels of NA, DA, and 5-HT and the acid metabolites HVA, DOPAC, and 5-HIAA. Also, since FTCP is structurally related to p-chloroamphetamine, a known

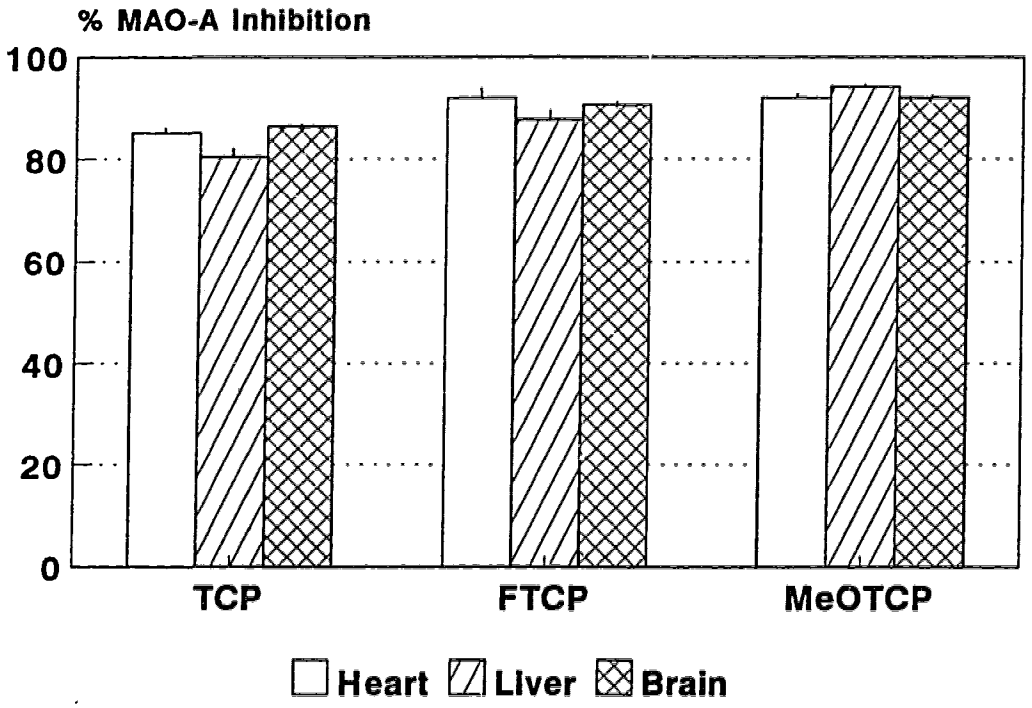


Figure 10: Effects of 28 d administration of TCP and analogues (dose=3.7 μmol/kg) on MAO-A activity in brain, heart and liver. Results expressed as % inhibition of vehicle MAO-A activity (N=4-8). Control MAO-A activity (pmol min^{-1} mg^{-1} tissue, mean ± SEM) = 78 ± 5 (brain), 165 ± 19 (heart), and 234 ± 28 (liver). All values are significantly different from control values.

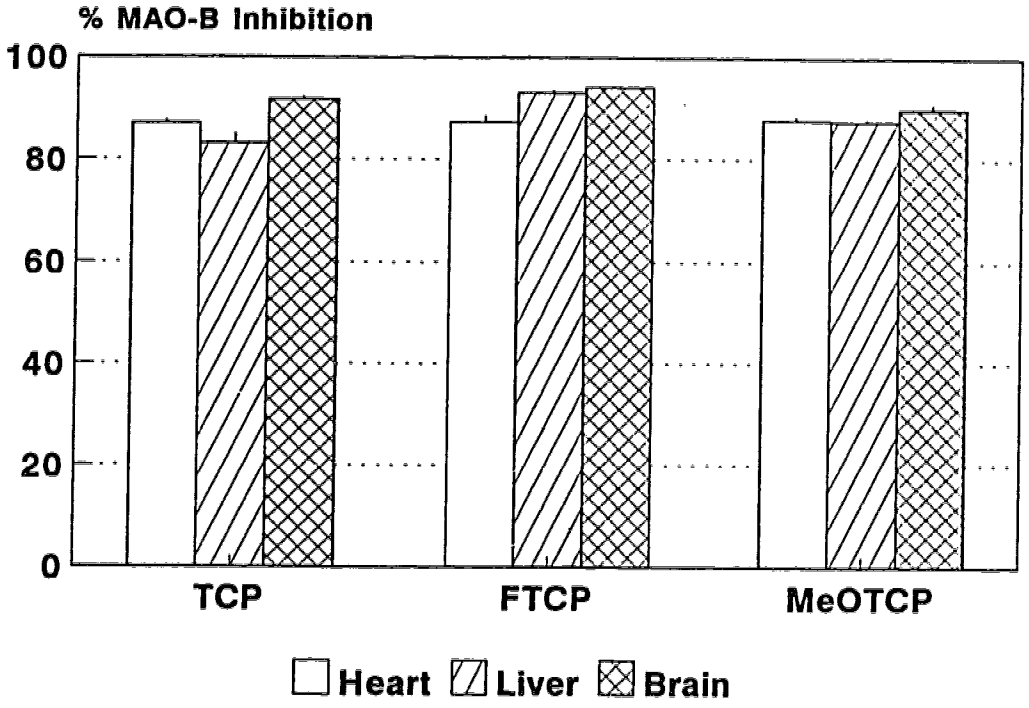


Figure 11: Effects of 28 d administration of TCP and analogues (dose=3.7 μmol/kg) on MAO-B activity in brain, heart and liver. Results expressed as % inhibition of vehicle MAO-B activity (N=7-8). Control MAO-B activity (pmol min^{-1} mg^{-1} tissue, mean ± SEM) = 70 ± 3 (brain), 71 ± 6 (heart), and 624 ± 24 (liver). All values are significantly different from control values.

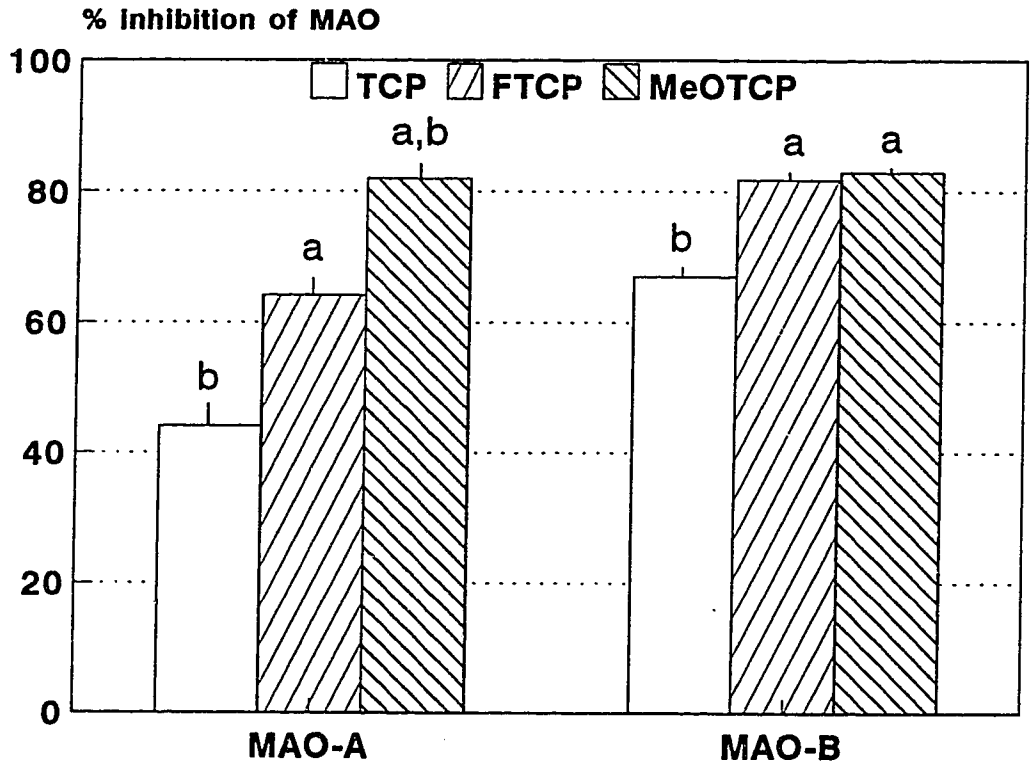


Figure 12: Effects of chronic administration (28 d) of TCP and its analogues at a dose of 1.2 μmol/kg on MAO activity in rat brain. Values represent mean % inhibition ± SEM (N=8). All values are significantly different from control values. a = significantly different from TCP-treated values; b = significantly different from FTCP-treated values.

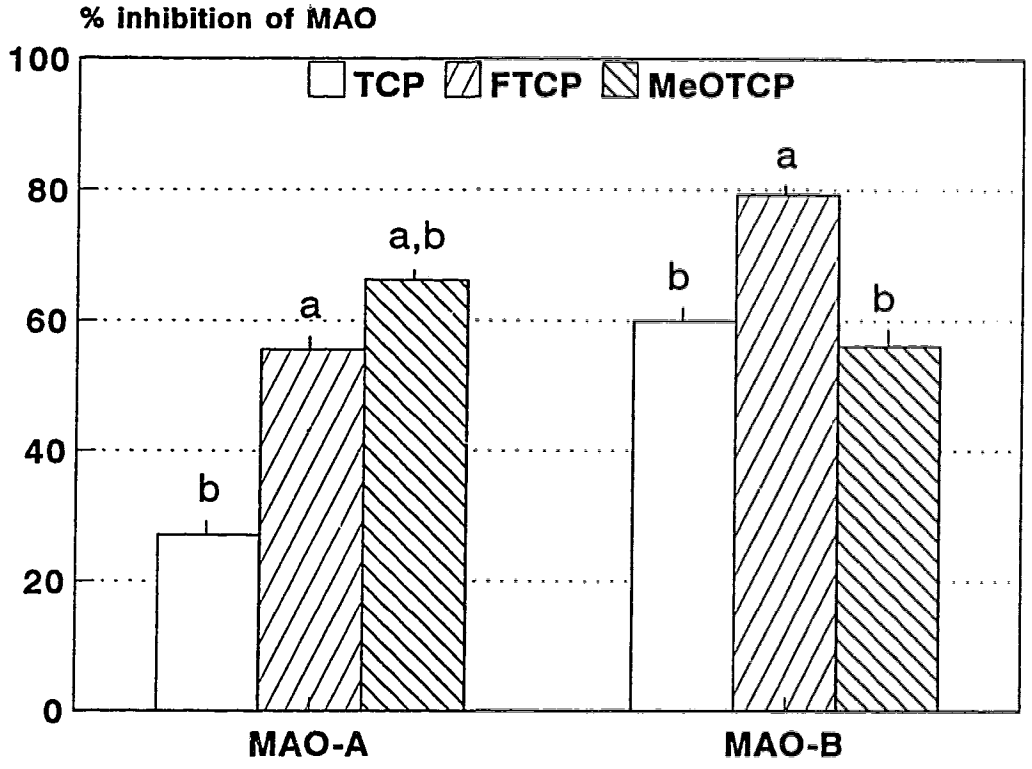


Figure 13: Effects of chronic administration (28 d) of TCP and its analogues at a dose of 1.2 μmol/kg on MAO activity in rat liver. Values represent mean % inhibition ± SEM (N=8). All values are significantly different from control values. a = significantly different from values in TCP-treated rats; b = significantly different from values in FTCP-treated rats.

neurotoxin to 5-HT-containing neurones (Fuller and Snoddy, 1991) and since MeOTCP is structurally related to 3,4-methoxymethamphetamine (MDMA) as well as 3,4-methylenedioxyamphetamine (MDA), drugs considered to be 5-HT neurotoxins (Schmidt et al., 1987; McKenna and Peroutka, 1990), 5-HT concentrations in the pons-medulla, hypothalamus and hippocampus of rats administered TCP, FTCP or MeOTCP were measured (Figures 14-19). These specific brain areas are known to contain high concentrations of serotoninergic cell bodies (pons-medulla) or terminals (hypothalamus and hippocampus).

Concentrations of NA, DA, and 5-HT were measured in the three specified brain regions following administration of the dose of 3.7 μmol/kg of TCP, FTCP, and MeOTCP. The major findings are summarized in Figures 14-16. In hippocampus, all three drugs caused a similar small increase in DA levels; TCP and FTCP, but not MeOTCP produced a small increase in NA levels. All three drugs elevated 5-HT levels, but this effect was greater with FTCP and MeOTCP than with TCP (Figure 14). In pons medulla, all three drugs caused a similar increase in NA and DA levels; the increase in 5-HT concentrations produced was considerably greater with MeOTCP than with FTCP or TCP (Figure 15). In hypothalamus, all three drugs increased NA and DA levels to a similar extent; as in the hippocampus the increases in 5-HT levels were in the order MeOTCP > FTCP > TCP (Figure 16).

Thus neither FTCP nor MeOTCP caused a decrease in brain levels of 5-HT. In fact, both drugs elevated brain concentrations of 5-HT to an even greater extent

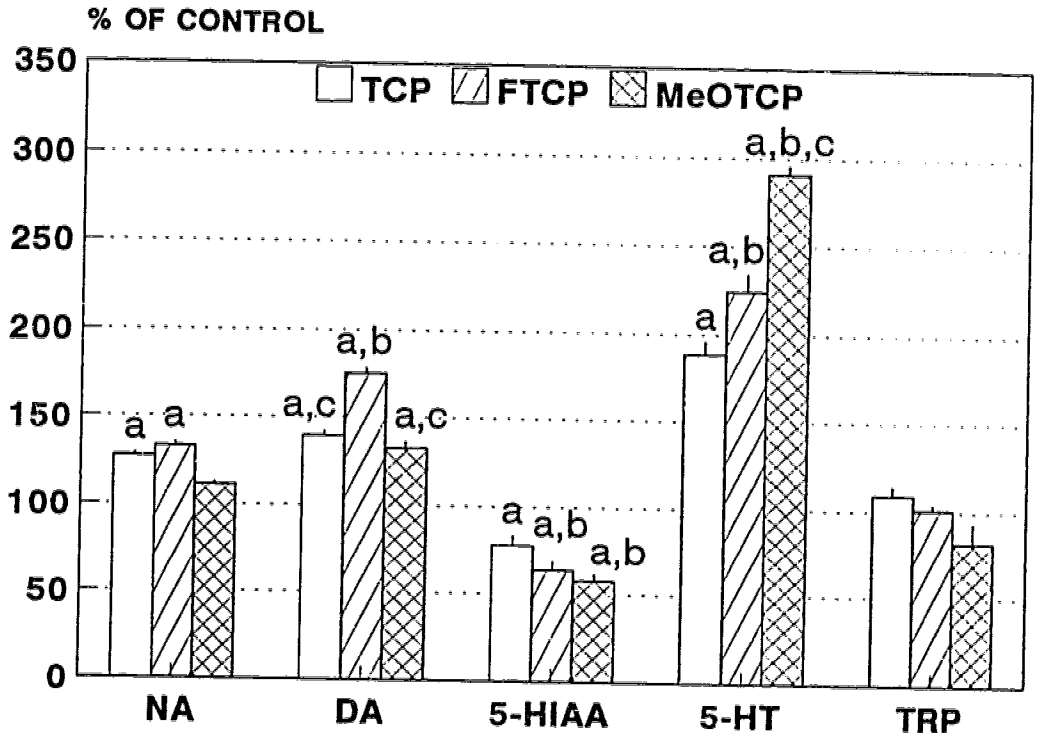


Figure 14: Levels of neurotransmitter amines and metabolites and tryptophan in hippocampus after chronic administration of TCP, FTCP or MeOTCP (3.7 μmol/kg/d for 28 d). Values represent mean % ± SEM of vehicle-treated controls (N=8). a,b,c = significantly different from values in vehicle-treated, TCP-treated and FTCP-treated rats, respectively.

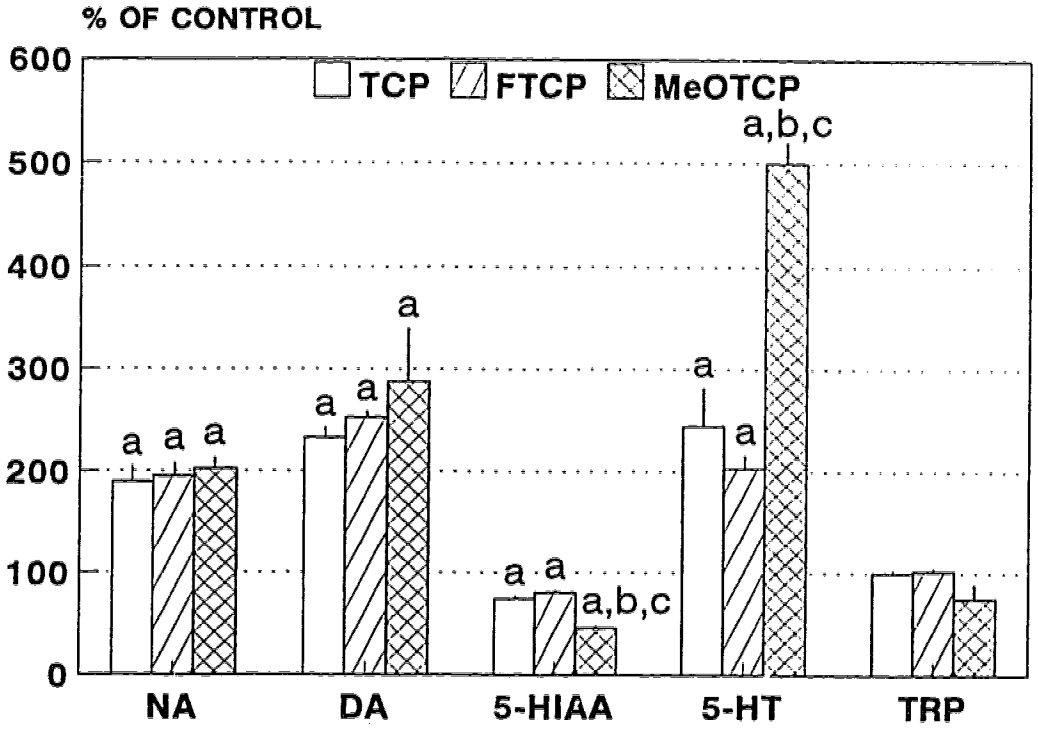


Figure 15: Levels of neurotransmitter amines and metabolites and tryptophan in pons medulla after chronic administration of TCP, FTCP or MeOTCP (3.7 μmol/kg/d for 28 d). Values represent mean % ± SEM of vehicle-treated controls (N=8). a,b,c = significantly different from values in vehicle-treated, TCP-treated and FTCP-treated rats, respectively.

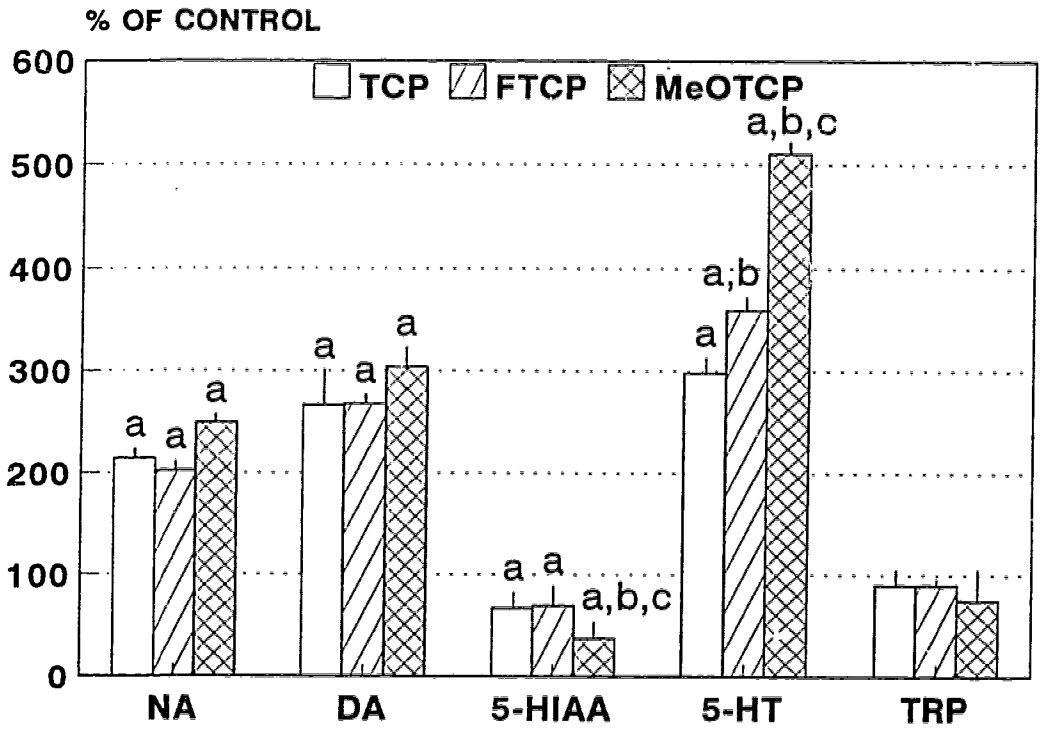


Figure 16: Levels of neurotransmitter amines and metabolites and tryptophan in hypothalamus after chronic administration of TCP, FCTP or MeOTCP (3.7 μmol/kg/d for 28 d). Values represent mean % ± SEM of vehicle-treated controls (N=8). a,b,c = significantly different from values in vehicle-treated, TCP-treated and FTCP-treated rats, respectively.

than did TCP. This pattern was also evident in all three brain regions at a drug dose of 1.2 μmol/kg/day (Figures 17-19).

C.3 Effects on Tryptophan Levels in Brain

As shown in Figures 14-16 and Table 8, chronic administration of TCP, FTCP or MeOTCP did not result in any change in Trp levels from control values in the brain regions studied. Included in this Table are the results of similar studies with three other MAO inhibitors, namely PLZ, N^2-acetylPLZ and (-)-deprenyl; none of these drugs demonstrated an effect on Trp levels either.

C.4 Effects on Receptor Binding

C.4.1 β-Adrenergic Receptors

Single point binding with ^{3}H-DHA (a ligand for β-adrenergic receptors) revealed that TCP, FTCP and MeOTCP caused decreased ^{3}H-DHA binding to membrane fractions prepared from whole cortex (Table 9). The percentage decrease in binding to β-adrenergic receptors was similar with all three drugs.

C.4.2 Tryptamine Receptors

All three drugs decreased binding of ^{3}H-tryptamine to membrane fractions prepared from whole cortex (Table 10), and FTCP was significantly stronger than MeOTCP and TCP in this regard.

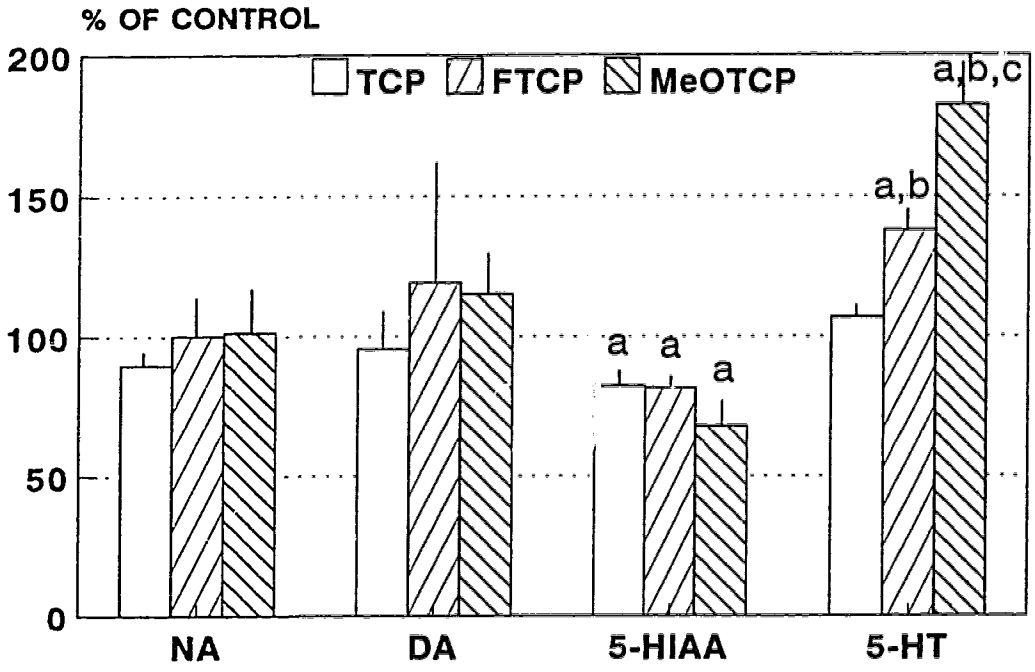


Figure 17: Levels of neurotransmitter amines and metabolites in hippocampus after chronic administration of TCP, FTCP or MeOTCP (1.2 μmol/kg/d for 28 d). Values represent mean % ± SEM of vehicle-treated controls (N=8). a,b,c = values significantly different from controls, TCP-treated rats and FTCP-treated rats, respectively.

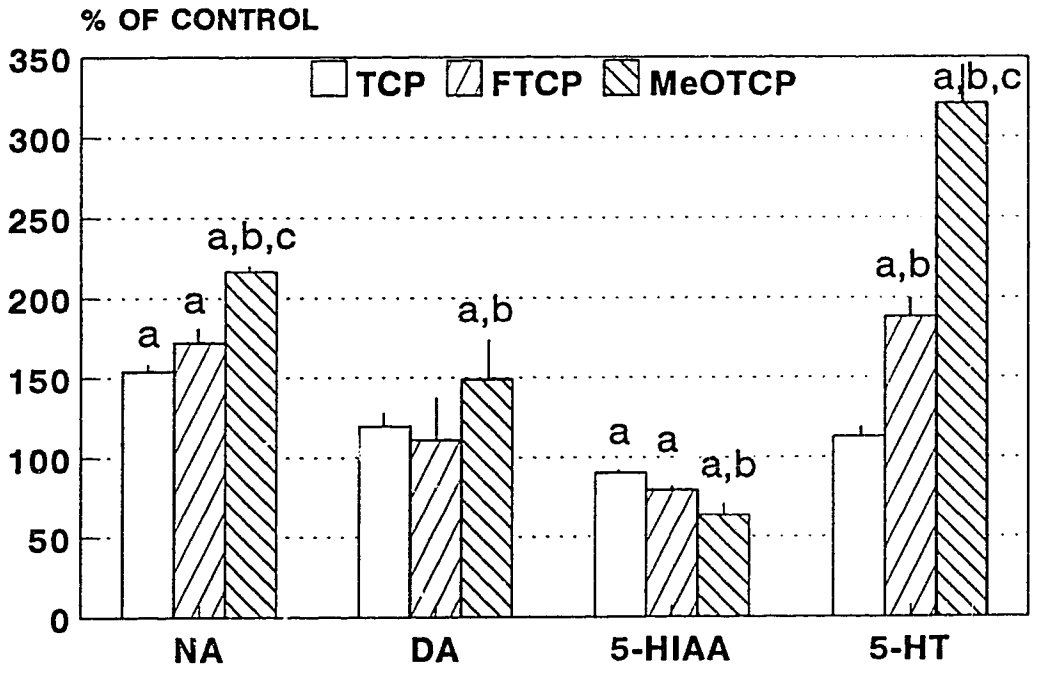


Figure 18: Levels of neurotransmitter amines and metabolites in hypothalamus after chronic administration of TCP, FTCP or MeOTCP (1.2 μmol/kg/d for 28 d). Values represent mean % ± SEM of vehicle-treated controls (n=8). a,b,c = values significantly different from controls, TCP-treated rats and FTCP-treated rats, respectively.

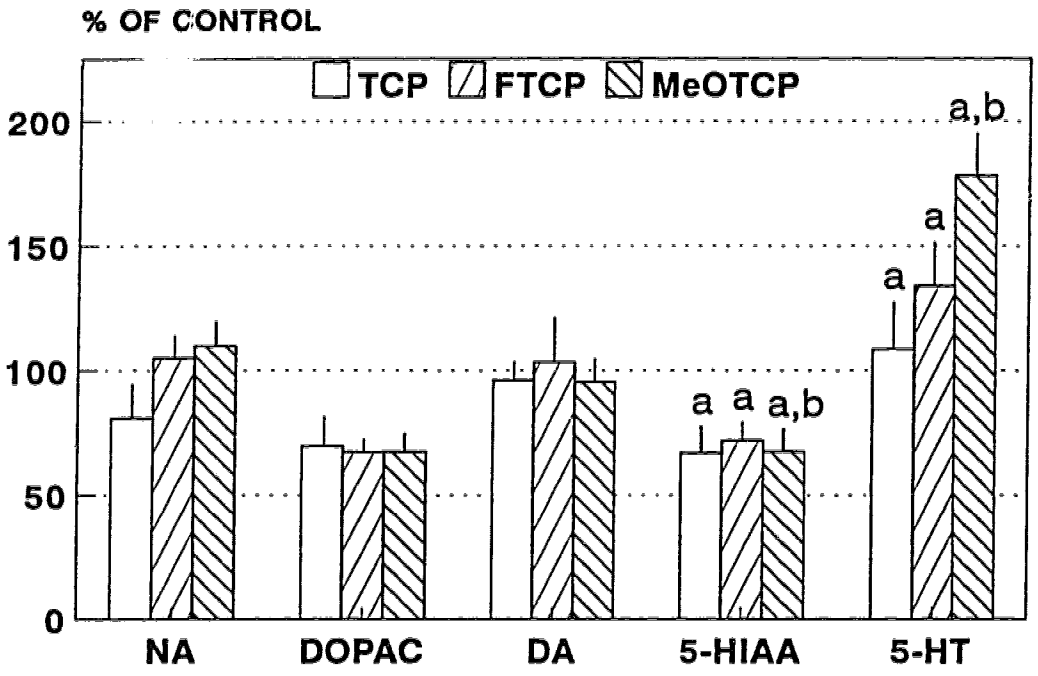


Figure 19: Levels of neurotransmitter amines and metabolites in pons medulla after chronic administration of TCP, FTCP or MeOTCP (1.2 μmol/kg/d for 28 d). Values represent mean % ± SEM of vehicle-treated controls (N=8). a,b,c = values significantly different from controls, TCP-treated rats and FTCP-treated rats, respectively.

Drug	Daily Dose (mg/kg)	Brain Region (N)	Brain Tryptophan (Trp) (% of Control)
TCP	0.5	Whole (6)	97 ± 6[a]
TCP	0.5	Pons medulla (8)	100 ± 4[b]
TCP	0.5	Hypothalamus (8)	90 ± 15[c]
TCP	0.5	Hippocampus (8)	109 ± 9[d]
FTCP	0.57	Pons medulla (9)	102 ± 4[b]
FTCP	0.57	Hypothalamus (9)	90 ± 7[c]
FTCP	0.57	Hippocampus (9)	101 ± 6[d]
MeOTCP	0.61	Hypothalamus (8)	92 ± 4[e]
MeOTCP	0.61	Pons Medulla (8)	75 ± 30
MeOTCP	0.61	Hippocampus (8)	82 ± 21
PLZ	5.8	Whole (8)	108 ± 6[f]
PLZ	13.6	Whole (8)	94 ± 5[f]
N^2-AcetylPLZ	18.0	Whole (8)	94 ± 9[f]
(-)-Deprenyl	0.83	Whole minus cerebral cortex (7)	94 ± 3[g]
(-)-Deprenyl	1.7	Whole minus cerebral cortex (8)	88 ± 2[g]
(-)-Deprenyl	3.3	Whole minus cerebral cortex (8)	92 ± 3[g]
(-)-Deprenyl	6.6	Whole minus cerebral cortex (8)	93 ± 3[g]

Table 8: Effects of chronic administration (28 d) of several MAO inhibitors on rat brain concentrations of Trp. Doses of all drugs are expressed as those of the free base. Control values (μg/g, means ± SEM) for Trp for each of the studies: (a) 3.5 ± 0.5; (b) 3.2 ± 0.5; (c) 4.9 ± 0.3; (d) 4.0 ± 0.3; (e) 5.1 ± 0.7; (f) 3.2 ± 0.2; (g) 4.7 ± 0.1. Values for PLZ, N^2-acetylPLZ and (-)-deprenyl are taken from Sherry-McKenna et al. (1994). In the whole minus cortex brain, only the cerebral cortex had been removed (for binding studies), and the remaining brain regions were analyzed for Trp levels in combination.

Drug	Radioligand	% of Control	N
TCP	^{3}H-DHA	81.4 ± 4.6	15
FTCP	^{3}H-DHA	80.5 ± 1.3	9
MeOTCP	^{3}H-DHA	73.2 ± 3.3	8

Table 9: Effects of long-term administration of TCP, FTCP and MeOTCP (3.7 μmol/kg) on ^{3}H-dihydroalprenolol (^{3}H-DHA) binding in rat cerebral cortex. Results are expressed as % of control values and represent means ± SEM. The specific binding was determined using concentrations of radiolabelled DHA at its K_d value, as determined in separate experiments using Scatchard analyses. Control values (dpm/mg protein; mean ± SEM) = 10,204 ± 521. All three values are significantly lower than control values.

Drug	Radioligand	% of Control	N
TCP	^{3}H-T	53.3 ± 5.6[b]	15
FTCP	^{3}H-T	37.0 ± 2.0[a]	9
MeOTCP	^{3}H-T	64.7 ± 4.7[a,b]	8

Table 10: Effects of long-term administration of TCP, FTCP and MeOTCP (3.7 μmol/kg) on ^{3}H-tryptamine (^{3}H-T) binding in rat cerebral cortex. Results are expressed as % of control values and represent means ± SEM. The specific binding was determined using concentrations of radiolabelled T at its K_d value, as determined in separate experiments using Scatchard analyses. Control values (dpm/mg protein; mean ± SEM) = 4,684 ± 607. a = significantly different from results in TCP-treated rats; b = significantly different from results in FTCP-treated rats. All values are significantly lower than control values.

D. The Differential Effects of TCP, FTCP, MeoTCP, and OHTCP on Neurotransmitter Uptake and Release

4-Hydroxytranylcypromine (OHTCP) was compared with TCP to ascertain whether or not the 4-hydroxy group affects the ability of the parent drug to inhibit the uptake of NA, DA or 5-HT by brain tissue. The novel TCP analogues, FTCP and MeOTCP, were compared in this respect also. The results are shown in Table 11. As described by Hampson (1984), OHTCP was more potent at inhibiting the uptake of NA than of DA or 5-HT. This hydroxylated metabolite was slightly more potent than the parent TCP at inhibiting uptake of NA and 5-HT but was somewhat weaker as a DA uptake inhibitor. FTCP had stronger effects on uptake of NA and 5-HT than of DA. It is interesting to note that the pattern for MeOTCP was markedly different from those of TCP, OHTCP and FTCP. Its strongest effect was on 5-HT inhibition and it was much weaker than the other drugs in inhibiting uptake of NA and DA. With regard to inhibition of the individual neurotransmitter amines, the patterns of inhibition of uptake for the drugs were as follows: NA: 4-OH-TCP > FTCP ≈ TCP >> MeOTCP; DA: TCP > FTCP ≈ 4-OH-TCP >> MeOTCP; and 5-HT: MeOTCP ≈ FTCP > OHTCP > TCP.

FTCP, MeOTCP, OHTCP, and TCP were compared to TCP at a concentration of 10^{-5} M for their ability to facilitate the release of tritiated NA, DA, and 5-HT from brain prisms (Table 12). TCP and the three analogues are all relatively modest releasers of the biogenic amines. At this relatively high concentration, only FTCP produced a significant release of NA. A significant, but weak release of DA was observed with all four drugs. Neither TCP nor MeOTCP caused a release of 5-HT

DRUG	IC_{50} (μM)		
	^{3}H-NA (hyp.)	^{3}H-DA (str.)	^{3}H-5-HT (Str.)
TCP	0.63 ± 0.14	0.95 ± 0.44	7.76 ± 3.42[b,c]
FTCP	0.51 ± 0.08	2.33 ± 0.84[b]	0.38 ± 0.05[a]
MeOTCP	3.95 ± 0.92[a]	17.71 ± 4.63[a,b]	0.80 ± 0.20[a,b,c]
OHTCP	0.27 ± 0.04	3.95 ± 0.75[a,b]	3.80 ± 0.91[a,b]

Table 11: The effects of TCP and analogues on uptake of radiolabelled neurotransmitter amines in prisms prepared from hypothalamus (NA) or striatum (DA and 5-HT). Results are expressed as means ± SEM (N=6). a = significantly different from TCP in columns; b = significantly different from NA in rows; c = significantly different from NA and DA in rows. Data were analyzed using the Mann-Whitney U-Test.

	Conc. (M)	NA	DA	5-HT
TCP	10^{-5}	114 ± 8.9 (N=8)	*120 ± 5.2 (N=11)	116 ± 11.2 (N=10)
OHTCP	10^{-5}	115 ± 3.6 (N=8)	*127 ± 6.3 (N=13)	*158 ± 12.5 (N=8)
FTCP	10^{-5}	*127 ± 9.9 (N=8)	*117 ± 2.3 (N=8)	*142 ± 7.5 (N=6)
MeOCTP	10^{-5}	105.6 ± 1.2 (N=8)	*116 ± 1.2 (N=5)	114 ± 3.1 (N=6)

Table 12: Effects of TCP, FTCP, MeOTCP and OHTCP on ^{3}H-labelled neurotransmitter release from hypothalamic (NA) or striatal (DA, 5-HT) prisms. Results are expressed as % control ± SEM. * = significantly different from controls.

at 10^{-5}M, while a significant effect was noted with FTCP and OHTCP.

E. Failure to Detect Amphetamine(AMP) as a Metabolite of TCP

Typical GC traces of extracts of urine samples from human subjects taking TCP and of extracts of brains from rats treated with TCP are shown on Figures 20 and 21, respectively.

The concentrations of AMP and TCP in rat brain, heart, liver, and plasma are shown in Table 13. The sensitivity of the assay was 5 ng/g, but AMP was not detected in any of the tissues or plasma sampled, despite the fact that animals were administered a high dose of TCP (20 mg/kg i.p.). Levels of AMP and TCP in extracts of human urine are represented in Table 14. The sensitivity was 10 μg/24 h (< 10 ng/ml), but AMP was not detected in urine from any of the patients sampled. As mentioned in the Introduction to this thesis, two other possible metabolites which could result from opening of the cyclopropyl ring of TCP are 2-phenylpropylamine and 3-phenylpropylamine. 3-Phenylpropylamine was not detectable in the TCP-treated samples from rats or humans despite a sensitivity level similar to that for AMP. An authentic sample of 2-phenylpropylamine was not commercially available, so this potential metabolite was not investigated.

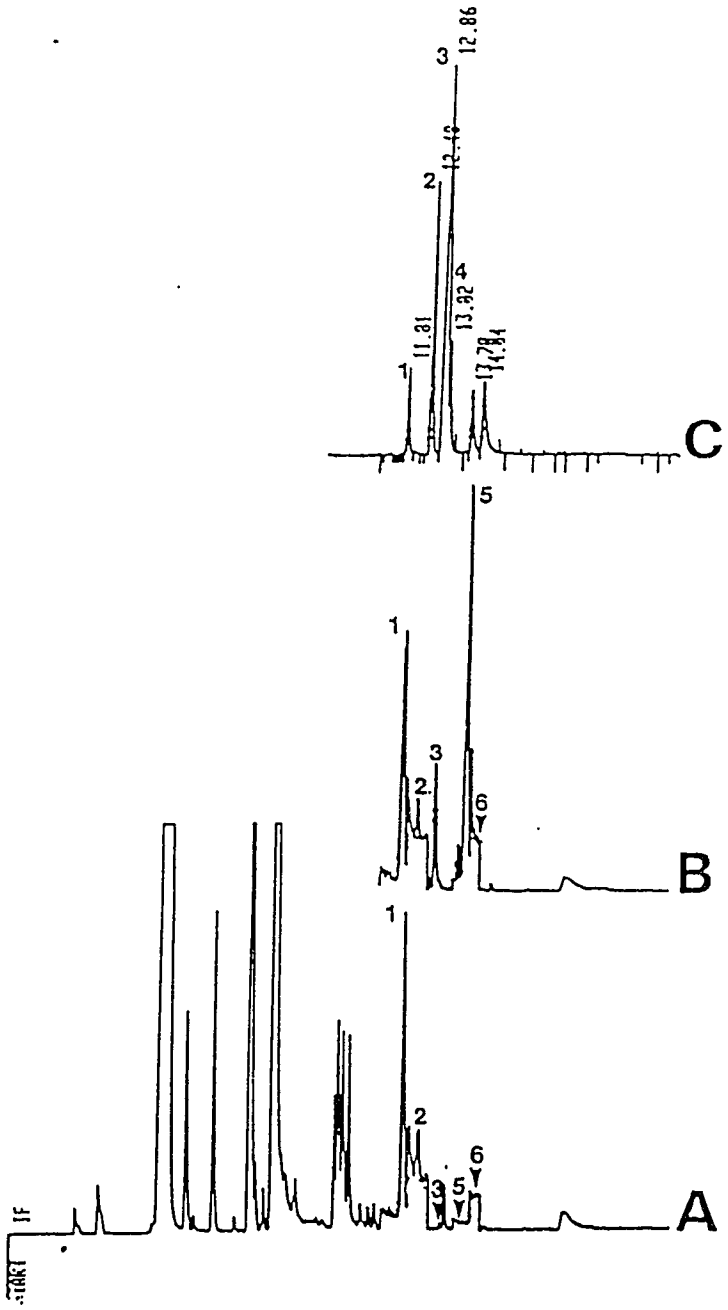


Figure 20: GC traces of: (A) a derivatized extract of a urine sample from a patient pretreatment; (B) a derivatized extract of a urine sample from the same patient after treatment with TCP for 2 weeks; and (C) derivatives of authentic standards of benzylamine [internal standard] (1); amphetamine (2); β-phenylethylamine, an endogenous amine (3); N-methylamphetamine (4); tranylcypromine (5); and 3-phenylpropylamine (6). All amines were derivatized with pentafluorobenzenesulfonyl chloride.

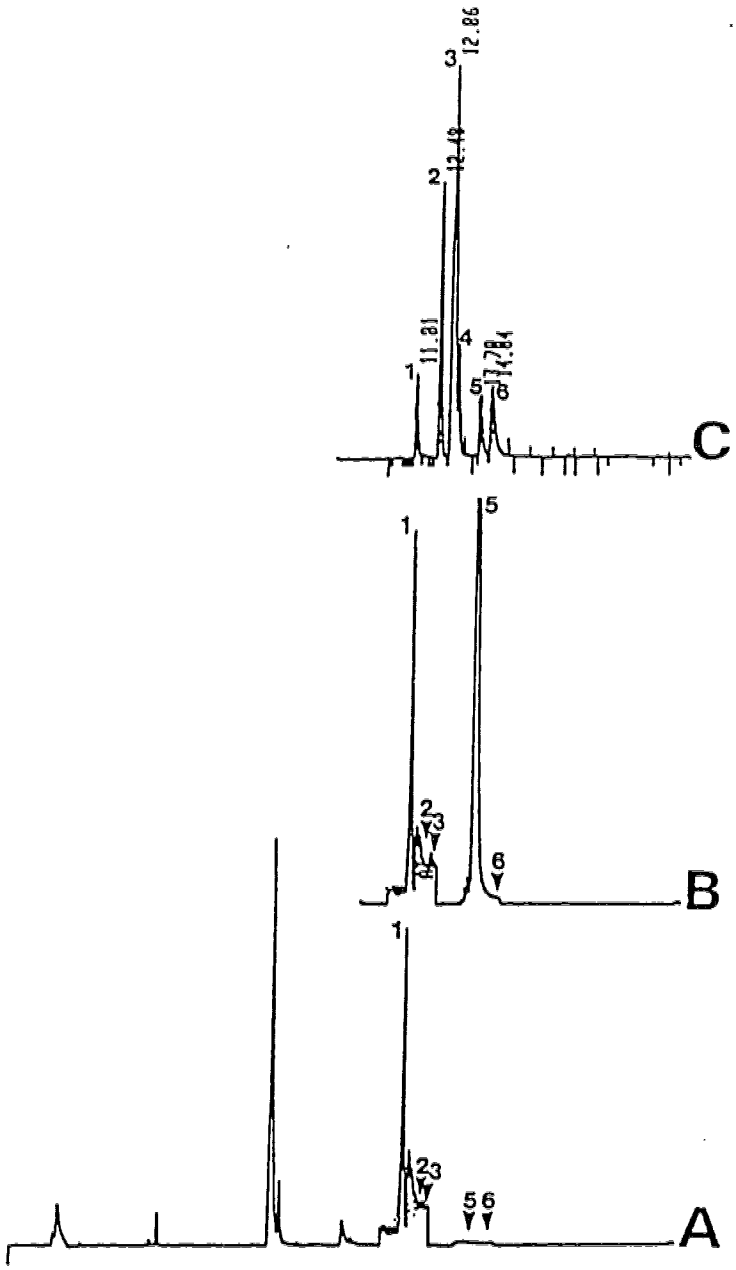


Figure 21: GC traces of: (A) a derivatized extract of a brain from a rat treated with normal saline vehicle and killed 1.5 h later; (B) a derivatized extract of a brain from a rat treated with TCP (20 mg/kg i.p.) and killed 1.5 h later; and (C) derivatives of authentic standards of benzylamine [internal standard] (1); amphetamine (2); β-phenylethylamine, an endogenous amine (3); N-methylamphetamine (4); tranylcypromine (5); and 3-phenylpropylamine (6). All amines were derivatized with pentafluorobenzenesulfonyl chloride.

	AMP	TCP
Brain	n.d.	16.5 ± 1.9
Liver	n.d.	17.2 ± 1.9
Heart	n.d.	6.6 ± 0.3
Plasma	n.d.	5.4 ± 1.2

Table 13: Concentrations of TCP and AMP in rats 2 h after treatment with TCP (20 mg/kg i.p.). Results are expressed as μg/g (tissues) or μg/ml (plasma) and represent means ± SEM (N=6). n.d.=not detectable (<5 ng/g).

Patient	AMP	TCP
TCP001	n.d.	143
TCP003	n.d.	991
TCP004	n.d.	2947
TCP005	n.d.	1996
TCP006	n.d.	1729
TCP007	n.d.	308

Table 14: Urinary levels (μg/24 h) of TCP and AMP in human subjects following 2 weeks of TCP administration (10-20 mg, bid). n.d. (not detectable) = <10 μg/24 h.

F. Gas Chromatographic Assay for TCP, FTCP and MeOTCP

The assay procedure developed was suitable for analysis of TCP, FTCP and MeOTCP. The peaks of the derivatives had excellent chromatographic properties (see Figure 22). Plots of drug/internal standard *versus* amount of drug added were linear, giving correlation coefficients of > 0.99 routinely. Mean recoveries of the three drugs (N=6) were 69.8% (TCP), 72.9% (FTCP) and 76.4% (MeOTCP). Using 250 ng standards, the mean intra-assay coefficients of variation for the three drugs were 0.68, 3.1, and 2.1% (N=6), respectively. At the same concentration, the interassay coefficients of variation were 8.1, 7.3 and 6.9%, respectively (N=6). The structures of the derivatives were confirmed using combined GC-MS, and the proposed fragmentation patterns are shown in Figure 23.

G. Levels of TCP and MeOTCP in the Brain, Heart and Liver

The levels of TCP and MeOTCP were measured at 1, 2 and 4 h following an i.p. injection of an equimolar dose (18 μmol/kg). FTCP was not investigated since a previous study had shown that this analogue attained higher levels in brain than did TCP after i.p. injection of equimolar doses (Coutts et al., 1987). In contrast, the present study demonstrated that MeOTCP maintained significantly lower concentrations than TCP at each time interval in whole brain samples of the rat (Figure 24). The same trend was also observed in the liver and heart (Figures 25 and 26).

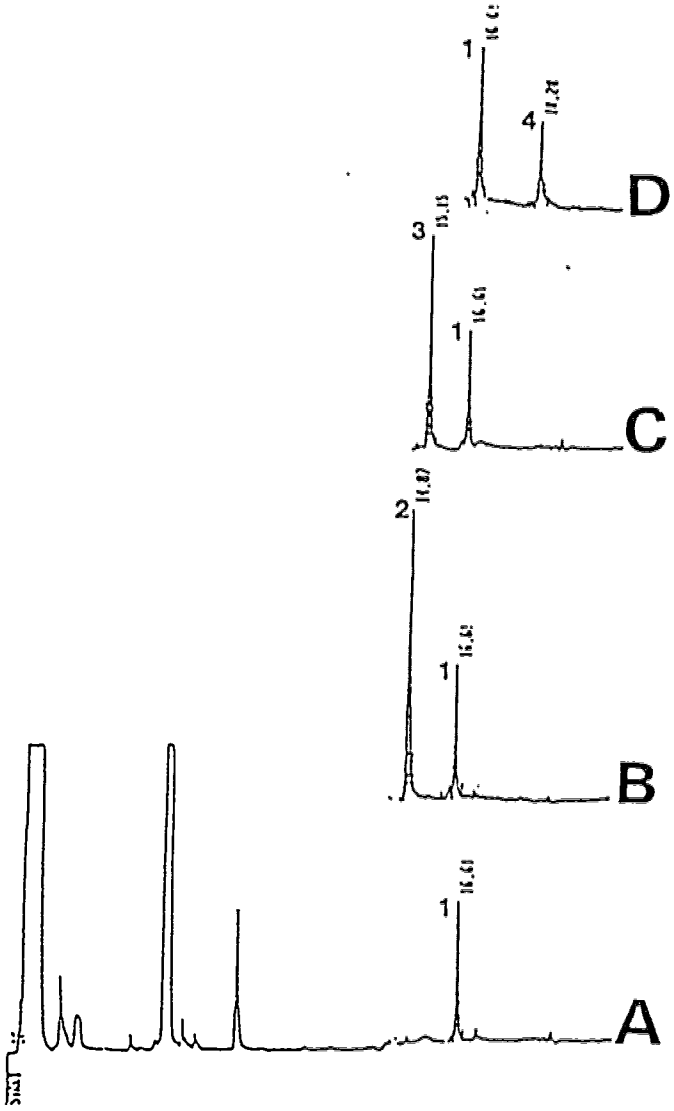


Figure 22: Typical traces of extracts from brains of rats treated with (A) vehicle, (B) TCP, (C) FTCP or (D) MeOTCP. Each drug was administered at a dose of 18 μg/kg i.p. 1 h before killing the rats. Derivatives of: (1) internal standard; (2) TCP; (3) FTCP; and (4) MeOTCP.

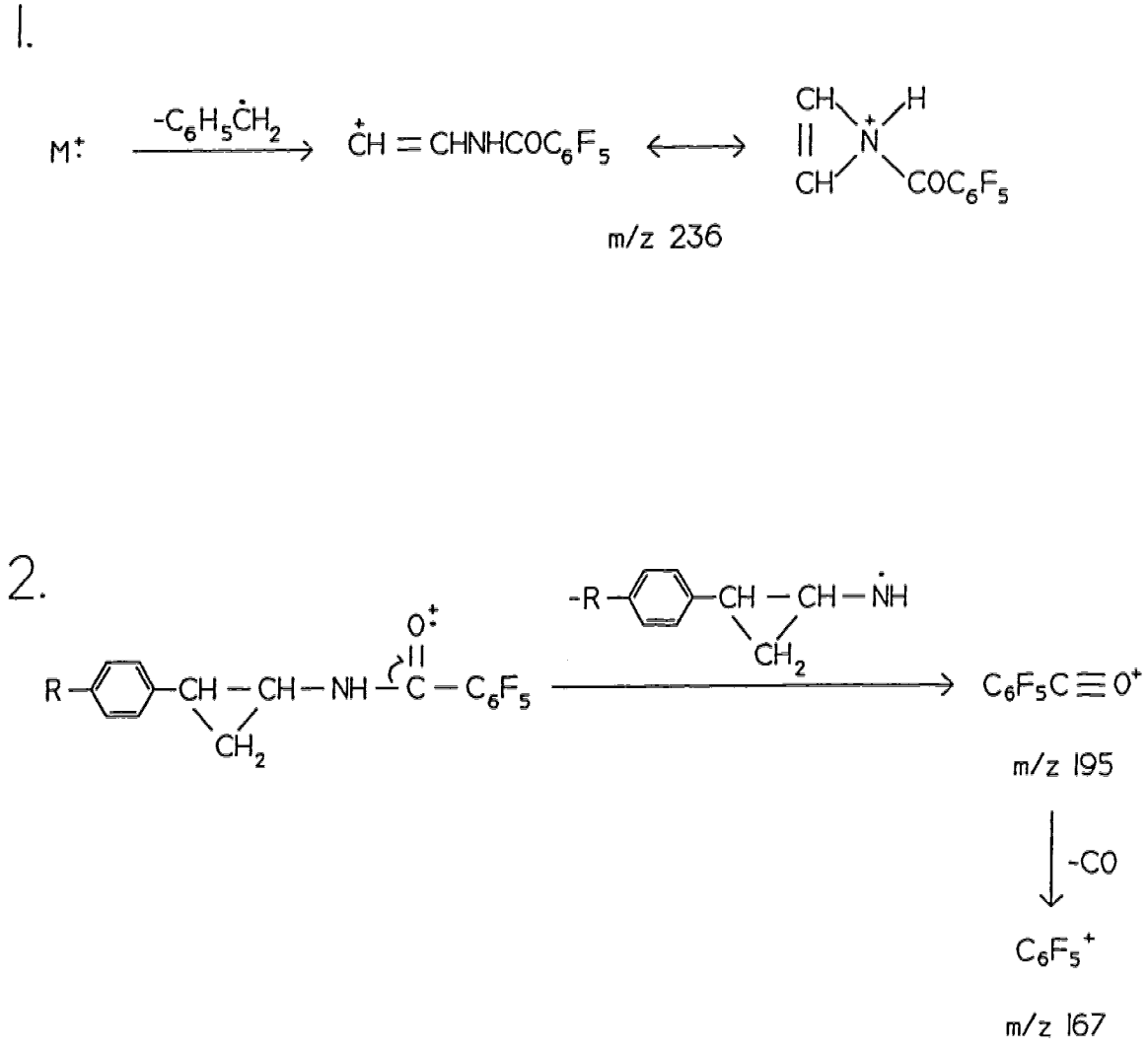
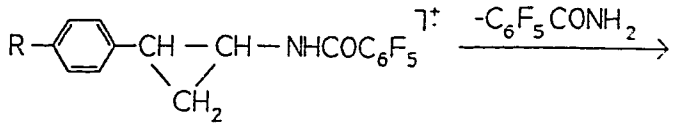


Figure 23: Proposed mass spectral fragmentation pathways for the pentafluorobenzoyl derivatives of TCP (R=H), FTCP (R=F) and MeOTCP (R=MeO) [continued on next page].

3.



	R	m/z
$M^{+\cdot}$	H	327
	CH_3O	357
	F	345

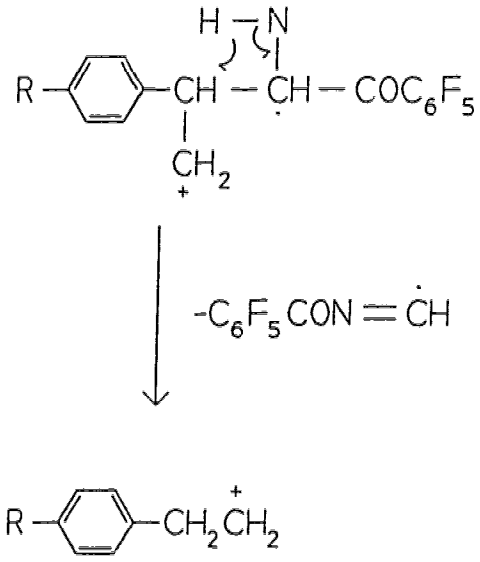
R	m/z
H	116
CH_3O	146
F	134

-H·

R	m/z
H	115
CH_3O	145
F	133

Figure 23 (con't): Proposed mass spectral fragmentation pathways for the pentafluorobenzoyl derivatives of TCP (R=H), FTCP (R=F) and MeOTCP (R=MeO).

4.



R	m/z
H	105
CH_3O	135
F	123

Figure 23 (cont'd): Proposed mass spectral fragmentation pathways for the pentafluorobenzoyl derivatives of TCP (R=H), FTCP (R=F) and MeOTCP (R=MeO).

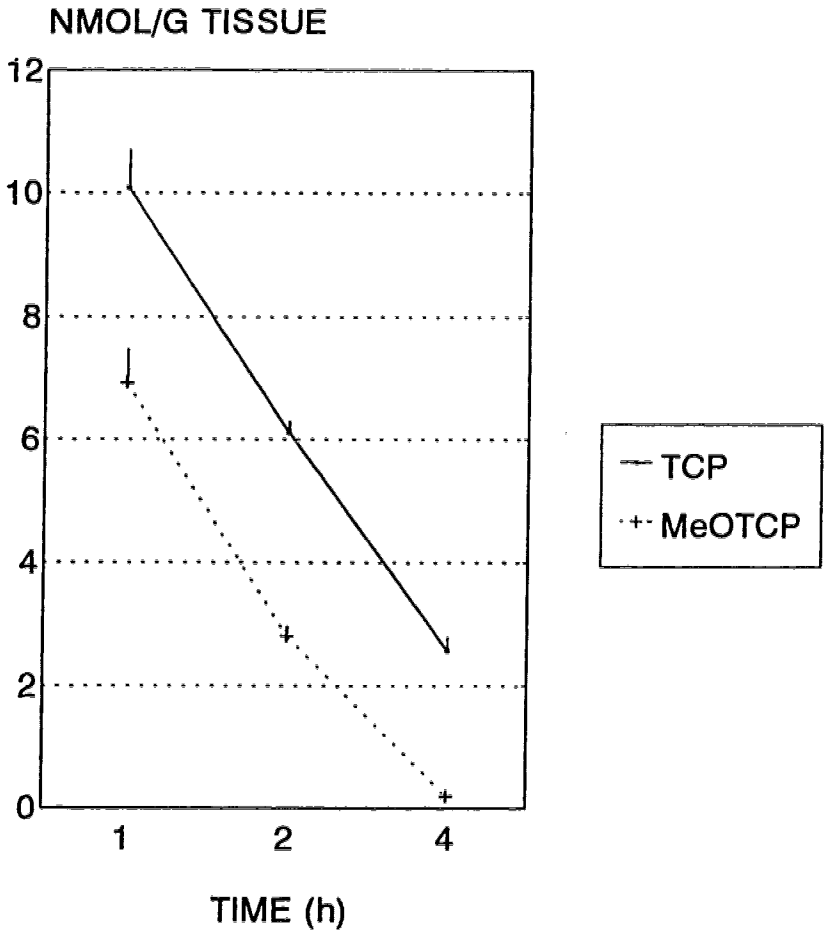


Figure 24: Levels of TCP and MeOTCP in rat brain at 1, 2 and 4 h after injection of equimolar doses (18 μmol/kg i.p.) of the drugs. Results are expressed as nmol/g (means ± SEM, N=6). At all 3 time intervals, MeOTCP levels were significantly lower than TCP levels.

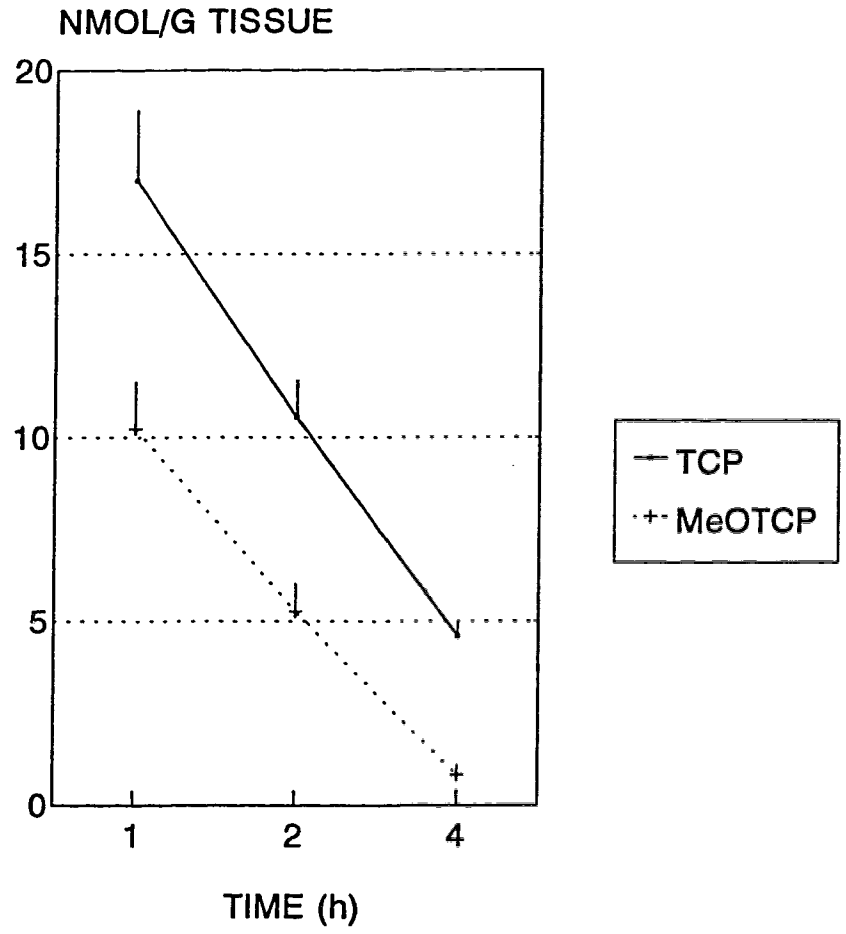


Figure 25: Levels of TCP and MeOTCP in rat liver at 1, 2 and 4 h after injection of equimolar doses (18 μmol/kg i.p.) of the drugs. Results are expressed as nmol/g (means ± SEM, N=6). At all 3 time intervals, MeOTCP levels were significantly lower than TCP levels.

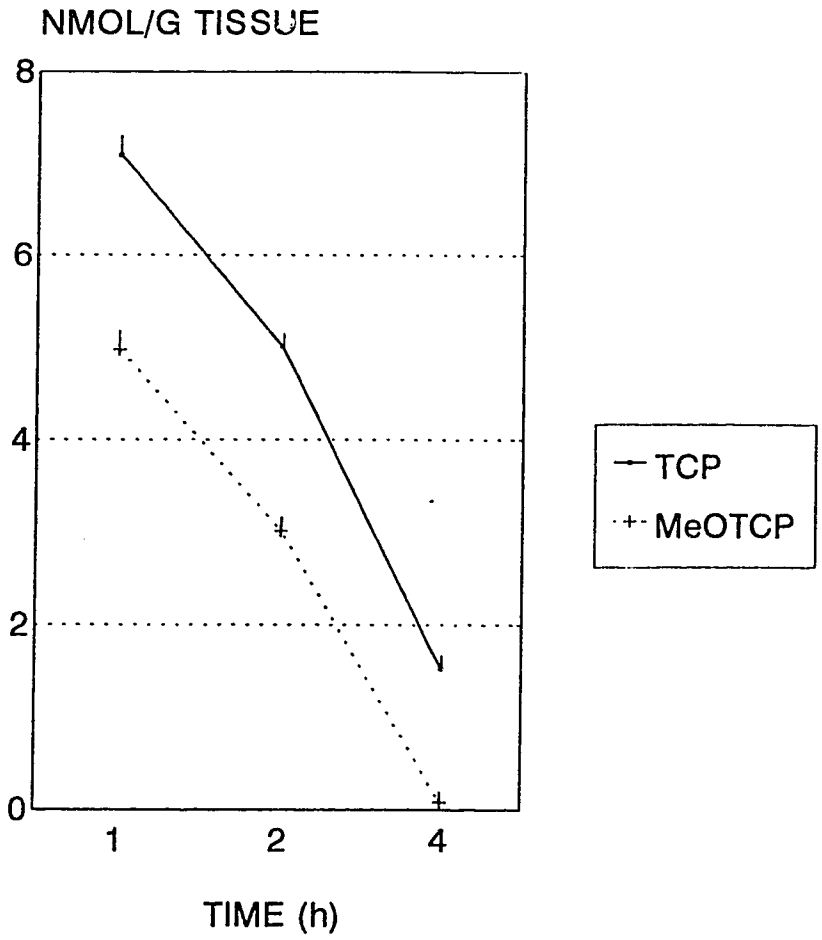


Figure 26: Levels of TCP and MeOTCP in rat heart at 1, 2 and 4 h after injection of equimolar doses (18 μmol/kg i.p.) of the drugs. Results are expressed as nmol/g (means ± SEM, N=6). At all 3 time intervals, MeOTCP levels were significantly lower than TCP levels.

H. Lack of Effect of Iprindole or Trifluoperazine on Brain Levels of FTCP and MeOTCP

Pretreatment with iprindole or trifluoperazine prior to injection of FTCP or MeOTCP did not alter brain levels of either drug 2 h later when compared with values in rats pretreated with saline vehicle and then given FTCP or MeOTCP. Values for FTCP were 93.3 ± 4.7 and 88.9 ± 7.7% of vehicle-pretreated values, respectively, while the corresponding values for MeOTCP were 111.1 ± 27.5 and 124.5 ± 18.4% (mean ± SEM, N=5). In previous experiments in the Neurochemical Research Unit, Baker et al. (1986) had demonstrated that at the same dose and time intervals used here, iprindole and trifluoperazine pretreatment doubled and tripled, respectively, TCP levels in brain compared to values in TCP-treated rats which had been pretreated with normal saline vehicle.

DISCUSSION

A. Acute Studies on the Effects of Novel TCP Analogues on MAO Activity and Brain Levels of Neurotransmitter Amines and Their Metabolites

The demonstration that the MAO-inhibiting antidepressant TCP undergoes ring hydroxylation in the para or 4-position of the phenyl ring (Baker et al., 1986) led members of the Neurochemical Research Unit to synthesize analogues of TCP (Rao et al., 1986; Coutts et al., 1987) substituted in that position. It was anticipated that such analogues might have improved pharmacokinetic properties and side effect profiles compared to TCP but still retain MAO-inhibitory properties. Initial in vitro studies indicated that FTCP, MeOTCP, MeO_2TCP and NCP were as potent as or more potent than TCP at inhibiting brain MAO in vitro (Rao et al., 1986). These workers reported that FTCP was ten times more potent in than TCP in this regard. The studies reported in this thesis indicate that all five drugs are also relatively potent inhibitors of MAO ex vivo. Based on the preliminary acute screen done on MAO-inhibiting properties, the two most potent analogues, namely FTCP and MeOTCP, were chosen for further comparative studies with TCP. In this same initial acute study, both analogues were shown to be more potent inhibitors of MAO-A and MAO-B than was the parent drug, TCP.

It is apparent from these experiments that substitution at the 4-position of the phenyl ring with fluorine or a methoxy group does not reduce the MAO-inhibiting properties of TCP. When given to rats at doses equivalent to TCP, the two analogues produced a similar degree of inhibition of MAO-A and MAO-B as the

parent drug at 3.7 μmol/kg, but produced a greater degree of inhibition of MAO-A and -B than did TCP at 1.2 μmol/kg. At the dose of 3.7 μmol/kg, FTCP and MeOTCP resulted in a more pronounced elevation of brain levels of the neurotransmitter amines, particularly NA and 5-HT, than did TCP. It is important to note that the dose of 3.7 μmol/kg is similar, on a mg/kg basis, to the usual clinical dose of TCP. Because the analogues showed such good activity at these clinically relevant doses, it was felt that they were worthy of further investigation in chronic studies.

B. The *Ex Vivo* Effects of Chronic Administration of TCP and Its Novel Analogues FTCP and MeOTCP

B.1 MAO Activity

The analogues were also effective inhibitors of MAO-A and MAO-B in chronic experiments. Studies in rat brain, liver and heart at a dose of 3.7 μmol/kg showed very little difference in MAO-inhibiting abilities among TCP, FTCP and MeOTCP, as all three drugs inhibited MAO-A and MAO-B by greater than 80%. At the lower dose of 1.2 μmol/kg, the stronger inhibitory effects of the analogues on MAO activity become very obvious. These results support previous *in vitro* results obtained in our laboratories (Rao et al., 1986) which indicate that substitution of TCP in the 4-position of the phenyl ring by a fluorine or methoxy group does not reduce the MAO-inhibiting ability of TCP and in fact increases this activity to some extent.

B.2 Levels of Neurotransmitter Amines and Their Acid Metabolites

It was important to conduct the chronic studies on the effects of the drugs on levels of the amine neurotransmitters and their metabolites for two reasons. First, TCP, like other antidepressants, must be administered to most patients for 2-3 weeks or more before clinical improvement becomes obvious. Second, it was important to ascertain whether the ring-substituted analogues caused a depletion of brain levels of 5-HT when administered chronically. The analogues are closely related structurally to p-chloroamphetamine (pCA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA), drugs which have been reported to be neurotoxic to 5-HT systems in brain (Harvey et al., 1977; Schmidt et al., 1988; McKenna and Peroutka, 1990; Green et al., 1995; McCann and Ricaurte, 1995; Lew et al., 1996; Sabol et al., 1996).

Neither FTCP nor MeOTCP caused a decrease in levels of 5-HT in the brain regions studied. In fact, both drugs caused elevations of 5-HT levels which were higher than those observed with equimolar doses of TCP. It is of interest in this regard that a structurally related drug, the 4-methoxy analogue of AMP, has been reported to be more potent than AMP after acute administration in elevating brain 5-HT (Hitzemann et al., 1971) and to be a more potent inhibitor of MAO-A than is AMP (Green and Hait, 1980). Martin-Iverson and Lodge (1992) recently reported that chronic administration (14 days) of 4-methoxyAMP to rats also produced a marked increase in concentrations of 5-HT in the brain areas studied, and Hegadoren et al. (1995) more recently demonstrated that acute (3 h) administration of 4-methoxyAMP to rats resulted in a significant elevation of 5-HT in several brain

areas. It has been suggested that an as yet unidentified metabolite (or metabolites) may be responsible for the serotoninergic neurotoxicity of pCA, MDA and MDMA (Fuller, 1978; McKenna and Peroutka, 1990) and it is possible that MeOTCP and FTCP do not undergo such metabolism. Indeed, MDMA and MDA are known to be metabolized, at least in part by cytochrome P450IID6 (CYP2D6) [Tucker et al., 1994], but the preliminary studies in this thesis showing the lack of effect of the CYP2D6 inhibitors iprindole and trifluoperazine on FTCP and MeOTCP suggest that FTCP and MeOTCP do not undergo such metabolism.

Higher levels of 5-HT were observed in the brains of the chronic MeOTCP-treated rats than in those of FTCP-treated rats. Interestingly, MeOTCP produced higher levels of 5-HT in hippocampus, hypothalamus and pons medulla even though the two drugs inhibited MAO-A to a similar extent at this dose. This difference suggests that MeOTCP may have other effects on 5-HT turnover in addition to inhibition of MAO. Such effects could include stimulation of Trp hydroxylase and/or aromatic amino acid decarboxylase, inhibition of Trp pyrrolase and/or increases in Trp brain concentrations through facilitation of transport of this amino acid into the brain and/or a reduction in the firing rate of serotoninergic neurones [as has been reported after acute administration of (+)-amphetamine (Schubert and Sedvall, 1972; Trulson and Jacobs, 1980)]. Inhibition of Trp hydroxylase and/or Trp pyrrolase and stimulation of Trp transport into the brain would be expected to cause an increase in brain levels of Trp but, as discussed elsewhere in this thesis, such an increase in Trp brain levels was not observed after administration of MeOTCP. Bradberry (1994) has recently shown that MDA, which is structurally similar to

MeOTCP, inhibits firing of 5-HT neurones, and such a mechanism could also be contributing to the 5-HT elevating actions of MeOTCP.

B.3 Tryptophan (Trp) Concentrations in the Rat Brain

No changes were observed in brain region levels of Trp after chronic administration of TCP, FTCP or MeOTCP at a dose of 3.7 μmol/kg. This finding is similar to those observed by other workers in the Neurochemical Research Unit using the MAO inhibitors phenelzine (PLZ), N^2-acetylPLZ and (-)-deprenyl (Paetsch and Greenshaw, 1991; Sherry-McKenna et. al., 1994). Although Badawy and Evans (1981, 1982) reported that TCP at both high and low doses (10 and 0.5 mg/kg) elevated rat brain Trp after acute drug administration, such an elevation was observed in our laboratories only after administration of very high doses of TCP (Wong, 1990) and it was determined, in agreement with observations by Grahame-Smith (1971), that this elevation was very short-lived (Sherry-McKenna et al., 1994). In any case, my results suggest that at clinically relevant doses, chronic administration of TCP, FTCP or MeOTCP does not cause an increase in brain Trp levels.

B.4 Binding to β-Adrenergic Receptors and Tryptamine Receptors

Although antidepressants have been employed for the treatment of affective disorders since the early 1950s, researchers are still probing into the mechanism of their actions. Changes in neuroreceptor number as a function of antidepressant use have been the focus of intensive investigation since the mid 1970s (Vetulani et

al., 1976; Sugrue, 1983; Baker and Greenshaw, 1989). Researchers are still attempting to understand and explain the mysterious delayed onset of clinical improvement despite the immediate change in the neuroenvironment (e.g. inhibition of uptake of 5-HT and/or NA) produced by antidepressants. The process of receptor down-regulation is not instantaneous and may in part account for the delayed therapeutic efficacy of antidepressant drugs. The effects of 28 day administration of TCP, FTCP and MeOTCP on tryptaminergic and β_2-adrenergic receptors were investigated and results are now reported.

Tryptamine has been implicated in the etiology of a variety of psychiatric illnesses, including the affective disorders (Dewhurst, 1968a,b; Slingsby and Boulton, 1976; Mousseau, 1993). There is a great deal of evidence indicating that treatment with MAOIs dramatically increases brain tryptamine levels (Marsden and Curzon, 1974; Philips and Boulton, 1979; Philips et al., 1980; Juorio and Durden, 1984; Baker et al., 1988). Several MAO-inhibiting antidepressants have also been demonstrated to produce a reduction in the number of cortical tryptamine binding sites following chronic administration (Wood et al., 1984; Mousseau et al., 1993). These reports agree with the current pharmacological knowledge of receptor theory, namely that continued stimulation of tryptamine neuroreceptors following the initiation of MAOI treatment and the presumed increased availability of tryptamine in the synapse should serve to decrease the number of tryptamine receptors. It appears that tryptamine receptor down-regulation is a characteristic feature of several MAO inhibitors (Mousseau, 1993). In the experiments undertaken during the course of this thesis, chronic administration of TCP, FTCP and MeOTCP at an

equimolar dose of 3.7 μmol/kg resulted in a significant decrease in cortical ^{3}H-tryptamine binding *ex vivo*. Since it appears that decreased ^{3}H-tryptamine binding is a characteristic feature of chronic administration of several MAOI antidepressants, the fact that both FTCP and MeOTCP mediated this effect provides further evidence that they may have potential antidepressant action.

Down-regulation (usually a decrease in B_{max} or receptor number) of β-adrenoceptors has also been chronicled as an emergent modification to the neuronal molecular environment following treatment with numerous antidepressant drugs (Snyder and Peroutka, 1984; Spyraki and Fibiger,1980; Przegalinski et al., 1983 and 1984), including MAO inhibitors (Vetulani et al., 1976; Cohen et al., 1982; Frazer and Lucki, 1982; Murphy et al., 1987). The present studies indicate that MeOTCP and FTCP, like TCP and other antidepressants, result in a decrease of binding to β-adrenergic receptors after chronic administration of the drugs. This effect occurs at the clinically relevant dose (based on the usual mg/kg dose of TCP) of 3.7 μmol/kg/day.

C. The Differential Effects of TCP, FTCP, MeOTCP and OHTCP on Neurotransmitter Uptake and Release

TCP, which is structurally similar to amphetamine, has been reported to have effects on uptake and release of the catecholamines in brain tissue (Hendley and Snyder, 1968; Schildkraut, 1970; Baker et al., 1980, 1992; Reigle et al., 1980), and several researchers have suggested that such actions may contribute to some of the cardiovascular side effects of TCP (Mallinger et al., 1986; Keck et al., 1991).

In this thesis is reported a comparison of the effects of TCP, FTCP and MeOTCP on uptake and release of ^{3}H-labelled NA, DA and 5-HT in prisms prepared from discrete brain areas. 4-Hydroxy-TCP (OHTCP), the ring-hydroxylated metabolite of TCP, was also included in this study. The addition of the 4-hydroxy or 4-fluoro group to TCP had little effect on the NA-uptake inhibiting properties of the parent drug, while the addition of the 4-MeO group caused a marked diminution of the NA uptake-inhibiting properties. At the relatively high concentration of 10 μM, only FTCP caused a significant release of ^{3}H-NA. The lack of effect of OHTCP on NA release was somewhat surprising since ring hydroxylation of the related compound β-phenylethylamine (PEA) to TA results in a marked increase in release of NA (Raiteri et al., 1977) relative to that produced by PEA. In agreement with the new findings with OHTCP and TCP reported in this thesis, Raiteri et al. (1977) found that TA was approximately twice as potent as PEA at inhibiting NA uptake. The pattern of effects of TCP and MeOTCP on NA uptake and release was similar to that observed by Raiteri et al. (1977) and Baker et al. (1976) when comparing PEA and 4-chloro-PEA; i.e. the addition of the chlorine moiety had little effect on NA-releasing ability but dramatically reduced the ability to inhibit NA uptake. Workers in the Neurochemical Research Unit (Baker, Martin-Iverson and Hegadoren, unpublished) also found that 4-methoxyAMP was much weaker than AMP with regard to ability to inhibit NA uptake.

The analogues MeOTCP and OHTCP were weaker inhibitors of DA release than the parent drug and all four drugs caused a similar, albeit weak, release of DA at a drug concentration of 10 μM. Again, the relatively weak effect of OHTCP was

surprising since TA is much more potent than PEA at inhibiting uptake of DA and stimulating its release (Raiteri et al., 1977). However, 4-chloro-PEA has been reported to be a weaker inhibitor of DA uptake than is PEA (Raiteri et al., 1977), similar to the pattern reported here with MeOTCP and OHTCP and the parent drug. All three analogues were more potent than the parent compound at inhibiting 5-HT uptake, and OHTCP and FTCP were more potent than TCP in stimulating 5-HT release. The effects of OHTCP and FTCP on release were not unexpected given the reported comparisons of PEA with 4-TA and 4-chloro-PEA (Baker et al., 1976; Raiteri et al., 1977). Hegadoren et al. (1994) also recently reported that 4-methoxyAMP was more potent than (+)-AMP at inhibiting the uptake of ^{3}H-5-HT in striatal prisms. Surprisingly, it was observed in the current study that MeOTCP had a weaker effect than OHTCP and FTCP on 5-HT release despite the fact that it was more potent than the other two analogues at inhibiting 5-HT uptake. This finding was also unexpected given the stronger 5-HT releasing effect of 4-MeOAMP relative to AMP (Hegadoren et al., 1994).

The dose of drugs (0.37 μmol/kg) used in the chronic studies in this thesis is similar to those used in the clinical study by Keck et al. (1991). Those authors found that such a dose resulted in plasma TCP levels of about 1 μM and a significant reduction of plasma NA levels in the patients. TCP is often given at much higher doses (37-185 μmol) in animal studies reported in the literature, and at those doses TCP in brain and heart could reach levels at which it would be having marked effects on neurotransmitter amine uptake and release (Fuentes et al., 1976; Calverley et al., 1981). Several reports also indicate that some otherwise

refractory depressed patients respond well to doses of TCP that are up to 4-5 times the usual dose (Robinson, 1983; Guze and Baxter, 1987; Pearlman, 1987; Amsterdam and Berwish, 1989). Based on the drug level findings of Keck et al (1991) and those shown in the Results section of this thesis and the results of Coutts et al. (1987) and Goodnough (1994), at such doses levels of TCP (and FTCP and MeOTCP) in brain and other tissues would be sufficient to be affecting uptake and/or release of neurotransmitter amines in the clinical situation.

If, as has been suggested by Mallinger et al. (1986) and Keck et al. (1991), effects of TCP on NA other than inhibition of MAO may contribute to cardiovascular effects of TCP, from the results shown in Tables 11 and 12 of this thesis FTCP should have similar such actions as TCP. MeOTCP, on the other hand, will have weaker actions on NA, particularly since it reaches lower concentrations in tissues than does TCP (Figures 24-26). However, both FTCP and MeOTCP are much more potent inhibitors of 5-HT uptake than is TCP, and the possibility of the production of a serotonin syndrome may be more likely with these analogues than with the parent drug. However, the combined effects on MAO inhibition and inhibition of 5-HT reuptake of FTCP and MeOTCP could also prove beneficial from a therapeutic standpoint since the selective MAO-A inhibitor brofaromine also has both of these properties (Waldmeier et al., 1994) and has proven effective in treating not only depression (Chouinard et al., 1994; Verhoeven, 1994; Volz et al., 1994), but other psychiatric disorders such as panic disorder and social phobia (Priest et al., 1995).

As mentioned previously in this thesis, there have been several reports in the

literature indicating that high doses of TCP may be effective in treating refractory depression. It has been speculated that both the efficacy and side effects of TCP at these doses may be due, at least in part, to its AMP-like activity (review: Briggs et al., 1990). There is now some concern that the tolerance and addiction associated with AMP may occur when TCP is taken in large doses (Dilsaver et al., 1988; Briggs et al., 1990). It is feasible that such effects may not be as much of a problem with MeOTCP and FTCP since they have weaker effects than TCP on DA reuptake (see Table 11).

D. Failure to Detect Amphetamine as a Metabolite in both Human and Rat Following Administration of TCP

The results of the study on the metabolism of TCP reported here are in agreement with those of others (Baselt et al., 1977; Bailey and Baron, 1980; Reynolds et al., 1980; Calverley et al., 1981; Mallinger et al., 1986; Jefferson, 1992) who indicated that at normal therapeutic doses AMP is not a metabolite of TCP. The study reported in this thesis has the advantage that AMP levels in both human urine samples and rat brain samples were investigated. The doses of TCP administered to human subjects were normal therapeutic doses. The dose given to the rats was very high, but even so, AMP was not detectable in the brain, liver or heart tissue despite the very high concentrations of TCP. In summary, AMP could not be detected in any of the rat tissues sampled or in the urine of psychiatric patients administered TCP despite the fact that large amounts of TCP were available for metabolism. Given the fact that this particular assay is so sensitive,

one can be confident that the metabolic fate of TCP does not involve the formation of AMP. The fact that 3-phenylpropylamine, another potential product of cleavage of TCP, could not be detected provides further strong evidence that cleavage of the cyclopropyl ring of TCP is not an important route of metabolism for this drug. In the original paper by Youdim et al. (1979), both AMP and N-methylAMP were found in the plasma of the patient who had taken an overdose of TCP. Presumably the N-methylAMP was the result of N-methylation in the body of the AMP proposed by those workers to be formed from TCP, although this was not clarified in that publication. In the present study it was not possible to separate derivatized N-methylAMP completely from derivatized PEA for accurate quantitation, but the results in rat brain (Figure 19), where the interference from PEA is low, indicate that the formation of N-methylAMP after TCP administration is minimal. It is possible that the subject described in the paper by Youdim et al. (1979) could have been taking AMP and/or methAMP in addition to TCP.

E. The Use of Extraction Followed by Pentafluorobenzoylation for Analysis of TCP and Its Ring-Substituted Analogues

Pentafluorobenzoyl chloride (PFBC) is a derivatizing reagent with two very important properties. It imparts excellent sensitivity for analysis by gas chromatography with electron-capture detection (Moffat et al., 1972; Matin and Rowland, 1972; Midha et al., 1979; Durden, 1991) and will react with amines and phenols under aqueous conditions, facilitating extraction of drugs containing these functional groups from aqueous media (Baker et al., 1982; Cristofoli et al., 1982; Nazarali et al., 1987). In the procedure described in the present thesis, there were

no phenol groups on the drugs of interest, therefore the pentafluorobenzoylation could be conducted under aqueous or anhydrous conditions. In the first case, the supernatant from the brain homogenate was basified and shaken with a solution of PFBC in ethyl acetate. The organic layer was retained and taken to dryness and the residue taken up in a small volume of toluene and injected on the gas chromatograph. In the second case, the supernatant from the brain homogenate was basified and shaken with ethyl acetate to extract the drugs. The organic layer was retained and taken to dryness; the residue was then reacted with PFBC under anhydrous conditions. The reaction mixture was then partitioned between borate buffer and toluene and a portion of the toluene layer used for GC analysis. Both situations resulted in formation of PFBC derivatives, but the latter procedure provided cleaner chromatograms and was chosen as the preferred assay method in this thesis.

F. Levels of TCP and MeOTCP in Rat Brain, Liver and Heart

Since TCP undergoes ring hydroxylation (Baker et al., 1986) in the para position, it was proposed that both FTCP and MeOTCP might provide for longer-lasting, more consistent brain levels than TCP since they were substituted in this position. As expected, blockade of the para position by the fluorine molecule resulted in higher brain levels of TCP analogues than were evidenced with TCP; this phenomenon had been reported previously with FTCP by Coutts et al. (1987), and thus was not investigated in this thesis. Unexpectedly, MeOTCP produced lower concentrations than did the parent compound in rat brain, liver and heart, suggesting that this analogue is more poorly absorbed, is cleared more rapidly from tissues, and/or is more extensively metabolized than TCP. Related compounds such as MDMA and MDA are known to undergo metabolic reactions such as side

chain hydroxylation, ortho ring hydroxylation and/or deamination (Lim and Foltz, 1988; Johnson et al., 1992; Zhao et al., 1992). However, if MeOTCP is extensively metabolized, it is unlikely to be by CYP2D6, since the CYP2D6 inhibitors iprindole and trifluoperazine did not increase brain levels of MeOTCP (see Section G of this Discussion). It is of interest that Hegadoren et al. (1995) also found that 4-methoxy-AMP achieved much lower levels in rat brain than did (+)-AMP after i.p. injection of an equimolar dose of both drugs.

G. Effects of Iprindole and Trifluoperazine on Brain Levels of FTCP and MeOTCP

Pretreatment of rats with iprindole and trifluoperazine (drugs known to interfere with cytochrome P450-mediated metabolic reactions such as hydroxylation) had no effect on brain levels of FTCP or MeOTCP. These findings have important implications for possible drug-drug interactions with TCP and its analogues. TCP is given to some refractory depressed patients in combination with tricyclics such as amitriptyline and desipramine (review: Schmauss et al., 1988), drugs also known to compete for and inhibit cytochrome P450 isozymes responsible for ring hydroxylation (Coutts, 1994). A combination of TCP with trifluoperazine, which like other phenothiazine antipsychotics, blocks cytochrome CYP2D6, is marketed under the trade name Parstelin in the United Kingdom. Despite this use of combination therapy, there is a paucity of information about metabolic drug-drug interactions with TCP. However, Baker et al. (1986) found that pretreatment of rats with iprindole, chlorpromazine or trifluoperazine resulted in a significant increase in rat brain levels of TCP compared to those observed in rats pretreated with saline vehicle. Aspeslet et al. (1992) also recently reported that pretreatment with iprindole, a drug which is known to block ring hydroxylation of AMP (Freeman and

Sulser, 1972; Hemrick-Luecke, 1980; Steranka, 1982), resulted in increased brain levels of TCP in the rat.

The question of metabolic drug-drug interactions has become a particularly active area of interest in psychiatry in recent years with the introduction of fluoxetine (FLU) as one of the most frequently prescribed antidepressants. This drug and its metabolite, norfluoxetine (NFLU) inhibit oxidative enzymes which also act on many other drugs. Both FLU and NFLU are potent inhibitors of CYP 2D6 (Brosen and Skjelbo, 1991), and there are now numerous reports (e.g. Vaughan, 1988; Aranow et al., 1989; Ciraulo and Shader, 1990a,b; Fuller and Snoddy, 1991; Rosenstein et al., 1991; Bergstrom et al., 1992; Messiha, 1993; DeVane 1994; Preskorn, 1996; Taylor and Lader, 1996) of alterations in plasma levels of drugs which are coadministered with FLU. Recent studies in the Neurochemical Research Unit also indicate that desipramine and iprindole also both increase brain levels of FLU when coadministered with FLU in the rat (Aspeslet et al., 1994).

Since TCP undergoes ring hydroxylation, it is also presumably prone to metabolic interactions with other drugs which undergo similar metabolism and/or are inhibitors of hydroxylating enzymes, as has been suggested by the results of Baker et al. (1986) mentioned above. In the study of Baker et al. (1986), pretreatment of rats with iprindole or trifluoperazine resulted in a doubling and tripling, respectively of TCP brain levels compared to values in vehcile pretreated rats. The preliminary findings with iprindole and trifluoperazine (at the same doses and time intervals) reported in this thesis suggest that FTCP and MeOTCP may be free from such interactions, which could be a useful property if these drugs do prove to be effective antidepressants.

CONCLUSIONS

The findings in this thesis have provided important information about TCP as well as about two analogues which may also be potential antidepressants:

- FTCP and MeOTCP are more potent than TCP with regard to inhibiting MAO *ex vivo*.
- At doses of 3.7 and 1.2 μmol/kg administered acutely and chronically, FTCP and MeOTCP caused greater elevations in rat brain levels of 5-HT than did TCP.
- When administered chronically (28 day) at a clinically relevant dose of 3.7 μmol/kg/day, FTCP and MeOTCP, like TCP, caused decreased binding of ^{3}H-tryptamine and ^{3}H-dihydroalprenolol (radioligand for β-adrenergic receptors) to receptors in rat brain cortex.
- At a dose of 3.7 μmol/kg/day administered for 28 days, none of the three drugs, TCP, FTCP and MeOTCP, caused any effects on rat brain levels of tryptophan.
- *In vitro* studies in brain prisms prepared from rat brain regions showed that TCP, FTCP and MeOTCP differed considerably in their effects on uptake and release of neurotransmitter amines. These effects could contribute to the overall pharmacological profile of these drugs, particularly at higher doses.
- Neither AMP nor 3-phenylpropylamine, potential products of cleavage of the cyclopropyl ring, are metabolites of TCP in humans or rats.
- Extraction followed by pentafluorobenzoylation and subsequent GC analysis

with electron-capture detection provides a useful means for analysis of TCP, FTCP and MeOTCP in rat brain, liver and heart tissue.

- Studies in rat brain, liver and heart at 1, 2 and 4 h showed that levels of MeOTCP were lower than those of TCP in all tissues and at all time intervals following administration of equimolar doses of the two drugs.
- Pretreatments of rats with iprindole and trifluoperazine suggest that metabolic drug-drug interactions may not be a concern with FTCP or MeOTCP.

POSSIBLE FUTURE WORK

1. *Behavioural Testing of MeOTCP and FTCP*

The findings reported in this thesis indicate that these analogues are potent MAO inhibitors and, like TCP, after chronic administration produce down-regulation of tryptamine receptors in rat brain. In common with many other antidepressants, they also produce a reduction in the number of β-adrenergic receptors. These results suggest that MeOTCP and FTCP may be effective antidepressants, and it would now be logical to test them in an animal model of depression. One such model is the forced swimming test (Porsolt et al., 1977) in which mice are dropped into cylinders containing water. A mouse is judged to be immobile when it floats in the water and makes only small movements to keep its head above water. Antidepressants cause an increase in the time to reach immobility.

If these analogues are identified as potential antidepressants in the animal model, it would then be worthwhile to pursue further their possible mechanisms of action. Recent research findings on TCP indicate that appropriate avenues to pursue would be: to investigate the importance of chirality in the overall action; to study the effects of these analogues on signal transduction mechanisms (e.g. those involving G proteins and protein kinases) and on expression of mRNAs of isoforms of $GABA_A$ receptor subunits; and to examine the possible effects of the analogues on activity of the cytochrome P450 isozymes.

2. *Importance of Chirality in Actions of MeOTCP and FTCP*

As mentioned earlier in this thesis, TCP is a racemate, i.e. a mixture of (+) and (-) enantiomers. The individual enantiomers differ not only in their

pharmacological activity [the (+) enantiomer is a much stronger inhibitor of MAO and the (-) enantiomer is the more potent inhibitor of catecholamine uptake], but also in their pharmacokinetic properties (Hampson et al., 1986; Aspeslet et al., 1992; Spahn-Langguth et al., 1992; Aboul-Enein and Serignese, 1995). It would be very useful to synthesize the individual enantiomers of MeOTCP and FTCP (which are currently available only as racemates) and to compare them with regard to pharmacological activity and to conduct comprehensive pharmacokinetic studies on the individual enantiomers in tissues and body fluids.

3. Effects of TCP Analogues on G Proteins and Protein Kinases

Radioligand binding methodologies have been useful for investigating the effects of antidepressants on receptors such as β-adrenergic and 5-HT_2 receptors, but they presumably represent a fairly early stage in the action of antidepressants and, by themselves, are limited in value for interpreting functional changes in neuronal function produced by the drug of interest (Hrdina, 1993). The need to investigate more closely transduction mechanisms occurring "downstream" of the interaction at the receptor has lead to a great deal of recent interest in the effects of drugs such as antidepressants on G proteins and associated protein kinases (Bourin and Baker, 1996).

The actions of G protein-linked receptors are mediated through two principal second messenger-generating systems, namely the adenylyl cyclase (AC) and phosphoinositide (PI) systems. The resultant second messengers, cAMP and diacylglycerol (DAG), activate the respective protein kinases [cAMP-dependent protein kinase (PKA), calcium/phospholipid-dependent protein kinase C (PKC) and

also the calcium/calmodulin-dependent kinase (CaM-kinase II)] and these kinases subsequently phosphorylate a range of cellular proteins. β-Adrenergic receptors and some subtypes of 5-HT receptors (5-$HT_{1A, 1B, 1D}$, 5-HT_4) are linked to the AC-PKA cascade. Receptors of the 5-HT_{1C}, and 5-HT_2 subtypes are linked to the PI-PKC cascade (Hrdina, 1993 for review).

Li et al. (1993) found that chronic administration of TCP to rats did not alter the levels of mRNAs on or immunoreactivity of several G protein subunits in cortex, but Nestler et al. (1989) reported that long-term administration of TCP to rats increases PKA activity in the particulate and decreases it in the cytosolic fractions of frontal cortex. It would be of considerable interest to extend these studies to MeOTCP and FTCP and include studies not only on protein kinase activity but on expression of mRNAs for these protein kinases.

4. Effects of TCP, MeOTCP and FTCP on mRNAs for Isoforms of the $GABA_A$ Receptor Subunits

As mentioned previously in this thesis, there has been a great deal of controversy about the effects of antidepressants on GABA receptors in brain. Much of this work has been conducted using radioligand binding studies, and recent studies on benzodiazepines and antidepressants have demonstrated that more refined molecular biological techniques may reveal changes in expression of some mRNAs for isoforms of $GABA_A$ receptor subunits under drug administration conditions in which changes in radioligand binding were not obvious (Heninger et al., 1990; Kang and Miller, 1991; Primus and Gallager, 1992; Tanay et al., 1996).

The $GABA_A$ receptor is an oligomeric structure composed of 5 subunits (Nayeem et al., 1994). The subunits are from 4 distinct classes, at least 3 of which have a number of isoforms. It is possible that drugs such as antidepressants may result in the substitution of one subunit isoform in a given functional receptor with another, changing the characteristics of the drug response without necessarily changing binding capacity. Such changes in $GABA_A$ receptor subunit mRNAs have recently been reported in rat brainstem after administration of the MAO inhibitor phenelzine (Tanay et al., 1996), and it would be of value to extend these studies to TCP, MeOTCP and FTCP.

5. Effects of TCP and Its Analogues on CYP2C19

As mentioned in the Discussion section of this thesis, there is indirect evidence that: TCP may be metabolized by CYP2D6; coadministration of drugs which inhibit CYP2D6 may alter tissue levels of TCP; and MeOTCP and FTCP will be much less susceptible than TCP to metabolic drug-drug interactions with inhibitors of CYP2D6. However, another CYP isozyme is worthy of further investigation with TCP and its analogues. It has been known for some time that TCP is a potent inhibitor of mephenytoin hydroxylase (Inaba et al., 1985), a CYP isozyme now termed CYP2C19 (Goldstein et al., 1994). This isozyme is now commercially available, and it will be of great interest to determine if the analogues are as potent as TCP at inhibiting it. If the analogues have reduced ability (relative to TCP) to inhibit CYP2C19, this would suggest further that these are potentially safer drugs to use than TCP because of the possibility of reduced incidence of metabolic interactions with coadministered drugs.

REFERENCES

Aboul-Enein H.Y. and Serignese V. (1995) Direct separation of tranylcypromine enantiomers and their profile in an atypical depressive patient. Biomed. Chromatogr. 9: 98-101.

Alemany R., Olmos G. and García-Sevilla J.A. (1995) The effects of phenelzine and other monoamine oxidase inhibitor antidepressants on brain and liver I_2 imidazoline-preferring receptors. Br. J. Pharmacol. 114: 837-845.

Ali B.H. (1985) Effect of some monoamine oxidase inhibitors on the thiamin status of rabbits. Br. J. Pharmacol. 86: 869-875.

Alleva J.J. (1965) Metabolism of tranylcypromine-^{14}C and *d,l*-amphetamine-^{14}C in the rat. J. Med. Chem. 6: 621-624.

Altar C.A., Wasley A.M. and Martin I.L. (1986) Autoradiographic localization and pharmacology of unique ^{3}H-tryptamine binding sites in rat brain. Neurosci. 17: 263-273.

Amsterdam J.D. and Berwish N.J. (1989) High dose tranylcypromine therapy for refractory depression. Pharmacopsychiat. 22: 21-25.

Amsterdam J.D., Kaplan M., Potter L., Bloom L. and Rickels K. (1986) Adinazolam, a new triazalobenzodiazepine, and imipramine in the treatment of major depressive disorder. Psychopharmacol. (Berl.). 88: 484-488.

Ananth J. and Luchins D. (1977) A review of combined tricyclic and MAOI therapy. Compr. Psychiat. 18: 221-230.

Angst J., Amrein R. and Stabl M. (1995) Moclobemide and tricyclic antidepressants in severe depression: meta-analysis and prospective studies. J. Clin. Psychopharmacol. 15: 16S-23S.

Antelman S.M. and Chiodo L.A. (1981) Dopamine autoreceptor subsensitivity: a mechanism common to the treatment of depression and the induction of amphetamine psychosis? Biol. Psychiat. 16: 717-727.

Aranow R.B., Hudson J.I., Pope H.G., Grady T.A., Laage T.A., Bell I.R. and Cole J.O. (1989) Elevated antidepressant plasma levels after addition of fluoxetine. Am. J. Psychiat. 146: 911-913.

Arnt J., Overo K.R., Hyttel J. and Olsen R. (1984) Changes in rat dopamine and serotonin function *in vivo* after prolonged administration of the specific 5-HT uptake inhibitor citalopram. Psychopharmacol. 84: 457-465.

Aspeslet L.J., Baker G.B., Coutts R.T. and Mousseau D.D. (1992) A gas chromatographic procedure for separation and quantitation of the enantiomers of the antidepressant tranylcypromine. Biochem. Pharmacol. 44: 1894-1897.

Aspeslet L.J., Baker G.B., Coutts R.T. and Torok-Both G.A. (1994) The effects of desipramine and iprindole on levels of enantiomers of fluoxetine in rat brain and urine. Chirality 6: 86-90.

Avery D.H. (1987) REM sleep and temperature regulation in affective disorders. In: Chronobiology and Psychiatric Disorders, Halaris A. (ed.), Elsevier, New York, pp. 75-102.

Badawy A.A. and Evans M. (1981) Inhibition of rat liver tryptophan pyrrolase activity and elevation of brain tryptophan concentration by administration of antidepressants. Biochem. Pharmacol. 30: 1211-1216.

Badawy A.A. and Evans M. (1982) Inhibition of rat liver tryptophan pyrrolase activity and elevation of brain tryptophan concentration by acute administration of small doses of antidepressants. Br. J. Pharmacol. 77: 59-67.

Badawy A.A. and Morgan C.J. (1991) Effects of acute paroxetine administration on tryptophan metabolism and disposition in the rat. Br. J. Pharmacol. 102: 429-433.

Bailey E. and Barron E.J. (1980) Determination of tranylcypromine in human plasma and urine by high-resolution gas-liquid chromatography with nitrogen-sensitive detection. J. Chromatogr. 183: 25-31.

Baker G.B., Bertollini A., del Carmine R., Martin I.L. and Raiteri M. (1976) Effects of *p*-chloro-β-phenylethylamine on the uptake and release of putative amine neurotransmitters in rat brain. Br. J. Pharmacol. 48: 420P-421P.

Baker G.B. and Dewhurst W.G. (1985) Biochemical theories of affective disorders. In: The Pharmacotherapy of Affective Disorders: Theory and Practice, Dewhurst W.G. and Baker G.B. (eds.), Croom Helm Ltd., London, pp. 1-59.

Baker G.B. and Coutts R.T. (1982) Amines of biological interest and their analysis. In: Analysis of Biogenic Amines, Baker G.B. and Coutts R.T. (eds.), Elsevier, Amsterdam, pp. 1-13.

Baker G.B. and Coutts R.T. (1989) Metabolism of monoamine oxidase inhibitors. Prog. Neuropsychopharmacol. Biol. Psychiat. 13: 395-403.

Baker G.B., Coutts R.T. and LeGatt D.F. (1982) Gas chromatographic analysis of

amines in biological systems. In: Analysis of Biogenic Amines, Baker G.B. and Coutts R.T. (eds.), Elsevier Science Publishing Co., Amsterdam, pp. 109-128.

Baker G.B., Coutts R.T. and Martin I.L. (1981) Measurement of amines in the central nervous system using gas chromatography with electron-capture detection. Prog. Neurobiol. 17: 1-24.

Baker G.B., Coutts R.T., McKenna K.F. and Sherry-McKenna R.L. (1992) Insights into the mechanisms of action of the MAO inhibitors phenelzine and tranylcypromine: a review. J. Psychiat. Neurosci. 17: 206-214.

Baker G.B., Coutts R.T. and Rao T.S. (1987) Neuropharmacological and neurochemical properties of N-(2-cyanoethyl)-2-phenylethylamine, a prodrug of 2-phenylethylamine. Br. J. Pharmacol. 92: 243-255.

Baker G.B. and Greenshaw A.J. (1989) Effects of long-term administration of antidepressants and neuroleptics on receptors in the central nervous system. Cell. Mol. Neurobiol. 9: 1-44.

Baker G.B., Greenshaw A.J. and Coutts R. T. (1988) Chronic administration of MAO inhibitors: implications for interactions between trace amines and catecholamines. In: Progress in Catecholamine Research, Part A: Basic Aspects and Peripheral Mechanisms, Dahlstrom A., Belmaker R. H. and Sandler M. (eds), Alan R. Liss, New York, NY, pp. 569-572.

Baker G.B., Hampson D.R., Coutts R.T., Micetich R.G., Hall T.W. and Rao T.S. (1986) Detection and quantitation of a ring-hydroxylated metabolite of the antidepressant drug tranylcypromine. J. Neural Transm. 65: 233-244.

Baker G.B., Hiob L.E. and Dewhurst W.G. (1980) Effects of monoamine oxidase inhibitors on release of dopamine and 5-hydroxytryptamine from rat striatum *in vitro*. Cell. Mol. Biol. 26: 182-186.

Baker G.B., Wong J.T.F., Coutts R.T. and Pasutto F.M. (1987) Stimultaneous extraction and quantitation of several bioactive amines in cheese and chocolate. J. Chromatogr. 392: 317-331.

Baldessarini R.J. (1980) Drugs and the treatment of psychiatric disorders. In: The Pharmacological Basis of Therapeutics, 6th Edn., Gilman A.G., Goodman L.S. and Gilman A. (eds.), MacMillan, New York, pp. 391-447.

Baldessarini R.J. (ed.) [1985] Chemotherapy in Psychiatry: Principles and Practice, Harvard University Press, Boston.

Baldwin D. and Rudge S. (1993) Moclobemide: a reversible inhibitor of monoamine oxidase type A. Br. J. Hosp. Med. 49: 497-499.

Barbaccia M.L., Ravizza L. and Costa E. (1986) Maprotiline: an antidepressant with an unusual pharmacological profile. J. Pharmacol. Exp. Ther. 236: 307-312.

Baselt R.C., Stewart C.G. and Shaskan E. (1977) Determination of serum and urine concentrations of tranylcypromine by electron-capture gas-liquid chromatography. J. Anal. Toxicol. 1: 215-217.

Bel N. and Artigas F. (1996) Reduction of serotonergic function in rat brain by tryptophan depletion: effects in control and fluvoxamine-treated rats. J. Neurochem. 67: 669-676.

Bélanger P.M. and Atitsé-Gbeasson A. (1982a) Inhibitory effect of phenelzine on oxidative microsomal enzymes systems of rat liver. Can. J. Physiol. Pharmacol. 61: 524-529.

Bélanger P.M. and Atitsé-Gbeasson A. (1982b) Inhibitory effect of tranylcypromine on hepatic drug metabolism in the rat. Biochem. Pharmacol. 31: 2679-2683.

Bergstrom R.F., Peyton A.L. and Lemberger L. (1992) Quantification and mechanism of the fluoxetine and tricyclic antidepressant interaction. Clin. Pharmacol. Ther. 51: 239-248.

Berry M.D., Juorio A.V. and Paterson I.A. (1994) The functional role of monoamine oxidases A and B in the mammalian central nervous system. Progr. Neurobiol. 47: 375-391.

Bieck P.R., Antonin K.H. and Schmidt E. (1993) Clinical pharmacology of reversible monoamine oxidase-A inhibitors. Clin. Neuropharmacol. 16: S34-S41.

Bieck P.R., Antonin K.H. and Schulz R. (1993) Clinical pharmacology of MAO inhibitors. In: Monoamine Oxidase: Basic and Clinical Aspects, Yasuhara H., Parvez S.H., Oguchi K. et al. (eds.), Utrecht, The Netherlands, VSP, pp. 177-196.

Blaschko H. (1952) Amine oxidase and amine metabolism. Pharmacol. Rev. 4: 415-425.

Bloch R.G., Dooneief A.S., Buchberg A.S. and Spellman S. (1954) The clinical effects of isoniazid and iproniazid in the treatment of pulmonary tuberculosis. Ann. Intern. Med. 40: 881-900.

Blier P. and de Montigny C. (1994) Current advances and trends in the treatment

of depression. Trends Pharmacol. Sci. 15: 220-226.

Blier P., de Montigny C. and Chaput Y. (1990) A role for the serotonin and the mechanism of action of antidepressant treatments, preclinical evidence. J. Clin. Psychiat. 51: 14-20.

Bond P.A. and Dundall R.L. (1977) Properties of monoamine oxidase (MAO) in human blood platelets, plasma, lymphocytes and granulocytes. Clin. Chim. Acta, 80: 317-326.

Boulton A.A. (1984) Trace amines and the neurosciences: an overview. In: Neurobiology of the Trace Amines, Boulton A.A., Baker G.B., Dewhurst W.G. and Sandler M. (eds.), Humana Press, Clifton, N.J., pp. 13-24.

Bourin M. and Baker G.B. (1996) Do G proteins have a role in antidepressant treatment? Eur. Neuropsychopharmacol. 6: 49-53.

Bouthillier A. and de Montigny C. (1987) Long-term antidepressant treatment reduces neuronal responsiveness to flurazepam: an electrophysiological study in the rat. Neurosci. Lett. 73: 271-275.

Bradberry C.W. (1994) Microdialysis assessment of the impact of (+)-3,4-methylenedioxymethamphetamine, cocaine and cocaethylene on serotonergic neurons. Drug Dev Res. 33: 1-9.

Brady K.T., Lydiard R.B. and Kellner C. (1991) Tranylcypromine abuse. Am. J. Psychiat. 148: 1268-1269.

Braestrup C., Andersen H. and Randrup A. (1975) A monoamine oxidase-inhibitor deprenyl potentiates phenylethylamine behaviour in rat without inhibition of catecholamine metabolite formation. Eur. J. Pharmacol. 34: 181-187.

Briggs N.C., Jefferson J.W. and Koenecke F.H. (1990) Tranylcypromine addiction: a case report and review. J. Clin. Psychiat.,51: 426-429.

Brodie B.B., Olin J.S., Kuntzman R.G. and Shore P.A. (1957) Possible interrelationship between release of brain norepinephrine and serotonin by reserpine. Science 125: 1293-1294.

Brosen K. and Skjelbo E. (1991) Fluoxetine and norfluoxetine are potent inhibitors of P450IID6 - the source of the sparteine/debrisoquine oxidation polymorphism. Br. J. Clin. Pharmacol. 32: 137-138.

Browne M., Lapierre Y.D., Hrdina P.D., Horne E. (1990) Lithium as an adjunct in the treatment of major depression. Int. Clin. Psychopharmacol. 5: 103-110.

Bylund D.B. and Snyder S.H. (1976) Beta adrenergic binding in membrane preparations from mammalian brain. Mol. Pharmacol. 12: 568-580.

Caldwell J. (1996) Importance of stereospecific bioanalytical monitoring in drug development. J Chromatrogr A 719: 3-13.

Calverley D.G., Baker G.B., Coutts R.T. and Dewhurst W.G. (1981) A method for measurement of tranylcypromine in rat brain regions using gas chromatography with electron capture detection. Biochem. Pharmacol. 30: 861-867.

Campbell I.C., Durcan M.J., Cohen R.M., Pickar D., Chugani D. and Murphy D.L. (1985) Chronic clorgyline and pargyline increase apomorphine-induced stereotypy in the rat. Pharmacol. Biochem. Behav. 23: 921-925.

Campbell I.C., Gallager D.W., Hamburg M.A., Tallman J.F. and Murphy D.L. (1985) Electrophysiological and receptor studies in rat brain: effects of clorgyline. Eur. J. Pharmacol. 3: 355-364.

Carpéné C., Collon P., Remaury A., Cordi A., Hudson A., Nutt D. and Lafontan M. (1995) Inhibition of amine oxidase activity by derivatives that recognize imidazoline I_2 sites. J. Pharmacol. Exp. Ther. 272: 681-688.

Cascio C.S. and Kellar K.J. (1983) Characterization of ^{3}H-tryptamine binding sites in brain. Eur. J. Pharmacol. 94: 31-39.

Cawthon R.M. and Breakefield X.O. (1979) Differences in A and B forms of monoamine oxidase revealed by limited proteolysis and peptide mapping. Nature, 281: 692-694.

Chouinard G., Saxena B.M., Nair N.P.V., Kutcher S.P., Bakish D., Bradwejn J., Kennedy S.H., Sharma V., Remick R.A., Kukha-Mohamad S.A., Belanger M.C., Snaith J., Beauclair L., Pohlmann H. and D'Souza J. (1994) A Canadian multicentre placebo-controlled study of a fixed dose of brofaromine, a reversible selective MAO-A inhibitor, in the treatment of major depression. J. Affec. Dis., 32: 105-114.

Chouinard G., Young S.N., Annable L. and Sourkes T.L. (1979) Tryptophan, nicotinamide, imipramine and their combination in depression. Acta Psychiat. Scand. 59: 395-414.

Ciraulo D.A. and Shader R.I. (1990a) Fluoxetine drug-drug interactions: I. Antidepressants and antipsychotics. J. Clin. Psychopharmacol. 10: 48-50.

Ciraulo D.A. and Shader R.I. (1990b) Fluoxetine drug-drug interactions: II. J. Clin.

Psychopharmacol. 10: 213-217.

Clark B., Thompson J.W. and Widdington G. (1972) Analysis of the inhibition of pethidine N-demethylation by monoamine oxidase inhibitors and some other drugs with special reference to drug interactions in man. Br. J. Pharmacol. 44: 89-99.

Cohen R.M., Ebstein R.P., Daly J.W. and Murphy D.L. (1982) Chronic effects of a monoamine oxidase-inhibiting antidepressant: decreases in functional β-adrenergic autoreceptors precede the decrease in norepinephrine-stimulated cyclic adenosine 3,5-monophosphate systems in rat brain. J. Neurosci. 2: 1588-1595.

Coppen A. (1967) The biochemistry of affective disorders. Br. J. Psychiat. 113: 1237-1264.

Coppen A. and Abou-Saleh M.T. (1988) Lithium therapy: from clinical trials to practical management. Acta Psychiat. Scand. 78: 754-762.

Coppen A., Shaw D.M. and Farrell J.P. (1963) Potentiation of the antidepressive effect of a monoamine oxidase inhibitor by tryptophan. Lancet i: 79-81.

Coutts R.T. (1994) Polymorphism in the metabolism of drugs, including antidepressant drugs: comments on phenotyping. J. Psychiat. Neurosci. 19: 30-44.

Coutts R.T. and Baker G.B. (1989) Implications of chirality and geometric isomerism in some psychoactive drugs and their metabolites. Chirality 1: 99-120.

Coutts R.T., Baker G.B. and Pasutto F.M. (1986) Foodstuffs as sources of psychoactive amines and their precursors: content, significance and identification. In: Advances in Drug Research, Vol. 15, Testa B. (ed), Academic Press, London, UK, pp. 170-232.

Coutts R.T., Rao T.S., Baker G.B., Micetich R.G. and Hall T.W.E. (1987) Neurochemical and neuropharmacological properties of 4-fluorotranyl-cypromine. Cell. Mol. Neurobiol. 7: 271-290.

Crane G.E. (1957) Iproniazid (Marsilid) phosphate, a therapeutic agent for mental disorders and debilitating disease. Psychiat. Res. Rep. Am. Psychiat. Assoc. 8: 142-152.

Cristofoli W.A., Baker G.B., Coutts R.T. and Benderly A. (1982) Analysis of a monofluorinated analogue of amphetamine in brain tissue using gas chromatography with electron-capture detection. Prog. Neuropsycho-

pharmacol. Biol. Psychiat. 6: 373-376.

Cross J.A. and Horton R.W. (1987) Are increases in $GABA_B$ receptors consistent findings following chornic antidepressant administration? Eur. J. Pharmacol. 141: 159-162.

Da Motta T. and Cordás T.A. (1990) Autoinduction of hypertensive reactions by tranylcypromine. J. Clin. Psychopharmacol. 10: 232.

Da Prada M., Kettler R., Keller H.H., Burkard W.P. and Haefely W.E. (1989) Preclinical profiles of the novel reversible MAO-A inhibitors, moclobemide and brofaromine, in comparison with irreversible MAO inhibitors. J. Neural Transm., 28(Suppl): 5-20.

Davidson J. and Pelton S. (1986) Forms of atypical depression and their response to antidepressant drugs. Psychiat. Res. 17: 87-95.

De Ceballos M.L., Benedi A., De Felipe C. and Del Rio J. (1985) Prenatal exposure of rats to antidepressant enhances agonist affinity of brain dopamine receptors and dopamine-mediated behaviour. Eur. J. Pharmacol. 116: 257-262.

De La Tore J.C. [ed.] (1972) In: Dynamics of Brain Monoamines, Plenum Press, New York, pp. 87-106, as quoted in Tipton (1986).

Delgado P.L., Charney D.S., Price L.H., Aghajanian G.K., Landis H. and Heninger G.R. (1990) Serotonin function and the mechanism of antidepressant action: reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. Arch. Gen. Psychiat. 47: 411-418.

DeMeyer M.K., Shea P.A., Hendrie H.C. and Yoshimura N.N. (1981) Plasma tryptophan and five other amino acids in depressed and normal subjects. Arch. Gen. Psychiat. 38: 642-646.

de Montigny C., Cournoyer G., Morrissette R., Langleis R. and Caille G. (1983) Lithium carbonate addition in tricyclic antidepressant-resistant depression: correlation with the neurobiologic actions of tricyclic antidepressant drugs and lithium ion on the serotonin system. Arch. Gen. Psychiat. 40: 1327-1334.

de Montigny C., Grunberg F., Mayer A. and Deschenes J.P. (1981) Lithium induces rapid relief of depression in tricyclic antidepressant drug non-responders. Br. J. Psychiat. 138: 252-256.

Denny R.M., Fritz R.R., Patel N.T. and Abell C.W. (1982a) Human liver MAO-A and

MAO-B separated by immunoaffinity chromatography with MAO-B specific monoclonal antibody. Science 215: 1400-1403.

Denny R.M., Patel N.T., Fritz R.R. and Abell C.W. (1982b) A monoclonal antibody elicited to human platelet monoamine oxidase. Isolation and specificity for human monoamine oxidase-B but not for A. Mol. Pharmacol. 22: 500-508.

DeVane C.L. (1994) Pharmacokinetics of the newer antidepressants: clinical relevance. Am. J. Med. 97: 6A-13S.

Dewhurst W.G. (1968a) Cerebral amine functions in health and disease. In: Studies in Psychiatry, Shepherd M. and Davies D.L. (eds.), Oxford University Press, pp. 289-317.

Dewhurst W.G. (1968b) New theory of cerebral amine function and its clinical application. Nature (Lond.) 218: 1130-1133.

Dilsaver S.C. (1988) Monoamine oxidase inhibitor withdrawal phenomenon: symptoms and pathophysiology. Acta. Psychiat. Scand. 78: 1-7.

Dilsaver S.C. and Jaeckle R.S. (1990) Winter depression responds to an open trial of tranylcypromine. J. Clin. Psychiat. 51: 326-329.

Donnelly C.H. and Murphy D.L. (1977) Substrate- and inhibitor-related characteristics of human platelet monoamine oxidase. Biochem. Pharmacol., 26: 853-858.

Dowling G.P. (1990) Human deaths and toxic reactions attributed to MDMA and MDE. In: The Clinical, Pharmacological and Neurotoxicological Effects of the Drug MDMA, Peroutka S.J. (ed.), Kluver Academic Publishers, Boston, pp. 63-75.

Dupont H., Davies D.S. and Strolin-Benedetti M. (1987) Inhibition of cytochrome P-450-dependent oxidation reactions by MAO inhibitors in rat liver microsomes. Biochem. Pharmacol. 36: 1651-1657.

Durden D.A. (1991) An evaluation of the negative ion mass spectra of the electron capturing derivatives of the biogenic trace amines. I. Phenylethylamine. Biol. Mass Spectrom. 20: 367-374.

Eade N.R. and Renton K.W. (1970) Effect of monoamine oxidase inhibitors on the N-demethylation and hydrolysis of meperidine. Biochem. Pharmacol. 19: 2243-2250.

Edwards D.J., Mallinger A.G., Knofp S. and Himmelhoch J. (1985) Determination

of tranylcypromine in plasma using gas chromatography-chemical ionization mass spectrometry. J. Chromatogr. Biomed. Appl. 344: 356-361.

Edwards D.J. and Sorisio D.A. (1988) Effects of imipramine on tyrosine and tryptophan are mediated by beta-adrenoceptor stimulation. Life Sci. 42: 853-862.

Edwards J.G., Long S.K., Sedgewick E.M. and Wheal H.V. (1986) Antidepressants and convulsive seizures: clinical, electroencephalographic and pharmacological aspects. Clin. Neuropharmacol. 9: 329-360.

Eison A.S., Yocca F.D. and Gianutsos G. (1991) Effect of chronic administration of antidepressant drugs on 5-HT_2-mediated behavior in the rat following noradrenergic or serotoninergic denervation. J. Neural Transm. 84: 19-32.

Enna S.J. and Mohler H. (1987) γ-Aminobutyric acid (GABA) receptors and their association with benzodiazepine receptor sites. In: Psychopharmacology: The Third Generation of Progress, Meltzer H.Y. (ed.), Raven Press, New York, pp. 265-272.

Everett G.M. and Tomon J.E.P. (1959) Mode of action of Rauwolfia alkaloids and motor activity. In: Biological Psychiatry, Masserman J.H. (ed.), Grune & Stratton, New York, pp. 75-81.

Fallon B., Foote B., Walsh B.T. and Roose S.P. (1988) "Spontaneous" hypertensive episodes with monoamine oxidase inhibitors. J. Clin. Psychiat. 49: 163-165.

Fibiger H.C. and Phillips A.G. (1987) Behavioral pharmacology of neuroleptic drugs: possible mechanisms of action. In: Behavioral Pharmacology: The Current Status, Seiden L.S. and Balseir R.L. (eds.), Alan R. Liss, New York, pp. 243-259.

Fischer W. (1991) The effects of (+)- and (-)-tranylcypromine as an anticonvulsive agent. Pharmazie 46: 357-358.

Foldes A. and Costa E. (1975) Relationship of brain monoamine and locomotor activity in rats. Biochem. Pharmacol. 24: 1617-1621.

Folks D.G. (1983) Monoamine oxidase inhibitors: reappraisal of dietary considerations. J. Clin. Psychopharmacol. 3: 249-252.

Foster B.C., Litster D.L., Zamecnik J. and Coutts R.T. (1991) The biotransformation of tranylcypromine by Cunninghamella echinulata. Can. J. Microbiol. 37: 791-795.

Frazer A. and Lucki I. (1982) Antidepressant drugs: effects on β-adrenergic and serotoninergic receptors. In: Advances in Biochemical Psychopharmacology, Vol. 31, Costa E. and Racagni G. (eds.), pp. 69-90.

Freeman J.J. and Sulser F. (1972) Iprindole-amphetamine interactions in the rat: the role of aromatic hydroxylation of amphetamine in its mode of action. J. Pharmacol. Exp. Ther. 183: 307-315.

Fuentes J.A., Oleshansky M.A. and Neff N.H. (1976) Comparison of the apparent antidepressant activity of (-) and (+) tranylcypromine in an animal model. Biochem. Pharmacol. 25: 801-804.

Fuller R.W. (1978) Structure-activity relationships among the halogenated amphetamines. Ann. NY Acad. Sci. 305: 147-159.

Fuller R. and Hemrick-Leucke S. (1980) Long-lasting depletion of striatal dopamine by a single injection of amphetamine in iprindole-treated rats. Science 209: 305-307.

Fuller R.W. and Snoddy H.D. (1991) Role of fluoxetine in the inhibition of desipramine metabolism and in the inhibition of serotonin uptake after fluoxetine administration to rats. Res. Comm. Chem. Path. Pharmacol. 73: 31-40.

Fuxe K., Ogren S.O., Agnati L.F., Benfenati F., Bredholm B., Andersson K., Zini I. and Eneroth P. (1983) Chronic antidepressant treatment and central 5-HT synapses. Neuropharmacol. 22: 389-400.

Garbutt J.C., Mayo J.P. Jr., Gillette G.M., Little K.Y. and Mason G.A. (1986) Lithium potentiation of tricyclic antidepressants following lack of T3 potentiation. Am. J. Psychiat. 143: 1038-1039.

Gaultieri C.T. and Powell S.F. (1978) Psychoactive drug interactions. J. Clin. Psychiat. 39: 720-729.

Glassman A.H. and Platman S.R. (1969) Potentiation of a monoamine oxidase inhibitor by tryptophan. J. Psychiat. Res. 7: 83-88.

Glue P., Coupland N. and Nutt D.J. (1994) Pharmacologic basis for the therapeutic activity of MAOIs. In: Clinical Advances in Monoamine Oxidase Therapies, Kennedy S.H. (ed), Am. Psychiatric Press, Washington, DC, pp. 1-32.

Goldstein J.A., Faletto M.B., Ramdses-Sparks M., Sullivan T., Kitaruwan S., Raucy T.L., Lasker J.M. and Ghanyem B.I. (1994) Evidence that CYP2C19 is the major (S)-mephenytoin 4-hydroxylase in humans. Biochem. 33: 1743-1752.

Goodman W.K. and Charney D.S. (1985) Therapeutic applications and mechanisms of action of monoamine oxidase inhibitor and heterocyclic antidepressant drugs. J. Clin. Psychiat. 46: 6-22.

Goodnough D.B. (1994) Mechanisms of action of selected drug therapies used in the treatment of refractory depression. Ph.D. Thesis, University of Alberta.

Goodnough D.B. and Baker G.B. (1992) Comparision of the actions of high and low doses of the MAO inhibitor tranylcypromine on 5-HT_2 binding sites in rat cortex. J. Neural Transm. 41(Suppl): 127-134.

Goodnough D.B., Baker G.B., Mousseau D.D., Greenshaw A.J. and Dewhurst W.G. (1992) Effects of low- and high-dose tranylcypromine on ^{3}H-tryptamine binding sites in the rat hippocampus. Proc. 5th Amine Oxidases Workshop, Galway, Ireland.

Gordis C. and Neff N.H. (1971a) Monoamine oxidase in sympathetic nerves: a transmitter specific enzyme type. Br. J. Pharmacol., 43: 814-818.

Gordis C. and Neff N.H. (1971b) Monoamine oxidase: an approximation of turnover rates. J. Neurochem. 18: 1673-1682.

Gouzoulis E., Borchardt D. and Hermle L. (1993) A case of toxic psychosis induced by Eve (3,4-methylenedioxyethylamphetamine). Arch. Gen. Psychiat. 50: 75.

Grahame-Smith D.G. (1971) Studies *in vivo* on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. J. Neurochem. 18: 1053-1066.

Gray J.A. and Green A.R. (1987) Increased $GABA_B$ receptor function in mouse frontal cortex after repeated administration of antidepressant drugs or electroconvulsive shocks. Br. J. Pharmacol. 92: 357-362.

Green A.L. and El Hait M.A.S. (1980) p-Methoxyamphetamine, a potent reversible inhibitor of type-A monoamine oxidase *in vitro* and *in vivo*. J. Pharm. Pharmacol. 32: 262-266.

Green A.R., Cross A.J. and Goodwin G.M. (1995) Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy"). Psychopharmacol. 119: 247-260.

Greenshaw A.J. (1986) Osmotic minipumps: a convenient program for weight-adjusted filling concentrations. Brain Res. Bull. 16: 759-761.

Greenshaw A.J. and Dewhurst W.G. (1987) Tryptamine receptors: fact, myth or misunderstanding? Brain Res. Bull. 18: 253-256.

Greenshaw A.J., Nazarali A.J., Rao T.S., Baker G.B. and Coutts R.T. (1988) Chronic tranylcypromine treatment induces functional α_2-adrenoceptor down-regulation in rats. Eur. J. Pharmacol. 154: 67-72.

Grimsby J., Lan N.C., Neve R., Chen K. and Shih J.C. (1990) Tissue distribution of human monoamine oxidase A and B mRNA. J. Neurochem. 55: 1166-1169.

Gupta B.D., Dandiya P.C., Gupta M.L. and Gabba A.K. (1971) An examination of the effects of central nervous system stimulant and antidepressant drugs on open field performance in rats. Eur. J. Pharmacol. 13: 341-346.

Guze B.H. and Baxter Jr. L.R. (1987) Refractory depression treated with high doses of a monoamine oxidase inhibitor. J. Clin. Psychiat. 48: 31-32.

Halle M.H., Del Medico V.J. and Dilsaver S.C. (1991) Symptoms of major depression: acute effect of withdrawing antidepressants. Acta Psychiat. Scand. 83: 238-239.

Hampson D.R., Baker G.B. and Coutts R.T. (1986) A comparison of the neurochemical properties of the stereoisomers of tranylcypromine in the central nervous system. Cell. Mol. Biol. 32: 333-341.

Hare M.L.C. (1928) Tyramine oxidase I. A new enzyme system in liver. Biochem. J. 22: 968-972.

Harvey J.A., McMaster S.E. and Fuller R.W. (1977) Comparison between the neurotoxic and serotonin-depleting effects of various halogenated derivatives of amphetamine in the rat. J. Pharmacol. Exp. Ther. 202: 581-589.

Hauger R.L. and Paul S.M. (1983) Neurotransmitter receptor plasticity: alterations by antidepressants and antipsychotics. Psychiat. Ann. 13: 399-407.

Hegadoren K.M., Greenshaw A.J., Baker G.B., Martin-Iverson M.T., Lodge B.A. and Soin S. (1994) 4-Ethoxyamphetamine: effects on intracranial self-stimulation and *in vitro* uptake and release of ^{3}H-dopamine and ^{3}H-serotonin in rat brain. J. Psychiat. Neurosci. 19: 57-62.

Hegadoren K.M., Martin-Iverson M.T. and Baker G.B. (1995) Comparative behavioural and neurochemical studies with a psychomotor stimulant, an hallucinogen and 3,4-methylenedioxy analogues of amphetamine. Psychopharmacol. 118: 295-304.

Hendley E.D. and Snyder S.H. (1968) Relationship between the action of monoamine oxidase inhibitors on the noradrenaline uptake system and their antidepressant efficacy. Nature (Lond.) 220: 1330-1331.

Heninger C., Saito N., Tallman J.F., Garrett K.M., Vitek M.P., Duman R.S. and Gallager D.W. (1990) Effects of continuous diazepam administration on $GABA_A$ subunit mRNA in rat brain. J. Mol. Neurosci. 2: 101-107.

Heninger G.R. and Charney D.S. (1987) Mechanism of action of antidepressant treatments: implications for the etiology and treatment of depressive disorders. In: Psychopharmacology: The Third Generation of Progress, Meltzer H.Y. (ed.), Raven Press, New York, pp. 535-544.

Heninger G.R., Charney D.S. and Sternberg D.E. (1983) Lithium carbonate augmentation of antidepressant treatment. Arch. Gen. Psychiat. 40: 1335-1342.

Himmelhoch J.M., Detre T., Kupfer D.J. Swartzburg M. and Byck R. (1972) Treatment of previously intractable depressions with tranylcypromine and lithium. J. Nerv. Ment. Dis. 155: 216-220.

Himmelhoch J.M., Fuchs C.Z. and Symons B.J. (1982) A double-blind study of tranylcypromine treatment of major anergic depression. J. Nerv. Ment. Dis. 170: 628-634.

Himmelhoch J.M., Thase M.E., Mallinger A.G. and Houck P. (1991) Tranylcypromine versus imipramine in anergic bipolar depression. Am. J. Psychiat. 148: 910-916.

Hitzemann R.J., Loh H.H. and Domino E.F. (1971) Effect of para-methoxyamphetamine on catecholamine metabolism in mouse brain. Life Sci. 10: 1087-1095.

Holt A. and Baker G.B. (1995) Metabolism of agmatine (clonidine-displacing substance) by diamine oxidase and the possible implications for studies of imidazoline receptors. In: Progress in Brain Research, Yu P.M., Tipton K.F. and Boulton A.A. (eds.), Elsevier Sciences BV, Amsterdam, pp. 187-197.

Horn A.S. and Snyder S.H. (1972) Steric requirements for catecholamine uptake by rat brain synaptosomes: studies with rigid analogs of amphetamine. J. Pharmacol. Exp. Ther. 180: 523-530.

Hrdina P.D. (1993) Is there action beyond receptors? J. Psychiat. Neurosci. 18: 57-59.

Hsu Y-P.P., Weyler W., Chen S., Sims K.B., Rinehart W.B., Utterback M.C., Powell J.F. and Breakefield X.O. (1988) Structural features of human monoamine oxidase A elucidated from cDNA and peptide sequences. J. Neurochem. 51: 1321-1324.

Hyman S.E., Arana G.W. and Rosenbaum J.F. (1995) Handbook of Psychiatric Drug Therapy, Third Edition, Little, Brown & Company, Boston, pp. 43-92.

Hyttel J. (1994) Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs) Int. Clin. Psychopharmacol. 9 (Suppl. 1): 19-26.

Inaba T., Jurima M., Mahon W.A. and Kalow W. (1985) Mephenytoin and sparteine pharmacogenetics in Canadian Caucasians. Drug Metab. Dispos. 13: 443-448.

Jamali F., Mehvar R. and Pasutto F.M. (1989) Enantioselective aspects of drug action and disposition: therapeutic pitfalls. J. Pharm. Sci. 78: 695-715.

Jandhyala B.S., Steenberg M.L., Perel J.M., Manian A.A. and Buckley J.P. (1977) Effects of tricyclic antidepressants on the hemodynamics and myocardial contractibility of the anesthesized dog. Eur. J. Pharmacol. 42: 403-410.

Jefferson J.W. (1992) Is tranylcypromine really metabolized to amphetamine? J. Clin. Psychiat. 53: 450-451.

Jenike M.A., Surman O.S., Cassem N.H., Zusky P. and Anderson W.H. (1983) Monoamine oxidase inhibitors in obsessive-compulsive disorder. J. Clin. Psychiat. 44: 131-132.

Joffe R.T. (1988) T3 and lithium potentiation of tricyclic antidepressants (letter). Am. J. Psychiat. 145: 317-318.

Joffe R.T. (1990) Afternoon fatigue and somnolence associated with tranylcypromine treatment. J. Clin. Psychiat. 51: 192-193.

Johnson M., Elayan I., Hanson G.R., Foltz R.L., Gibb J.W. and Lim H.K. (1992) Effects of 3,4-dihydroxymethamphetamine and 2,4,5-trihydroxymeth-amphetamine, two metabolites of 3,4-methylenedioxymethamphetamine, on central serotonergic and dopaminergic systems. J. Pharmacol. Exp. Ther. 261: 447-452.

Johnson M.R., Lydiard R.B. and Ballenger J.C. (1994) In vitro studies of two isozymes of human liver cytochrome P-450, mephenytoin p-hydroxylase and sparteine monooxygenase In: Clinical Advances in Monoamine Oxidase Inhibitor Therapies, Kennedy S.H. (ed), Am. Psychiatric Press, Washington,

DC, pp. 205-224.

Johnston J.P. (1968) Some observations upon a new inhibitor of monoamine oxidase in brain tissues. Biochem. Pharmacol. 17: 1285-1297.

Joyce P.R., Hewland H.R. and Jones A.V. (1983) Rapid response to lithium in treatment resistant depression. Br. J. Psychiat. 142: 204-206.

Juorio A.V. and Durden D.A. (1984) The distribution and turnover of tryptamine in the brain and spinal cord. Neurochem. Res. 9: 1283-1293.

Kahn D. (1988) Mysterious MAOI hypertensive episodes. J. Clin. Psychiat. 49: 38-39.

Kanazawa I. (1994) Short review on monoamine oxidase and its inhibitors. Eur. Neurol. 34: 36-39.

Kang G.I. and Chung S.Y. (1984) Identification of N-acetyl and hydroxylated N-acetyltranylcypromine from tranylcypromine-dosed rat urine. Arch. Pharm. Res. (Korea) 7: 65-68.

Kang I. and Miller L.G. (1991) Decreased $GABA_A$ receptor subunit mRNA concentrations following chronic lorazepam administration. Br. J. Pharmacol. 103: 1285-1287.

Kaplan H.I., Sadock B.J. and Grebb J.A. [Eds.] (1994) Kaplan and Sadock's Synopsis of Psychiatry: Behavioral Sciences -- Clinical Psychiatry, Williams and Wilkins, Baltimore, 1257 pp.

Katona C.L.E. (1995) Refractory depression: a review with particular reference to the use of lithium augmentation. Eur. Neuropsychopharmacol. 5: 109-113.

Katona C. and Bill G. (1988) Suicide risk in depression (letter). Br. J. Hosp. Med. Feb. 39: 170.

Keck P., Pope H.G. and Nierenberg A. (1989) Autoinduction of hypertensive reactions by tranylcypromine? J. Clin. Psychopharmacol. 9: 48-51.

Keck P.E., Carter W.P., Nierenberg A.A., Cooper T.B., Potter W.Z. and Rothschild A.J. (1991) Acute cardiovascular effects of tranylcypromine: correlation with plasma drug, metabolite, norepinephrine, and MHPG levels. J. Clin. Psychiat. 52: 250-254.

Kellar K.J. and Cascio C.S. (1982) ^{3}H-Tryptamine: high affinity binding sites in rat brain. Eur. J. Pharmacol. 78: 475-478.

Kelly D. (1973) Phenelzine in phobic states. Proc. R. Soc. Med. 66: 37-38.

Kennedy S.H. and Goldbloom D.S. (1994) MAOIs in the treatment of bulimia nervosa. In: Clinical Advances in Monoamine Oxidase Inhibitor Therapies, Kennedy S.H. (ed), Am. Psychiat. Press, Washington, DC, pp. 255-264.

Ketter T.A., Post R.M., Parekh P.I. and Worthington K. (1995) Addition of monoamine oxidase inhibitors to carbamazepine: preliminary evidence of safety and antidepressant efficacy in treatment-resistant depression. J. Clin. Psychiat. 56: 471-475.

Kielholz P., Terzani S. and Gastpar M. (1979) Treatment for therapy resistant depressions. Pharmacopsychiat. 14: 94-100.

Kimber J.R., Cross J.A. and Horton R.W. (1987) Benzodiazepine and $GABA_A$ receptors in rat brain following chronic antidepressant drug administration. Biochem. Pharmacol. 36: 4173-4175.

Kinemuchi H., Arai Y., Oreland L., Tipton K.F. and Fowler C.J. (1982) Time-dependent inhibition of monoamine oxidase by β-phenylethylamine. Biochem. Pharmacol. 23: 47-56.

Klein D.F. and Davis J.M. (1969) Diagnosis and Drug Treatment of Psychiatric Disorders, Williams & Wilkins, Baltimore.

Klein D.F., Gittelman R., Quitkin F. and Rifkin A. (1980) Diagnosis of affective disorders: clinical considerations. In: Diagnosis and Drugs Treatment of Psychiatric Disorders: Adults and Children, 2nd Edn., Williams & Wilkins, Baltimore, pp. 223-248.

Kline N.S. (1958) Clinical experience with iproniazid (Marsilid). J. Clin. Exp. Psychopathol. 19: 72-78.

Kline N.S., Cooper I.B., Suchow R.F., Hallstrom C. and Brebbia D.R. (1981) Protection of patients on MAOIs against hypertensive crises. J. Clin. Psychopharmacol. 1: 410-411.

Knoll J. and Magyar K. (1972) Some puzzling pharmacological effects of monoamine oxidase inhibitors. Adv. Biochem. Psychopharmacol., 5: 393-408.

Kramlinger K.G. and Post R.M. (1989) Adding lithium carbonate to carbamazepine: antidepressant efficacy in treatment-resistant depression. Arch. Gen. Psychiat. 46: 794-800.

Kraus R. (1989) Hypertensive episodes with tranylcypromine treatment. J. Clin. Psychopharmacol. 9: 232-233.

Krishnan K.R.R. (1995) Monoamine oxidase inhibitors. In: The American Psychiatric Press Textbook of Psychopharmacology, Schatzberg A.F. and Nemeroff C.B. (eds.), Am. Psychiat. Press, Washington, DC, pp. 183-193.

Kutcher S.P., Reid K., Dubbin J.D. and Shulman K.I. (1985) Electrocardiogram changes and therapeutic desipramine and 2-hydroxydesipramine concentrations in elderly depressives. Br. J. Psychiat. 148: 676-679.

Lafaille F., Welner S.A. and Suranyi-Cadotte B.E. (1991) Regulation of serotonin type 2 (5-HT_2) and β-adrenergic receptors in rat cerebral cortex following novel and classical antidepressant treatment. J. Psychiat. Neurosci. 16: 209-214.

Lang F.A., Geissler H.E. and Mutschler E. (1979) Bestimmung und Vergleich der Plasma und Urinkonzentrationen Nach Gabe Von (+)- und (-)-Tranylcypromin. Arzneimittel-Forschung 29: 154-157.

Lapin I.P. and Oxenkrug G.F. (1969) Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. Lancet i: 132-136.

Lauer J.W., Inskip W.M., Bernsohn J. and Zeller E.A. (1958) Observations on schizophrenic patients after iproniazid and tryptophan. AMA Arch. Neurol. Psychiat. 80: 122-130.

Lavin M.R., Mendelowitz A. and Kronig M.H. (1993) Spontaneous hypertensive reactions with monoamine oxidase inhibitors. Biol. Psychiat. 34: 146-151.

Le Gassicke J., Ashcroft G.W., Eccleston D., Evans J.I., Oswald I. and Ritson E.G. (1965) The clinical state, sleep and amine metabolism of a tranylcypromine ('Parnate') addict. Br. J. Psychiat. 111: 357-365.

Lee C.M., Javitch J.A. and Snyder S.H. (1983) Recognition sites for norepinephrine uptake: regulation by neurotransmitters. Science 220: 626-629.

Lew R., Sabol K.E., Chou C., Vosmer G.L., Richards J. and Seiden L.S. (1996) Methylenedioxymethamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. 2. Radioligand binding and autoradiography studies. J. Pharmacol. Exp. Ther. 276: 855-865.

Lewis J. and Winokur G. (1982) The induction of mania. Arch. Gen. Psychiat. 39: 303-306.

Li P., Emanghoreishi M., Sibony D. and Warsh J.J. (1993) Chronic antidepressant treatment does not alter the levels of Gs and Gi α-subunit transcript and immunoreactivity in rat cortex. Soc. Neurosci. Abstr. 19: 1868.

Lim H.K. and Foltz R.L. (1988) In vivo and in vitro metabolism of 3,4-(methylenedioxy)methamphetamine in the rat: identification of metabolites using an ion trap detector. Chem. Res. Toxicol. 1: 370-378.

Lingjaard O. (1973) Synergistic effect of tricyclic antidepressants and lithium carbonate in endogenous depression (letter). Lancet ii: 1260.

Lloyd K.G., Thuret F. and Pilc A. (1985) Upregulation of γ-aminobutyric acid (GABA) "B" binding sites in rat frontal cortex: a common action of repeated administration of different classes of antidepressants and electroshock. J. Pharmacol. Exp. Ther. 235: 191-199.

Lloyd K.G., Sivkovic B., Scatton B., Morselli P.L. and Bartholini G. (1989) The GABAergic hypothesis of depression. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 13: 341-351.

Lopez-Ibor Alino J.J., Ayuso Gutierrez J.L. and Montejo Iglesias M.L. (1973) Tryptophan and amitriptyline in the treatment of depression: a double-blind study. Int. Pharmacopsychiat. 8: 145-151.

Lovecock J.E. and Lipsky S.R. (1960) Electron-affinity spectroscopy. A new method for the identification of functional groups in chemical compounds separated by gas chromatography. J. Am. Chem. Soc. 82: 431-441.

Lowry O.H., Rosenbrough N.J., Farr A.L. and Randall R.J. (1951) Protein measurements with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Lundberg D., Ahlfors H.G., Dencker S.J., Fruensgaard K., Hansten S., Jensen K., Ose E. and Pihkanen T.A. (1979) Symptom reduction in depression after treatment with L-tryptophan or imipramine: item analysis of Hamilton rating scale for depression. Acta Psychiat. Scand. 60: 287-294.

Maes M., De Ruyter M. and Suy E. (1987) Prediciton of subtype and severity of depression by means of dexamethasone suppression test, L-tryptophan: competing amino acids ratio, and MHPG flow. Biol. Psychiat. 22: 177-188.

Maj J., Papp M., Skuza B., Bigajska K. and Zazula M. (1989) Chronic treatment with antidepressant drugs: potentiation of apomorphine-induced agressive behaviour in rats. Neurosci. Lett. 13: 337-341.

Mallinger A.G., Edwards D.J., Himmelhoch J.M., Knopf S. and Elher J. (1986)

Pharmacokinetics of tranylcypromine in patients who are depressed: relationship to cardiovascular effects. Clin. Pharmacol. Ther. 40: 444-450.

Mallinger A.G., Himmelhoch J.M., Thase M.E., Edwards D.J. and Knopf S. (1990) Plasma tranylcypromine: relationship to the pharmacokinetic variables and clinical antidepressant actions. J. Clin. Psychopharmacol. 10: 176-183.

Marley E. and Blackwell B. (1970) Interactions of monomine oxidase inhibitors, amides, and foodstuffs. Adv. Pharmacol. Chemother. 8: 226-232.

Marsden C.A. and Curzon G. (1974) Effects of lesions and drugs on brain tryptamine. J. Neurochem. 23: 1171-1176.

Martin I.L., Baker G.B. and Mitchell P.R. (1978) The effect of viloxazine hydrochloride on the transport of noradrenaline, dopamine, 5-hydroxytryptamine and γ-aminobutyric acid in rat brain tissue. Neuropharmacol. 17: 421-423.

Martin I.L. (1987) The benzodiazepines and their receptors. Neuropharmacol. 26: 957-970.

Martin I.L., Bakish D. and Joffe R. (1994) MAOI treatment of depression. In: Current Advances in Monoamine Oxidase Inhibitor Therapies, Kennedy S.H. (ed), Am. Psychiatric Press, Washington, pp. 147-180.

Martin I.L. and Candy J.M. (1978) Facilitation of benzodiazepine binding by sodium chloride and GABA. Neuropharmacol. 17: 993-998

Martin K.F., Phillips I., Cheetham S.C. and Heal D.J. (1995) Dopamine D2 receptors: a potential pharmacological target for nomifensine and tranylcypromine but not other antidepressant treatments. Pharmacol. Biochem. Behav. 51: 565-569.

Martin-Iverson M.T., Leclere J-F. and Fibiger H.C. (1983) Cholinergic-dopaminergic interactions and the mechanisms of action of antidepressants. Eur. J. Pharmacol. 94: 193-201.

Martin-Iverson M.T. and Lodge B.A. (1992) Effects of chronic treatment of rats with "designer" amphetamines on brain regional monoamines. Can. J. Physiol. Pharmacol. 69: 1825-1832.

Matin S.B. and Rowland M. (1972) Electron-capture sensitivity comparison of various derivatives of primary and secondary amines. J. Pharm. Sci. 61: 1235-1240.

McCann U.D. and Ricaurte G.A. (1995) On the neurotoxicity of MDMA and related amphetamine derivatives. J. Clin. Psychopharmacol. 15: 295-296.

McDaniel K.D. (1986) Clinical pharmacology of monoamine oxidase inhibitors. Clin. Neuropharmacol. 9: 207-234.

McGrath P.J., Stewart J.W., Harrison W., Wager S. and Quitkin F.M. (1986) Phenelzine treatment of melancholia. J. Clin. Psychiat. 47: 420-422.

McKenna D.J. and Peroutka S.J. (1990) Neurochemistry and neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). J. Neurochem. 54: 14-22.

McKenna K.F., McManus D.J., Baker G.B. and Coutts R.T. (1994) Chronic administration of the antidepressant phenelzine and its N-acetyl analogue: effects on GABAergic funcion. J. Neural Transm. 41: 115-122.

McManus D.J. and Greenshaw A.J. (1991a) Differential effects of chronic antidepressants in behavioural tests of β-adrenergic and $GABA_B$ receptor function. Psychopharmacol. (Berl.) 103: 204-208.

McManus D.J. and Greenshaw A.J. (1991b) Differential effects of antidepressants on $GABA_B$ and β-adrenergic receptors in rat cerebral cortex. Biochem. Pharmacol. 42: 1525-1528.

McPherson G.A. (1987) Kinetic, EBDA, Ligand, Lowry. A Collection of Radioligand Binding Analysis Programs, Elsevier-Biosoft, U.K.

Meana J.J., Barturen F., Martin I. and García-Sevilla J.A. (1993) Evidence of increased non-adrenoceptor [^{3}H]idazoxan binding sites in the frontal cortex of depressed suicide victims. Biol. Psychiat. 34: 498-501.

Messiha F.S. (1993) Fluoxetine: adverse effects and drug-drug interactions. Clin. Toxicol. 31: 603-630.

Midha K.K., McGilveray I.J. and Cooper J.K. (1979) A GLC-ECD assay for simultaneous determination of fenfluramine and norfenfluramine in human plasma and urine. Can. J. Pharm. Sci. 14: 18-21.

Modigh K. and Svensson T.H. (1972) On the role of central nervous system catecholamines and 5-hydroxytryptamine in the nialamide-induced behavioural syndrome. Br. J. Pharmacol. 46: 32-45.

Moffat A.C., Horning E.C., Matin S.B. and Rowland M. (1972) Perfluorobenzene derivatives as derivatizing agents for the gas chromatography of primary and

secondary amines using electron capture detection. J. Chromatogr. 66: 255-260.

Møller S.E., Kirk L. and Honoré P. (1980) Relationship between plasma ratio of tryptophan to competing amino acids and the response to L-tryptophan treatment in endogenously depressed patients. J. Affec. Dis. 2: 47-59.

Mousseau D.D. (1993) Tryptamine: a metabolite of tryptophan implicated in various neuropsychiatric disorders. Metab. Brain Dis. 8: 1-44.

Mousseau D.D., McManus D.J., Baker G.B., Juorio A.V., Dewhurst W.G. and Greenshaw A.J. (1993) Effects of age and of chronic antidepressant treatment on ^{3}H-tryptamine and ^{3}H-dihydroalprenolol binding to rat cortical membranes. Cell Mol. Neurobiol. 13: 3-13.

Murphy D.L., Aulakh C.S., Garrick N.A. and Sunderland T. (1987) Monoamine oxidase inhibitors as antidepressants: implications for the mechanism of action of antidepressants and the psychobiology of the affective disorders and some related disorders. In: Psychopharmacology: The Third Generation of Progress, Meltzer H.Y. (ed.), Raven Press, New York, pp. 545-552.

Murphy D.L., Garrick N.A., Aulakh C.S. and Cohen R.M. (1984) New contributions from basic science to understanding the effects of monoamine oxidase inhibiting antidepressants. J. Clin. Psychiat. 45: 37-43.

Murphy D.L. and Donnelly C.H. (1974) Monoamine oxidase in man: enzyme characteristics in platelets, plasma and other human tissues. Adv. Biochem. Psychopharmacol., 12: 71-85.

Murphy D.L. and Kalin N.H. (1980) Biological and behavioral consequences of alterations in monoamine oxidase activity. Schizophr. Bull., 6: 355-367.

Murphy D.L., Lipper S., Slater S. and Shiling D. (1979) Selectivity of clorgyline and pargyline as inhibitors of monoamine oxidases A and B in vivo in man. Psychopharmacol. 62, 129-132.

Murphy D.L., Pickar D., Jimerson D., Cohen R.M., Garrick N.A., Karoum F. and Wyatt R.J. (1981) Biochemical indices of the effects of selective MAO inhibitors (clorgyline, pargyline and deprenyl) in man. In: Clinical Pharmacology in Psychiatry, Usdin E., Gram L.F. and Lingjaerde O. (eds.), Macmillan Press, London, pp. 307-316.

Mutschler D. and Mohrke W. (1983) Kinetics of MAO inhibitors. Mod. Probl. Pharmacopsychiat. 19: 126-134.

Mutschler E., Gietl Y., Krauss D., Martin E., Pflugmann G. and Weber H. (1990) Stereospecific analysis and human pharmacokinetics of the enantiomers of drugs administered as racemates. In: Chirality and Biological Activity, Holmstedt B., Frank H. and Testa B. (eds.), Alan R. Liss, New York, pp. 199-219.

Nasser B.A., Juang Y.S., McDonald A.T., Jenkins K.D. and Horrobin D.F. (1988) The influence of phenelzine and tranylcypromine on the release of prostaglandins from the rat mesenteric vascular bed. Can. J. Physiol. Pharmacol. 66: 1206-1209.

Nayeem N., Green T.P., Martin I.L. and Barnard E.A. (1994) Quaternary structure of the native $GABA_A$ receptor determined by electron microscopic image analysis. J. Neurochem. 62: 815-818.

Nazarali A.J., Baker G.B., Coutts R.T. and Greenshaw A.J. (1987) *Para*-hydroxytranylcypromine: presence in rat brain and heart following administration of tranylcypromine and an N-cyanoethyl analogue. Eur. J. Drug Metab. Pharmacokinet. 12: 207-214.

Nelson J.C. and Byck R. (1982) Rapid response to lithium in phenelzine non-responders. Br. J. Psychiat. 142: 85-86.

Nestler E.J., Terwilliger R.Z. and Duman R.S. (1989) Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. J. Neurochem. 53: 1644-1647.

Nierenberg A.A., Price L.H., Charney D.S., Heninger G.R. (1990) After lithium augmentation: a retrospective follow-up of patients with antidepressant-refractory depression. J. Affec. Dis., 18: 167-175.

Nies A. (1984) Differential response patterns to MAO inhibitors and tricyclics. J. Clin. Psychiat. 45: 70-77.

Nolen W.A., Hoencamp E., Bouvy P.F. and Haffmans P.M.J. (1993) Reversible monoamine oxidase-A inhibitors in resistant major depression. Clin. Neuropharmacol. 16: S69-S76.

Nolen W.A., van de Putte J.J., Kijken W.A., Kamp J.S., Blansjaar B.A., Kramer H.J. and Haffmans J. (1988) Treatment strategy in depression. I. Non-tricyclic and selective reuptake inhibitors in resistant depression: a double-blind partial crossover study on the effects of oxaprotiline and fluvoxamine. Acta Psychiat. Scand. 78: 668-675.

Nutt D.J. and Glue P. (1989) Monoamine oxidase inhibitors: rehabilitation from

recent research. Br. J. Psychiat. 154: 287-291.

O'Brien S., McKeon P. and Oregan M. (1993) The efficacy and tolerability of combined antidepressant treatment in different depressive subgroups. Br. J. Psychiat. 162: 363-368.

Olmos G., Gabilondo A.M., Miralles A., Escriba P.V. and García-Sevilla J.A. (1993) Chronic treatment with the monoamine oxidase inhibitors clorgyline and pargyline down-regulates non-adrenoceptor [^{3}H]-idazoxan binding sites in the rat brain. Br. J. Pharmcol. 108: 597-603.

Osward I., Brezinova V. and Dunleavy D.L.F. (1972) On the slowness of action of tricyclic antidepressant drugs. Br. J. Psychiat. 120: 673-677.

Ozelius L., Hsu Y-P.P., Bruns G., Powell J.F., Chen. S., Weyler W., Utterback M., Zicker D., Haines J., Trofatter J.A., Conneally P.M., Gusella J.F. and Breakefield X.O. (1988) Human monoamine oxidase gene (MAOA); chromosome position (Xp21-011) and DNA polymorphism. Genomics 3: 53-58.

Paetsch P.R., Baker G.B., Caffaro L.E., Greenshaw A.J., Rauw G.A. and Coutts R.T. (1992) An electron-capture gas chromatographic procedure for simultaneous determination of amphetamine and N-methylamphetamine. J. Chromatogr. Biomed. Appl. 573: 313-317.

Paetsch P.R. and Greenshaw A.J. (1992) Effects of chronic antidepressant treatment on dopamine related [^{3}H]-SCH23390 and [^{3}H]-spiperone binding in the rat striatum. Cell. Mol. Neurobiol., 12: 597-606.

Paetsch P.R. and Greenshaw A.J. (1993) Effects of chronic antidepressant treatment of β-adrenoceptor subtype binding in the rat cerebral cortex and cerebellum. Mol. Chem. Neuropathol. 20: 21-31.

Pallanti S. and Mazzi D. (1992) MDMA (Ecstasy) precipitation of panic disorder. Biol. Psychiat. 32: 91-95.

Pare C.M. (1985) The present status of monoamine oxidase inhibitors. Br. J. Psychiat. 156: 576-584.

Pare C.M.B. (1963) Potentiation of monoamine-oxidase inhibitors by tryptophan. Lancet ii: 527-528.

Pare C.M.B., Kline N., Hallstrom C. and Cooper T.B. (1982) Will amitriptyline prevent the "cheese" reaction of monoamine oxidase inhibitors? Lancet ii: 183-186.

Pare C.M.B. and Sandler M.J. (1959) A clinical and biochemical study of a trial of iproniazid in the treatment of depression. J. Neurol. Neurosurg. Psychiat. 22: 247-251.

Paul S.M., Janowsky A. and Skolnick P. (1985) Monoamine neurotransmitters and antidepressant drugs. In: Psychiatry Update, APA Annual Review, Vol. 4, Hales R.E. and Frances A.J. (eds.), American Psychiatric Press, Washington, D.C., p. 37.

Paykel E.S. (1979) Predictors on treatment response. In: Psychopharmacology of Affective Disorders, Paykel E.S. and Coppen A. (eds.), Oxford University Press, New York, pp. 193-200.

Paykel E.S. (1995) Clinical efficacy of reversible and selective inhibitors of monoamine oxidase A in major depression. Acta Psychiat. Scand. 91: 22-27.

Paykel E.S., Parker R.R., Rowan P.R., Rao B.M. and Taylor C.N. (1983) Nosology of atypical depression. Psychol. Med. 13: 131-139.

Pearlman C. (1987) High dose tranylcypromine in refractory depression. J. Clin. Psychiat. 48: 423-424.

Pérez de Francisco C. (1979) Therapy resistant depression. Pharmacopsychiat. 14: 71-78.

Peroutka S.J. and Snyder S.H. (1980) Long term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. Science 210: 88-90.

Pilc A. and Lloyd K.G. (1984) Chronic antidepressant and GABA "B" receptors: a GABA hypothesis of antidepressant drug action. Life Sci. 35: 2149-2154.

Plaznik A. and Kostowski W. (1987) The effects of antidepressants and electroconvulsive shock on the functioning of the mesolimbic dopaminergic system: a behavioural study. Eur. J. Pharmacol. 135: 389-396.

Philips S.R., Baker G.B and McKim H.R. (1980) Effects of tranylcypromine on the concentrations of some trace amines in the diencephalon and hippocampus of the rat. Experientia 36: 241-242.

Philips S.R. and Boulton M. (1979) The effects of monoamine oxidase inhibitors on some arylalkylamines in the rat striatum. J. Neurochem. 33: 159-167.

Porsolt R.D., Bertin A. and Jalfre M. (1977) Behavioural despair in mice: a primary

screening test for antidepressants. Arch. Int. Pharmacodyn. 229: 327-336.

Potter W.Z. and Manji H.K. (1990) Antidepressants, metabolites and apparent drug resistance. Clin. Neuropharmacol. 13: S45-S53.

Powell J.F., Hsu Y-P.P., Weyler W., Chen S., Salach J.I., Anadrikopoulos K., Mallet J. and Breakefield X.O. (1988) The primary structure of bovine monoamine oxidase type A: comparison with peptide sequences of bovine monoamine oxidase type B and other flavoenzymes. Biochem. J. 250: 547-555.

Preskorn S.H. (1996) Clinical Pharmacology of Selective Serotonin Reuptake Inhibitors, First Edition. Professional Communications Inc., Caddo, Oklahoma.

Preskorn S.H., Janicak P.G., Davis J.W. and Ayd F.J. (1995) Advances in the pharmacotherapy of depressive disorders. In: Principles and Practice of Psychopharmacology, Vol. 1, Janicak P.G., Davis J.M., Preskorn S.H. and Ayd F.J. Jr. (eds), Williams & Wilkins, Baltimore, pp. 1-24.

Price L.H., Charney D.S. and Heninger G.R. (1985) Efficacy of lithium-tranylcypromine treatment in refractory depression. Am. J. Psychiat. 142: 619-623.

Priest R.G., Gimbrett R., Roberts W. and Steinert J. (1995) Reversible and selective inhibitors of monoamine oxidase A in mental and other disorders. Acta Psychiat. Scand. 91: 40-43.

Primus R.J. and Gallager D.W. (1992) $GABA_A$ receptor subunit mRNA levels are differentially influenced by chronic FG7142 and diazepam exposure. Eur. J. Pharmacol. 226: 21-28.

Przegalinski E., Baran L. and Siwanowicz J. (1983) The effect of chronic treatment with antidepressant drugs on salbutamol-induced hypoactivity in rats. Psychopharmacol. 80: 355-359.

Przegalinski E., Baran L., Siwanowicz J. and Bigajska K. (1984) Repeated treatment with antidepressant drugs prevents salbutamol-induced hypoactivity in rats. Pharmacol. Biochem. Behav. 21: 695-698.

Przegalinski E. and Jurkowska T. (1990) Repeated treatment with antidepressants does not modify the locomotor effect of dopaminergic stimulants injected into the rat hippocampus. Arch. Int. Pharmacodyn. 305: 152-162.

Rabkin J., Quitkin F., Harrison W., Tricamo E. and McGrath P. (1984) Adverse reactions to monoamine oxidase inhibitors. Part I. A comparative study. J.

Clin. Psychopharmacol. 4: 270-278.

Rabkin J., Quitkin F., McGrath P., Harrison W., and Tricamo E. (1985) Adverse reactions to monoamine oxidase inhibitors. Part II. Treatment correlates and clinical management. J. Clin. Psychopharmacol. 5: 2-9.

Ramsey A. and Mendels J. (1982) Lithium ion as an antidepressant. In: Antidepressants: Neurochemical, Behavioral and Clinical Perspectives, Enna S.J., Malick J.B. and Richelson E. (eds.), Raven Press, New York, pp. 175-182.

Raiteri M., del Carmine R., Bertollini A. and Levi G. (1977) Effect of sympathomimetic amines on synaptosomal transport of noradrenaline, dopamine 5-hydroxytryptamine. Eur. J. Pharmacol. 41: 133-143.

Rao T.S., Coutts R.T., Baker G.B., Hall T.W. and Micetich R.G. (1986) Analogues of tranylcypromine: synthesis and comparison of effects on inhibiton of monoamine oxidase *in vitro*. Proc. West Pharmacol. Soc. 29: 279-281.

Razani J., White K.L., White J., Simpson G., Sloane K.B., Rebal R. and Palmer R. (1983) The safety and efficacy of combined amitriptyline and tranylcypromine antidepressant treatment: a controlled clinical trial. Arch. Gen. Psychiat. 40: 657-661.

Reigle T.G., Orsulak P.J., Avni J., Platz P.A. and Schildkraut J.J. (1980) The effects of tranylcypromine isomers on norepinephrine-H^3 metabolism in rat brain. Psychopharmacol. 69: 193-199.

Reynolds G.P., Rausch W-D. and Riederer P. (1980) Effects of tranylcypromine stereoisomers on monoamine oxidation in man. Br. J. Clin. Pharmacol. 9: 521-523.

Richards G., Schoch P. and Haefely W. (1991) Benzodiazepine receptors: new vistas. Sem. Neurosci. 3: 191-203.

Rickels K., Feighner J.P. and Smith W.T. (1985) Alprazolam, amitriptyline, doxepin and placebo in the treatment of depression. Arch. Gen. Psychiat. 42: 134-141.

Rizack M.A. (ed) [1995] The Medical Letter Handbook of Adverse Drug Interactions. The Medical Letter, Inc., New Rochelle, NY, pp. 300-301.

Robinson D.S., Kayser A., Corcella J., Laus D., Yingling K. and Howard D. (1985) Panic attacks in outpatients with depression: response to antidepressant treatment. Psychopharmacol. Bull. 21: 562-567.

Robinson D.S. (1983) High-dose monoamine oxidase-inhibitor therapy. JAMA, 250: 2212.

Rosenstein D.L., Takeshita J. and Nelson J.C. (1991) Fluoxetine-induced elevation and prolongation of tricyclic levels in overdose. Am. J. Psychiat. 148: 807.

Rosenthal N.E., Sack D.A., Gillin J.C., Levy A.J., Goodwin, F.K., Davenport Y., Mueller B., Newson D.A. and Wehr T.A. (1984) Seasonal affective disorders: a description of the syndrome and preliminary findings with light therapy. Arch. Gen. Psychiat. 41: 72-80.

Rosenthal N.E., Sack D.A. and Wehr T.A. (1987) Seasonal affective disorders. Psychiat. Ann. 17: 670-674.

Rudorfer M.V. and Potter W.Z. (1987) Pharmacokinetics of antidepressants. In: Psychopharmacology: The Third Generation of Progress, Melter H.Y. (ed.), Raven Press, New York, pp. 1353-1363.

Rudorfer M.V. and Potter W.Z. (1989) Antidepressants: a comparative review of the clinical pharmacology and therapeutic use of the "newer" versus the "older" drugs. Drugs 37: 713-738.

Sabol K.E., Lew R., Richards J.B., Vosmer G.L. and Seiden L.S. (1996) Methylene-dioxymethamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. 1. Synaptosomal uptake and tissue concentrations. J. Pharmacol. Exp. Ther. 276: 846-854.

Sackheim H.A., Devanand D.P. and Nobler M.S. (1995) Electroconvulsive therapy. In: Psychopharmacology: The Fourth Generation of Progress, Bloom F.E. and Kupfer D.J. (eds), Raven Press, New York, NY, pp. 1123-1142.

Salach J.I. and Detmer K. (1979) Chemical characterization of monoamine oxidase A from human placental mitochondria. In: Monoamine Oxidase: Structure, Function and Altered Functions, Singer T.P., Von Korff R.W. and Murphy D.L. (eds.), Academic Press, New York, p. 121.

Salomon R.M., Miller H.L., Delgado P.L. and Charney D. (1993) The use of tryptophan depletion to evaluate central serotonin function in depression and other neuropsychiatric disorders. Int. Clin. Psychopharmacol. 8: 41-46.

Sargant W. (1962) The treatment of anxiety states and atypical depressions by the monoamine oxidase inhibitor drugs. J. Neuropsychiat. 3: 96-103.

Saura J., Bleuel Z., Ulrich J., Mendelowitsch A., Chen K., Shih J.C., Malherbe P., DaPrada M. and Richards J.G. (1996) Molecular neuroanatomy of human monoamine oxidases A and B revealed by quantitative enzyme

radioautography and in situ hybridization histochemistry. Neurosci. 70: 755-774.

Saura J., Kettler R., Da Prada M. and Richards J.G. (1990) Molecular neuroanatomy of MAO-A and MAO-B. J. Neural Transm. 32: 49-53.

Schildkraut J.J. (1965) The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiat. 122: 509-522.

Schildkraut J.J. (1970) Tranylcypromine: effects on norepinephrine metabolism in rat brain. Am. J. Psychiat. 126: 925-931.

Schmauss M., Kapfhammer H., Meyr P. and Hoff P. (1988) Combined MAO-inhibitor and tri-(tetra)cyclic antidepressant treatment in therapy resistant depression. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 12: 523-532.

Schmidt C.J., Levin J.A. and Lovenberg W. (1987) *In vitro* and *in vivo* neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem. Pharmacol. 36: 747-755.

Schmidt L.G., Lammers V., Stockel M. and Muller-Oerlinghausen B. (1988) Recent trends in prescribing psychotropic drugs at a psychiatric university hospital. Pharmacopsychiat. 21: 126-130.

Schubert J. and Sedvall G. (1972) Effect of amphetamines on tryptophan concentrations in mice and rats. J. Pharm. Pharmacol. 24: 53-62.

Schuckit M., Robins E. and Feighner J. (1971) Tricyclic antidepressants and monoamine oxidase inhibitors: combined therapy in the treatment of depression. Arch. Gen. Psychiat. 24: 509-514.

Selikoff I.J., Robitzek E.H. and Ornstein G.G. (1952) Toxicity of hydrazine derivatives of isonicotinic acid in the chemotherapy of human tuberculosis. Q. Bull. Sea View Hosp. 13: 17.

Serra G., Argiolas A., Fadda F., Melis M.R. and Gessa G.L. (1981) Repeated electroconvulsive shock prevents the sedative effect of small doses of apomorphine. Psychopharmacol. 73: 194-196.

Sethna E.R. (1974) A study of refractory cases of depressive illness and their response to combined antidepressant treatment. Br. J. Psychiat. 124: 265-272.

Sheehan D.V., Ballenger J. and Jacobsen G. (1980) Treatment of endogenous anxiety with phobic, hysterical and hypochondrial symptoms. Arch. Gen.

Psychiat. 37: 51-59.

Sherry-McKenna R.L., Wong J.T.F., Paetsch P.R., Baker G.B., Mousseau D.D., McKenna K.F. and Greenshaw A.J. (1994) Monoamine oxidase inhibitors: effects on tryptophan concentrations in rat brain. J. Neural Transm. Suppl. 41: 155-163.

Shih J.C., Zhu Q.S., Grimsby J. and Chen K. (1994) Identification of human monoamine oxidase (MAO) A and B gene promoters. J. Neural Transm. Suppl. 41: 27-33.

Silverstone T. and Turner P. (1995) Drug Treatment in Psychiatry, 5th Ed, Rutlegde, London, UK, pp. 140-189.

Sims K.B., de la Chapelle A., Norio R., Sankila E-M., Hsu Y-P.P., Rinehart W.B., Corey T.J., Ozelius L., Powell J.F., Bruns G., Gusella J.F., Murphy D.L. and Breakefield X.O. (1989) Monoamine oxidase deficiency in males with an X chromosome deletion. Neuron 2: 1069-1076.

Slingsby J.M. and Boulton A.A. (1976) Separation and quantitation of some urinary arylalkylamines. J. Chromatogr. 123: 51-56.

Smith D.F. (1980) Tranylcypromine stereoisomers, monoaminergic transmission and behaviour. A review. Pharmacopsychiat. 13: 130-136.

Smith D.F. (1989) Lithium alters the stereoselectivity of monoamine oxidase in rat brain. J. Neural Transm. 76: 205-210.

Snyder S.H. and Peroutka S.J. (1984) Antidepressants and neurotransmitter receptors. In: Neurobiology of Mood Disorders, Post R.M. and Ballenger J.C. (eds.), Williams and Wilkins, Baltimore, M.D., pp. 686-697.

Sovner R.D. (1981) The clinical characteristics and treatment of atypical depression. J. Clin. Psychiat. 42: 285-289.

Sovner R.D. (1990) Amphetamine and tranylcypromine in treatment-resistant depression. Biol. Psychiat. 28: 1011-1012.

Spahn-Langguth H., Hahn G., Mutschler E., Möhrke W. and Langguth P. (1992) Enantiospecific high-performance liquid chromatographic assay with fluorescence detection for the monoamine oxidase inhibitor tranylcypromine and its applicability in pharmacokinetic studies. J. Chromatogr. 584: 229-237.

Spyraki C. and Fibiger H.C. (1981) Behavioural evidence for supersensitivity of

postsynaptic dopamine receptors in the mesolimbic system after chronic administration of desipramine. Eur. J. Pharmacol. 74: 195-206.

Srinivas N.R., Shyu W.C. and Barbhaiya R.H. (1995) Gas chromatographic determination of enantiomers as diastereomers following pre-column derivatization and applications to pharmacokinetic studies: a review. Biomed. Chromatogr. 9: 1-9.

Steranka L.R. (1982) Long term decreases in striatal dopamine, 3,4-dihydroxy-phenylacetic acid and homovanillic acid after a single injection of amphetamine in iprindole-treated rats: time course and time-dependent interactions with amfonelic acid. Brain Res. 234: 123-126.

Sternbach H. (1991) The serotonin syndrome. Am. J. Psychiat. 148: 705-713.

Sugrue M.F. (1983) Some effects of chronic antidepressant treatments on rat brain monoaminergic systems. J. Neural Transm. 57: 281-288.

Sulser F., Vetulani J. and Mobley P.L. (1978) Mode of action of antidepressant drugs. Biochem. Pharmacol. 27: 257-271.

Suranyi-Cadotte B.E., Bodnoff S.R. and Welner S.A. (1990) Antidepressant-anxiolytic interactions: involvement of the benzodiazepine-GABA and serotonin systems. Prog. Neuropsychopharmacol. Biol. Psychiat. 14: 633-654.

Suranyi-Cadotte B.E., Dam T.V. and Quirion R. (1985) Antidepressant-anxiolytic interaction: decreased density of benzodiazepine receptors in rat brain following chronic administration of antidepressants. Eur. J. Pharmacol. 106: 673-675.

Suzdak P.D. and Gianutsos G. (1986) Effect of chronic imipramine or baclofen on $GABA_B$ binding and cyclic AMP production in cerebral cortex. Eur. J. Pharmacol. 131: 129-133.

Szekely A.M., Barbaccia M.L. and Costa E. (1987) Effect of a protracted antidepressant treatment on signal transduction and ^{3}H-(-)-baclofen binding at $GABA_B$ receptors. J. Pharmacol. Exp. Ther. 243: 155-159.

Tabakoff B. and Moses F. (1976) Differential effects of tranylcypromine and pargyline on indoleamines in brain. Biochem. Pharmacol. 25: 2555-2560.

Talbott J.A., Hales R.E. and Yudofsky S.C. (1988) Textbook of Psychiatry, American Psychiatric Press, Washington, D.C.

Tanay V.A-M.I., Glencorse T.A., Greenshaw A.J., Baker G.B. and Bateson A.N. (1996) Chronic administration of antipanic drugs alters rat brain stem $GABA_A$ receptor subunit mRNA levels. Neuropharmacol. (in press).

Taylor D. and Lader M. (1996) Cytochromes and psychotropic drug interactions. Br. J. Psychiat. 168: 529-532.

Teicher M.H., Cohen B.M., Baldessarini R.J. and Cole J.O. (1988) Severe daytime somnolence in patients treated with an MAOI. Am. J. Psychiat. 145: 1552-1556.

Testa B. (1979) Principles of Organic Stereochemistry, Dekker, New York.

Testa B. (1982) The geometry of molecules: basic principles and nomenclature. In: Stereochemistry, Tamm Ch. (ed.), Elsevier Biomedical Press, Amsterdam, pp. 1-7.

Thase M.E., Himmelhoch J.M., Mallinger A.G., Jarrett D.B. and Kupfer D.J. (1989) Sleep EEG and DST findings in anergic bipolar depression. Am. J. Psychiat. 146: 329-333.

Thase M.E. and Rush A.J. (1995) Treatment-resistant depression. In: Psychopharmacology the Fourth Generation of Progress, Bloom F.E. and Kupfer D.J. (eds), Raven Press, New York, NY, pp. 1081-1097.

Thomson J., Rankin H., Ashcroft G.W., Yates G.M., McQueen J.K. and Cummings S.W. (1982) The treatment of depression in general practice: a comparision of L-tryptophan, amitriptyline and a combination of L-tryptophan and amitriptyline with placebo. Psychol. Med. 12: 741-751.

Thorpe L.W., Westlund K.N., Kochersperger L.M., Abell C.W. and Denney R.M. (1987) Immunocytochemical localization of monoamine oxidase A and B in human peripheral tissues and brain. J. Histochem. Cytochem., 35: 23-32.

Tipton K.F. (1975) Monoamine oxidase. In: Handbook of Physiology, Sec. 7, Vol. 2, Smith A.D. and Blaschko H. (eds.), Am. Physiol. Soc., Washington, pp. 667-691.

Todd K.G., Sherry-McKenna R.L., Mousseau D.D., McKenna K.F., Baker G.B. and Coutts R.T. (1992) Lack of effect of chronically administered monoamine oxidase inhibitors on ^{3}H-flunitrazepam binding sites in rat cortex. Proc. 15th Ann. Meet. Can. Coll. Neuropsychopharmacol., Saskatoon, Sask.

Tollefson G.D. (1983) Monoamine oxidase inhibitors: a review. J. Clin. Psychiat. 44: 280-288.

Trulson M.E. and Jacobs B.L. (1976) Behavioural evidence for the rapid release of CNS serotonin by PCA and fenfluramine. Eur. J. Pharmacol. 36: 149-154.

Tucker G.T., Lennard M.S., Ellis S.W., Woods H.F., Cho A.K., Lin L.Y., Miratsuka A., Schmitz D.A. and Chu T.Y. (1994) The demethylenation of methylenedioxymethamphetamine (Ecstasy) by debrisoquine hydroxylase (CYP2D6). Biochem. Pharmacol. 47: 1151-1156.

Tuomisto J. and Smith D.F. (1986) Effects of tranylcypromine enantiomers on monoamine uptake and release and imipramine binding. J. Neural Transm. 65: 135-145.

Tyrer P. (1979) Clinical use of monoamine oxidase inhibitors. In: Psychopharmacology of Affective Disorders, Paykel E.S. and Coppen A. (eds.), Oxford University Press, New York, pp. 159-178.

Valzelli I., Bernasconi S., Coen E. and Petkov V.V. (1980) Effects of different psychoactive drugs on serum and brain tryptophan levels. Neuropsychobiol. 6: 224-229.

van Nguyen T. and Juorio A.V. (1989a) Binding sites for brain trace amines. Cell. Mol. Neurobiol. 9: 297-311.

van Nguyen T. and Juorio A.V. (1989b) Down-regulation of tryptamine binding sites following chronic molindone administration. Naunyn-Schmied. Arch. Pharmacol. 340: 366-371.

van Praag H.M. (1984a) Depression, suicide and serotonin metabolism in the brain. In: Neurobiology of Mood Disorders, Post R.M. and Ballenger J.C. (eds.), Williams & Wilkins, Baltimore, pp. 601-618.

van Praag H.M. (1984b) Studies in the mechanism of action of serotonin precursors in depression. Psychopharmacol. Bull. 20: 599-602.

Vaughan D.A. (1988) Interaction of fluoxetine with tricyclic antidepressants. Am. J. Psychiat. 145: 1478.

Verhoeven W.M.A. (1994) Brofaromine in endogenous depression. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 18: 235-241.

Vetulani J., Stawarz R.J., Dingell J.V. and Sulser F. (1976) A possible common mechanism of action of antidepressant treatments. Reduction in the sensitivity of noradrenergic cyclic AMP generating system in the rat limbic forebrain. Naunyn-Schmied. Arch. Pharmacol. 293: 109-114.

Vetulani J. and Sulser F. (1975) Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP generating systems in limbic forebrain. Nature (Lond.) 257: 495-496.

Volosin M., Cancela L.M., Laino C., Massei M. and Molina V.A. (1991) Adrenocorticotropic hormone influences the development of adaptive changes in dopamine autoreceptors induced by chronic administration of desipramine. Neuropharmacol. 30: 719-725.

Volz H-P., Faltus F., Magyar I. and Moller H.J. (1994) Brofaromine in treatment-resistant depressed patients -- a comparative trial versus tranylcypromine. J. Affect. Dis. 30: 209-217.

Walsh B.T., Stewart J.W., Roose S.P., Gladis M. and Glassman A.H. (1984) Treatment of bulimia with phenelzine. Arch. Gen. Psychiat. 41: 1105-1109.

Weber H., Spahn H., Möhrke W. and Mutschler E. (1984) Pharmacokinetics of tranylcypromine enantiomers in healthy volunteers. J. Pharm. Pharmacol. 36: 50W.

Westlund K.N., Denney R.M., Kochersperger L.M., Rose R.M. and Abell C.W. (1985) Distinct monoamine oxidase A and B populations in primate brain. Science, 230: 181-183.

Weyler W. and Salach J.I. (1985) Purification and properties of mitochondrial monoamine oxidase type A from human placenta. J. Biol. Chem. 260: 13199-13207.

White K. and Simpson G. (1981) Combined MAOI tricyclic antidepressant treatment, a reevaluation. J. Clin. Psychopharmacol. 1: 264-282.

Wolf W.A., Youdim M.B.H. and Kuhn D.M. (1985) Does brain 5-HIAA indicate serotonin release or monoamine oxidase activity? Eur. J. Pharmacol. 109: 381-387.

Wong J.T.F. (1990) Analogues of β-phenylethylamine: effects on amino acids in the brain. Ph.D. Thesis, University of Alberta.

Wood P.L., Martin L.L. and Altar A.A. (1985) ^{3}H-Tryptamine receptors in rat brain. In: Neuropsychopharmacology of the Trace Amines, Boulton A.A., Maitre L., Bieck P.R. and Riederer P. (eds.), Humana Press, Clifton, N.J., pp. 101-114.

Wood P.L., Pilapil C., La Faille F., Nair N.P.V. and Glennon R.A. (1984) Unique ^{3}H-tryptamine binding sites in rat brain: distribution and pharmacology. Arch. Int. Pharmacodyn. Ther. 268: 194-201.

Wurtman R.J. and Axelrod J. (1963) A sensitive and specific assay for the estimation of monoamine oxidase. Biochem. Pharmacol. 12: 1439-1441.

Youdim M.B.H., Aronson J.K., Blau K., Green A.R. and Grahame-Smith D.G. (1979) Tranylcypromine ('Parnate') overdose: measurement of tranylcypromine concentrations and MAO inhibitory activity and identification of amphetamine in plasma. Psychol. Med. 9: 377-382.

Youdim M.B.H. (1975) Monoamine deaminating system in mammalian tissues. In: M.T.P. International Review of Science: Biochemistry, Blaschko H. (ed.), M.T.P., London, pp. 169-209.

Young S.N. (1984) Monoamine precursors in the affective disorders. In: Advances in Human Psychopharmacology, Vol. 3, Burrows G.D. and Werry J.W. (eds.), JAI Press Inc., Greenwich, CT, pp. 251-285.

Young S.N. (1991) Use of tryptophan in combination with other antidepressant treatments: a review. J. Psychiat. Neurosci. 16: 241-246.

Yu P.H. (1986) Monoamine oxidase. In: Neuromethods: Neurotransmitter Enzymes, Boulton A.A., Baker G.B. and Yu P.H. (eds.), Humana Press, Clifton, NJ, chapter 7.

Zall H. (1971) Lithium carbonate and isocarboxazid - an effective approach in severe depressions. Am. J. Psychiat. 127: 1400-1403.

Zeller E.A., Barsky J., Fouts J.R., Kircheimer W.F. and Van Orden L.S. (1952) Influence of isonicotinic acid hydrazide (INH) and 1-isonicotinyl-2-isopropyl hydrazide (IIH) on bacterial and mammalian enzymes. Experientia 8: 349-350.

Zhao Z., Castagnoli Jr. N., Ricaurte G.A., Steele T. and Martello M. (1992) Synthesis and neurotoxicological evaluation of putative metabolites of the serotonergic neurotoxin 2-(methylamino)-1-[3,4-(methylenedioxy)phenyl]-propane [(methylenedioxy)methamphetamine]. Chem. Res. Toxicol. 5: 89-94.

Zirkle C.L., Kaiser C., Tedeschi D.H., Tedeschi R.E. and Burger A. (1962) 2-Substituted cyclopropylamines. II. Effect of structure upon monoamine oxidase inhibitory activity as measured in vivo by potentiation of tryptamine convulsions. J. Med. Chem. 5: 1265-1284.