NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

UNIVERSITY OF ALBERTA

MECHANISMS OF ACTION OF SELECTED DRUG THERAPIES USED
IN THE TREATMENT OF REFRACTORY DEPRESSION

by

DAYAN BURKE GOODNOUGH

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
MEDICAL SCIENCES (PSYCHIATRY)

EDMONTON, ALBERTA
SPRING, 1994

ISBN 0-612-11222-5

Canada

UNIVERSITY OF ALBERTA

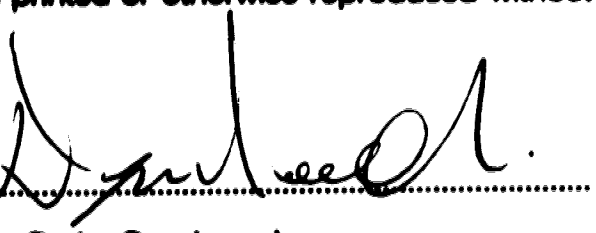
RELEASE FORM

NAME OF AUTHOR: Dayan Burke Goodnough

TITLE OF THESIS: Mechanisms of action of selected drug therapies used in the treatment of refractory depression

DEGREE FOR WHICH THESIS WAS PRESENTED: Doctor of Philosophy

YEAR THIS DEGREE GRANTED: 1994



Dayan Burke Goodnough
3808 - 107 Street
Edmonton, AB
T6J 2L4

DATED January 31, 1994

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 Mechanisms of action of selected drug therapies used in the treatment of refractory depression by DAYAN BURKE GOODNOUGH in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Medical Sciences (Psychiatry).

.....

W. McBlain

.....

G.B. Baker

.....

A.J. Greenshaw

.....

R.T. Coutts

.....

I.L. Martin

.....

D.F. LeGatt

.....

A. Byrne

.....

A.A. Boulton (External Examiner)

Date January 31, 1994

NOV 11-93 THU 13:27 P.02

University of Alberta
Edmonton

Canada T6G 2R7

Neurochemical Research Unit
Department of Psychiatry and
Faculty of Pharmacy and Pharmaceutical Sciences

Mailing Address: Department of Psychiatry
1E7.44 W Mackenzie Health Sciences Centre
Telephone (403) 492-7604/4891 Fax (403) 492-6841

November 8, 1993

Mr. Ronald E. McMillen
Director, Publications and Marketing
American Psychiatric Association
1400 K Street NW
Washington, DC 20005
U.S.A.

Dear Mr. McMillen,

By this letter I am requesting permission to include the following information from the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised* (DSM-III-R) in the introduction of my Ph.D. thesis entitled "Mechanisms of action of selected drug therapies used in the treatment of refractory depression". I will, of course, acknowledge the source of this material in my thesis.

1. Diagnostic Criteria for Major Depressive Episode (pages 222 and 223 of DSM-III-R).

2. Diagnostic Criteria for Melancholic Type and for Seasonal Pattern (page 224 of DSM-III-R).

Thank you for your consideration of this matter. I look forward to your reply.

Yours sincerely,

[signature]

Dayan B. Goodnough

Permission granted for one-time use. English language only. The correct citation for this book is: American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised, Washington, DC, American Psychiatric Association, 1987.

Ronald E. McMillen [signature] 11-19-93 N/A
Ronald E. McMillen — Date — Fee
Dir., Publications and Marketing

Please send a copy of this letter/form along with check payable to APA,
Attn: Publications and Marketing

ABSTRACT

Approximately 20% of depressed patients are refractory to conventional therapy, but some treatment strategies [*e.g.* high-dose tranylcypromine (TCP); TCP in combination with amitriptyline (AMI); and desipramine (DMI) in combination with fluoxetine (FLU)] have been shown to be relatively effective in the treatment of these refractory depressed patients. Chronic studies were performed to investigate some of the biochemical changes that occurred in rats treated with these novel treatments. High dose TCP (2.5 mg/kg/day) produced significantly greater levels of noradrenaline (NA) and serotonin (5-HT) in brain and a greater and more rapid decrease in ^{3}H-tryptamine binding in striatum and hippocampus, respectively, than did the low dose (0.5 mg/kg/day). Furthermore, the high, but not the low, dose resulted in a down-regulation of cortical 5-HT$_2$ receptors. When the combination of AMI (3.5 mg/kg/day) and TCP (0.5 mg/kg/day) was compared to the drugs alone, an additive effect was observed. TCP alone elevated brain levels of NA and 5-HT but had no effect on 5-HT$_2$ receptor density in cortex and AMI alone down-regulated cortical 5-HT$_2$ receptors but had no effect on NA and 5-HT levels in brain. In the combination an elevation of NA and 5-HT and a down-regulation of 5-HT$_2$ receptors were observed, but these changes were no greater than those expected from the actions of the individual drugs alone. The combination of DMI (5 mg/kg/day) and FLU (10 mg/kg/day) resulted in higher cortical levels of DMI, FLU, and norfluoxetine (NFLU) than when the drugs were administered alone. DMI alone down-regulated 5-HT$_2$ and β-adrenergic receptors in cortex, but FLU alone had no effect on the density of either receptor. When the drugs were administered in combination the 5-HT$_2$ down-regulation produced by DMI was blocked and the β-adrenergic down-regulation produced by DMI was enhanced. The higher cortical DMI levels in the combination appears to be responsible for the enhanced down-regulation of β-adrenergic receptors, and it is proposed that high levels of FLU present in the combination may displace DMI from the 5-HT$_2$ receptor and block DMI from down-regulating the 5-HT$_2$ receptor.

ACKNOWLEDGEMENTS

There are many individuals and agencies who played key roles in the completion of this thesis.

First and foremost I would like to thank my family. Without the love, patience, selflessness, and understanding of my wife Catherine and the love and joy of our children Jessica, Kya, and Alyx this endeavor would have been a much less enjoyable one.

I thank Dr. Glen B. Baker for allowing me the freedom to learn, make mistakes, and work towards this goal in a flexible manner. I would also like to thank Dr. Baker for being accessable, attentive, constructive and expedient in all matters relating to the research and writing of this thesis.

Drs. Andrew J. Greenshaw, Ronald T. Coutts, and Mathew T. Martin-Iverson were very helpful in many aspects of the work presented in this thesis. Their contributions are much appreciated.

The technical and computer orientated aspects of this thesis were learned from the individuals who know the inner workings of the laboratory and the equipment well enough to answer the many questions asked of them. These individuals are: Gail Rauw; Jordyce van Muyden; and Sally Omura.

Funding for this work was provided by generous research scholarships administered by Alberta Mental Health Research Fund and The Faculty of Medicine's 75th Anniversary Scholarship Fund. Travel funds to attend scientific meetings were provided by the Department of Psychiatry and the Faculty of Graduate Studies and Research.

Finally, I would like to wish the best of fortune on the graduate students currently working towards their Ph.D. degrees and those who are just beginning their work. I hope that they experience the satisfaction that I have been able to enjoy.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMI	amitriptyline
ANOVA	analysis of variance
B_{max}	maximum density of binding sites (in fmol mg^{-1} protein)
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
CSF	cerebrospinal fluid
d	day
DA	dopamine
DAG	diacylglycerol
DMI	desmethylimipramine; desipramine
DOPAC	3,4-dihydroxyphenylacetic acid
5,7-DHT	5,7-dihydroxytryptamine
DSP4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine
ECD	electron capture detection(or)
ECT	electroconvulsive shock therapy
eV	electron volt
FLU	fluoxetine
fmol	femtomole (10^{-15} moles)
g	gram
G protein	guanine nucleotide binding regulatory protein
GABA	γ-aminobutyric acid
GC	gas chromatography
h	hour
5-HIAA	5-hydroxyindole-3-acetic acid
HP	Hewlett Packard
HPLC	high-pressure liquid chromatography
5-HT	5-hydroxytryptamine; serotonin
HVA	homovanillic acid
i.d.	internal diameter
i.p.	intraperitoneally
IP_3	inositol triphosphate

K_d	dissociation constant (in nM)
kg	kilogram
K_i	inhibition constant (in nM)
KPa	kilopascals
l	liter
M	molar
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MAP	maprotiline
mg	milligram
MHPG	3-methoxy-4-hydroxyphenylglycol
min	minute
ml	milliliter
mm	millimeter
mM	millimolar
ms	millisecond
MS	mass spectrometry
NA	noradrenaline
ng	nanogram
nM	nanomolar
NFLU	norfluoxetine
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetraline
PEA	2-phenylethylamine
s	second
SSUI	selective serotonin uptake inhibitor
TCA	tricyclic antidepressant
TCP	tranylcypromine
Tris	Tris buffer
VEH	vehicle
VMA	vanillylmandelic acid
µl	microliter
µM	micromolar
°C	degree Celsius

1 INTRODUCTION

Lifetime prevalence of major depression has been reported to be between 3.7 and 6.7 percent in the general population (Robins *et al.* 1984). Furthermore, upwards of 21 percent of patients with major depression who seek treatment have not recovered after two years of treatment (Keller *et al.* 1984). This high non-response rate represents a substantial proportion of the depressed population, and many strategies have been used in the treatment of these refractory depressives, with varying degrees of success. The biochemical changes elicited by these novel treatment regimes which are not observed with traditional treatments may give meaningful insights into the biochemical deficits present in refractory depression.

1.1 DEPRESSION

Depression was classified as part of manic-depressive insanity by Kraepelin in 1921. This classification was obviously vague as it combined mild mood swings with recurring mania and melancholia. Later the American Psychiatric Association published the Diagnostic and Statistical Manual of Mental Disorders (DSM) in 1952. This manual was later updated in 1968, 1980, and 1987. The latest version is a revised version of the third printing and is thus called DSM-III-R. In the DSM-III-R, depression is placed in the category of mood disorders. The disorders listed in this category are identified in Table 1. Mood disorders are subdivided into Bipolar Disorders and Depressive Disorders. If a patient satisfies the criteria for depression with no history of mania then that patient is classified as having a Depressive Disorder. Depressive disorder is further divided into Major Depression or Dysthymia. Dysthymia is considered a milder, but more chronic disorder, with patients having a depressed mood more days than not

Table 1. MOOD DISORDERS

BIPOLAR DISORDERS

- Bipolar Disorder
- Cyclothymia
- Bipolar Disorder Not Otherwise Specified

DEPRESSIVE DISORDERS

- Major Depression
- Dysthymia
- Depressive Disorder Not Otherwise Specified

(Based on the classification scheme of *The Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised,* Washington, D.C., American Psychiatric Association, 1987.)

for at least a two year period without satisfying the criteria for a Major Depressive Episode over that period. Major Depression is classified as either recurrent or single episode. The criteria used to diagnose a Major Depressive Episode are listed in Table 2. A Major Depressive Episode can be further characterized as: 1. Mild (only a minor impairment of regular daily activities); 2. Moderate (impairment somewhere between mild and severe); 3. Severe, without psychotic features (symptoms are in excess of those needed for diagnosis and markedly interfere with regular daily functioning); 4. Severe, with mood-congruent psychotic features (delusions or hallucinations consistent with depressive themes); 5. Severe, with mood-incongruent psychotic features (delusions or hallucinations inconsistent with depressive themes); 6. In partial remission (between mild and in full remission); 7. In full remission (no significant symptoms over the last 6 months). The classification scheme for a Major Depressive Episode also allows for chronic, melancholic, and seasonal variations; these classifications are identified in Table 3.

There are four major classes of antidepressants used in the treatment of depression: tricyclic antidepressants (TCAs); monoamine oxidase inhibitors (MAOIs); selective serotonin uptake inhibitors (SSUIs); and novel antidepressants (*i.e.* antidepressant drugs which do not fit into the above categories). These treatments are often augmented with thyroid hormones, tryptophan, or lithium. Studies employing these drug classes have led to a much greater understanding of the biochemical changes associated with depression and the drugs are invaluable tools in the search for common etiological factors associated with depression.

Table 2. DIAGNOSTIC CRITERIA FOR A MAJOR DEPRESSIVE EPISODE

A. At least five of the following symptoms have been present during the same two-week period and repre nt a change from previous functioning; at least one of the symptoms is either (1) depressed mood, or (2) loss of interest or pleasure.
   1. depressed mood most of the day, nearly every day
   2. markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day
   3. significant loss or gain in weight when not dieting
   4. insomnia or hypersomnia nearly every day
   5. psychomotor retardation or agitation nearly every day
   6. fatigue or loss of energy nearly every day
   7. feelings of worthlessness or excessive or inappropriate guilt nearly every day
   8. diminished ability to think or concentrate, or indecisiveness, nearly every day
   9. recurrent thoughts of death, recurrent suicidal ideation

B. 1. It cannot be established that an organic factor initiated and maintained the disturbance.
   2. The disturbance is not a normal reaction to the death of a loved one.

C. At no time during the disturbance have there been delusions or hallucinations for as long as two weeks in the absence of prominent mood symptoms.

D. Not superimposed on Schizophrenia, Schizophreniform Disorder, Delusional Disorder, or Psychotic Disorder.

(Taken from *The Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised*, Washington, D.C., American Psychiatric Association, 1987.)

Table 3. SUBCLASSIFICATION OF A MAJOR DEPRESSIVE EPISODE

Chronic: Current episode has lasted longer than two consecutive years without a period of two months or more during which there were no significant depressive symptoms.

Melancholic: The presence of at least five of the following:

1. loss of interest or pleasure in all, or almost all, activities
2. lack of reactivity to usually pleasurable stimuli
3. depression regularly worse in the morning
4. early morning awakening
5. psychomotor retardation or agitation
6. significant anorexia or weight loss
7. no significant personality disorders before first Major Depressive Episode
8. one or more previous Major Depressive Episodes followed by complete, or nearly complete, recovery
9. previous good response to specific and adequate somatic anti-depressant therapy

Seasonal:

A. There has been a regular temporal relationship between the onset of Recurrent Major Depression and a particular 60 day period of the year.

B. Full remissions also occur within a particular 60 day period of the year

C. There have been at least three episodes of mood disturbance in three separate years that demonstrated the temporal seasonal relationship; at least two of the years were consectutive.

D. The seasonal episodes outnumber any nonseasonal episodes by more than three to one.

(Taken from *The Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised*, Washington, D.C., American Psychiatric Association, 1987.)

1.2 TRICYCLIC ANTIDEPRESSANTS

The first tricyclic antidepressant (TCA) introduced as a drug for the treatment of depression was originally synthetized and tested for antipsychotic applications. Imipramine, a derivative of chlorpromazine, was found to be ineffective in quieting agitated psychotic patients but was of great benefit to the depressed patients of the study (Kuhn, 1957). There are nine compounds structurally related to imipramine currently being used as antidepressants; desipramine (DMI), amitriptyline (AMI), clomipramine, nortriptyline, doxepin, trimipramine, protriptyline, maprotiline (a tetracyclic antidepressant), and amoxapine.

The major routes of metabolism of the TCAs are: 1. N-demethylation; 2. hydroxylation of the ring aliphatic and phenyl components; 3. glucuronide formation at hydroxylation sites (Gram, 1974); and 4. side chain N-oxidation (Crammer *et al.*, 1969). Glucuronide formation is the major inactivation/excretion mechanism (Potter *et al.*, 1984). The other three major routes produce metabolites that retain uptake inhibition properties (Bertilsson *et al.*, 1979; Potter *et al.*, 1979).

Most metabolism of the TCAs occurs in the liver, and accordingly liver function plays an important role in the regulation of levels of these drugs (Wilkinson and Shand, 1975). Alcohol (Iber, 1977), tobacco (Vahakangas *et al.*, 1983), barbiturates (Gillette, 1971), and anticonvulsants (Eichelbaum *et al.*, 1975) may stimulate liver enzymes, resulting in decreased plasma levels of the parent TCAs when these drugs are administered concomitantly. Phenothiazines (Gram, 1977), methylphenidate (Wharton *et al.*, 1971), cimetidine (Miller and Maldin,

1984), and oral contraceptives (O'Malley *et al.*, 1972) are metabolized by the same liver enzymes as the TCAs, and when administered in combination this metabolic competition can result in elevated levels of both drugs. Factors which influence TCA levels may have an important impact on the clinical efficacy of treatment with these antidepressants.

Most of the classical TCAs potentiate the action of the neurotransmitter amines noradrenaline (NA) and/or 5-hydroxytryptamine (5-HT) by inhibiting the uptake of these amines from the synaptic cleft into the presynaptic neuron (Carlsson *et al.*, 1969a, b; Shaskan and Snyder, 1970; Lidbrink *et al.*, 1971; Ross *et al.*, 1972), the primary mechanism of their inactivation (Iversen, 1971). However, the TCAs possess different uptake inhibition profiles. The tertiary amines (imipramine, amitriptyline, and clomipramine) inhibit both NA and 5-HT but are relatively more potent at inhibiting 5-HT uptake. The secondary amine metabolites (DMI, nortriptyline, and desmethylclomipramine) are much more potent than the corresponding tertiary amines at inhibiting NA uptake (Baker and Greenshaw, 1989).

The effects of the TCAs are not limited to their actions on the uptake of 5-HT and NA. Many are potent 5-HT_2 receptor antagonists (Baker and Greenshaw, 1989), a factor which may contribute to their overall efficacy (Deakin, 1991). Side effects commonly seen due to muscarinic cholinergic receptor blockade are blurred vision, constipation, urinary retention, dry mouth, and excessive sweating (Snyder and Yamamura, 1977). α_2-Adrenergic receptor blockade can cause tachycardia, hypo/hypertension, arrythmias, and sedation (Klein *et al.*, 1980; Snyder and Peroutka, 1984), and H_1-histaminergic receptor blockade can cause

sedation and hypotension (Snyder and Peroutka, 1984; Paul *et al.*, 1985; Richelson, 1978). Skin rashes and an increased sensitivity to sunlight have also been reported (Abramowicz, 1980).

1.3 MAO INHIBITORS

As with the TCAs, the efficacy of MAO inhibitors (MAOIs) in depression was discovered serendipitously. The antituberculosis drug iproniazid was found to have mood elevating effects in depressed tuberculosis patients (Selikoff *et al.*, 1952). Based on this discovery other MAOIs were synthesized. These included: phenelzine, tranylcypromine (TCP), nialamide, and pheniprazine. The two most commonly prescribed MAOIs are phenelzine and tranylcypromine (Dubovsky, 1987); both are irreversible and nonselective. Other MAOIs were later developed which exploited the fact that there are two isozymes of MAO, denoted A and B (Johnson, 1968). Clorgyline and deprenyl are selective, irreversible MAO-A and -B inhibitors respectively (Johnson, 1968; Knoll *et al.*, 1965), while brofaromine and moclobemide are selective, reversible inhibitors of MAO-A (Delini-Stula *et al.*, 1988).

MAO was first characterized in 1928 when an enzyme that oxidized tyramine was found in rabbit liver and named tyramine oxidase (Hare, 1928). It was later noted that other amine-containing compounds could also be oxidized and the name was changed to amine oxidase (Blaschko *et al.*, 1937). The present name was introduced by Zeller in 1951 who categorized the amine oxidases into diamine oxidase and MAO. MAO was subsequently subdivided into MAO-A and MAO-B according to the affinity of these isozymes for certain substrates. MAO-A preferentially oxidizes 5-HT and to a lesser extent NA and adrenaline, whereas

MAO-B is more specific for benzylamine and β-phenethylamine (Murphy *et al.*, 1985). The characterization of MAO-A and -B extends further than substrate specificities alone. While most tissues contain both MAO-A and -B, regional differences are present. In the brain, MAO-A is preferentially located in areas containing catecholamine cell bodies (Westlund *et al.*, 1985; Thorpe *et al.*, 1987; Saura Marti *et al.*, 1990) and MAO-B is preferentially located in areas rich in 5-HT-containing cell bodies (Saura Marti *et al.*, 1990), astrocytes and radial glia cells (Westlund *et al.*, 1985; Thorpe *et al.*, 1987). Peripherally, only MAO-A is found in placental tissue (Salach and Detmer, 1979) and only MAO-B is found in platelets (Donnelly and Murphy, 1977) and lymphocytes (Bond and Dundell, 1977). Clearly, inhibition of MAO subtypes will play different roles in different areas depending on the subtype inhibited.

The most effective MAO-inhibiting antidepressants are inhibitors of MAO-A or MAO-A and -B (Laux, 1993). Deprenyl, a selective MAO-B inhibitor is not a particularly effective antidepressant at doses where only MAO-B is inhibited (Mann *et al.*, 1980), but has been shown to have some efficacy in depressive disorders at higher doses where both MAO-B and MAO-A are inhibited (Mann and Gershon, 1980; Mendlis *et al.*, 1981; Mann *et al.*, 1982; Mendlewicz and Youdim, 1983; Quitkin *et al.*, 1984; Mann *et al.*, 1989; McGrath *et al.*, 1989). Inhibition of MAO results in the elevation of brain levels of 5-HT, NA, and dopamine (DA) and a concomitant reduction in the metabolites of these biogenic amines [5-HIAA (5-hydroxyindoleacetic acid), DOPAC (3,4-dihydroxyphenylacetic acid), HVA (homovanillic acid), MHPG (3-methoxy-4-hydroxyphenylglycol), and VMA (vanillylmandelic acid)] (Campbell *et al.*, 1979; O'Regan *et al.*, 1987). Increased

levels of these biogenic amines presynaptically results in higher levels of neurotransmitter being released upon neuronal stimulation, ultimately leading to a greater postsynaptic response.

The side-effect profile of MAOIs consists of orthostatic hypotension with dizziness, weight gain, sexual dysfunction, edema, insomnia, daytime sedation, myoclonus, and dry mouth (Murphy *et al.*, 1985). Potentially lethal hypertensive reactions and headaches have also been documented in patients concomitantly taking foods containing sympathomimetic amines and led to the expulsion of these agents from the market for a short period of time in the mid sixties (review: McDaniel, 1986). When MAOIs reentered the market, strict dietary restrictions were placed upon those patients taking the drugs.

In addition to inhibiting the metabolism of 5-HT, NA, and DA, MAOIs also inhibit the metabolism of trace amines and may cause profound elevations in brain levels of these amines (β-phenethylamine, octopamine, tryptamine, tyramine, and N-methylhistamine) (Philips and Boulton, 1979; Philips *et al.*, 1980; Baker *et al.*, 1985). Tyramine can be especially dangerous as high levels of this trace amine can displace NA from storage vesicles. This effect combined with the already higher levels of NA present due to inhibition of MAO can result in headaches and in severe cases a hypertensive crisis (Baldessarini, 1989). The classical MAOIs TCP and phenelzine and the selective irreversible MAO-A inhibitor clorgyline all evoke an increased sensitivity to tyramine in healthy volunteers. Deprenyl (an irreversible MAO-B inhibitor) and the reversible MAO-A inhibitors brofaromine and moclobemide have a much lower sensitivity to ingested tyramine (Da Prada *et al.*, 1989; Rudorfer and Potter, 1989). The potentially dan-

gerous effects can result from the ingestion tyramine-rich foods (aged cheese, red wine, chocolate, pickled or smoked meat or fish, fabe beans, and yeast products) since the deamination of tyramine by MAO in the gut is inhibited by MAOIs. Proper dietary considerations can effectively eliminate the occurrence of these side effects. It is important to note that although hypertensive side effects are a possibility, they occur very rarely (Nies, 1983; Raskin, 1972; Laux, 1993) and that overall side effect incidence for MAOIs is similar to that of the TCAs (Robinson *et al.*, 1978; Nies, 1983).

MAOIs have come to be used predominantly for atypical depression (Himmelhoch *et al.*, 1982, 1991; Thase *et al.*, 1992) even though their efficacy in typical depression has been suggested to be equal to that of the TCAs (Murphy *et al.*, 1987). Atypical depression is generally characterized by reversed vegetative symptoms (*i.e.* hypersomnia, weight gain, and elevation of mood in the morning) (Sovner, 1981; Murphy *et al.*, 1987). Anxiety, panic, anergia, and hysteroid dysphoria can also be present in "atypical depression" (Sargent, 1962; Kelly, 1973; Himmelhoch *et al.*, 1982).

MAOIs have also been proposed to be effective in the treatment of other psychiatric disorders such as: seasonal affective disorder (Rosenthal *et al.*, 1987); obsessive compulsive disorder (Jenike *et al.*, 1983); phobic disorders and panic attacks (Sheehan *et al.*, 1980; Pohl *et al.*, 1992; Nutt and Glue, 1989); general anxiety (Sargent and Daly, 1962; Sheehan, 1994); bulimia (Walsh *et al.*, 1984); and migraine (Anthony and Lance, 1989).

1.4 SELECTIVE 5-HT UPTAKE INHIBITORS

The mounting evidence supporting decreased 5-HT function in depression fueled the search for compounds that selectively enhanced 5-HT function. Zimelidine entered the market in 1982 as the first selective 5-HT uptake inhibitor (clomipramine, imipramine, and AMI cannot be considered selective because their major metabolites are NA uptake inhibitors). It was taken off the market in 1983 due to a high incidence of hypersensitivity reactions (Bech, 1988). Many SSUIs are currently being marketed or are still under clinical investigation, such as: fluoxetine (FLU), fluvoxamine, paroxetine, sertraline citalopram and femoxetine (Feighner and Boyer, 1991; Warrington, 1992; Gram *et al.*, 1993). These selective 5-HT uptake inhibitors differ from the 5-HT uptake inhibiting TCAs in that neither they nor their metabolites (*i.e.* the N-demethylated metabolite of FLU, norfluoxetine (NFLU), is also a selective 5-HT uptake inhibitor) have an appreciable effect on NA uptake (Wong *et al.*, 1974; Claassen *et al.*, 1976; Squires, 1974; Hyttel, 1982). The popularity of these agents rests not only in their clinical efficacy but in the lower incidence of side effects (Warrington, 1992).

1.5 NOVEL ANTIDEPRESSANTS

Novel antidepressants are antidepressants which do not fit into the above-mentioned categories. These compounds do not inhibit neuronal uptake of neurotransmitter amines or MAO activity appreciably, yet are effective in treating depression. A lower incidence of anticholinergic side effects and cardiotoxicity is commonly observed (Damiouji *et al.*, 1985), making novel antidepressants popular for use in the elderly. Iprindole, mianserin, alprazolam,

adinazolam, and trazodone are novel antidepressants currently being marketed. Rudorfer and Potter (1989) have recently reviewed the actions and side effects of novel antidepressants.

1.6 TREATMENT STRATEGIES FOR REFRACTORY DEPRESSION

Depression affects a large number of systems in the central nervous system which are important for normal everyday functioning. Whether the expression of symptoms in depressed patients results from a single biological disturbance or many is not clear. What is clear is that the antidepressants that are available achieve their results by a variety of mechanisms. Refractory depression has been defined as a depressive episode which has failed to respond to two 4-week trials of different antidepressants at full therapeutic doses (Davidson, 1985), although others have suggested that a longer duration of treatment is necessary before a drug trial can be considered to ineffective (Quitkin *et al.*, 1988). The following treatment strategies have been used in the treatment of refractory depression.

1.6.1 THE COMBINATION OF TCAS AND MAOIS

There exists a considerable body of literature on the combination of TCAs and MAOIs in the treatment of depression. White and Simpson (1981) has provided an excellent review on the reports from which the fear over this combination originated.

"The major cause for alarm over the hazards of combining MAOIs with tricyclics derives from a body of about 40 clinical reports, generally of single cases, involving more or less severe adverse reactions experienced by patients receiving the two types of drugs together or in rapid sequence. The

preponderance of these reports appeared in the early and middle 1960's, generally preceding the publication of large clinical series on the outcome of such treatment, and preceding the publication of guidelines for its use. Therefore, it remains unclear what manner of common clinical practice gave rise to many of these adverse experiences. Moreover, useage of these combinations has continued, particularly in England, whereas case reports of adverse reactions have ceased to appear since 1973."

There are 7 major published studies involving MAOIs in combination with TCAs (Davidson *et al.*, 1978; Gander and Lond, 1965; Razani *et al.*, 1983; Schmaues *et al.*, 1968; Sethna, 1974; White *et al.*, 1980; Young *et al.*, 1979).

Davidson *et al.* (1978) compared the efficacy of a phenelzine-AMI combination with electroconvulsive therapy (ECT) in 17 patients. The average dose in the drug combination was 71 mg of AMI and 34 mg of phenelzine per day. The criterion for refractory depression was for the patient to have been "treated unsuccessfully with conventional psychotropic drugs in clinical doses" in the past. Of the 17 patients, 9 received ECT while 8 received drug therapy. Only one of patients in the drug group improved enough to be discharged from the hospital. No mention was made of how many of the ECT patients were discharged. Depression score ratings showed that ECT was far superior to the drug combination. However, it can be argued that the dose of TCA was inadequate.

Sethna (1974) studied 12 patients who had not responded, or who had consistently relapsed after MAOI, TCA, and ECT treatment alone. The patients were treated with a combination of AMI (75mg/day) and phenelzine (45mg/day). Of the 12, 9 became virtually free of depressive symptoms.

Gander and Lond (1965) presented a paper in which 90 patients who, in the last year, had not responded to antidepressant treatment (MAOI, TCA, ECT, or psychotherapy). The patients were treated with a variety of combinations. The three most common combinations were: 1. phenelzine and AMI (45 patients); 2. isocarboxazid and AMI (18); and 3. ipronazid and AMI (12). The average dose, although not specified, was stated to be just slightly less than the individual therapeutic dosage, *i.e.* 150 mg/day for AMI and 45mg/day for phenelzine. Of the patients, 49 showed considerable to complete resolution, 13 showed some improvement, and 28 showed no improvement.

The most robust study on TCA-MAOI combination therapy in refractory depression was conducted by Schmauss *et al.* (1983). A total of 94 patients were given one of nine TCA-tranylcypromine (TCP) combinations. The patients had previously not responded to two TCA trials of three to four weeks each. The three most common TCAs used were AMI (37 patients), imipramine (20 patients), and dibenzepine (14 patients). The TCA was administered for at least three weeks and then TCP was started at 10mg/day up to a maximum of 30mg/day. The TCA dosage remained constant throughout the study. The average doses were AMI (180mg/day), imipramine (222mg/day), dibenzepine (467mg/day), and TCP (13mg/day). Of the patients, 31% demonstrated very good improvement (no depressive symptomatology) and 37% demonstrated

good improvement (little depressive symptomatology) with the combined treatment. The AMI-TCP combination proved to be the best combination, with 78% showing good to very good improvement.

The four papers cited above are the only studies in which the patients had to satisfy criterion for refractory depression. MAOI treatment was added to an ongoing TCA treatment and a period when the patients received only MAOI treatment was not present. We do not know what the response to the MAOI alone would have been. These four studies, therefore, do not strictly concern combined treatment with MAOIs and TCAs, but actually the augmentation of TCA treatment with MAOIs.

Of these four studies, the combination of a MAOI and a TCA was considered effective in the treatment of refractory depression in three. The only study in which the combination showed no benefit (Davidson *et al.*, 1978) an inadequate dose of the TCA was used. It appears that augmentation of a TCA treatment (in the traditional dose range) with MAOIs may be a successful therapy in the treatment of refractory depression.

In the three other studies on the TCA-MAOI combination (Razani *et al.*, 1983; White *et al.*, 1980; Young *et al.*, 1979), the patient samples did not, unfortunately, have to meet a criterion for refractory depression; however, in each study groups receiving only a TCA, only a MAOI and both a TCA and a MAOI were present. Each study showed that the drug combination was no more effective than each of the drugs alone in the general depressed population. This is not unexpected in that there is a high success rate of the

drugs alone in the general depressed patient population. The combination of the antidepressants is not commonly used until the drugs alone prove to be ineffective.

MAOIs and TCAs operate by two different mechanisms at the biochemical level. MAOIs inhibit the metabolic breakdown of amines, while TCAs inhibit the uptake of these amines (most commonly NA and 5-HT) into the presynaptic neuron. Although different mechanistically, the action of the two drug classes is to enhance those processes that result from the action of these amines on the postsynaptic neuron. When a MAOI and a TCA are administered together there is both inhibition of biogenic amine uptake and higher biogenic amine concentrations. There is also evidence that the combination of AMI with TCP may actually decrease the tyramine pressor response observed when TCP is administered to healthy volunteers (Pare *et al.*, 1982). The ability of TCAs to act as antagonists at 5-HT_2 receptors (Baker and Greenshaw, 1988) is often overlooked and may be an important contributing factor in the efficacy of the above-mentioned combination.

Much of the literature has been concerned with the side effects seen when the antidepressants are used in combination. In the three studies in which patients received one of the drugs used in the drug combination alone or the combination of these drugs (*i.e.* the drug combination was compared to suitable controls), no significant difference in side effects between the drugs alone and the combination was observed (Razani *et al.*, 1983; White *et al.*, 1980; Young *et al.*, 1979). In the other studies, 4/94 patients in the Schmaus *et al.* (1988) study, 0/8 in the Davidson *et al.* (1978) study, 7/90 in the Gander

and Lond (1965) study, and 0/12 in the Sethna (1974) study discontinued treatment due to side effects, in no instance were the side effects considered life-threatening.

"The typical picture of the adverse MAOI-TCA reaction involves agitated delirium, often progressing to coma, generalized hypertonicity, seizures, hyperpyrexia, and variable elevation of pulse and respiratory rate. This pattern is non-specific and can readily result from overdosage of either type of drug alone." (White and Simpson, 1961).

1.6.2 THE COMBINATION OF TCAs AND SSUIs

There are only two major studies which address the efficacy of combining a SSUI and a TCA in depression (Wellburg *et al.*, 1989b; Nelson *et al.*, 1991); several case reports (Seth *et al.*, 1992; Schrami *et al.*, 1989; Downs *et al.*, 1989; Bell and Cole, 1988; de Maso and Hunter, 1990; Elson, 1989) are also present in the literature.

The Wellburg *et al.* (1989b) study involved the addition of FLU to ongoing, but clinically unsatisfactory TCA treatment. Retrospectfully, it was found that 26 of 30 patients improved with the combination. The study was not controlled and a group receiving FLU alone was not present. However, when the TCA was discontinued in 12 of the 26 responders, 8 relapsed; these 8 recovered when the TCA was reinstated.

Seth *et al.* (1992) reported 8 case studies in which the combination of a predominantly NA uptake inhibiting TCA (nortriptyline) with a SSUI (sertraline or FLU) proved more effective than either drug alone. Overall, the other case reports a good therapeutic response with the drug combination.

The lag between initiation of antidepressant treatment and therapeutic response has been difficult to explain and is definitely a hindrance to patient compliance. A study by Nelson *et al.* (1991) examines this issue. The combination of FLU and DMI was compared to DMI alone. Decreases in Hamilton depression ratings were compared each week for 4 weeks. The combination was approximately twice as effective as DMI alone each week; furthermore, the decrease in depression scores seen at week 1 in the combination was not attained in the DMI alone group until week 4.

Inhibition of biogenic amine uptake is considered to be the major biochemical effect of TCA treatment, and often the potency of TCAs as 5-HT_2 antagonists is overlooked. The 5-HT receptor imbalance theory of affective disorder proposed by Deakin *et al.* (1991) suggests that a successful antidepressant has to enhance 5-HT_{1A} receptor-mediated effects and/or inhibit 5-HT_2 receptor-mediated effects. Drugs which increase 5-HT levels in the synapse (uptake inhibitors and MAOIs) selectively enhance 5-HT_1 receptor-mediated effects because 5-HT has higher affinity for 5-HT_1 receptors than most other 5-HT receptors. To inhibit 5-HT_2 neurotransmission a drug must be an antagonist at that site or create chronically high concentrations that desensitize the 5-HT_2 receptor over time (*i.e.* MAOIs). Most of the TCAs have been shown to be potent 5-HT_2 antagonists (Baker and Greenshaw, 1989). Therefore the combination of DMI or nortriptyline with a SSUI should act synergistically, the former by inhibiting 5-HT_2 mediated responses and the latter by enhancing 5-HT_1 mediated responses. This postulated synergism may be responsible for the rapid antidepressant

response observed.

1.6.3 LITHIUM AUGMENTATION OF ANTIDEPRESSANT TREATMENT

Lithium continues to be one of the most studied drugs used in psychiatry. There are many studies which explore the augmentation of TCA treatment with lithium carbonate in refractory depression, 4 of which (Price *et al.*, 1986; de Montigny *et al.*, 1985; Heninger *et al.*, 1983; Lingjaerde *et al.*, 1974) are discussed in some detail below. de Montigny *et al.* (1988) and Schopf (1989) provide excellent reviews on this topic.

Price *et al.* (1986) studied 84 patients who were classified as major depressive and had previously not responded to TCA treatment. Lithium (900-1500mg/day) was added to ongoing TCA treatment, with blood lithium levels being between 0.5-1.3 meq/liter. No mention was made as to whether a change in TCA dosage occurred. Of the patients, 56% showed partial to marked improvement and 44 % showed no improvement.

Heninger *et al.* (1983) treated 32 patients in a double blind manner with AMI, DMI, or mianserin. Of these patients, 15 proved to be refractory. These 15 were then treated in a double blind manner with either lithium or placebo. Significant decreases in depression scores were seen on days 1 and 2 and after day 7. At the end of the study lithium was administered to the placebo group and the response was identical to that of the original lithium group.

De Montigny *et al.* (1985) studied 7 patients from an original sample of 30. These 7 had not responded to iprindole treatment. Lithium was added (mean serum level of 0.74meq/liter). All 7 patients showed marked decreases in their Hamilton depression scores, with an average reduction of 68%.

The study by Lingjaerde *et al.* (1974) involved 45 patients from 9 hospitals who were refractory to TCA treatment. The patients were given either lithium or placebo in addition to the TCA. The results were inconclusive and lithium enhancement of TCA treatment, if present, was slight.

The augmentation of TCA treatment with lithium is definitely effective in turning some TCA-nonresponders into responders. Smaller studies and case reports (reviews: de Montigny *et al.*, 1988; Schopf, 1989) also suggest that lithium is effective in augmenting TCA treatment.

There are many reports of lithium augmentation of MAOI treatment (Fein *et al.*, 1988; Himmelhoch *et al.*, 1972; Joyce *et al.*, 1983; Louie and Meltzer, 1984; Madakasira, 1986; Nelson and Byck, 1982; Price *et al.*, 1985; Tariot *et al.*, 1988; Zall, 1971). While many of these are case studies or collections of case studies, two are major studies (Himmelhoch *et al.*, 1972 and Price *et al.*, 1985). These two groups studied patients that had proven to be TCA nonresponders. In both studies TCP was added to ongoing lithium treatment, but was never given alone. In the Himmelhoch *et al.* (1972) report, 11/21 patients had complete remission, and 5/21 showed substantial improvement. In the Price *et al.* (1985) study, 11/12 showed improvement. We do not know whether the results obtained were due to the combination, or if the MAOI would have given a similar response on its own.

Collectively, the other 7 papers cite 15 case reports. Of these cases, 14 showed improvement. Eleven had not responded to the MAOI alone, 3 were not given the MAOI alone, and one did not respond to the addition of lithium.

1.6.4 TRYPTOPHAN AUGMENTATION OF ANTIDEPRESSANT TREATMENT

There are three studies that deal with the combination of tryptophan and TCAs (Lopez-Ibor *et al.*, 1973; Shaw *et al.*, 1972; Walinder *et al.*, 1976), but these three studies did not deal specifically with refractory depression. Lopez-Ibor *et al.* (1973) and Shaw *et al.* (1972) both found that tryptophan did not significantly improve the clinical status of the TCA-treated group. Walinder *et al.* (1976) reported that the tryptophan-treated group showed a 74 percent decrease in depression rating whereas the placebo group showed only a 46 percent decrease. The TCA used in the Shaw *et al.* (1972) and Walinder *et al.* (1976) studies was clomipramine. If augmentation of TCAs occurs with tryptophan, one would expect to see it with clomipramine, a potent 5-HT uptake inhibitor. However, the two reports are conflicting and it is unlikely that the tryptophan-TCA combination is helpful in treating general cases of depression. Reports of this combination in the treatment of refractory depression have yet to be published, so its efficacy in this area is unknown.

There are four papers on the efficacy of the MAOI-tryptophan combination in refractory depression (Coppen *et al.*, 1963; Pare, 1963; Glassman and Platman, 1969; Gutierrez and Lopez-Ibor, 1971). Coppen *et al.* (1963) reported a study involving 25 patients treated with either TCP + placebo or TCP + (DL)-tryptophan. The average dosages were 30 to 50mg of TCP in both groups and 214mg/kg tryptophan administered on a daily basis. The combination produced a 72 percent improvement in depression rating, whereas the MAOI alone produced only a 38 percent improvement. Pare

(1963) treated 14 patients. Six patients had to be discontinued due to adverse side-effects, and of the 8 remaining patients, 6 showed marked improvement while 2 did not respond. The doses were reduced doses of various MAOIs along with 7.5-15 grams of tryptophan daily. Glassman and Platman (1969) treated 20 patients with phenelzine (60mg/day) and either tryptophan (12-18g/day) or placebo. Sixty percent of those patients receiving tryptophan improved, but only 20% of those receiving the placebo improved. Gutierrez and Lopez-Ibor (1971) studied 30 patients. These patients received one of two treatments: Nialamide (500mg) + tryptophan (6g), or nialamide (500mg) + placebo. The combination was successful in 66% of the patients, whereas nialamide alone was successful in only 33%.

In all four of these papers the patients were selected on the basis that they had previously not responded to antidepressant treatment, most commonly TCAs. All four of the papers reported a positive response to the addition of tryptophan to MAOI treatment.

1.6.5 THYROID HORMONE AUGMENTATION OF TCA TREATMENT

The observation that clinical thyroid disease can manifest symptoms of depression led to the addition of thyroid hormone to the drug regimen of patients not responding to their current antidepressant therapy. There are several reports in the literature on the potentiation of TCAs by thyroid hormone (Nierenberg and White, 1980 and Joffe, 1990). Three reports are specific to TCA-resistant depression (Earle, 1970; Goodwin *et al.*, 1982; Joffe *et al.*, 1993). Earle (1970) reported a 61% response rate in AMI nonresponders,

Goodwin *et al.* (1982) reported a 50% response rate in AMI nonresponders and an 88% response rate in imipramine nonresponders, and Joffe *et al.* (1993) reported a 59% response rated in imipramine or DMI nonresponders.

1.6.6 HIGH DOSE TRANYLCYPROMINE

There are four reports of high dose TCP therapy in the treatment of refractory depression (Amsterdam and Berwish, 1989; Guze and Baxter, 1987; Pearlman, 1987; Robinson, 1983). Amsterdam and Berwish (1989) reported on the use of high dose TCP in the treatment of refractory depression in a controlled study involving 7 patients. All patients had failed to respond to at least three prior treatment regimes. The 7 patients were treated with TCP at doses ranging from 90 to 170 mg/day (compared to the conventional dose of 20-30 mg/day), the average dose being 112 mg/day. Four of the 7 patients had complete remission of their symptoms; one had partial remission; and 2 were nonresponders. The patients were kept at their maximum dose for a minimum of 2 weeks and a maximum of 4 months. Two of the patients had to discontinue treatment due to side-effects. One gained 28 pounds, and one had an episode of post-micturational syncope. None of the adverse reactions was life-threatening. The other 3 papers consist of 4 case reports all showing a positive response to high doses of TCP when other treatments had failed. As long as dietary precautions were adhered to and the drug dosage was increased slowly over a period of time no serious adverse reactions occurred.

It would seem likely that at such a high dose the action of TCP is not merely the inhibition of MAO, as 80-90 percent inhibition is obtained at normal

therapeutic doses of approximately 30 mg/day (Robinson *et al.*, 1978). It has been shown that the (-)-enantiomer inhibits the uptake of NA (Baker *et al.*, 1978; Horn and Snyder, 1972). TCP has also been shown to facilitate DA and 5-HT release and inhibit their uptake in rat striatal tissue (Baker *et al.*, 1990; Hampson *et al.*, 1988).

1.7 5-HT AND DEPRESSION

5-HT has been implicated in depression since the discovery that iproniazid inhibited MAO (Zeller *et al.*, 1952) and elevated brain 5-HT and NA levels in rats (Brodie *et al.*, 1956). Later, TCAs were found to inhibit neuronal uptake of 5-HT and NA. It was not until the 1970s that NA, although undisputedly important in depression, became questionable as a common etiological factor in antidepressant efficacy. The following data implicated a more pivotal role of 5-HT in depression: 1. precursors to 5-HT were reported to have mood elevating effects (Coppen *et al.*, 1963; Pare, 1963; Glassman and Platman, 1969; Gutierrez and Lopez-Ibor, 1971); 2. *para*-chlorophenylalanine (PCPA), a compound known to deplete 5-HT, was shown to reverse the antidepressant effects of imipramine and TCP (Shopsin *et al.*, 1975, 1976); 3. compounds which selectively inhibited 5-HT uptake were discovered (Wong *et al.*, 1974) and later found to be effective antidepressants (Benfield *et al.*, 1986); 4. electrophysiological reports of increased sensitivity of postsynaptic neurons to 5-HT following chronic antidepressant treatment (de Montigny and Aghajanian, 1978); and 5. receptor binding studies reporting a down-regulation of 5-HT_2 receptors produced by chronic administration of antidepressants (Peroutka and Snyder, 1980). The search for a common mechanism of action for all antidepressants shifted more towards

5-HT systems.

5-HT is synthesized from the essential amino acid tryptophan, which crosses the blood-brain barrier with ease and is readily available for metabolic action (Oldendorf, 1970a,b). In the brain tryptophan is hydroxylated in the 5 position to 5-hydroxytryptophan by tryptophan hydroxylase and then decarboxylated to 5-HT by aromatic amino acid decarboxylase (Brown, 1980). Neuronal 5-HT exists in two pools, one for storage and one which is functionally active (Mulder, 1982). 5-HT is released from the functional pool following depolarization of the cell membrane by a calcium-dependent mechanism, and 5-HT is actively taken up from the synapse into the presynaptic neuron by a 5-HT transporter. Uptake of 5-HT into the presynaptic neuron is the primary mechanism responsible for terminating the 5-HT response. This intraneuronal 5-HT is either restored in vesicles or is catabolized to 5-hydroxyindole-3-acetic acid (5-HIAA) by MAO-A. 5-HIAA is excreted from the body in both glucuronidated and free forms in the urine. 5-HT elicits its responses on the postsynaptic neuron by interacting with a variety of 5-HT receptors, and these responses can either be inhibitory or excitatory depending on the type of receptors present.

There are two distinct subdivisions of 5-HT neuronal pathways; a rostral and a caudal division. The rostral division has cell bodies in the caudal linear nucleus (midbrain), dorsal raphé nucleus (caudal midbrain and rostral pons), median raphé nucleus (rostral pons), and the B9 group (nucleus pontis oralis) and the caudal division has cell bodies in the raphé nuclei of the medulla oblongata (raphé magnus nucleus, raphé pallidus nucleus, raphé obscurus nucleus). The ascending projections of these divisions innervate regions of the

cerebral cortex, basal ganglia, limbic system and diencephalon. Descending projections innervate various regions of the spinal cord, principally the dorsal horn (Laminae I and II), the intermediolateral cell column, and the motor columns (Lamina IX) (Tork, 1990).

There are now proposed to be seven pharmacologically distinct families of 5-HT receptors (Shen *et al.*, 1993). These "families" have been designated 5-HT_1 to 5-HT_7. Although at least 7 families are proposed to exist, the majority of research has been conducted on the first three families. In simplistic terms, the 5-HT_1 family of receptors (5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D}) exhibits the highest affinity (nM range) for 5-HT and elicits an inhibitory response (via inhibition of cAMP formation and opening of K^+ channels). The 5-HT_2 receptor family (5-HT_{1C}, 5-HT_{2A}, and 5-HT_{2B}) has a lower affinity (μM range) for 5-HT (except for 5-HT_{1C} receptors which exhibit a nanomolar affinity for 5-HT) and elicit excitatory responses (via stimulation of phosphoinositide turnover and closing of K^+ channels). 5-HT has a mid-range affinity for the 5-HT_3 receptor (250nM). Activation of this receptor results in the opening of a cation channel and depolarization of the membrane. 5-HT_1 and 5-HT_2 receptors are linked to G proteins whereas 5-HT_3 receptors open a ligand-gated cation channel without a G protein link. The two 5-HT receptor systems which are believed to play major roles in the etiology of depression at this time are the 5-HT_{1A} and 5-HT_2. The pharmacology of these receptors is subsequently discussed. An indepth review of 5-HT receptors has been published (Peroutka, 1993).

The highest density of 5-HT_{1A} receptors is in limbic areas (hippocampus, lateral septum, frontal and entorhinal cortex and the central amygdala) and in

the dorsal and raphé nuclei (Marcinkiewicz *et al.*, 1984; Pazos and Palacios, 1985; Verge *et al.*, 1985). The 5-HT_{1A} receptor is believed to be a somato-dendritic autoreceptor in the brain stem (Lanfumey *et al.*, 1989; Goodwin *et al.*, 1985; Hall *et al.*, 1985) and a postsynaptic receptor in the hippocampus (Hall *et al.*, 1985; Verge *et al.*, 1986). This receptor is coupled to two effector systems through G proteins. One coupling involves inhibition of adenylate cyclase (De Vivo and Maayani, 1986; Weiss *et al.*, 1986) and the other involves activation of a K^+ channel (Andrade *et al.*, 1986; Innis *et al.*, 1988). The regulation of the 5-HT_{1A} receptor has been studied using three different methods: 1. radioligand binding; 2. measurement of adenylate cyclase activity; and 3. electrophysiologic cell recordings.

When the fact that the Km value for 5-HT for the synaptic uptake site is 47 nM (Baker and Greenshaw, 1989) is combined with the fact that the affinity for 5-HT at the 5-HT_{1A} receptor is 1-5 nM (Hamon *et al.*, 1990) and that the EC_{50} for 5-HT-inhibited forskolin-stimulated adenylate cyclase is 20 nM (Peroutka, 1993), the 5-HT_{1A} receptor can be assumed to interact with 5-HT continuously. If it is continuously active to some degree, increases in synaptic 5-HT concentrations would not be expected to have a strong effect on 5-HT_{1A} receptor regulation, but should result in an increased or prolonged response. In fact, it has been shown that chronic administration of the 5-HT_{1A} agonist ipsapirone for two weeks did not alter the effect of an acute dose of ipsapirone on adenylate cyclase activity 24h later (Hamon *et al.*, 1990), suggesting that postsynaptic 5-HT_{1A} function linked to adenylate cyclase activity was not altered by chronic agonist activity. Receptor binding studies following antidepressant treatment

support this finding. Chronic treatment with antidepressants of all classes resulted in cortical 5-HT_{1A} receptors being down-regulated (decreased receptor density) in 4/13; unchanged in 8/13; and up-regulated (increased receptor density) in 1/13 studies. 5-HT_{1A} receptors in the hippocampus were down-regulated in 3/16; unchanged in 12/16 and upregulated in 1/16 studies. Out of 7 studies in the dorsal raphé nucleus, 5-HT_{1A} receptor number was not changed in 6 of them and down-regulated in one (Newman *et al.*, 1993). Antidepressant treatment is not altering 5-HT_{1A} receptor density to any significant extent, however postsynaptically the activity of 5-HT_{1A}-mediated adenylate cyclase in the hippocampus has been reported to be attenuated in 10/14 studies and unchanged in the other 4. Therefore antidepressants appear to be increasing post-synaptic 5-HT_{1A} function without altering 5-HT_{1A} receptor number.

The 5-HT_{1A} receptor system is complicated in that these receptors are expressed both pre- and post-synaptically and they appear to be regulated differently. Chronic treatment with citalopram, a selective 5-HT uptake inhibitor, resulted in somatodendritic and terminal autoreceptors in the raphé nucleus being desensitized after 14 days, partially desensitized after 7 days and not at all after 2 days. Postsynaptic cells in the hippocampus showed no enhancement of response with direct microiontophoric application of 5-HT, but did show enhancement when the afferent 5-HT neuron was excited (de Montigny *et al.*, 1990). Previous studies with zimelidine (Blier and de Montigny, 1983), indalpine (Blier *et al.*, 1984), paroxetine (de Montigny *et al.*, 1989), and FLU (Blier *et al.*, 1988) have shown similar results. This effect is also observed with phenelzine and clorgyline (Blier *et al.*, 1985). The effect observed with TCAs and mianserin

is different. Desensitization of the terminal and somatodendritic autoreceptors is not observed but the sensitivity of the postsynaptic neuron to 5-HT is enhanced (de Montigny *et al.*, 1989; Blier *et al.*, 1984). This effect has been challenged by the report that chronic TCA treatment actually attenuated the actions of 5-HT on hippocampal neurons (Rowan and Anwyl, 1985). The effects observed in this latter study were, however, antagonized by the 5-HT_2 antagonist ketanserin, which raises doubts as to whether the cell recordings in this study were measuring 5-HT_{1A} activity or 5-HT_2 activity.

The increase in K^+ conductance following activation of the 5-HT_{1A} receptor is most likely the response measured in electrophysiologic experiments (Hamon *et al.*, 1990). This response does not appear to be affected by chronic treatment with SSUIs or MAOIs (de Montigny *et al.*, 1990), but following chronic TCA treatment the response appears to be enhanced.

The 5-HT_2 receptor is only active following a nerve impulse when concentrations of 5-HT reach sufficient concentrations to elicit a response (5-HT has an affinity for the 5-HT_2 receptor in the μM range). The density of this receptor would be expected to be more dependent on the amount of stimulation it receives than the 5-HT_{1A} receptor because it is less active. Antidepressants of all classes have been shown to decrease 5-HT_2 density and/or decrease the frequency of the 5-HT_2 receptor-mediated head-shake behavior (Peroutka and Snyder, 1980; Eison *et al.*, 1991). The mechanism by which this is attained is dependent on the type of antidepressant being studied. MAOIs accomplish this by greatly increasing 5-HT levels so that there is a prolonged response at the 5-HT_2 site which results in a down-regulation (decrease in receptor density) of the 5-HT_2

receptor chronically (Goodnough and Baker, 1993a; Peroutka and Snyder, 1980; Goodwin *et al.*, 1984; Sherry-McKenna *et al.*, 1992; Mousseau *et al.*, 1992). Drugs which are selective uptake inhibitors (*i.e.* FLU for 5-HT and nomifensine for NA and DA) are strongly dependent on the integrity of the presynaptic neuron for their ability to decrease 5-HT_2-mediated behavior, but TCAs, which act as direct antagonists at the 5-HT_2 site, decrease both 5-HT_2-mediated behavior and receptor density even though the presynaptic neurons had been lesioned with either DSP4 or 5,7-DHT (Elson *et al.*, 1991).

5-HT_2 agonists and antagonists have been shown to down-regulate the 5-HT_2 site by a similar degree (Leysen and Pauwels, 1990). However, the densities of receptors labelled by the antagonist, ^{3}H-ketanserin (22 fmol/mg tissue), and the agonist, ^{3}H-1-(2,5-dimethoxy-4-bromophenyl-2-aminopropane (^{3}H-DOB, 6 fmol/mg tissue), do not coincide. This suggests that there is heterogeneity in the 5-HT_2 receptor, with ^{3}H-ketanserin labelling one receptor (5-HT_{2B}) and ^{3}H-DOB another one (5-HT_{2A}) (Peroutka, 1993).

The 5-HT_2 receptor family is linked to the stimulation of phosphatidylinositol (PI) hydrolysis (Conn and Sanders-Bush, 1986) which results in the production of two important second messengers: inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 has the primary role of releasing calcium from intracellular stores, which can then act on many intracellular processes. DAG activates protein kinase C which regulates many intracellular proteins. Obviously, the hydrolysis of PI leads to many intracellular effects, the magnitudes of which are unknown. The 5-HT_2 receptor has also been shown to be linked to K^+ conductance in a manner opposite to that of 5-HT_{1A} receptors. Activation of the

5-HT_{2B} receptor results in a slow depolarization by decreasing the resting membrane conductance to K^+ (Vandermaelen and Aghajanian, 1980, 1982). This effect is blocked by the 5-HT_2 antagonist ritanserin (Leyson *et al.*, 1985)

There are many receptors on the cell membrane of a neuron, and it is the summation of the activities at these receptors which determines whether or not the neuron will send an impulse. Neurons postsynaptic to ascending 5-HT neurons exhibit 5-HT receptors which may act in a competitive manner. 5-HT_1 receptors inhibit the propagation of a nerve impulse (by inhibiting adenylate cyclase and increasing K^+ conductance) whereas 5-HT_2 receptors facilitate a nerve impulse (by stimulating PI hydrolysis and inhibiting K^+ conductance) (Vandermaelen and Aghajanian, 1980, 1982). The balance in activity between 5-HT_{1A} and 5-HT_2 receptors may be important in determining the overall effect nerve impulses from ascending 5-HT neurons will have on the postsynaptic neuron.

Close evaluation of receptor regulation and neuronal excitation following chronic antidepressant treatment, along with the acute pharmacological actions of various antidepressants indicate that the biogenic amine deficiency theory of affective disorder, which states that depression is the result of a functional deficiency of NA and/or 5-HT at central synapses (Baldessarini, 1975) requires reinterpretation. No studies have conclusively shown that brain levels of 5-HT or NA are changed in depression. Attempts to monitor metabolite levels have also been inconsistent (Meltzer, 1980). The effects of MAOI and uptake inhibiting drugs on synaptic amine levels are rapid, but clinical response is delayed. The processes which these amines mediate are undeniably altered in depression,

but these alterations may not be due to imbalances in their levels, but to imbalances in the many actions that these amines have. The most consistent findings involving antidepressant action involve 5-HT. If malfunctioning 5-HT-mediated responses are proposed to be the primary factor in depression, then effective antidepressant therapies should be consistent in their manipulation of these systems. 5-HT can elicit both excitatory and inhibitory responses in the postsynaptic neuron. A disruption of this balance has been proposed to be the perturbation responsible for depressive illness (Deakin and Pennel, 1986; Deakin, 1988). Antidepressants are therefore proposed to act by enhancing 5-HT_{1A} and/or inhibiting 5-HT_2 function. The data which support this proposal are:

1. Antidepressants of all types increase postsynaptic 5-HT_{1A} function (de Montigny *et al.*, 1989, 1990; Blier and de Montigny, 1985; Newman *et al.*, 1993)

2. Antidepressants of all types decrease 5-HT_2 density and/or decrease 5-HT_2 mediated behavior (Peroutka and Snyder, 1980; Eison *et al.*, 1991)

3. 5-HT_{1A} agonists are effective antidepressants (Robinson *et al.*, 1989; Schweizer *et al.*, 1986)

4. 5-HT_2 antagonists are effective antidepressants (Brogden *et al.*, 1978; Feighner *et al.*, 1983)

It has also been proposed that anxiety and depression are manifestations of a single disorder and that they exist on a continuum, with anxiety preceding depression (Deakin, 1988). The data which support this hypothesis are summarized as follows:

1. Symptoms of anxiety and depression usually coexist (Goldberg *et al.*, 1987; Keller, 1992)

2. Drugs effective at treating depression are also effective at treating anxiety (Klein, 1964; Khan *et al.*, 1988; Bodnoff *et al.*, 1988, 1989).

3. 5-HT_2 antagonists display significant anxiolytic properties (Deakin and Wang, 1990; Deakin, 1988).

There is growing evidence that changes in neuroendocrine function may be predisposing factors responsible for the occurrence of a depressive episode. In females the occurrence of depressive episodes are strongly correlated to periods in which levels of estrogen are the lowest (*i.e.* premenstral, post-partum, and menopause phases) (Belgon, 1990). It has also been shown that platelet 5-HT_2 binding at the beginning or end of the menstrual cycle (when estrogen is the lowest) is double that in mid cycle (when estrogen levels are the highest) (Belgon, 1990). Platelet (Arora and Meltzer, 1989) and post-mortem brain (Yates *et al.*, 1990; McKeith *et al.*, 1987) studies have shown that 5-HT_2 receptor number is upregulated in depression. Chronic corticosterone treatment has been reported to result in decreased 5-HT_{1A}-mediated behavior (Dickinson *et al.*, 1995; Bagdy *et al.*, 1989), decreased ^{3}H-5-HT receptor binding (Biegon *et al.*, 1985; De Kloet *et al.*, 1986), and an increase in 5-HT_2-mediated behavior (Buckett and Luscombe, 1994). Immobilization stress results in elevated corticosterone levels (Iase *et al.*, 1990) and upregulates 5-HT_2 receptor number (M. Meaney, unpublished observations). If an imbalance in 5-HT receptor function is responsible for a depressive episode, then stress and/or the resultant higher levels of corticosterone may be the enviromental factors which trigger a

depressive episode in males, and these factors as well as periods of low estrogen may be the triggering factors in females. This endocrine difference may also explain the higher incidence of depression in females than males.

1.8 EFFECTS OF NORADRENERGIC SYSTEMS ON 5-HT FUNCTION

β-Adrenergic receptors are consistently down-regulated with MAOIs and TCAs (review: Baker and Greenshaw, 1989), but only under very high doses is this effect seen with the SSRIs (Wamsley *et al.*, 1987; Byerley *et al.*, 1988). The lack of effect of SSRIs on β-adrenergic receptors (Mishra *et al.*, 1979; Maggi *et al.*, 1980; Snyder and Peroutka, 1982; Fuxe *et al.*, 1983; Stolz *et al.*, 1983; Wong *et al.*, 1985; Peroutka and Snyder, 1980; Goodnough and Baker, 1993b) diminishes regulation of this receptor system as a common etiological factor in depression, however interactions between noradrenergic and serotonergic systems may be relevant. When noradrenergic neurons were lesioned with DSP4, 5-HT_2-mediated head shakes increased in frequency to 150% of non-lesioned control values (Eison *et al.*, 1991), suggesting that NA influences 5-HT_2-mediated behavior. Furthermore the integrity of the presynaptic neuron affects the ability of nomifensine (a NA/DA uptake inhibiting antidepressant, Brogden *et al.*, 1979) to inhibit the frequency of 5-HT_2 receptor-mediated head-shakes. An 84% decrease in the head-shake frequency is observed in non-lesioned rats which received nomifensine chronically, but only a 21% decrease is observed in DSP4-lesioned rats treated chronically with nomifensine (Eison *et al.*, 1991). Flerobuterol, a β-adrenoceptor agonist, may enhance 5-HT neurotransmission, when administered chronically, by increasing the amount of 5-HT released following electrical stimulation (Bouthillier *et al.*, 1990). Salbutamol

and clenbuterol, β_2 adrenoceptor agonists, have been reported to increase 5-HT synthesis in rat brain (Waldmeier, 1981) and potentiate the 5-hydroxytryptophan-induced 5-HT syndrome in rats (Ortmann *et al.*, 1981).

1.9 TRYPTAMINE AND 5-HT FUNCTION

Tryptamine and 5-hydroxytryptamine (5-HT) are both derived from the amino acid tryptophan and both amines have been implicated in the etiology of depression (Mousseau, 1993; Coppen and Doogan, 1988). Only trace amounts of tryptamine (approximately 0.5 ng/g in whole brain) are normally found in rat brain (Philips *et al.*, 1974). However, animals treated with MAO inhibitors demonstrate large increases in tryptamine concentrations (Philips and Boulton, 1979; Philips *et al.*, 1980; Baker *et al.*, 1984). Large increases in urinary tryptamine levels have also been observed in patients receiving MAO inhibitors (Dewhurst, 1968; Bieck *et al.*, 1984; Baker *et al.*, 1985; McKenna *et al.*, 1992). Tryptamine is structurally related to 5-HT and also has marked effects on 5-HT systems. In addition to affecting uptake and release of 5-HT (Baker *et al.*, 1977), tryptamine has been reported to inhibit excitatory and potentiate inhibitory effects of 5-HT in electrophysiological studies in rat brain (Jones and Boulton, 1981).

Dewhurst (1968) suggested that tryptamine is a neurotransmitter in its own right, and, on the basis of behavioral studies in chicks (Dewhurst and Marley, 1965), proposed the existence of tryptamine receptors. The existence of specific tryptamine receptors has been confirmed using *in vitro* receptor binding studies (Keller and Cascio, 1992; Bruning and Rommelspacher, 1994; Van Nguyen *et al.*, 1999) and autoradiographic experiments (Perry *et al.*, 1992; Altar *et al.*, 1996; Kaulen *et al.*, 1998; McKormack *et al.*, 1998; Perry, 1999).

Drugs that inhibit MAO activity have been shown to decrease [^{3}H]-tryptamine binding sites in rat brain (Keller and Cascio, 1986; Martin *et al.*, 1987; Van Nguyen and Juorio, 1989; Mousseau *et al.*, 1992; Goodnough *et al.*, 1993).

1.10 ADAPTIVE CHANGES WITH CHRONIC ANTIDEPRESSANT TREATMENT

With the realization that the acute effects of antidepressant treatments were not sufficient to explain clinical outcome, researchers searched for changes in the brain that occurred after chronic, but not acute, treatment. Many were found: 1. Inhibition of NA-stimulated adenylate cyclase (Vetulani and Sulser, 1975); 2. Decreased number of β-adrenoceptors (Vetulani *et al.*, 1976; Peroutka and Snyder, 1980); 3. Decreased number of 5-HT$_2$ receptors (Peroutka and Snyder, 1980); 4. Decreased accumulation of inositol phosphate resulting from 5-HT-stimulated PI hydrolysis (Kendall and Nahorski, 1985); 5. Inhibition of the 5-HT$_2$-mediated head-shake response (Eison *et al.*, 1991); 6. Increased sensitivity of postsynaptic neurons to 5-HT (de Montigny, 1981); 7. Decreased inhibition of forskolin-stimulated adenylate cyclase (Newman and Lerer, 1988); 8. Subsensitivity of presynaptic dopamine receptors (Maj *et al.*, 1984); 9. Decreased D_1 and D_2 receptor number (Klimek and Nielsen, 1987); 10. Upregulation of GABA-B receptors (Lloyd *et al.*, 1985). The upregulation of the GABA-B site is included here, but has not been satisfactorily replicated (McManus and Greenshaw, 1991). For a more detailed examination and extensive reference listings see Baker and Greenshaw (1989).

With the extensive research being conducted on the newer SSRIs (*i.e.* FLU, paroxetine, sertraline, fluvoxamine, citalopram, and femoxetine) less credence

can be applied to some of the ten points mentioned above with regard to their consideration as a common etiological event necessary for clinical improvement. FLU has low affinity and controversial effects on those receptor systems (5-HT_2 and β-adrenergic) believed to be of primary importance in the mechanisms of action of the classical antidepressants. Unless high doses of FLU are used, receptor changes are not observed. However, two points are consistent between FLU and other antidepressants: 1. Inhibition of 5-HT_2-mediated head shake response (Eison *et al.*, 1991); and 2. Increased sensitivity of postsynaptic neurons to 5-HT (de Montigny, 1991; de Montigny *et al.*, 1989). This consistency supports the 5-HT hypothesis of depression, a focus of this study.

1.11 INTRODUCTORY SUMMARY

Why do some patients respond readily to antidepressant treatment while others do not? There are different subtypes of depression and some have been shown to respond to antidepressants better than others. Many reports have been published on biochemical differences between depression subtypes (for review see Leonard, 1988), yet not enough is known to be able to conclusively differentiate between depression subtypes on the basis of biological differences. As well, the same biochemical abnormality may manifest different symptoms in different patients. The receptor imbalance hypothesis of depression (Deakin *et al.*, 1991) attempts to satisfy the results of the mass of data accrued over the past decades of antidepressant research. Patients with depression who do not respond to traditional antidepressant therapy may have a more severe imbalance and require supplementation to the current therapy.

1.12 OBJECTIVES OF THIS STUDY

This thesis explores three drug therapies used successfully in the treatment of refractory depression and attempts to elucidate some of the reasons why they are more effective than traditional therapy. The drugs or drug combinations used and the possible mechanisms studied include:

a) TCP and AMI: the effect of combined treatment of these agents was compared to the drugs alone on 5-HT_2 receptor density and affinity, MAO inhibition, and biogenic amine concentrations.

b) TCP, high vs. low dose: the effects on 5-HT_2 receptor density and affinity, ^{3}H-tryptamine binding, MAO inhibition, TCP levels, and biogenic amine levels between the two treatments were compared.

c) FLU and DMI: the effects of the combination on 5-HT_2 and β-adrenergic receptor density and affinity were compared to the effects observed when the drugs were administered singly. Drug-drug metabolic interactions were also investigated.

2 MATERIALS AND METHODS

2.1 LIST OF CHEMICALS USED IN THE DESCRIBED STUDIES

acetic anhydride[1]
acetonitrile-HPLC grade[2]
alprenolol HCl[3]
amitriptyline HCl[4]
ascorbic acid[5]
bovine serum albumin[3]
[^{3}H]-CGP 12177[6]
p-chlorophentermine
cupric sulfate[5]
deoxycholate - Na salt[5]
desmethylimipramine HCl[3]
dopamine HCl[3]
ethyl acetate[2]
ethylenediamine tetraacetate-Na_2 salt[5]
fluoxetine HCl[12]
folin - phenol reagent[3]
hydrochloric acid[5]
5-[2-^{14}C]-hydroxytryptamine binoxalate[7]
5-hydroxytryptamine creatinine sulfate[3]
maprotiline HCl[3]
methoxyflurane[8]
mianserin HCl[3]
[^{3}H]-ketanserin HCl[7]noradrenaline HCl[3]
norfluoxetine HCl[3]
pargyline HCl[3]
pentafluorobenzoyl chloride
perchloric acid[5]
2-[ethyl-1-^{14}C]-phenylethylamine HCl[7]
2-phenylethylamine HCl[3]
phosphoric acid[5]

potassium carbonate[5]
scintillation fluid (Ready-Safe)[9]
sodium bicarbonate[5]
sodium tetraborate[5]
sodium carbonate[5]
sodium hydroxide[5]
sodium potassium tartrate[10]
toluene, glass distilled[2]
toluene, reagent grade[2]
(±)-tranylcypromine HCl[3]
Tris (hydroxymethyl) aminomethane[5]
tryptamine HCl[3]
[^{3}H]-tryptamine
L-tryptophan[11]

[1]Caledon Laboratories (Georgetown, ON); [2]British Drug Houses (Toronto, ON); [3]Sigma (St. Louis, MO); [4]Ciba-Geigy (Summit, NJ); [5]Fisher Scientific (Edmonton, AB); [6]Amersham Canada Ltd. (Oakville, ON); [7]New England Nuclear Products, Dupont (Boston, MA); [8]MTC Pharmaceuticals (Mississauga, ON); [9]Beckman Instruments Inc. (Edmonton, AB); [10]Allen & Hanbury's (Toronto ON); [11]Raylo Chemicals Ltd. (Edmonton, AB); [12]Eli Lilly Laboratories (Mississauga, ON)

2.2 INSTRUMENTATION

2.2.1 Gas-Liquid Chromatography

Two types of gas-liquid chromatographs were used.

1. Levels of tranylcypromine were determined with a Hewlett-Packard 5890 instrument (HP, Palo Alto, CA) equipped with an HP 7673A automated sampler and an electron-capture detector with a radioactive source of 15mCi ^{63}Ni. Separation was achieved using a fused silica capillary column (25m x 0.32 mm i.d.) coated with a 0.52 μm film thickness of 5% phenylmethylsilicone (HP Co., Palo Alto, CA). Peak heights were analysed with a HP 3392A integrator. Helium gas was used as carrier gas at a flow rate of 2 ml/min and 5% methane in argon was used as detector makeup gas at a flow rate of 35 ml/min. Temperature programs were adjusted to maximize resolution.

2. FLU, NFLU, AMI, and DMI levels were analysed with a HP 5890 equipped with a nitrogen-phosphorus detector. The column and integrator were of the same type as that mentioned directly above. The carrier gas was helium set for a flow rate of 30 ml/min. The detector was purged with hydrogen at 3.5 ml/min mixed with dry air at 80 ml/min. Temperature programs were adjusted to maximize resolution.

2.2.2 High Performance Liquid Chromatography (HPLC)

Levels of the neurotransmitter amines NA and 5-HT were determined using reverse-phase HPLC with an electrochemical detection. The specific components included a Waters WISP 710B automated sampler with a 15μl injection volume (Milford, MA), a Waters 510 pump, Econosphere-C_{18} (4.6mm x 250mm; 5μm particle size) column [Applied Science Labs (Avon-

dale, PA)], mobile phase containing 55mM $NaH_2PO_4H_2O$, 0.85mM sodium octyl sulfate, 0.37mM EDTA, 9% acetonitrile, pH 3.0 (adjusted with phosphoric acid), either a LC-4B amperometric detector [Bioanalytical Systems (West Lafayette, IN)] or a Waters M460 detector, and either a HP 3392A integrator, or a Waters 740 data module.

2.2.3 Liquid Scintillation Spectrometry

Two types of liquid scintillation counters were used to measure radioactivity: a Beckman LS 7500 (Fullerton, CA) equipped with a Datamex 43 printer and a Beckman LS 6000SC equipped with a Beckman printer and a WYSE monitor. Beckman Ready-Safe liquid scintillation cocktail was used for all studies.

2.2.4 Ultraviolet Spectrophotometry

Determination of the protein content present in the binding assays was accomplished with a Unicam (Pye-Unicam, Cambridge, U.K.) SP1700 set at a wavelength of 660 nm.

2.2.5 Mass Spectrometry

Verification of peak identity for FLU, NFLU, and DMI derivatives was performed with a GC-MS system (electron impact, EI) consisting of a HP5840A GC inlet, HP 7920 disc drive and data system, HP 2648A graphics terminal, HP 9876A printer, and a HP 21MX series E computer. The following MS operating conditions were employed: ion source temperature, 200°; interface temperature, 305°; column pressure, 34.5 kPa; accelerating voltage, 2200 eV; ionization voltage, 70eV; scan speed, 100 amu/sec; and dwell time, 200 msec.

2.3 APPARATUS

2.3.1 Glassware

Non-radioactive test-tubes were rinsed with tap water, sonicated for 1 h in a 2-5% solution of Contrad-70 (Baxter/Canlab, Mississauga, ON), and subsequently rinsed with tap water and distilled water in a Miele dishwasher. Other non-radioactive glassware was rinsed with tap water then washed and rinsed in the abovementioned dishwasher with Sparkleen soap (Fisher Scientific, Fairlawn, NJ). Radioactive glassware was allowed to soak for a minimum of 24 h in a 2-5% solution of Contrad-70 (see above), and then cleaned as mentioned above. All glassware was air-dried at 250-300 ^{o}C in one of two Precision Scientific Group convection ovens (model 18EM or 28).

2.3.2 Homogenizer

Tissue samples were homogenized using a S63C Tri-R Stir-R homogenizer equipped with a Teflon pestle and a glass mortar/tube.

2.3.3 Shaker-Mixers

Individual samples were mixed using a Thermolyne Maxi Mix vortex mixer (Thermolyne Corp., Dubuque, IO). Multiple samples were mixed with a Ika-Vibrax VXR2 shaker (Janke and Kunkel, Staufen, Germany). Large volume mixing was accomplished with a Thermolyne 1000 magnetic stirrer/hot plate.

2.3.4 pH Meter

The pH value of various solutions was determined with a Fisher Scientific (Fairlawn, NJ) Accumet 610 pH meter.

2.3.5 Centrifuges

Low-speed centrifugation (up to 1500 g) was done with a Sorvall GLC-2B or GLC-1 (Dupont Instruments, Wilmington, DE) benchtop centrifuge. High-speed centrifugation (up to 40,000 g) was done with a Beckman L755 (Palo Alto, CA) refrigerated vacuum ultracentrifuge and with a MSE Micro-Centaur (Baxter/Canlab, Mississauga, ON) benchtop centrifuge for small volumes.

2.3.6 Filtration

The filtration step in the radioligand binding assays was carried out with a 48 sample Brandel Cell Harvester (Gathersburg, MD). Whatman GF/C filters were used.

2.3.7 Weighing Balance

Chemicals and tissue were weighed with a Mettler AE 160 (Zurich, Switzerland) electronic balance.

2.4 ANIMALS

2.4.1 Strain

Male Sprague-Dawley rats (Bioscience Animal Services, Ellerslie, AB) (200-300g) were used in all experiments.

2.4.2 Housing

Animals were housed two per cage on cedar chip bedding. Food [Lab-Blox feed, Wayne Feed Division, Continental Grain Co., Chicago, IL; 4.0% crude fat (min), 4.5% crude fibre (max), and 24% crude protein (min)] and tap water were freely available. The environment was maintained at 21 ± 1°C with a 12 h day/night cycle.

2.4.3 Drug Administration

Drugs were administered in one of two ways: 1. Alzet 2ML2 and 2ML4 osmotic minipumps were implanted subcutaneously in the dorsal thoracic area. Animals were anesthetized with the inhalant methoxyflurane. Operations were carried out under aseptic conditions. Pumps were loaded to administer drugs on a mg/kg/day free drug basis. Individual pump drug concentrations were determined using a basic computer program developed by Greenshaw (1986). 2. Animals were injected with the appropriate dose intraperitoneally once daily. These animals were killed 24 h after the last injection.

2.4.4 Sample Collection and Storage

Animals were killed by guillotine decapitation after which the brain was rapidly removed, immersed in ice-cold saline, and dissected over ice. Other tissue samples were collected in parallel. All tissue samples were immediately frozen on solid carbon dioxide as collected. When tissue collection was complete, samples were transferred to a low-temperature freezer and stored at -80°C until time of analysis.

2.4.5 Ethical Considerations

All procedures in this thesis involving the use of animals were approved by the University of Alberta Health Sciences Animal Welfare Committee and conducted according to the guidelines established by the Canadian Council on Animal Care.

2.5 ANALYSIS OF 5-HT AND NA CONCENTRATIONS

The rest of brain (whole brain minus cortex, hippocampus, and striatum) was homogenized in ice-cold 0.1N perchloric acid and centrifuged to remove the protein precipitate. Portions (150 μl) of the supernatant were placed in tubes in the WISP tray and aliquots (15 μl) of the supernatent were then injected onto a HPLC equipped with an electrochemical detector with the reference electrode set at 0.8 volt as described by Baker *et al.* (1987). Amine concentrations were determined by comparing peak heights obtained with the samples to a standard curve obtained using concentrations of NA and 5-HT ranging from 25-1000 ng. The standard curve was made up in 0.1N perchloric acid and run in parallel to the samples on each assay day.

2.6 [^{3}H]-TRYPTAMINE BINDING

The assay for [^{3}H]-tryptamine binding was identical to the procedure of Kellar and Cascio (1982) as modified by Mousseau *et al.* (1992). Cortical tissue was homogenized in 10 vol of ice-cold 50 mM TRIS buffer (washing buffer, pH 7.4). The homogenates were centrifuged (40,000xg for 10 min, 4^{o}C). The supernatant was discarded and the resultant pellet was quickly re-homogenized in the same volume of TRIS, and re-centrifuged. The final pellet was suspended in 10 vols of 50 mM TRIS buffer [incubation buffer, pH 7.4, containing the MAO inhibitor pargyline (10 μM) and the antioxidant ascorbic acid (5.6 mM)]. This homogenate was pre-incubated at 35-37^{o}C for 40 min. Single point determinations using a concentration of [^{3}H]-tryptamine of 2.0 nM were performed in parallel sets (each set in triplicate) of tubes in which a fixed amount of tissue homogenate (in a final volume of 1 ml) was incubated for 1 h at 0-4 ^{o}C with the

radioligand in the presence (non-specific binding) or absence (total binding) of an excess of unlabelled tryptamine (10 μM). Specific binding was defined as the difference between total and non-specific binding at each concentration. The incubation step was terminated by addition of 3 ml of ice-cold washing buffer followed by rapid filtration through Whatman GF/B filters. The filters were rapidly washed 3 times with 5 ml of ice-cold washing buffer, and were placed into counting vials. Five ml of scintillation cocktail (Ready Safe™) were added to the vials, which were allowed to sit overnight before being put into the scintillation counter for determination of radioactive content.

2.7 [^{3}H]-KETANSERIN BINDING

The 5-HT$_2$ binding assay described here is a modification of the assay described by Schotte *et al.* (1983). Rat cortex was homogenized in 10 volumes of ice-cold TRIS-HCl buffer, 50 mM, pH 7.5, and centrifuged twice at 40,000g for 10 min. The final tissue pellet was resuspended in 10 volumes as per original weight and used for binding. Saturation curves (8-points) were obtained using the 5-HT$_2$ antagonist, ^{3}H-ketanserin (Leysen *et al.*, 1982), at concentrations between 0.1 and 8.0 nM. The membrane preparation was incubated at 37°C for 15 min after which it was filtered rapidly through poly(ethenimine)-pretreated Whatman GF/B filter paper (using a Brandel cell harvester) and washed with 5 ml ice-cold TRIS buffer. Displaceable ^{3}H-ketanserin binding (specific binding) was determined from the difference between total binding and binding in the presence of the selective 5-HT$_2$ antagonist mianserin (10μM).

2.8 [^{3}H]-CGP 12177 BINDING

The β-adrenergic binding decribed here is a modification of the assay developed by Riva and Creese (1989). Rat cortex was homogenized in 10 volumes of Tris-HCl buffer, 50 mM pH 7.5, and centrifuged twice at 40,000g for 10 min. The final tissue pellet was resuspended in 10 volumes as per original weight and used for binding. Saturation curves (6-point) were obtained using concentrations of ^{3}H-CGP 12177 between 0.05 and 2.0 nM. The membrane preparation was incubated at 25^{o}C for 120 min after which it was filtered rapidly through Whatman GF/B filter paper (using a Brandel cell harvester) and washed three times with 5 ml ice-cold Tris buffer. Displaceable ^{3}H-CGP 12177 binding was determined from the difference between total binding and binding in the presence of 10μM alprenolol (Paetsch and Greenshaw, 1993).

Protein content was determined by the method of Lowry *et al.* (1951) for each of the three binding assays decribed above (see section 2.11).

2.9 MAO ACTIVITY

Monoamine oxidase activity was determined using a modification of the procedure of Wurtman and Axelrod (1963). Rat brain tissues were homogenized in 6 volumes of distilled water. A portion (100 μl) of this homogenate was added to 400 μl of isotonic potassium chloride and vortexed, and 25 μl of this dilute homogenate was added to each tube (for blank controls 25 μl of potassium chloride was added instead). All tubes were placed on ice; to each was added 250 μl 0.5 M sodium phosphate buffer (pH=7.4). Aliquots (25 μl) of solutions of [^{14}C]-5HT (substrate for MAO-A) or [^{14}C]-phenylethylamine (substrate for MAO-B), appropriately diluted with respective unlabelled compounds, were

added to each tube. Tubes were then incubated at 37°C for 20 min; after cooling to room temperature, 2 M HCl (200 µl) was added to stop the reaction. Toluene (6 ml) was added to all tubes and the mixtures were vortexed for 5 min. After a brief centrifugation, the tubes were placed at -80°C until the aqueous layer was frozen (at least 1h). The toluene layer was decanted into a vial containing 9 ml of scintillation fluid. The mixtures were shaken thoroughly and radioactivity counted by liquid scintillation spectrometry. The amount of radioactivity in blank tubes was subtracted from all samples, and the values from sample vehicle controls averaged. The radioactivity in the sample was divided by that in controls and the value multiplied by 100 to give % activity. Percent inhibition was determined by subtracting % activities from 100.

2.10 LEVELS OF ANTIDEPRESSANTS

2.10.1 AMI

A modification of the procedure of Drebit *et al.* (1966) was used. Rat brain tissue was homogenized in 5 volumes of distilled water and a portion (2 ml) of this homogenate was used for analysis. Maprotiline [internal standard] (1000 ng) was added to the homogenate and carried through the procedure. Acetylation was then carried out on the homogenate using the procedure of Martin and Baker (1977). The acetylated maprotiline and underivatized AMI were then extracted by shaking with ethyl acetate (5 ml) for 10 min on a vortex mixer. After a 5 min centrifugation at 1,000 x g, the organic phase was transferred to another set of tubes and evaporated to dryness under a stream of nitrogen. The samples were reconstituted by the addition of toluene (200 µl). A portion (1 µl) of this solution was injected onto

a gas chromatograph equipped with a fused silica capillary column (see Section 2.2.1) and a nitrogen-phosphorus detector. Chromatographic operating parameters were as follows: initial temperature 105°C; initial time 0.5 min; rate 25°C/min; final temperature 295°C; final time 5 min. A calibration curve was run in parallel to samples on each assay day and contained 25-1000 ng of AMI dissolved in brain homogenates obtained from untreated rat brain.

2.10.2 FLU, NFLU, and DMI

A 1 ml aliquot of the original cortical homogenate in the procedure for the preparation of a membrane fraction for 5-HT_2 binding (see section 2.7) was used for analysis. Maprotiline [internal standard] (1000 ng) was added to the homogenate and carried through the procedure. The homogenate was basified with K_2CO_3 (25% w/v) and extracted with 4 ml of ethyl acetate. After centrifugation to separate the phases, the organic layer was collected and taken to dryness using a Savant Speed Vac (model SC 110, Farmingdale NY). The residue was then taken up in 1 ml of distilled water and acetylated according to the the procedure of Martin and Baker (1977). The acetylated FLU, NFLU, DMI, and maprotiline were extracted by shaking with ethyl acetate (4 ml) for 10 min on a vortex mixer. After a 5 min centrifugation at 1,000 x g, the organic phase was transferred to another set of tubes and evaporated to dryness using the Savant Speed Vac. The samples were reconstituted by the addition of toluene (100 µl). A portion (1 µl) of this solution was injected onto a gas chromatograph equipped with a fused silica capillary column and a nitrogen-phosphorus detector. A calibration curve was run in parallel to

samples on each assay day and contained 25-1000 ng of FLU, DMI, and NFLU dissolved in brain homogenates obtained from untreated rat brain (see section 2.2.1 for running parameters).

2.10.3 Tranylcypromine

A modification of the procedure reported by Nazarali *et al.* (1987) was used for the determination of TCP. Rat tissue was homogenized in 5 volumes of distilled water. A 1 ml aliquot of tissue homogenate or urine was used for determination. The internal standard (100ng), p-chlorophentermine, was added at the beginning of the assay. Calibration curves were run on each assay day in parallel with the sample tubes. Perchloric acid (0.4 M, 2 ml) was added to the sample tubes. The tubes were then vortexed and centrifuged to precipitate the protein. The supernatants were decanted into another set of test tubes and basified with K_2CO_3 (25% w/v) and extracted with 4 ml of ethyl acetate. The organic layer was collected and brought to dryness under a gentle stream of dry nitrogen gas. The residue was then reacted with a solution containing 300 µl of toluene and 2 µl of pentafluorobenzoyl chloride (PFBC) for 1 h at 60 °C. After derivitization was complete, the toluene layer was collected and an aliquot injected onto a gas chromatograph equipped with a fused silica column and an electron capture detector. A calibration curve was run in parallel to samples on each assay day and contained 5-1000 ng of TCP dissolved in brain homogenates obtained from untreated rat brain.

2.11 PROTEIN CONTENT

Protein concentrations in rat brain homogenates were determined according to Lowry *et al.* (1951). To an aliquot (50 µl) of brain homogenate were

added 750 µl distilled water and 200 µl of membrane digestor (1:1 v/v 1 N sodium hydroxide and 1% sodium deoxycholate). The mixture was vortexed and incubated at room temperature for 10 min, and 5 ml of reagent A (1/.01/.01 v/v/v 2% sodium carbonate, 1% cupric sulfate and 2% sodium potassium tartrate) were added; the tubes were vortexed and incubated for another 10 min. Folin reagent (1:1 v/v 2 N folin and distilled water) [500 µl] was then added, and the tubes were vortexed and incubated for a minimum of 30 min. A standard curve was run in parallel with the tissue samples, using bovine serum albumin as protein standard. All tubes were placed in an ultraviolet-visible spectrophotometer (wave length = 660 nm) to determine absorbance values.

2.12 STATISTICAL ANALYSIS

Principal components analysis was carried out using the Statistical Package for the Social Sciences (SPSS). All other analyses used parametric ANOVA followed by Newman-Keuls multiple comparisons when appropriate. A two-tailed probability distribution was used for all statistical analyses. Treatment groups were considered to be significantly different when $p<0.05$.

3 RESULTS

3.1 HIGH VS. LOW DOSE TCP

3.1.1 ^{3}H-Tryptamine Binding in Hippocampus

Both the high (2.5 mg/kg/day) and the low (0.5 mg/kg/day) doses of TCP produced a significant decrease in ^{3}H-tryptamine binding after 10 and 28 days (Figure 1), but only the high-dose of TCP had a significant effect after only 4 days. With the low-dose, there was a significantly greater down-regulation at day 28 compared to days 10 and 4 and a significantly greater down-regulation at day 10 compared to day 4. The high dose resulted in a significant difference in ^{3}H-tryptamine binding between 4 and 28 days and between 10 and 28 days.

3.1.2 ^{3}H-Tryptamine Binding in Striatum

Unlike the hippocampus, ^{3}H-tryptamine binding in the striatum was decreased by both doses at all time intervals (Figure 2). Furthermore, the high-dose of TCP produced a significantly greater reduction than the low-dose at each time interval. The decrease in binding observed with the high-dose is greater at 28 days than at 4 days, and the low-dose showed a greater reduction at 28 days than at 4 or 10 days.

3.1.3 5-HT$_2$ Receptor Density and Affinity

A typical isotherm and Scatchard plot of ^{3}H-ketanserin binding in rat cortex are illustrated in Figures 3 and 4, respectively. The low (0.5 mg/kg/day) dose of TCP did not result in a significant down-regulation of the 5-HT$_2$ receptor site at any time interval. The high (2.5 mg/kg/day) dose of TCP produced a down-regulation after 10 and 28 days of chronic treatment, but

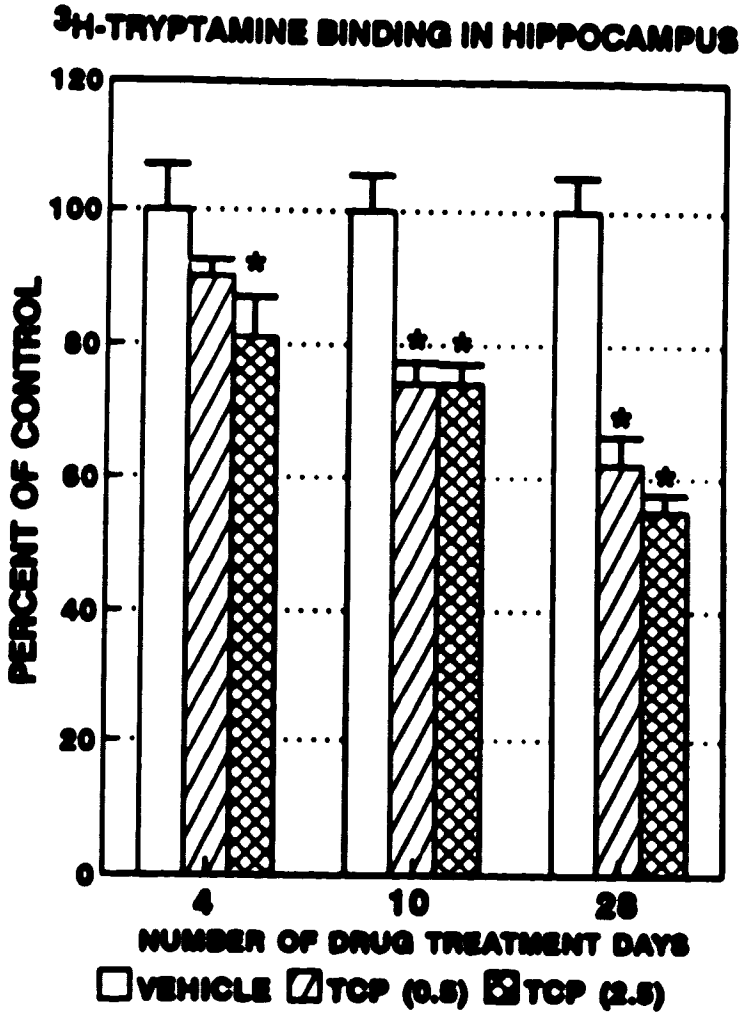


Figure 1. ^{3}H-Tryptamine binding in rat hippocampus following chronic treatment with high (2.5 mg/kg/day)- and low (0.5 mg/kg/day)-dose TCP. Values represent means ± SEM (n=8-10) and are expressed as % of values obtained in rats treated with vehicle for the same number of days. Control (vehicle-treated) values = 3,763 ± 279 dpm/mg protein. * denotes significant difference ($p<0.05$) from vehicle-treated controls.

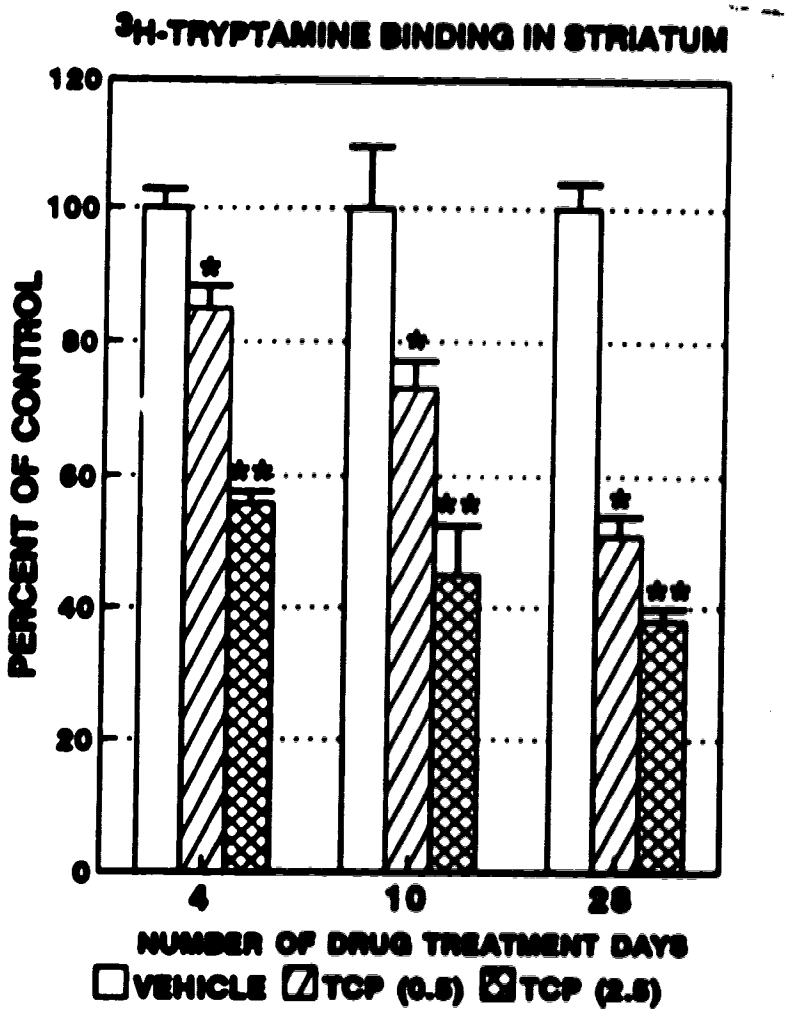


Figure 2. ^{3}H-Tryptamine binding in rat striatum following chronic treatment with high (2.5 mg/kg/day)- and low (0.5 mg/kg/day)-dose TCP. Values represent means ± SEM (n=8-10) and are expressed as % of values obtained in rats treated with vehicle for the same number of days. Control (vehicle-treated) values = 4,930 ± 267 dpm/mg protein. * denotes significant difference ($p<0.05$) from vehicle-treated controls. ** denotes significant difference ($p<0.05$) from TCP (0.5 mg/kg)-treated animals.

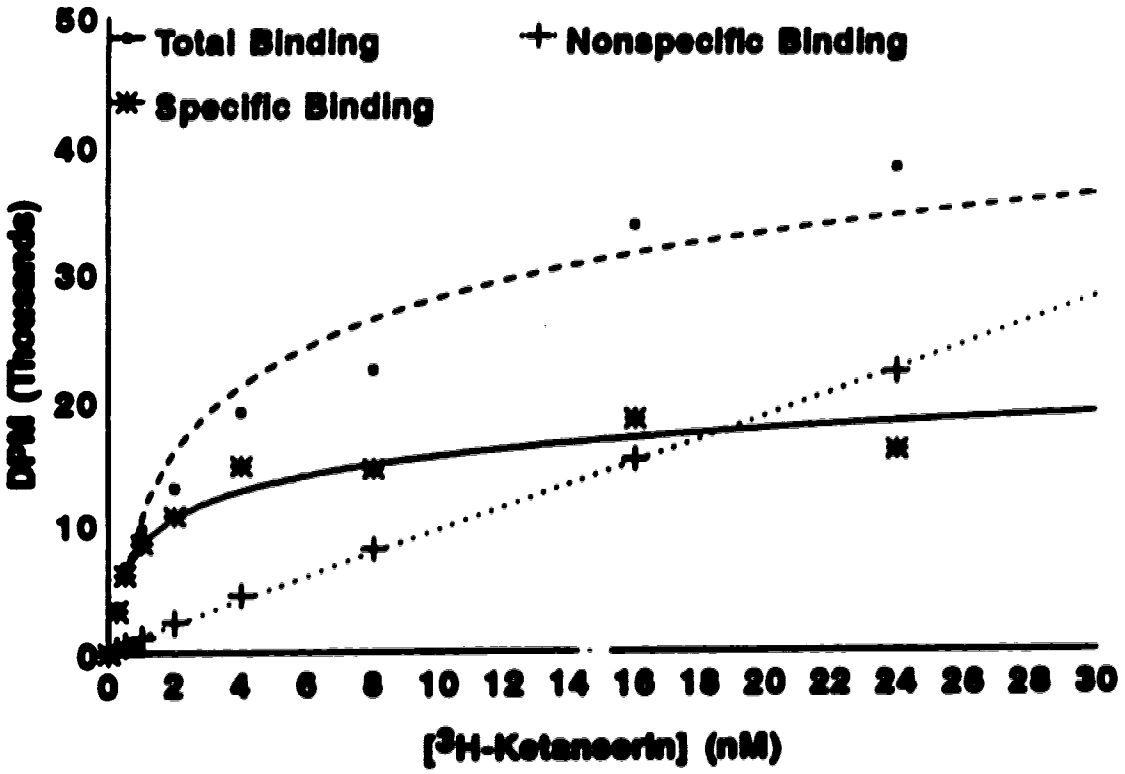


Figure 3. Typical binding isotherm for ^{3}H-ketanserin binding to rat cortical membranes.

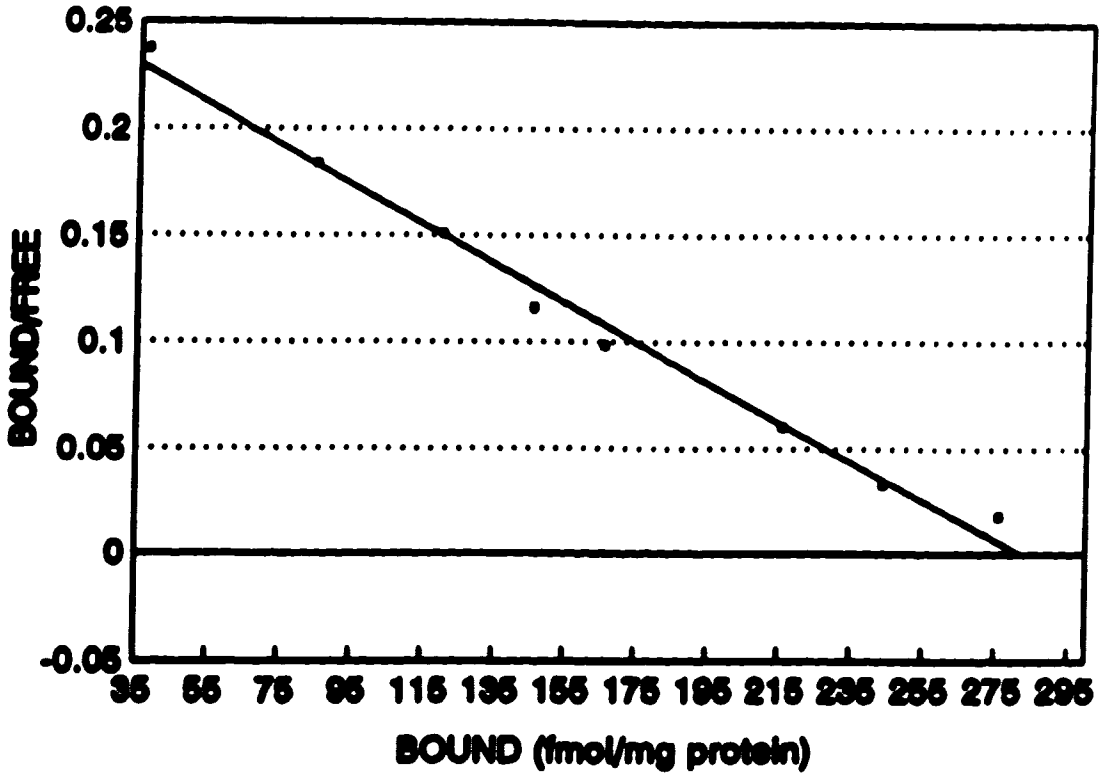


Figure 4. Typical Scatchard plot for ^{3}H-ketanserin binding to rat cortical membranes.

Table 4. 5-HT_2 Receptor Density and Affinity Following Chronic Treatment with High- and Low-Dose TCP

Drug Treatment	Duration	Bmax (fmol/mg protein)	Kd (nM)
Vehicle (dist. water)	4 days	198 ± 7	0.50 ± .01
TCP (0.5mg/kg/day)	4 days	185 ± 6	0.49 ± .02
TCP (2.5mg/kg/day)	4 days	186 ± 5	0.52 ± .02
Vehicle (dist. water)	10 days	252 ± 15	0.58 ± .03
TCP (0.5mg/kg/day)	10 days	235 ± 9	0.58 ± .04
TCP (2.5mg/kg/day)	10 days	207 ± 15 *	0.64 ± .04
Vehicle (dist. water)	28 days	203 ± 11	0.54 ± .03
TCP (0.5mg/kg/day)	28 days	195 ± 13	0.58 ± .02
TCP (2.5mg/kg/day)	28 days	147 ± 11 *	0.65 ± .05

Values represent mean ± SEM (n=8-10). * denotes significant difference ($p<0.05$) from control.

not after only 4 days (Table 4). A significant change in the affinity (Kd) of ^{3}H-ketanserin for the 5-HT$_2$ site did not occur after drug treatment at any time interval. 5-HT$_2$ receptor density in vehicle treated controls was elevated at day 10 compared to the densities observed in these controls at days 4 and 28.

3.1.4 MAO Activity

Although both doses of TCP produced significant MAO inhibition, the high-dose of TCP inhibited MAO to a greater extent than the low-dose at all time intervals (Figures 5 and 6).

3.1.5 Levels of Neurotransmitter Amines

Both doses of TCP produced significantly greater levels of the neurotransmitter amines NA and 5-HT in rat brain than were observed in the vehicle-treated rats at the 4, 10 and 28 day intervals (Figures 7 and 8). Animals receiving the high dose of TCP had increases in these amines which were significantly higher than those observed in animals receiving the low-dose over the time intervals studied.

3.1.6 Tissue and Fluid Levels of TCP

Levels of TCP were determined in urine, liver, heart, and brain remainder (Table 5) at the 10-day interval. Levels of TCP were significantly higher in the high-dose than in the low-dose animals. Levels of TCP in animals receiving the low dose were below the detectable limit (30ng/g) in brain remainder. Typical GC traces of derivatized TCP and *p*-chlorophentermine are shown in Figure 9.

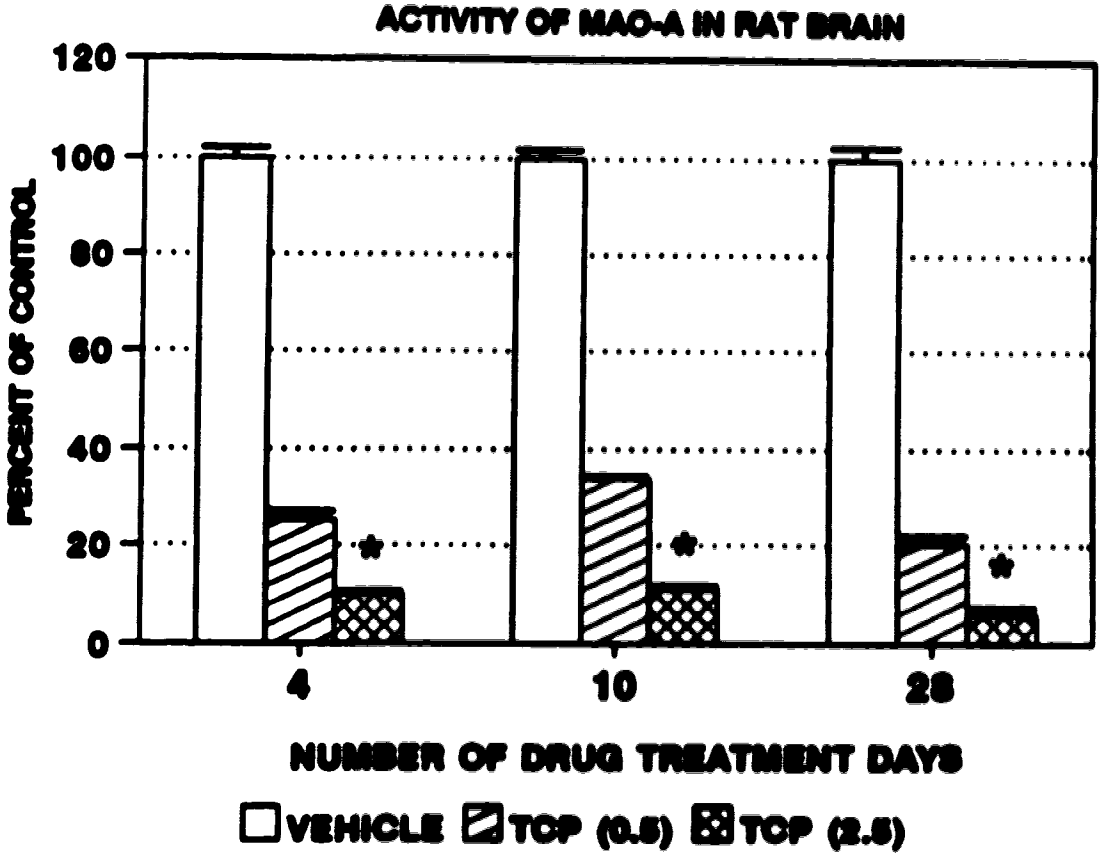


Figure 5. Activity of MAO-A in rat whole brain minus cortex, hippocampus and striatum following chronic treatment with high- and low-dose TCP. Values represent means ± SEM (n=8-10) and are expressed as % of values in rats treated with vehicle for the same number of days. Doses are expressed as mg/kg/day. All drug treatment groups were significantly different from vehicle. * denotes significant difference ($p<0.05$) from TCP (0.5 mg/kg/day).

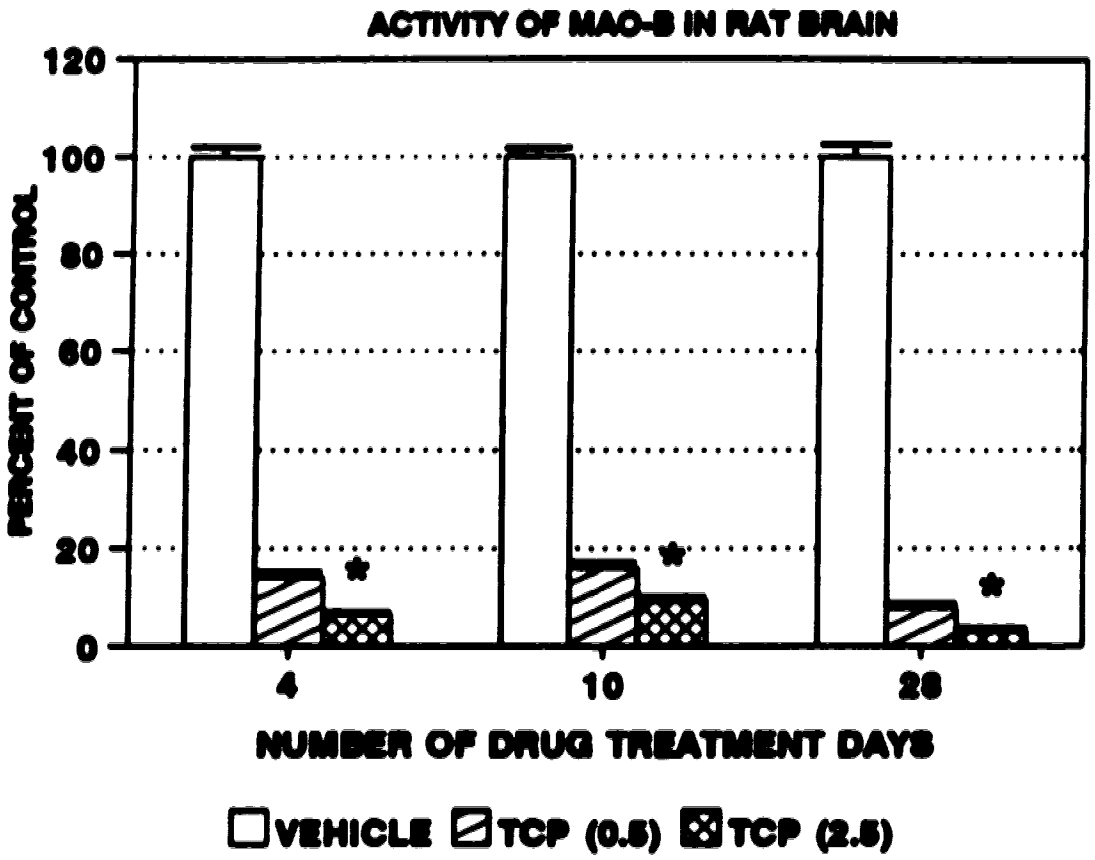


Figure 6. Activity of MAO-B in rat whole brain minus cortex, hippocampus and striatum following chronic treatment with high- and low-dose TCP. Values represent means ± SEM (n=8-10) and are expressed as % of values in rats treated with vehicle for the same number of days. Doses are expressed as mg/kg/day. All drug treatment groups were significantly different from vehicle. * denotes significant difference ($p<0.05$) from TCP (0.5 mg/kg/day).

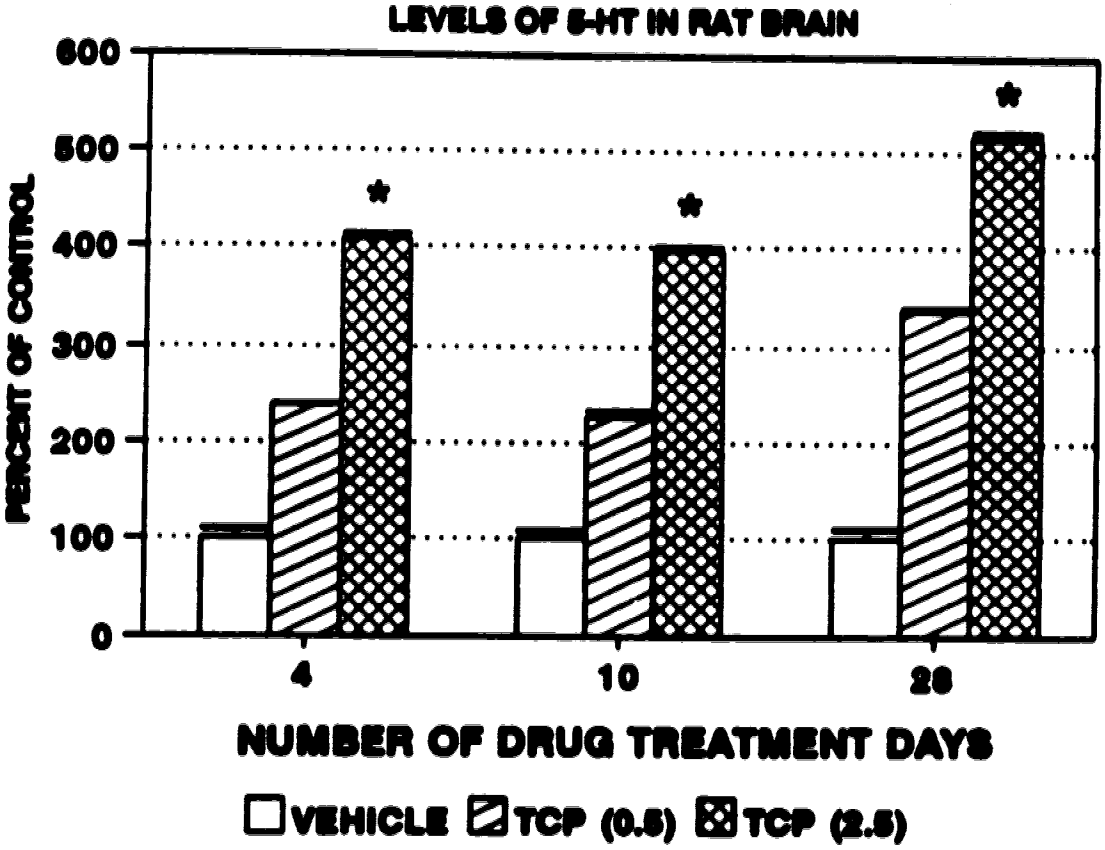


Figure 7. Levels of 5-HT in rat whole brain minus cortex, hippocampus and striatum following chronic treatment with high- and low-dose TCP. Values represent means ± SEM (n=8-10) and are expressed as % of values in rats treated with vehicle for the same number of days. Doses are expressed as mg/kg/day. All drug treatment groups were significantly different from control. * denotes significant difference ($p<0.05$) from TCP (0.5 mg/kg/day). Control values (in ng/g tissue): 210 ± 18, n=28.

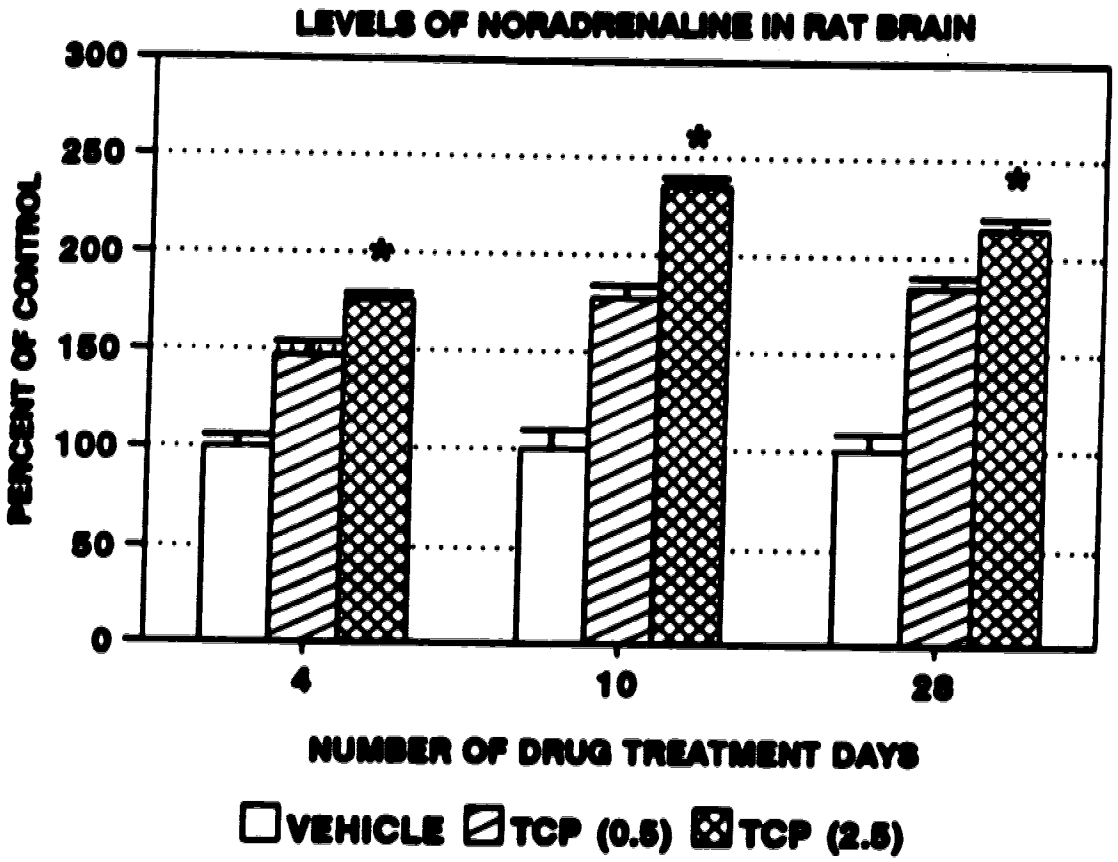


Figure 8. Levels of NA in rat whole brain minus cortex, hippocampus and striatum following chronic treatment with high- and low-dose TCP. Values represent means ± SEM (n=8-10) and are expressed as % of values in rats treated with vehicle for the same number of days. Doses are expressed as mg/kg/day. All drug treatment groups were significantly different from control. * denotes significant difference ($p<0.05$) from TCP (0.5 mg/kg/day). Control values (in ng/g tissue): 335 ± 28, n=28.

Table 5. Levels of TCP in Various Tissues and Fluids Following Chronic Treatment with TCP for Ten Days (Administration via Osmotic Mini-pumps)

TREATMENT	URINE (μg/day)	LIVER (ng/g)	HEART (ng/g)	REST OF BRAIN (ng/g)
TCP (0.5mg/kg/day)	1.7 ± .2	68 ± 9	29 ± 6	<30
TCP (2.5mg/kg/day)	11 ± 3	350 ± 57	59 ± 4	164 ± 30

Values represent mean ± SEM (n=10). All drug levels are significantly greater ($p<0.05$) in the TCP (2.5 mg/kg/day) treated group than in the TCP (0.5 mg/kg/day) group. Rest of brain consists of whole brain minus cortex, hippocampus, and striatum.

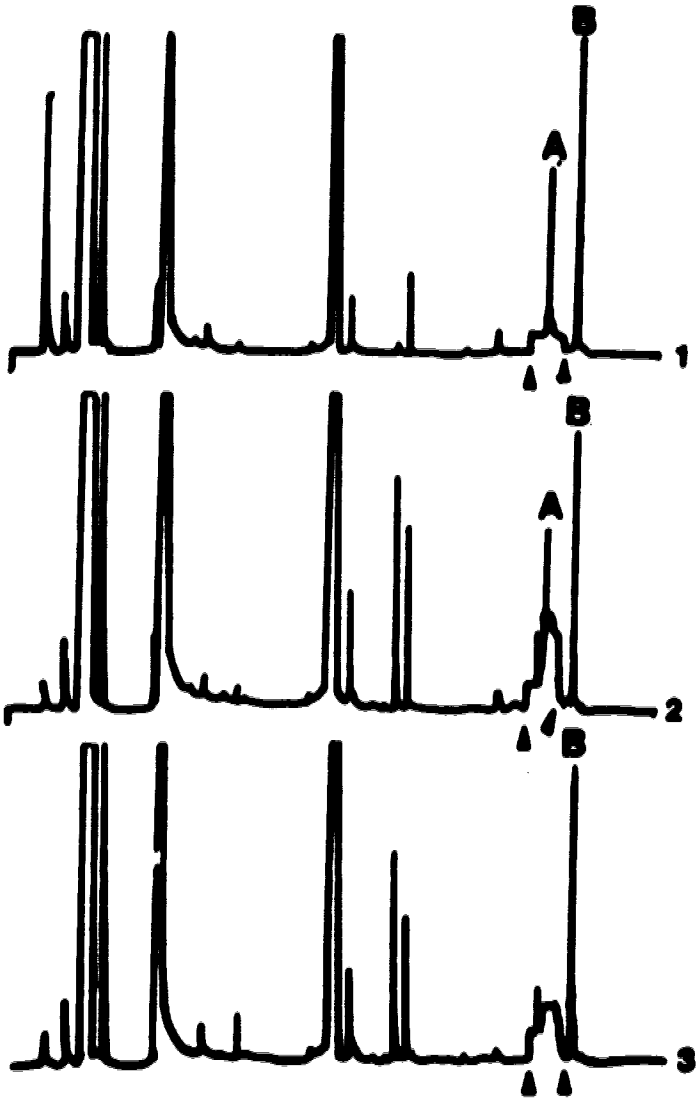


Figure 9. Representative gas chromatographic traces for the PFB derivatives of TCP (A) and *p*-chlorophentermine (internal standard, B). "1" represents a trace obtained from the analysis of standards dissolved in water; "2" represents a trace obtained from a brain tissue sample from an animal which had received TCP and to which internal standard had been added; and "3" represents a trace obtained from a brain tissue sample from an untreated animal to which internal standard had been added. Retention times for the peaks were 20.64 min (A) and 21.72 min (B). Δ indicates a change in attenuation.

3.2 AMI AND TCP VS. AMI ALONE

3.2.1 Residual AMI Levels in 5-HT_2 Binding Assay

Following the previously described tissue preparation for 5-HT_2 binding (Section 2.7) the concentration of AMI still remaining in the incubation medium was found to be 56 ± 10 nM. Typical GC traces for AMI and derivatized MAP are shown in Figure 10.

3.2.2 MAO Activity

AMI had no effect on MAO-A or MAO-B activity at any time interval, while the AMI-TCP combination inhibited MAO-A and -B by a similar degree at all time intervals (Figures 11 and 12). MAO inhibition with the combination at 28 days was significantly greater than that seen at the 4 and 10 day intervals.

3.2.3 Neurotransmitter Amine Levels

Treatment with AMI alone had no effect on levels of 5-HT or NA in rat brain. Those animals receiving the combination had the expected rise in the levels of these amines due to MAO inhibition by TCP (Figures 13 and 14). After 28 days of administration, the drug combination increased levels of 5-HT and NA to approximately 300% and 200% of control, respectively.

3.2.4 5-HT_2 Receptor Density and Affinity

Down-regulation of the 5-HT_2 receptor site was observed at all time intervals in those animals treated with AMI and the AMI-TCP combination. Table 6 indicates that although a small but significant difference between the AMI-TCP combination and AMI alone was observed at the 10 day interval, this effect was not observed at the 4 or 28 day interval. The average Kd value for the vehicle-treated animals over the three time intervals was 0.51 nM.

Figure 10. Representative gas chromatographic traces for AMI (A) and the acetylated derivative of MAP (internal standard, B). "1" represents a trace obtained from the analysis of a pure standards; "2" represents a trace obtained from a brain tissue sample from an animal which had received AMI and to which internal standard had been added; and "3" represents a trace obtained from a brain tissue sample from an untreated animal to which internal standard had been added. Retention times for the peaks were 18.25 min (A) and 28.67 min (B). ▲ indicates a change in attenuation.

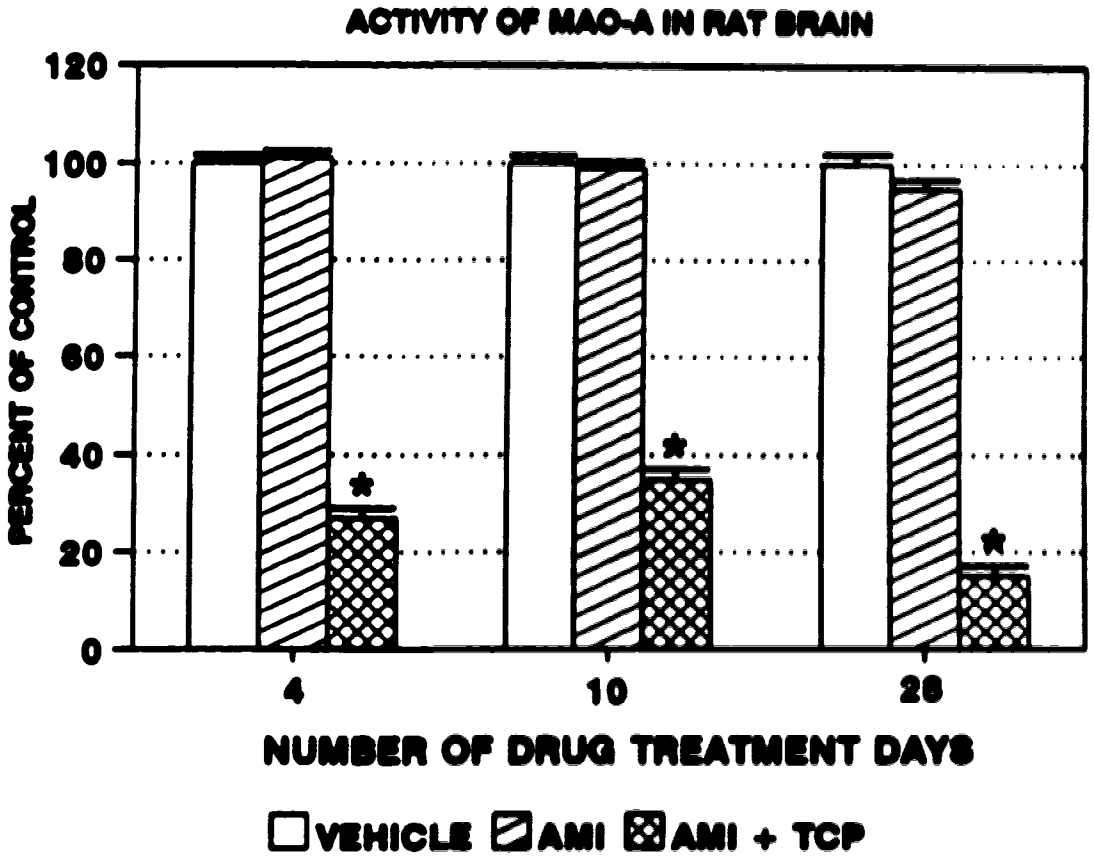


Figure 11. Activity of MAO-A in rat whole brain minus cortex, hippocampus and striatum. Values represent means ± SEM (n=8-10) and are expressed as % of values in rats treated with vehicle for the same number of days. Doses: AMI, 3.5 mg/kg/day; TCP, 0.5 mg/kg/day. * denotes significant difference ($p<0.05$) from control. Inhibition of MAO-A by AMI + TCP was not significantly different between days 4 and 10, but at day 28 inhibition was greater than that at the other two time intervals.

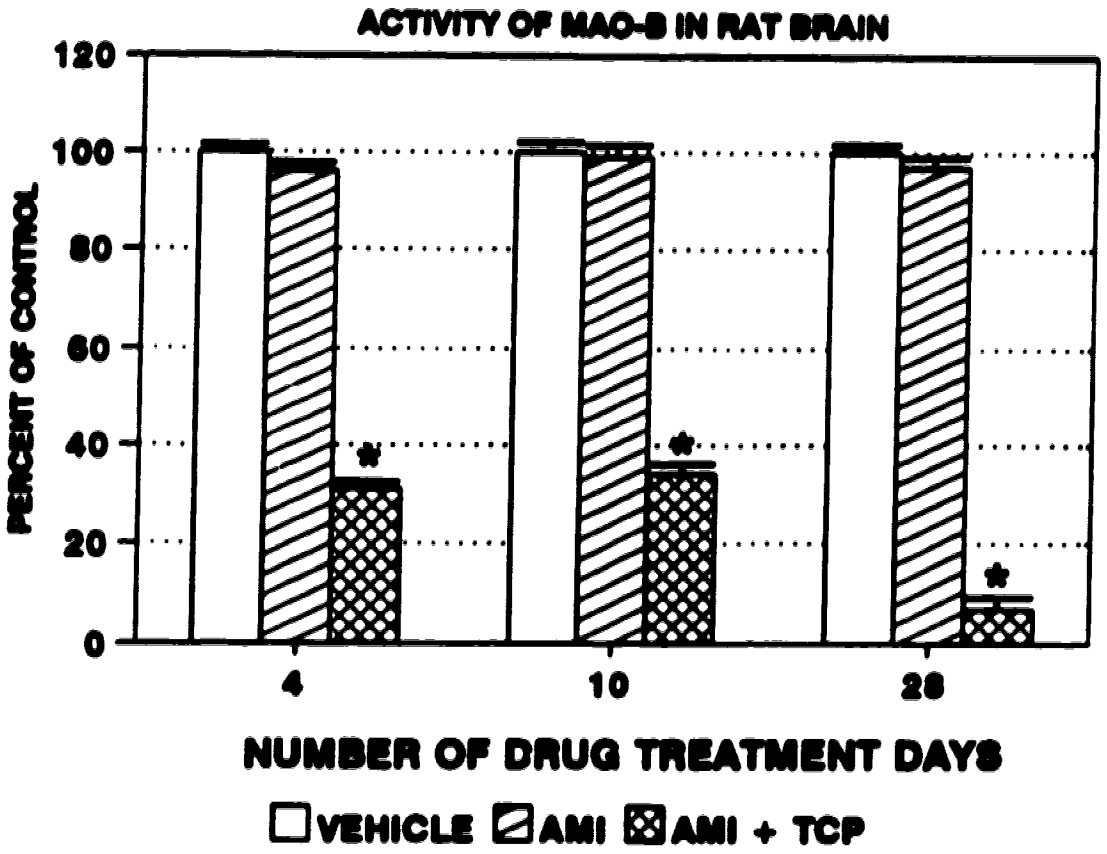


Figure 12. Activity of MAO-B in rat whole brain minus cortex, hippocampus and striatum. Values represent means ± SEM (n=8-10) and are expressed as % of values in rats treated with vehicle for the same number of days. Doses: AMI, 3.5 mg/kg/day; TCP, 0.5 mg/kg/day. * denotes significant difference ($p<0.05$) from control. Inhibition of MAO-B by AMI + TCP was not significantly different between days 4 and 10, but at day 28 inhibition was greater than that at the other two time intervals.

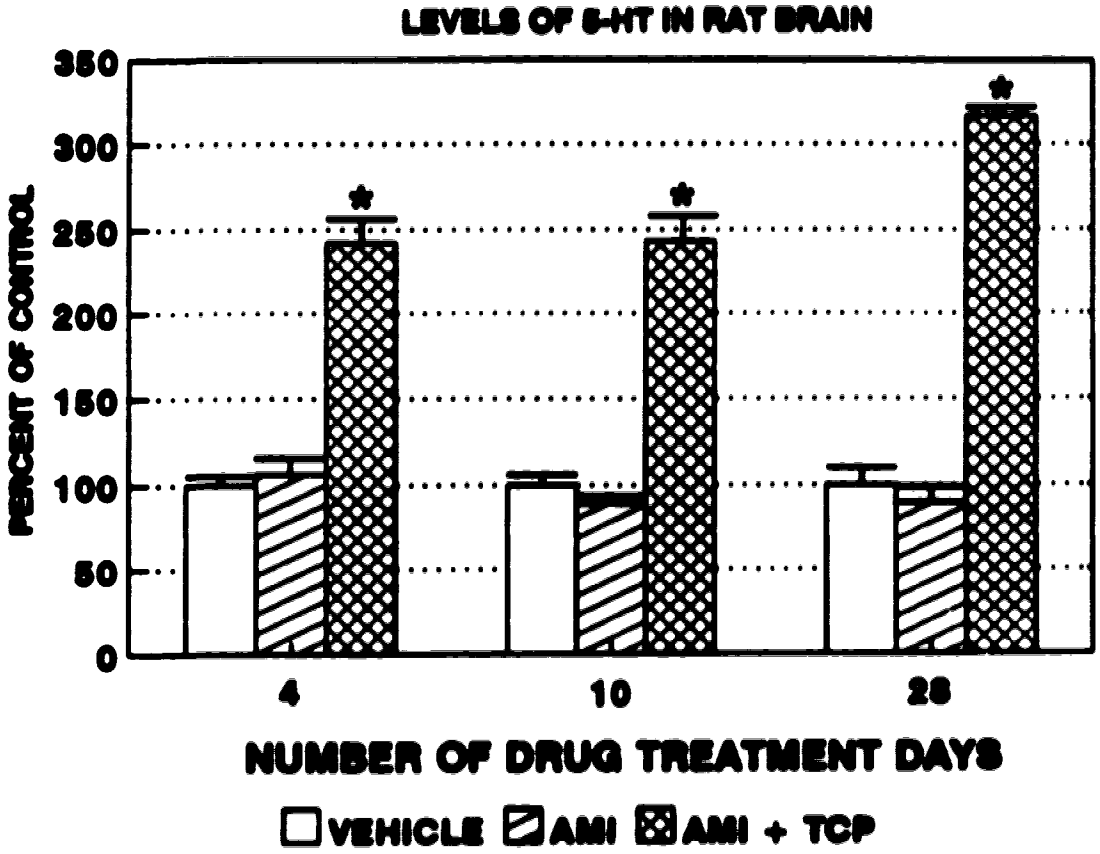


Figure 13. Levels of 5-HT in rat rest of brain. Values represent means ± SEM (n=8-10) and are expressed as % of values in rats treated with vehicle for the same number of days. Doses are expressed as mg/kg/day. * denotes significant difference ($p<0.05$) from control. Control values (in ng/g tissue): 233 ± 16, n=28.

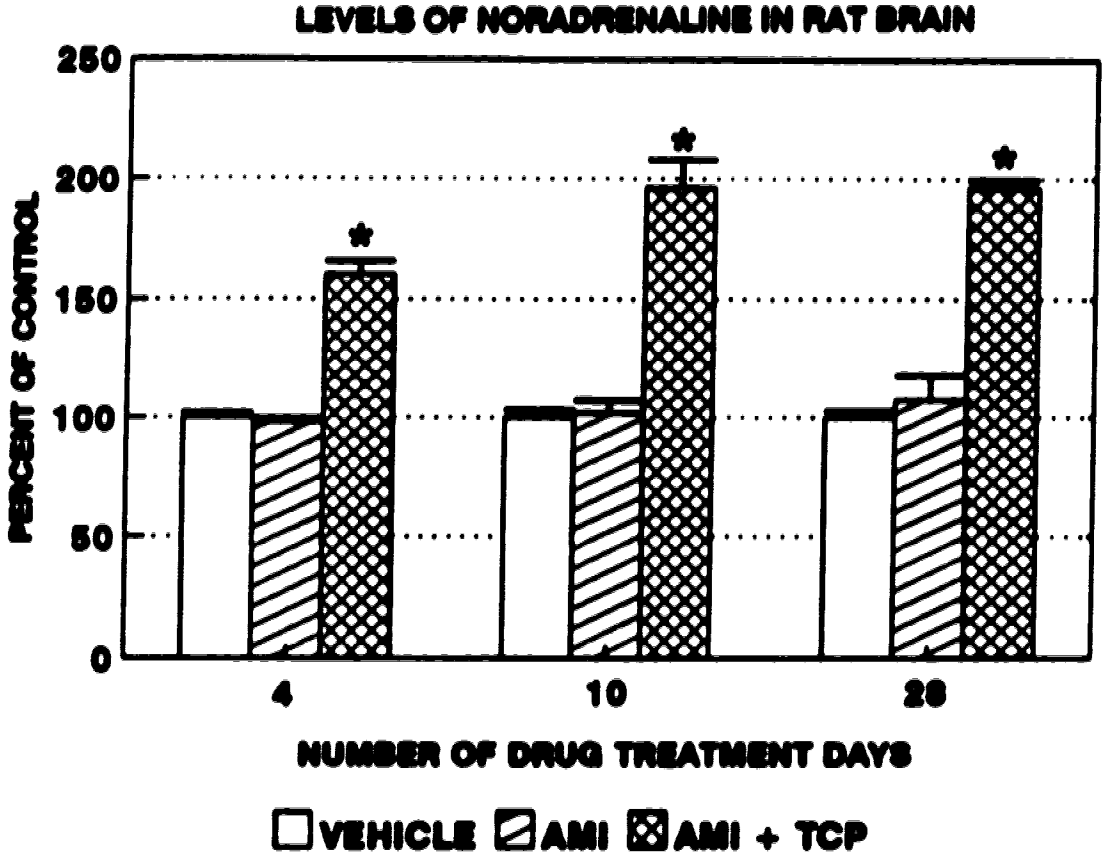


Figure 14. Levels of NA in rat rest of brain. Values represent means ± SEM (n=8-10) and are expressed as % of values in rats treated with vehicle for the same number of days. Doses are expressed as mg/kg/day. * denotes significant difference ($p<0.05$) from control. Control values (in ng/g tissue): 382 ± 21, n=28.

Table 6. 5-HT_2 Receptor Density and Affinity Following Chronic Treatment with AMI, TCP, or AMI + TCP

Drug Treatment	Duration	Bmax (fmol/mg protein)	Kd (nM)
Vehicle (dist. water)	4 days	190 ± 10	0.50 ± .03
AMI (3.5mg/kg/day)	4 days	106 ± 4 *	0.80 ± .05 *
AMI (3.5mg/kg/day) + TCP (0.5mg/kg/day)	4 days	114 ± 5 *	0.92 ± .05 *
Vehicle (dist. water)	10 days	231 ± 7	0.48 ± .02
AMI (3.5mg/kg/day)	10 days	157 ± 6 *	1.06 ± .09 *
AMI (3.5mg/kg/day) + TCP (0.5mg/kg/day)	10 days	137 ± 6 **	1.01 ± .05 *
Vehicle (dist. water)	28 days	203 ± 14	0.50 ± .02
AMI (3.5mg/kg/day)	28 days	131 ± 14 *	1.22 ± .14 *
AMI (3.5mg/kg/day) + TCP (0.5mg/kg/day)	28 days	117 ± 8 *	1.15 ± .14 *

Values represent mean ± SEM (n=8-10). * denotes significant difference ($p<0.05$) from control; ** denotes significant between control and AMI (3.5 mg/kg/day).

Those animals receiving AMI or the AMI-TCP combination had an average Kd of 1.04 nM. 5-HT_2 receptor density in vehicle-treated controls was slightly elevated at day 10 compared to the densities observed in these controls at days 4 and 28, similar to the situation found in the study reported in section 3.1.3.

3.3 FLU AND DMI VS. THE DRUGS ALONE (Administration by Osmotic Minipumps)

3.3.1 5-HT_2 Receptor Density and Affinity

The three doses of DMI (5, 10, and 15 mg/kg/day) each resulted in a significant down-regulation of the 5-HT_2 receptor site (Table 7), while neither FLU (10 mg/kg/day) nor the combination of FLU (10 mg/kg/day) and DMI (5 mg/kg/day) had any effect. A significant decrease in the affinity (increased Kd value) of ^{3}H-ketanserin for the 5-HT_2 site occurred between all groups and vehicle except DMI (5 mg/kg/day). The affinity of the group receiving the combination was also significantly less than groups receiving FLU (10 mg/kg/day) or DMI (5 mg/kg/day). This effect may be due to a residual presence of FLU, NFLU or DMI in the tissue preparation, as has been reported with AMI (Goodnough and Baker, 1993c and section 3.2.4 of this thesis).

3.3.2 β-Adrenergic Receptor Density and Affinity

A typical isotherm and a typical Scatchard plot of ^{3}H-CGP 12177 binding in rat cortex are illustrated in Figures 15 and 16, respectively. The three doses of DMI (5, 10, and 15 mg/kg/day) and the combination of FLU (10 mg/kg/day) and DMI (5 mg/kg/day) resulted in a significant down-regulation of the β-adrenergic receptor site in rat cortex. DMI (10, 15 mg/kg/day) and the

Table 7. 5-HT_2 Receptor Density and Affinity Following Chronic Treatment with FLU, DMI, or FLU + DMI (Administration via Osmotic Minipumps)

Drug Treatment	Duration	Bmax (fmol/mg protein)	Kd (nM)
Vehicle (dist. water)	14 days	259 ± 8 §	0.55 ± .01
DMI (5 mg/kg/day)	14 days	200 ± 11 *	0.56 ± .04
DMI (10 mg/kg/day)	14 days	190 ± 14 *	0.68 ± .04 *§
DMI (15 mg/kg/day)	14 days	171 ± 11 *§	0.77 ± .05 *§
FLU (10 mg/kg/day)	14 days	255 ± 17 §	0.63 ± .02 *§
FLU (10 mg/kg/day) +DMI (5 mg/kg/day)	14 days	246 ± 11 §	0.76 ± .03 *§

Values represent mean ± SEM (n=10). * denotes significant difference ($p<0.05$) from vehicle. § denotes significant difference ($p<0.05$) from DMI (5 mg/kg/day)

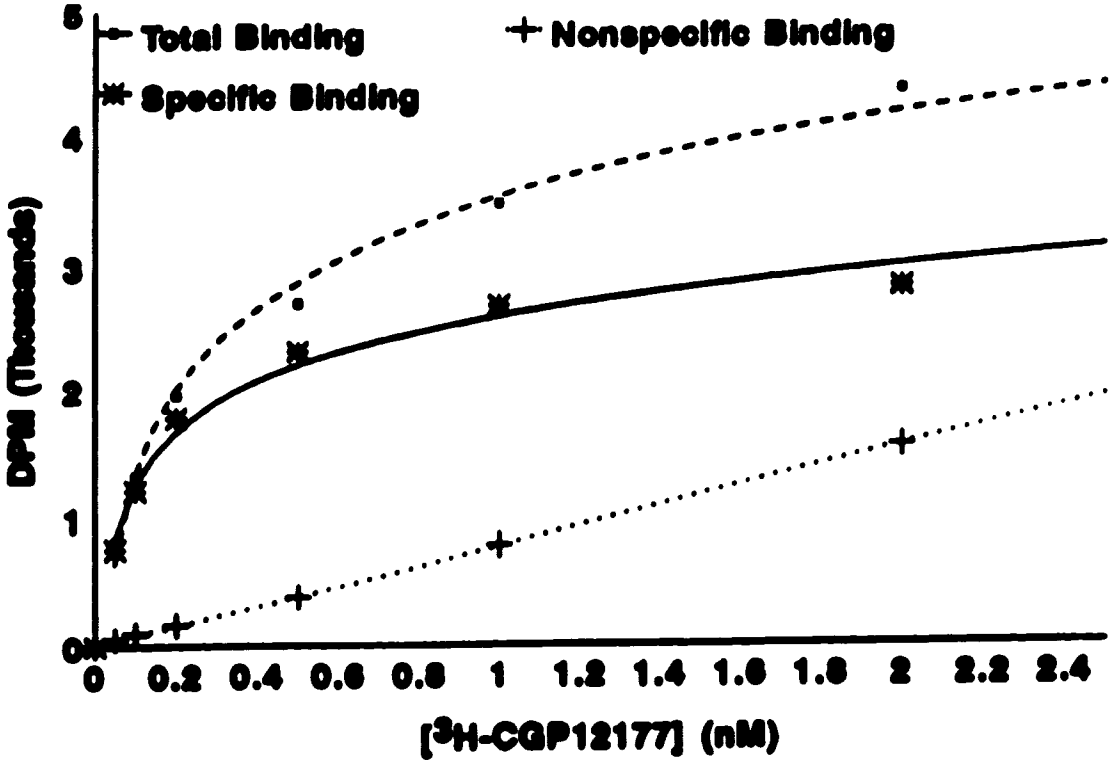


Figure 15. Typical binding isotherm for ^{3}H-CGP 12177 binding to rat cortical membranes.

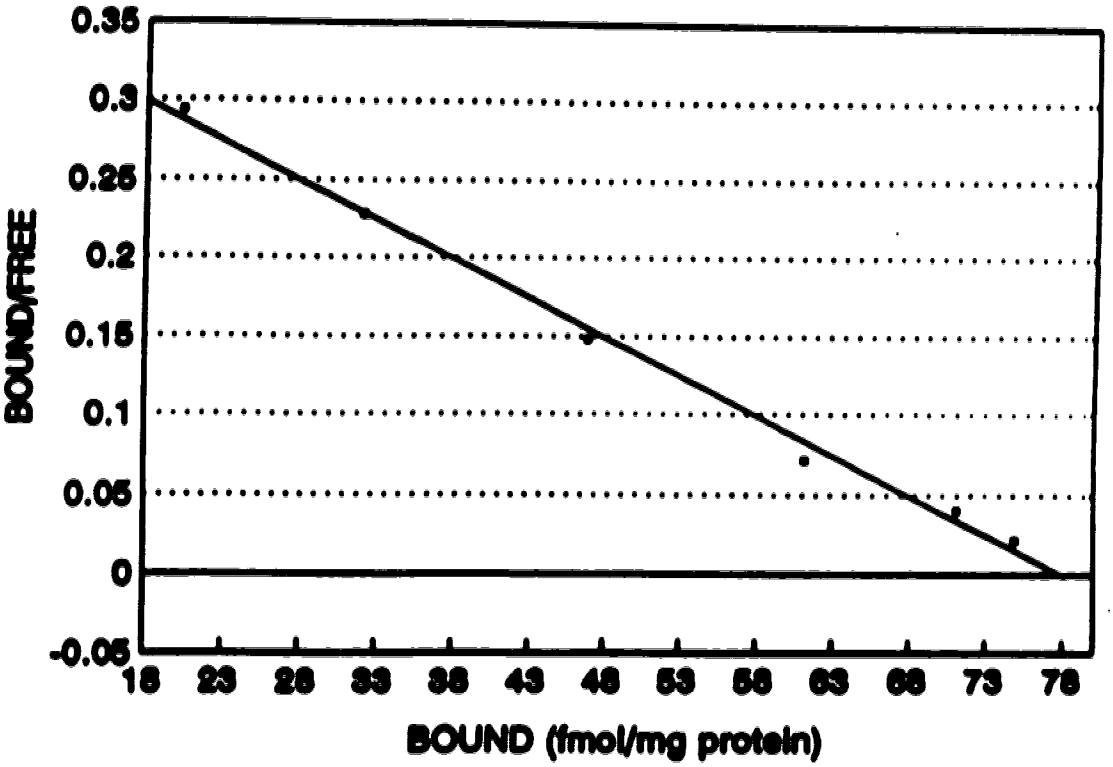


Figure 16. Typical Scatchard plot for ^{3}H-CGP12177 binding to rat cortical membranes.

combination of FLU (10 mg/kg/day) and DMI (5 mg/kg/day) produced a significantly greater down-regulation than did DMI (5 mg/kg/day) (Table 8). FLU (10 mg/kg/day) had no effect. There was no significant change in affinity between the drug treatment groups and vehicle treated controls.

The greater down-regulation observed with the combination was probably the result of greater levels of DMI in these animals. Principal components analysis of FLU, NFLU, DMI, and Bmax extracted two factors: 1. FLU and NFLU levels; and 2. DMI levels and Bmax. Rotated factor analysis showed that FLU and NFLU covaried with factor 1 by .990 and .985 and with factor 2 by .00573 and .0999 respectively. DMI and Bmax covaried with factor 1 by -.103 and -.0355 and with factor 2 by .994 and -.995 respectively. These findings indicate that there is a very strong relationship between DMI levels and Bmax and between FLU levels and NFLU levels and almost no relationship between FLU/NFLU and Bmax. A graphical representation of the relationship between DMI levels and Bmax is shown in Figure 17. Here the point corresponding to the combination lies directly on the line produced from DMI only-treated groups and vehicle.

3.3.3 Drug Levels

A simple gas chromatographic assay has been developed which can quantitate FLU, NFLU, and DMI simultaneously, enabling researchers to readily study the effects of combined FLU and DMI treatment on the levels of the parent drugs and NFLU.

Table 8. β-Adrenergic Receptor Density and Affinity Following Chronic Treatment with FLU, DMI, or FLU + DMI (Administration via Osmotic Minipumps)

Drug Treatment	Duration	Bmax (fmol/mg protein)	Kd (nM)
Vehicle (dist. water)	14 days	74.3 ± 1.6 §	0.121 ± .008
DMI (5 mg/kg/day)	14 days	52.4 ± 2.7 *	0.127 ± .016
DMI (10 mg/kg/day)	14 days	43.3 ± 1.9 *§	0.120 ± .006
DMI (15 mg/kg/day)	14 days	43.5 ± 1.8 *§	0.135 ± .009
FLU (10 mg/kg/day)	14 days	71.2 ± 2.8 §	0.133 ± .013
FLU (10 mg/kg/day) +DMI (5 mg/kg/day)	14 days	46.0 ± 2.0 *§	0.124 ± .008

Values represent mean ± SEM ($n=10$). * denotes significant difference ($p<0.05$) from control. § denotes significant difference ($p<0.05$) from DMI (5 mg/kg/day).

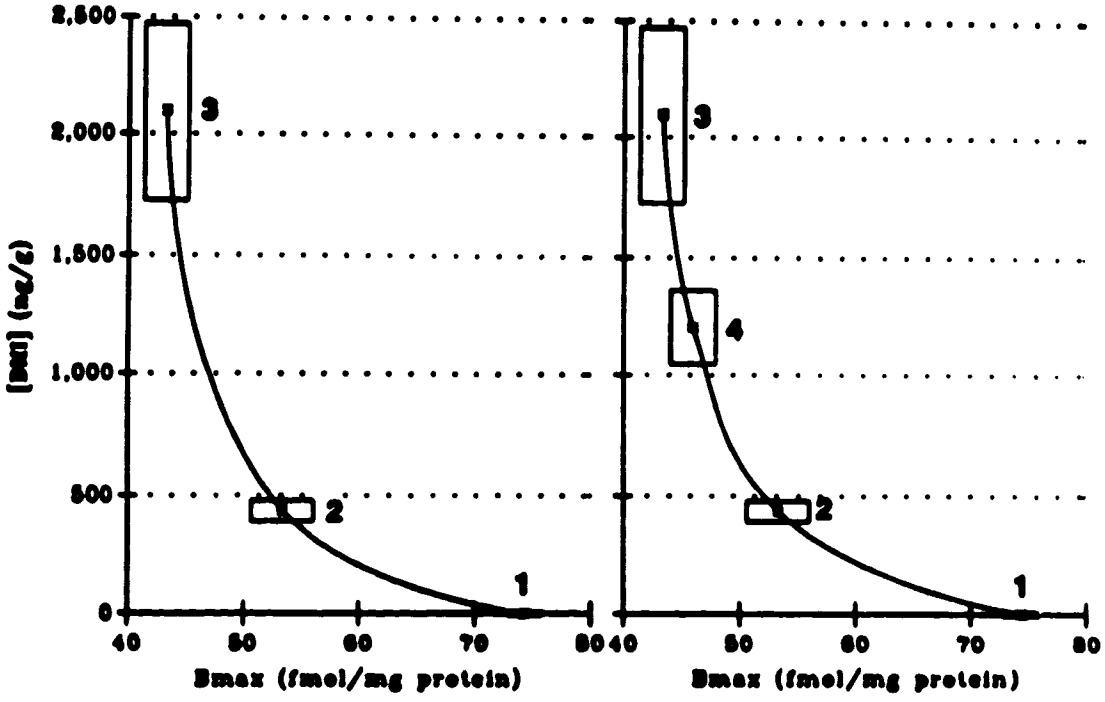


Figure 17. Graphical representation of the relationship between DMI levels in cortex and the density of β-adrenergic receptors in cortex. The figure on the left shows this relationship for animals receiving vehicle or DMI; the figure on the right shows this relationship for animals receiving vehicle, DMI, or the combination of DMI and FLU. Point #1 - Vehicle; Point #2 - DMI (5 mg/kg/day); Point #3 - DMI (10 mg/kg/day); Point #4 - DMI (5 mg/kg/day) + FLU (10 mg/kg/day). Boxes represent SEM for both Bmax and drug levels (horizontal and vertical portions, respectively).

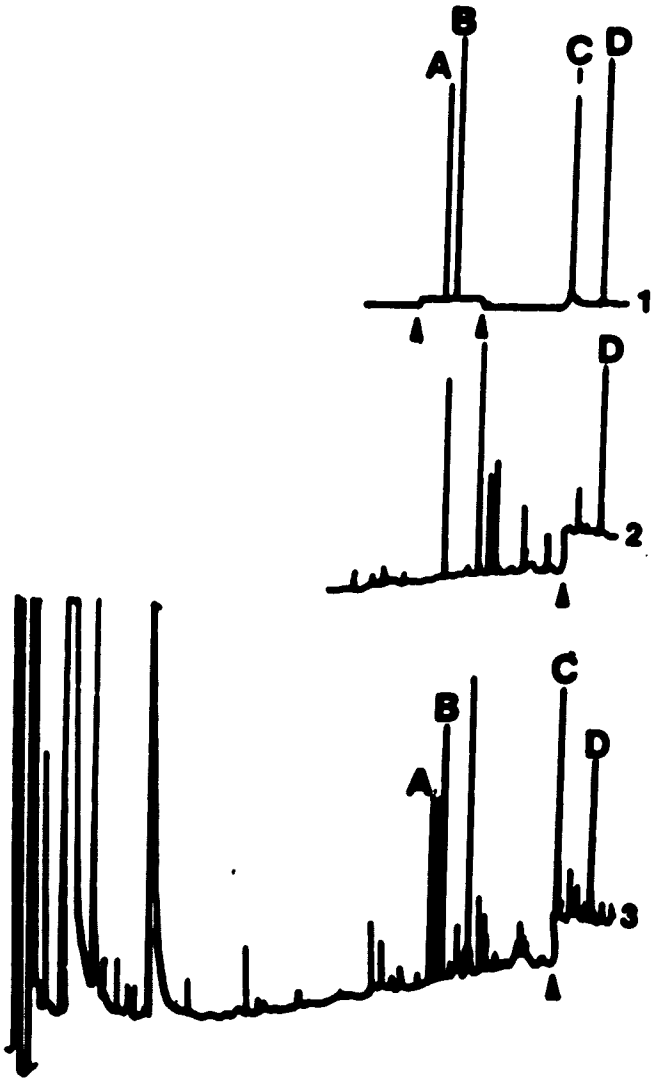


Figure 18. Representative gas chromatographic traces for the acetylated derivatives of NFLU (A), FLU (B), DMI (C), and maprotiline (internal standard, D). "1" represents a trace obtained from pure standards; "2" represents a trace obtained from the analysis of a brain tissue sample from a vehicle-treated animal to which internal standard had been added and "3" represents a trace obtained from the analysis of a brain tissue sample from an animal which had received the combination of FLU and DMI. Retention times for the peaks were 14.27 min (A), 14.67 min (B), 18.69 min (C), and 19.89 min (D). Δ indicates a change in attenuation.

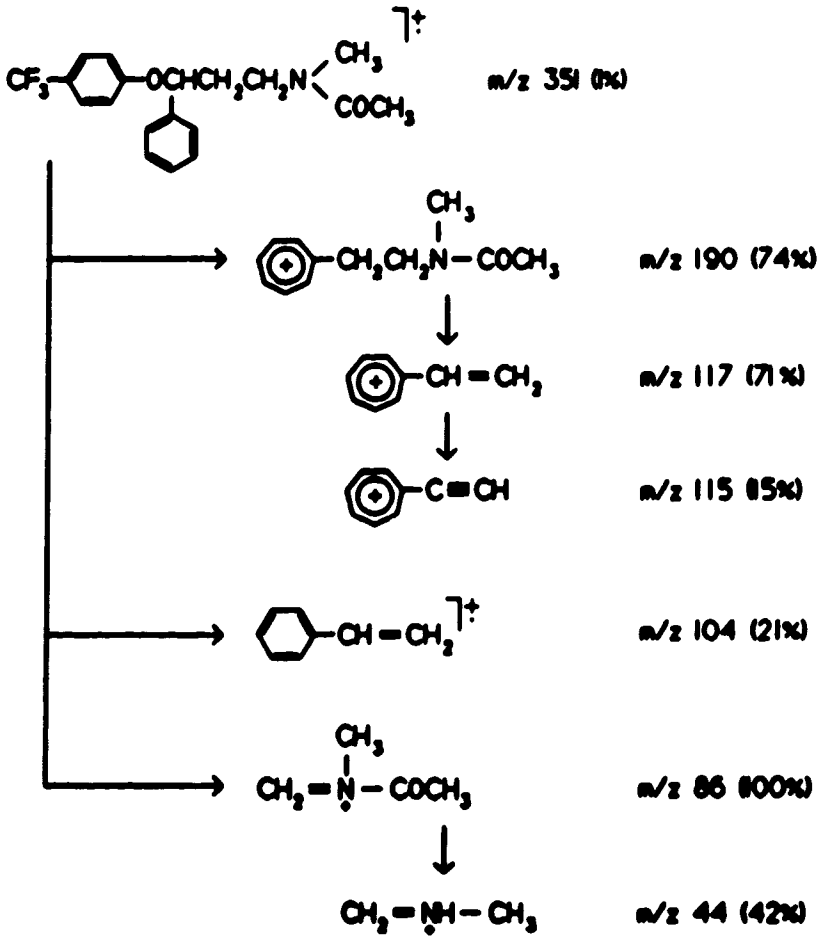


Figure 19. Proposed electron impact mass spectrometric fragmentation pattern for the acetylated derivative of FLU. Values in parentheses represent percent relative abundance of that ion as compared to the most prominent ion (100%).

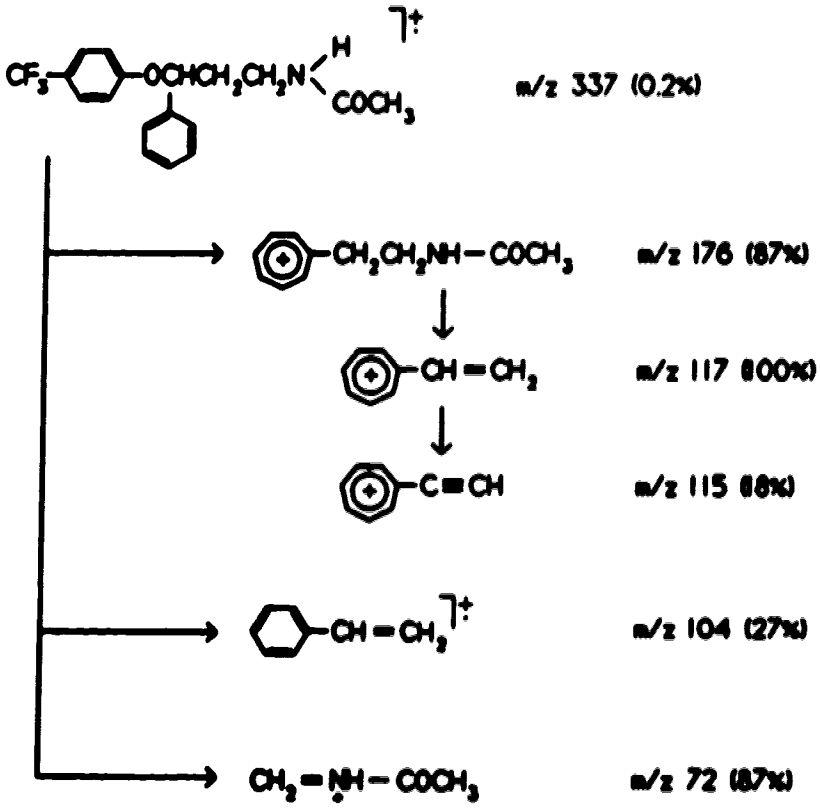


Figure 20. Proposed electron impact mass spectrometric fragmentation pattern for the acetylated derivative of NFLU. Values in parentheses represent percent relative abundance of that ion as compared to the most prominent ion (100%).

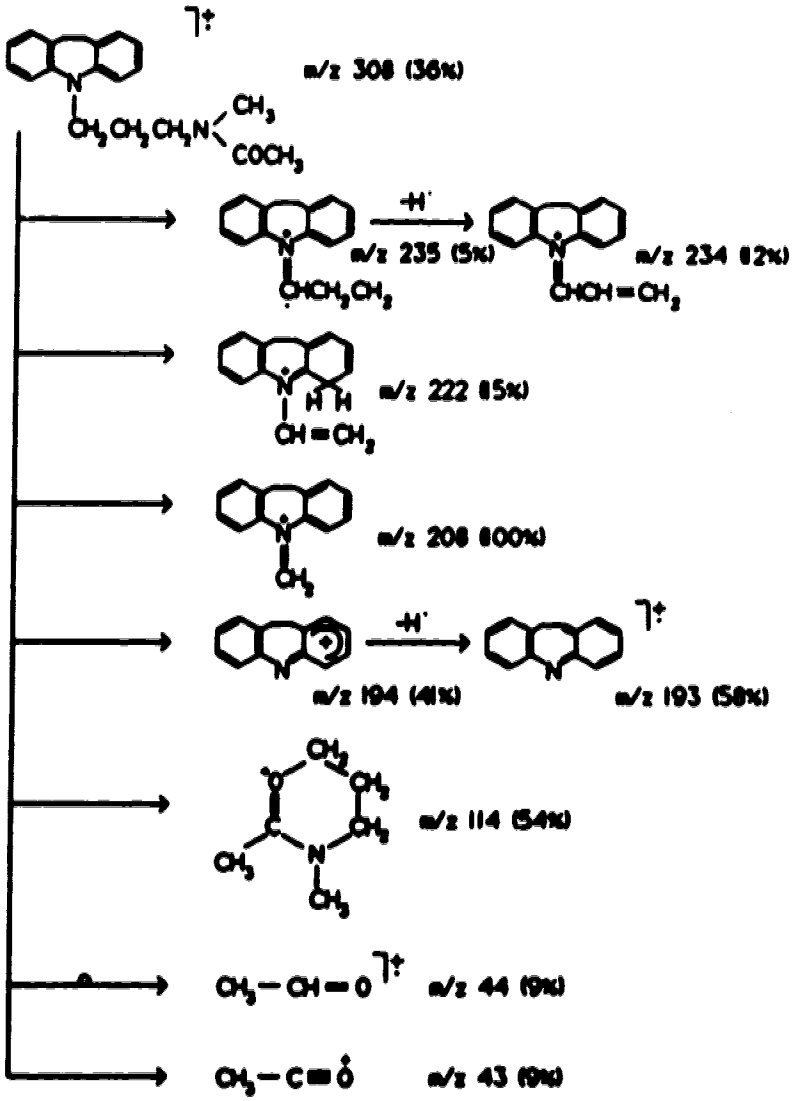


Figure 21. Proposed electron impact mass spectrometric fragmentation pattern for the acetylated derivative of DMI. Values in parentheses represent percent relative abundance of that ion as compared to the most prominent ion (100%).

The procedure is rapid and results in derivatives with excellent chromatographic properties (Figure 18). The structures of the derivatives, as confirmed by mass spectrometry, are shown in Figures 19-21. Calibration curves were linear, with correlation coefficients of > 0.99 and y-intercepts near zero obtained routinely. Mean recoveries (n = 10) for the three derivatives were as follows: FLU, 97; NFLU, 91; DMI, 68% with coefficients of variation < 10%. The procedure has been applied to liver and brain tissue taken from rats treated with FLU, DMI, or FLU + DMI for 14 days. The results are described below.

3.3.3.1 Cortex

Levels of DMI were significantly increased as the dose of DMI increased from 5 mg/kg/day to 15 mg/kg/day. The combination of FLU and DMI produced significantly greater levels of DMI than did DMI (5 mg/kg/day) alone (Table 9). Both FLU and NFLU levels were significantly elevated in the combination-treated rats compared to those in the FLU-treated rats (Table 9).

3.3.3.2 Rest of Brain

The relationship between drug levels in animals treated with the drugs singly and in combination was similar in the rest of brain and cortex. NFLU levels were tripled in the rest of brain, but only doubled in the cortex (Table 10) by administration of the drug combination.

3.3.3.3 Liver

The combination of FLU and DMI resulted in DMI levels being increased to approximately 2.5 times those observed when DMI was

TABLE 9. Levels of DMI, FLU, and NFLU in Cortex (nmoles/g ± SEM, n=10) Following Chronic Treatment With DMI, FLU or FLU + DMI (Administration via Osmotic Minipumps)

Drug Treatment	DMI	FLU	NFLU
DMI (5 mg/kg/day)	1.6 ± .2		
DMI (10 mg/kg/day)	7.6 ± 1.4		
DMI (15 mg/kg/day)	13.4 ± 2.6		
FLU (10 mg/kg/day)		7.9 ± .6	44.7 ± 4.2
FLU (10 mg/kg/day) + DMI (5 mg/kg/day)	4.4 ± .6	19.0 ± 2.3	90.7 ± 17.0

Drugs were administered for 14 days. Values represent mean ± SEM (n=10). All drug levels are significantly greater ($p<0.05$) in the combination treated group than in those groups receiving a single treatment.

TABLE 10. Levels of DMI, FLU, and NFLU in Rest of Brain (nmoles/g ± SEM, n=5-7) Following Chronic Treatment With FLU, DMI, or FLU + DMI (Administration via Osmotic Minipumps)

Drug Treatment	DMI	FLU	NFLU
DMI (5 mg/kg/day)	2.4 ± .5		
FLU (10 mg/kg/day)		5.2 ± 1.1	47.2 ± 8.2
FLU (10 mg/kg/day)+ DMI (5 mg/kg/day)	5.5 ± .7	11.6 ± 2.2	81.1 ± 18.1

Drugs were administered for 14 days. Values represent mean ± SEM (n=5-7). All drug levels are significantly greater ($p<0.05$) in the combination treated group than in those groups receiving a single treatment. Rest of brain consists of whole brain minus cortex, hippocampus, and striatum.

TABLE 11. Levels of DMI, FLU, and NFLU in Liver (nmoles/g ± SEM, n=5-7) Following Chronic Treatment With FLU, DMI, or FLU + DMI (Administration via Osmotic Minipumps)

Drug Treatment	DMI	FLU	NFLU
DMI (5 mg/kg/day)	3.4 ± .8		
FLU (10 mg/kg/day)		4.3 ± .5	45.3 ± 7.0
FLU (10 mg/kg/day) + DMI (5 mg/kg/day)	8.8 ± 2.2	14.6 ± 4.8	96.6 ± 25.9

Drugs were administered for 14 days. Values represent mean ± SEM (n=5-7). All drug levels are significantly greater ($p<0.05$) in the combination treated group than in those groups receiving a single treatment.

administered alone. FLU levels were approximately tripled and NFLU levels were doubled in the combination as compared to those in animals treated with FLU only (Table 11).

3.4 FLU AND DMI VS. THE DRUGS ALONE (Administration by i.p. injection)

Since Baron *et al.* (1988) did not find an effect of DMI on 5-HT_2 binding in their study, a study was conducted to determine if route of drug administration affected the actions of DMI and FLU on 5-HT_2 receptors. Rats were treated with DMI (5 mg/kg/day), FLU (10 mg/kg/day) or the combination, as described previously in section 2.4.3.

3.4.1 5-HT_2 Receptor Density and Affinity

Animals treated with DMI (5 mg/kg/day) alone had a decreased density of 5-HT_2 receptors relative to control values. This effect was markedly reduced in animals which received both DMI (5 mg/kg/day) and FLU (10 mg/kg/day). FLU-treated and combination-treated animals showed a decreased affinity of ^{3}H-ketanserin for the 5-HT_2 site (Table 12). These effects are similar to those observed using osmotic mini-pumps for drug delivery (Section 3.3.1).

Table 12. 5-HT_2 Receptor Density and Affinity Following Chronic Treatment With FLU, DMI, or FLU + DMI (Administration via *i.p.* Injections)

Drug Treatment	Duration	Bmax (fmol/mg protein)	Kd (nM)
Vehicle (dist. water)	14 days	277 ± 15 §	0.56 ± .02
DMI (5 mg/kg/day)	14 days	217 ± 14 *	0.54 ± .03
FLU (10 mg/kg/day)	14 days	277 ± 19	0.63 ± .02 *§
FLU (10 mg/kg/day) +DMI (5 mg/kg/day)	14 days	258 ± 17 *§	0.68 ± .03 *§

Values represent mean ± SEM (n=8). * denotes significant difference ($p<0.05$) from vehicle. § denotes significant difference ($p<0.05$) from DMI (5 mg/kg/day)

4 DISCUSSION

4.1 HIGH VS. LOW-DOSE TCP

The low (0.5 mg/kg/day) dose of TCP is similar, on a mg/kg basis, to that used in the clinical setting and has been demonstrated to cause down-regulation of β-adrenergic (Sherry-McKenna *et. al.*, 1992), α_2-adrenergic (Greenshaw *et, al.*, 1988), and tryptamine receptors (Mousseau *et. al.*, 1992) in brain tissue after chronic administration to rats. In the results reported in this thesis, the high (2.5 mg/kg/day) dose of TCP produced a significantly greater reduction of ^{3}H-tryptamine binding than that observed at the low-dose at each time interval in the striatum. In the hippocampus, the high dose resulted in a significant reduction by day 4, an effect not observed with the low-dose. Tryptamine has been implicated in the etiology and pharmacotherapy of depression (review: Mousseau, 1993), and it is possible that the more rapid hippocampal and/or greater striatal decrease in ^{3}H-tryptamine binding observed with the high-dose may be contributing factors in the effectiveness of this treatment in refractory depression.

Down-regulation of the 5-HT_2 receptor occurs after chronic treatment with many antidepressants (review: Baker and Greenshaw, 1989). TCP, at the 0.5 mg/kg/day dose used in this thesis, produced a level of inhibition of brain MAO thought to be required for antidepressant efficacy in the clinical situation (Ferris *et al.*, 1975; Robinson *et. al.*, 1976; Giller and Lieb, 1980; Giller *et al.*, 1982) but did not result in a significant down-regulation of the 5-HT_2 site at 4, 10, or 28 days. The same dose of TCP given for the same time interval had been shown previously to cause a down-regulation of β-adrenergic receptors (Sherry-McKenna *et al.*, 1992). The high dose of TCP caused a significantly greater

inhibition of MAO-A and -B than did the low dose, resulting in levels of 5-HT that are almost twice those seen at the low dose. The high dose of TCP had less of an effect on NA levels (approximately 20% more NA is seen at the high dose than at the low dose). The dramatic increase in 5-HT seen with the high dose relative to that seen at the low dose probably accounts for the 5-HT_2 down-regulation seen at 10 and 28 days. 5-HT_2 receptor density was elevated in vehicle-treated controls at day 10 compared to the densities observed in the vehicle-treated controls at days 4 and 28. A vehicle-treated control was present for each time interval of the study and although variance may occur between studies the presence of a proper control group for each time interval diminishes variability within the study.

TCP has been shown to possess neurochemical properties other than MAO inhibition (Keck *et al.*, 1991; Baker *et al.*, 1992). In addition to being a potent MAO inhibitor, TCP has moderate to strong effects on the uptake and release of catecholamines in nerve terminals (Hendley and Snyder, 1968; Schildkraut, 1970; Baker *et al.*, 1978; Baker *et al.*, 1980; Reigle *et al.*, 1980). Mallinger and coworkers (1986, 1990) investigated the relationships among side effects and therapeutic response to TCP and the plasma levels of the drug. These researchers found that mean orthostatic drop of systemic blood pressure and rise of pulse rate correlated with mean plasma TCP concentrations (Mallinger *et al.*, 1986). It was also reported that plasma levels of TCP 5 hours after dosing correlated with ultimate Hamilton Depression Rating Scale scores (Mallinger *et al.*, 1990). Keck *et al.* (1991), in a study with TCP in 13 depressed patients, suggested that the acute hypotensive effect of TCP may be due to an effect of

the drug itself on α-adrenergic receptors. Following administration of the 2.5 mg/kg/day dose, levels of TCP in the present study were found to be approximately 1 μM after 4 days and approximately 1.25 μM after 10 and 28 days in rest of brain. At this concentration TCP should have significant effects on NA and DA uptake (Baker *et al.*, 1978). It is unclear at this time if these effects on catecholamines are contributing to the change in 5-HT_2 receptors observed in the present study.

4.2 AMI VS. AMI + TCP

Down-regulation of the 5-HT_2 receptor occurs after chronic treatment with many antidepressants (Baker and Greenshaw, 1989). The overall effect of AMI combined with TCP on 5-HT_2 receptor density over that of AMI alone is minimal at the doses used in the study reported here. It is of importance to note that the down-regulation occurring in the AMI and AMI-TCP groups is not significantly different from 4 to 28 days, indicating a rapid and sustained effect on the 5-HT_2 receptor system. 5-HT_2 receptor density in vehicle-treated controls was slightly elevated at day 10 compared to the densities observed in the vehicle-treated controls for days 4 and 28. A similar observation was made in the high- versus low-dose TCP study. The reason for this is not clear, but emphasizes the importance of running vehicle controls with drug-treated animals at all time intervals in chronic studies.

A difference in Kd between vehicle-treated and AMI-treated animals has been reported in the past (Peroutka and Snyder, 1980). These workers reported that addition of cold competing ligand to the incubation mixture resulted in a Kd change but had no effect on the Bmax. AMI has an Ki value for the ^{3}H-ketanserin

binding site of 4.2 nM (Leysen *et al.*, 1982). AMI is also very strongly protein-bound and levels of AMI in the actual incubation mixture (*i.e.* after preparation and washing of the membrane fragment) were found in the present study to be 56 nM. Thus, there is sufficient AMI present to compete with ^{3}H-ketanserin for the 5-HT_2 site, resulting in a change in slope (*i.e.* Kd) of the Scatchard analysis. Competition of this nature has no effect on Bmax values (Peroutka and Snyder, 1980). Further studies in which there are varying washout periods combined with the analysis of residual AMI in the membrane pellet should clarify this situation. TCP at the dose used in this study had no effect on Kd values for ^{3}H-ketanserin binding after 4, 10, or 28 days of administration (Goodnough and Baker, 1993a).

The increase in brain levels of 5-HT and NA and the inhibition of MAO-A and -B in those animals which received the AMI-TCP combination was expected and comparable to previous work done in our laboratory with animals treated with the above dose of TCP alone (Sections 3.2.2 and 3.2.3; Goodnough and Baker, 1993a; Sherry-McKenna *et al.*, 1992; Hampson *et al.*, 1988; Baker *et al.*, 1988).

4.3 SIMULTANEOUS DETERMINATION OF FLU, NFLU, AND DMI

Extractive acetylation with acetic anhydride followed by GC-NPD resulted in an assay procedure which permitted simultaneous analysis of DMI, FLU, and NFLU, thus facilitating studies on metabolic interactions between DMI and FLU. Acetylation with acetic anhydride followed by GC analysis has been utilized in the past for separation of secondary amine TCAs (*e.g.* DMI) from their parent tertiary amine TCA drugs (*e.g.* imipramine), but the acetylation was performed

under anhydrous conditions after extraction of the drugs of interest into organic solvents and after a series of extractions and back-extractions (Jorgenson, 1975; Gupta *et al.*, 1983). Since acetic anhydride will react under basic aqueous conditions with amines (Chattaway, 1931; Welsh, 1955; Sherman, 1989), the acetylation step can be introduced earlier on in the extraction procedure (Drebit *et al.*, 1988). This methodology has been applied to extraction of a number of biogenic amines and drugs from biological media (Martin and Baker, 1977; Baker *et al.*, 1982; Durden *et al.*, 1991; Baker *et al.*, 1993), and as shown in the present thesis, works well for simultaneous extraction and quantification of DMI, FLU, and NFLU.

4.4 FLU OR DMI VS. FLU + DMI

The down-regulation of 5-HT_2 receptors following chronic DMI treatment is consistent with literature reports (Bergstrom and Kellar, 1979; Peroutka and Snyder, 1980; Eison *et al.*, 1991). The lack of effect of FLU on 5-HT_2 receptor density is also in agreement with several reports in the literature (Peroutka and Snyder, 1980; Fuxe *et al.*, 1983; Baron *et al.*, 1988; Todd *et al.*, 1993). The 1988 study by Baron *et al.* is the only other report, to our knowledge, that compared the combination of FLU and DMI on receptor densities in the rat. They compared DMI (5 mg/kg/day), FLU (10 mg/kg/day) and the drug combination but used ^{3}H-spiperone as the radioligand, made single point determinations (*i.e.* Bmax and Kd values were not determined), and administered the drugs *via* intraperitoneal injections once daily. After 14 days of administration they found that no drug group was significantly different from control. In contrast to the literature reports mentioned above and to our results, Baron *et al.* did not find an effect

of DMI on 5-HT_2 binding. This difference does not appear to be a result of the different routes of administration since other studies in our laboratories on 5-HT_2 binding in rats receiving *i.p.* injections of DMI (5 mg/kg/day), FLU (10 mg/kg/day) or the combination show similar results as were achieved with pumps (Sections 3.4.1)

The observation that the drug combination did not result in down-regulation of 5-HT_2 receptors equal to or greater than that observed with DMI alone and in fact led to a reversal of the 5-HT_2 receptor down-regulation produced by DMI may be explained if the mechanisms of action for FLU and DMI are considered. A mechanism by which DMI down-regulates 5-HT_2 receptors has been suggested by Lafaille *et al.* (1991) and involves an enhancement of noradrenergic transmission to the dorsal raphé nucleus. This would result in an increase in raphé cell firing and a concomitant increase in 5-HT released in cortical brain regions. After chronic use a subsensitivity, characterized by a decrease in 5-HT_2 receptor density, may occur (Lafaille *et al.*, 1991). However, this theory cannot explain why lesioning either noradrenaline or 5-HT neurons had no effect on DMI's ability to down-regulate 5-HT_2 receptor number and function (Elson *et al.*, 1991). It appears that DMI is directly acting at or near the 5-HT_2 receptor site.

Affinity values (Ki) of 78 and 160 nM of DMI for the ^{3}H-ketanserin binding site have been reported by Leysen *et al.* (1982) and Thomas *et al.* (1987), respectively. In a comparison of the affinities of several antidepressants for the 5-HT_2 binding site, IC_{50} values of 540 nM and 1300 nM were observed for DMI and FLU, respectively (Baldessarini, 1985). The average cortical concentrations of DMI and FLU in rats treated with the drug combination in the present study

were approximately 4.5 and 20 μM, respectively. These levels indicate that both drugs are present in concentrations sufficient to be interacting with the 5-HT_2 site. FLU (10 mg/kg/day) resulted in an average FLU concentration of approximately 10 μM, a level at which the 5-HT_2 site should be virtually saturated. At this dose FLU decreased 5-HT_2-mediated head-shake frequency, but had no effect on 5-HT_2 density (Eison *et al.*, 1991). FLU may block the down-regulation of 5-HT_2 receptor sites observed with DMI by competing with DMI for the 5-HT_2 receptor site. Both DMI and FLU are highly protein bound and even though the concentration of tissue in the binding assay is diluted by 10 a large proportion of the FLU and DMI may still be carried over to the incubation medium. The concentrations of FLU and DMI could still be at levels (*i.e.* around 400nM for DMI and around 1-2 μM for FLU) at which competition with ^{3}H-ketanserin for the 5-HT_2 binding site may occur. This carry-over of FLU in animals receiving i.p. injections (DMI levels 24 hrs after the last i.p. injection are below measureable levels) and of FLU and DMI in animals implanted with osmotic minipumps may be responsible for the observed decreases in affinity in animals receiving the combination and FLU alone (osmotic pumps and i.p. injections), and at the 10 and 15 mg/kg/day doses of DMI (osmotic minipumps). The higher concentrations of these drugs (due to the lack of washout) in animals implanted with osmotic minipumps may account for the observation that the decrease in affinity is greater in these animals than in i.p. injected animals.

Principal components analysis of all treatment groups with the concentrations of DMI, FLU, and NFLU and β-adrenergic receptor density (Bmax) treated as dependent variables indicated a significant correlation between Bmax and

DMI concentration. There was no significant correlation between FLU or NFLU concentrations and Bmax. This observation indicates that the greater decrease in Bmax observed in the combination as compared to DMI (5 mg/kg/day) alone is probably a result of higher DMI levels and not due to the presence of FLU or NFLU.

It has been reported that FLU is an inhibitor of cytochrome P450IID6 (CYP2D6) (Broeen and Skjelbo, 1991; Otton *et al.*, 1993). This isozyme is thought to be responsible for hydroxylation of several tricyclic antidepressants (Coutts, 1993), and the elevating effect of FLU on DMI levels has been well documented in clinical studies and in studies in rats (Aranow *et al.*, 1989; Fuller and Perry, 1989; Wellburg *et al.*, 1989a,b; Vandel *et al.*, 1992; Wilens *et al.*, 1992; Bergstrom *et al.*, 1992). The results of the study reported in this thesis support these findings. There is, however a paucity of information on the effects of DMI on FLU and NFLU levels. The increases in FLU and NFLU levels observed with the DMI-FLU combination in the present study raise important questions pertaining to FLU and NFLU metabolism, as DMI is a known inhibitor of ring hydroxylation (Freeman and Suiser, 1972) and a substrate for CYP2D6 (Otto *et al.*, 1993). Ring hydroxylation of FLU and/or NFLU has not been reported, but p-trifluoromethylphenol has been identified as a metabolite of FLU by Lilly Research Laboratories (Benfield *et al.*, 1986). This metabolite would be formed by O-dealky' ..tion, a process mediated by CYP2D6 in the case of drugs such as dextromethorphan (Schmid *et al.*, 1985). Since this isozyme is also involved in ring hydroxylation in the rat, the increased levels of FLU in the brain of rats receiving the drug combination may be the result of competitive inhibition by DMI and FLU

for a cytochrome P450 isozyme. Aspeslet *et al.* (1993), in our laboratories, has demonstrated that the combination of iprindole, a known inhibitor of CYP2D6, and FLU results in an increase in brain levels of FLU in rats over those observed when FLU is administered alone. At present, it is not clear if the increased levels of NFLU reported in the present studies are due to an inhibition of its metabolism by DMI or simply the result of increased FLU available for N-demethylation.

The results reported in this thesis emphasize the importance of measuring brain levels of the drugs under study. Such measurements confirm that the drugs are getting to the brain after administration by pumps or injection and aid in determining if the drugs levels are appropriate for the study of the neurotransmitter systems of interest. Determining drug levels also allows the researcher to determine if the observed effects of combined drug administration are likely due to metabolic interactions. FLU at 10 mg/kg/day is a dosage commonly used in the literature to study the effects of FLU in rats (Baron *et al.*, 1988, Elson *et al.*, 1991; Torok-Both *et al.*, 1992). The brain levels of FLU observed at this dose may be sufficient for FLU to interact with NA and DA uptake sites, as well as the 5-HT uptake site and with receptors such as muscarinic and 5-HT_2 receptors (Baldessarini, 1985; Thomas *et al.*, 1987). Clearly this dose may be inappropriate, and lower doses should be studied in future investigations with this drug: Such studies are now underway by other investigators in the Neurochemical Research Unit. It will also be of interest to conduct future studies on combinations of DMI with a SSUI which does not interact metabolically with DMI.

4.5 SUMMARY

The search for common biochemical abnormalities associated with and possibly the causal factors in depression is hindered by the variety of actions elicited by antidepressant drug therapies. This variability makes it difficult to prove or disprove any of the many theories which have arisen in an attempt to explain how antidepressants work. It is the opinion of this investigator that the receptor imbalance theory of affective illness introduced by Deakin *et al.* (1991) and expanded upon in the introductory section of this thesis best explains the available data at this time. This theory allows for the varying actions between different classes of antidepressants and attempts to identify possible enviromental factors which may trigger a depressive episode.

The balance between the post-synaptic activities of the 5-HT_{1A} and 5-HT_2 receptor systems can be altered by changes to either system alone or by both simultaneously. There is a limit by which conventional therapy can alter this balance. Side-effects (which may become intolerable before a dose sufficient enough to result in the appropriate change is attained) and specificity of action (*i.e.* only one receptor system may be affected and this may not create a great enough change) can be limiting factors of drug action. Combining antidepressants of differing mechanisms or using doses in which the antidepressant has a less specific mechanism are methods which can be used to more greatly influence this balance. Indeed, these actions may be neccessary to alleviate more severe cases of depression.

The combination of AMI and TCP results in an elevation of 5-HT and NA levels and a down-regulation of ^{3}H-tryptamine receptors. These are biochemical

effects not associated with AMI treatment alone but which can be accounted for by the actions of TCP. 5-HT_2 receptors are also down-regulated by the combination, an effect which is not observed at the usual clinical doses of TCP alone, but which is produced by AMI. The 5-HT_2 antagonistic and 5-HT uptake inhibition properties of AMI combined with the 5-HT- and NA-elevating properties of TCP may be biochemical factors contributing to the reported efficacy of this treatment in refractory depression, but the mechanism still remains unclear.

Treatment of rats with high-dose TCP results in higher 5-HT and NA levels and a greater down-regulation of ^{3}H-tryptamine receptors as compared to those produced by the low dose and also results in a down-regulation of 5-HT_2 receptors, which is not observed in the lower dose. Levels of TCP in the brains of rats treated with the high dose are also elevated to concentrations at which TCP may interact with the uptake and/or release of biogenic amines, particularly NA at the presynaptic neuron. It is unlikely that the levels of TCP observed in the lower dose would have any appreciable effect on these systems as they fall below the reported levels of TCP necessary to interact with these systems. Clearly several biochemical factors are present at the high dose of TCP which are not present or are present to a lesser degree than at the low dose and may be contributing to the efficacy of this treatment in refractory depression.

The combination of FLU and DMI results in higher concentrations of DMI, FLU, and NFLU in brain as compared to the situation when DMI or FLU are administered alone. At the doses used in this thesis, the combination resulted in a marked reduction in the 5-HT_2 down-regulation observed when DMI was administered alone. This finding was attributed to concentrations of FLU high

enough to compete with DMI for the 5-HT_2 receptor. At the doses studied in this thesis no clear biochemical change was observed which supported the receptor imbalance theory of depression stated earlier. Rather the blockade of 5-HT_2 receptor down-regulation observed in the combination contradicts this theory. It is important to note that the dose of FLU most commonly used in the literature (10 mg/kg/day) is 25 times the dose needed to inhibit 5-HT uptake by 50% (0.4 mg/kg, Fuller and Wong, 1985) in the rat. Doses of FLU in the 1-2 mg/kg/day range would presumably result in much lower concentrations of FLU and competition for the 5-HT_2 receptor site may not be present. Further study is needed to determine if the 5-HT_2 receptor blocking effect of FLU is dose-dependent and if there is any effect on 5-HT_2 receptors at the minimum concentration of FLU necessary for maximal inhibition of 5-HT uptake.

When higher doses of drugs and combinations of drugs are being used to treat refractory cases of depression or other illnesses, the metabolism of these drugs and the effects they may have on each other's metabolism become important issues. Studies involving the combination of FLU and DMI presented in this thesis confirm earlier reports that FLU inhibits the metabolism of DMI. The novel assay which was developed allowed simultaneous quantification of DMI, FLU, and NFLU and I was able to show that not only did FLU inhibit DMI's metabolism but DMI inhibited FLU's (NFLU levels were also significantly elevated but this elevation could just be the result of higher FLU levels), an effect not previously reported with TCAs. This interaction raises questions pertaining to the metabolism of FLU and emphasizes that further studies need to be carried

out to determine routes of FLU metabolism other than the demethylation to NFLU. Seventy percent of the metabolism of FLU is still unaccounted for (Lemberger *et al.*, 1985).

As we continue to dissect the receptor classes into more subtypes and as specific ligands and antagonists for these subtypes are developed, we begin to learn more about the actions of antidepressants and local enviromental factors on these systems. It is hopeful that as a result of this increased knowledge, drug therapy for the psychiatric disorders will become more focused. The theories of today are only as valid as the data they are based upon. We question the specificity of assays developed over a decade ago as will researchers question the specificity of the assays utilized in this thesis a decade from now.

5 CONCLUSIONS

1. A comparison of high-dose TCP (similar to that used in refractory depression) to low-dose TCP (similar to the usual clinical dose) revealed that the former inhibited MAO-A and B and increased NA and 5-HT levels to a greater extent than the latter in rat brain.
2. The ratio of TCP levels, as measured at 10 days in various body tissues, between the two doses reflects quite closely the ratio of the administered doses.
3. The different doses of TCP had differential effects on ^{3}H-tryptamine binding, with the high dose resulting in a faster down-regulation of ^{3}H-tryptamine binding sites in the hippocampus and a greater decrease in ^{3}H-tryptamine binding in the striatum than was produced by the low dose.
4. The high dose of TCP was sufficient to down-regulate the 5-HT$_2$ receptor whereas this effect was not observed with the low dose.
5. AMI in combination with TCP did not result in a greater down-regulation of 5-HT$_2$ receptors compared to that observed with AMI treatment alone.
6. AMI in combination with TCP did not enhance the ability of TCP to elevate 5-HT and NA levels nor did it enhance TCP's inhibition of MAO-A and -B.
7. Residual carry-over of highly protein bound drugs such as AMI may be sufficient to affect the observed receptor affinity in the radioligand binding assay for 5-HT$_2$ receptors.
8. Rapid and sensitive quantification of FLU, NFLU, and DMI simultaneously is possible utilizing simple extractive acetylation under basic aqueous conditions followed by GC-NPD analysis.

9. FLU and/or NFLU inhibit the metabolism of DMI, resulting in elevated brain levels of DMI when FLU and DMI are administered in combination.
10. DMI inhibits the metabolism of FLU and increases brain levels of FLU and NFLU.
11. The increased down-regulation of β-adrenergic receptors following combined treatment with FLU and DMI appears to be the result of increased levels of DMI.
12. FLU and/or NFLU block the down-regulation of 5-HT_2 receptors caused by DMI and have no effect on 5-HT_2 receptor density on their own.

6 REFERENCES

Abramowicz M. (1980) Drugs for psychiatric disorders. *Med. Lett.* 22: 77-83.

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

American Psychiatric Association (1952) Diagnostic and Statistical Manual of Mental Disorders. A.P.A., Washington.

American Psychiatric Association (1968) Diagnostic and Statistical Manual of Mental Disorders, Second Edition. A.P.A., Washington.

American Psychiatric Association (1980) Diagnostic and Statistical Manual of Mental Disorders, Third Edition. A.P.A., Washington.

American Psychiatric Association (1987) Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised. A.P.A., Washington.

Amsterdam J.D. and Berwish N.J. (1988) High dose tranylcypromine treatment for refractory depression. *Pharmacopsychiat.*, 22: 21-25.

Andrade R., Malenka R.C. and Nicoll R.A. (1986) A G protein couples serotonin and GABA$_B$ receptors to the same channels in hippocampus. *Science*, 234: 1261-1265.

Anthony M. and Lance J.W. (1969) Monoamine oxidase inhibitors in the treatment of migraine. *Arch. Neurol.*, 21: 263-268.

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

Arora R.C. and Meltzer H.Y. (1989) Increased serotonin$_2$ (5-HT$_2$) receptor binding as measured by ^{3}H-lysergic acid diethylamide (^{3}H-LSD) in the blood platelets of depressed patients. *Life Sci.*, 44: 725-734.

Aspeslet L.J., Baker G.B., Coutts R.T. and Torok-Both G.A. (1993) The effects of desipramine and iprindole on levels of enantiomers of fluoxetine in rat brain and urine (manuscript submitted).

Bagdy G., Calogero A.E., Aulakh C.S., Szeeredi K. and Murphy D.L. (1989) Long-term cortisol treatment impairs behavioral and neuroendocrine responses to 5-HT$_1$ agonists in the rat. *Neuroendocrinol.*, 50: 241-247.

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 Holt A. (1993) Derivitization with acetic anhydride: Applications to the analysis of biogenic amines and psychiatric drugs by gas chromatography and mass spectrometry. *J. Pharmacol. Toxicol. Meth.*, (in press).

Baker G.B., Coutts R.T. and LeGatt D.F. (1992) Gas chromatographic analysis of amines in biological systems. In: *Analysis of Biogenic Amines* (Eds. Baker G.B. and Coutts R.T.), Elsevier, New York, pp. 109-128.

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

Baker G.B., Coutts R.T., Yeung J.M., Hampson D.R., McIntosh G.J.A. and McIntosh M. (1985) Chronic administration of monoamine oxidase inhibitors: Basic and clinical investigations. In: *Neuropsychopharmacology of the Trace Amines: Experimental and Clinical Aspects* (Eds. Boulton A.A., Maitre L., Bieck P.R. and Riederer P.), Humana Press, Clifton, N.J., pp. 317-328.

Baker G.B. and Greenshaw A.J. (1988) *In vitro* and *ex vivo* neurochemical screening procedures for antidepressants, neuroleptics, and benzodiazepines. In: *Neuromethods, Vol. 10* (Eds. A.A. Boulton, G.B. Baker, and R.T. Coutts), Humana Press, Clifton, NJ, pp. 327-377.

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

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

Baker G.B., Hlob 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. Molec. Biol.*, 26: 182-186.

Baker G.B., LeGatt D.F. and Coutts R.T. (1984) A comparison of the effects of acute and chronic administration of phenelzine and tranylcypromine on brain concentrations of 2-phenylethylamine, *p*-tyramine and tryptamine in the rat. In: *Neurobiology of the Trace Amines* (Eds. Boulton A.A., Baker G.B., Dewhurst W.G. and Sandler M.), Humana Press, Clifton, N.J., pp. 277-282.

Baker G.B., Martin I.L. and Mitchell P.R. (1977) The effects of some indolalkylamines on the uptake and release of 5-hydroxytryptamine in rat striatum. *Brit. J. Pharmacol.*, 61: 151-152.

Baker G.B., McKim H.R., Calverley D.G. and Dewhurst W.G. (1978) Effects of the monoamine oxidase inhibitors tranylcypromine, phenelzine, and pheniprazine on the uptake of catecholamine in slices from rat brain regions. *Proc. Eur. Soc. Neurochem.*, 1: 536.

Baldessarini R.J. (1975) Biogenic amine hypothesis of affective disorders. In: *The Nature and Treatment of Depression* (Eds. Flack F.F. and Draghi S.C.), John Wiley and Sons, New York, pp. 347-365.

Baldessarini R.J. (1985) *Chemotherapy in Psychiatry: Principles and Practice*, Harvard University Press, Cambridge, Massachusetts, pp. 156-157.

Baldessarini R.J. (1989) Current status of antidepressants: Clinical pharmacology and therapy. *J. Clin. Psychiat.*, 50: 117-126.

Baron B., Ogden A., Siegel B., Stegeman J., Urello R., and Dudley M. (1988) Rapid down-regulation of beta-adrenoceptors by coadministration of desipramine and fluoxetine. *Eur. J. Pharmacol.*, 154: 125-134.

Bech P. (1988) A review of the antidepressant properties of serotonin reuptake inhibitors. *Adv. Biol. Psychiat.*, 17: 59-69.

Belgon A. (1990) Effects of steroid hormones on the serotonergic system. In: *The Neuropharmacology of Serotonin* (Eds. Whitaker-Azmitia P.M. and Peroutka S.J.), Annals of the New York Academy of Sciences, Vol. 600, New York, pp. 427-434.

Belgon A., Rainbow T.C. and McEwen B.S. (1985) Corticosterone modulation of neurotransmitter receptors in rat hippocampus: a quantitative autoradiographic study. *Brain Res.*, 332: 309-314.

Bell I.R. and Cole J.O. (1986) Fluoxetine induces elevation of desipramine levels and exacerbation of geriatric nonpsychotic depression. *J. Clin. Psychopharmacol.*, 6: 447-448.

Benfield P., Heel R.C. and Lewis S.P. (1986) Fluoxetine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. *Drugs*, 32: 481-508.

Bergstrom D.A. and Keller K.J. (1979) Adrenergic and serotonergic receptor binding in rat brain after chronic desmethylimipramine treatment. *J. Pharmacol. Exp. Ther.*, 209: 256-261.

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.

Bertilsson L., Mellstrom B. and Sjoqvist F. (1979) Pronounced inhibition of noradrenaline uptake by 10-hydroxy-metabolites of nortriptyline. *Life Sci.*, 25: 1285-1292.

Black P.R., Nilsson E., Schick C., Waldmeier P.C. and Lauber J. (1984) Urinary excretion of tryptamine in comparison to normetanephrine and beta-phenethylamine in human volunteers after subchronic treatment with different monoamine oxidase inhibitors. In: *Neurobiology of the Trace Amines* (Eds. Boulton A.A., Baker G.B., Dewhurst W.G. and Sandler M.), Humana Press, Clifton, N.J., pp. 525-539.

Blaschko H., Richter D. and Schlossman H. (1937) The oxidation of adrenaline and other amines. *Biochem. J.*, 31: 2187-2196.

Blier P., Chaput Y. and de Montigny C. (1988) Long-term 5-HT reuptake blockade, but not monoamine oxidase inhibition, decreases the function of the terminal 5-HT autoreceptors: an electrophysiological study in the rat brain. *Naunyn Schmiedebergs Arch. Pharmacol.*, 337: 246-254.

Blier P. and De Montigny C. (1983) Electrophysiological investigations on the effect of repeated zimelidine administration on serotonergic neurotransmission in the rat. *J. Neurosci.*, 3: 1270-1278.

Blier P., de Montigny C. and Azzaro A.J. (1986) Modification of serotonergic and noradrenergic neurotransmission by repeated administration of monoamine oxidase inhibitors: electrophysiological studies in the rat central nervous system. *J. Pharmacol. Exp. Ther.*, 237: 987-994.

Blier P., de Montigny C. and Tardif D. (1984) Effects of two antidepressant drugs, mianserin and indalpine, on the serotonergic system: single cell studies in the rat. *Psycopharmacol.*, 84: 242-249.

Bodnoff S.R., Suranyi-Cadotte B., Aitken D., Quirion R. and Meaney M.J. (1988) The effects of chronic antidepressant treatment in an animal model of anxiety. *Psychopharmacol.*, 95: 298-302.

Bodnoff S.R., Suranyi-Cadotte B., Quirion R. and Meaney M.J. (1989) A comparison of the effects of diazepam versus several typical and atypical anti-depressant drugs in an animal model of anxiety. *Psychopharmacol.*, 97: 277-279.

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.

Bouthillier A., Blier P. and de Montigny C. (1990) Fierobuterol, a β-adrenoceptor agonist, enhances serotonergic neurotransmission: an electrophysiological study in the rat brain. *Psychopharmacol.*, 103: 357-365.

Brodie B.B., Pletscher A. and Shore P.A. (1956) Possible role of serotonin in brain function and in reserpine action. *J. Pharmacol. Exp. Ther.*, 116: 9.

Brogden R.N., Heel R.C., Speight T.M. and Avery G.S. (1978) Maneerin: a review of its pharmacological properties and therapeutic efficacy in depressive illness. *Drugs*, 16: 273-301.

Brogden R.N., Heel R.C., Speight T.M. and Avery G.S. (1979) Nomifensine: a review of its pharmacological properties and therapeutic efficacy in depressive illness. *Drugs*, 18: 1-24.

Broesn K. and Sidelbo E. (1991) Fluoxetine and norfluoxetine are potent inhibitors of P450IID6 - the source of the sparteine/debrisoquine oxidation polymorphism. *Br. J. Clin. Pharmac.*, 32: 136-137.

Brown R.R. (1980) Tryptophan metabolism in humans: Perspectives and predictions. In: *Biochemical and Medical Aspects of Tryptophan Metabolism* (Eds. Hayaishi O., Ishimura Y. and Kido R.), Elsevier/North Holland Biomedical Press, Amsterdam, pp. 227-235.

Bruning G. and Rommelspacher H. (1984) High affinity [^{3}H]tryptamine binding sites in various organs of the rat. *Life Sci.*, 34: 1441-1446.

Buckett W.R. and Luscombe G.P. (1984) Chronic hydrocortisone induces central serotonergic supersensitivity without affecting a behavioral paradigm for depression. *Br. J. Pharmacol.*, 81: 132.

Byerley W.F., McConnell E.J., McCabe R.T., Dawson T.M., Grosser B.I. and Wamsley J.K. (1988) Decreased beta-adrenergic receptors in rat brain after chronic administration of the selective serotonin uptake inhibitor fluoxetine. *Psychopharmacol.*, 94: 141-143.

Campbell I.C., Robinson D.S., Lovenberg W. and Murphy D.L. (1979) The effects of chronic regimens of clorgyline and pargyline on monoamine metabolism in the rat brain. *J. Neurochem.*, 12: 49-55.

Carlsson A., Corrodi H., Fuxe K. and Holdstt T. (1969a) Effects of some antidepressant drugs on the depletion of intraneuronal brain catecholamine stores caused by 4-α-dimethyl-*meta*-tyramine. *Eur. J. Pharmacol.*, 5: 367-373.

Carlsson A., Corrodi H., Fuxe K. and Holdstt T. (1969b) Effects of some antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-α-dimethyl-*meta*-tyramine. *Eur. J. Pharmacol.*, 5: 357-366.

Chattaway F.C. (1931) Acetylation in aqueous alkaline solutions. *J. Chem. Soc. (Lond)*, 1: 2495-2498.

Claassen V., Boschman Th.A.C., Dhaemana K.M., Hillen F.C., Vaststra W.J. and Zwagemakers J.M.A. (1978) Pharmacology of clovoxamine, a new non-tricyclic antidepressant. *Arzneim.-Forsch.*, 28: 1756-1766.

Conn P.J. and Sanders-Bush E. (1987) Central serotonin receptors: Effector systems, physiological roles and regulation. *Psychopharmacol.*, 92: 267-277.

Coppen A.J. and Doogan D.F. (1988) Serotonin and its place in the pathogenesis of depression. *J. Clin. Psychiat.*, 49:8(suppl): 4-11.

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

Coutts R.T. (1993) Polymorphism in the metabolism of drugs, including antidepressant drugs: comments on phenotyping. *J. Psychiat. Neurosci.* (in press).

Crammer J.L., Scott B. and Rolle B. (1969) Metabolism of ^{14}C-imipramine: II. Urinary metabolites in man. *Psychopharmacologie (Berl.)*, 15: 207-225

De 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.*, Suppl. 28: 5-20.

Damlouji N.F., Feighner J.P. and Rosenthal M.H. (1985) Recent advances in antidepressants. In: *Pharmacotherapy of Affective Disorders* (Eds. Dewhurst W.G. and Baker G.B.), Croom Helm, London, pp. 286-311.

Davidson J., McCleod M., Law-Yone B. and Linnoila M. (1978) A comparison of electroconvulsive therapy and combined phenelzine-amitriptyline in refractory depression. *Arch. Gen. Psychiat.*, 35: 639-642.

Davidson J.R.T. (1985) Non-response to tricyclic and MAOI drugs: what comes next? In: *Depression in Multidisciplinary Perspective* (Ed. Dean A.), Brunner, Maazel, pp. 171-193.

De Kloet E.R., Sybesma H. and Reul J.M.H.M. (1986) Selective control by corticosterone of serotonin receptor capacity in raphé-hippocampal system. *Neuroendocrinol.*, 42: 513-522.

De Maso D.R. and Hunter T.A. (1990) Combining fluoxetine with desipramine. *J. Am. Acad. Child Adolesc. Psychiat.*, 29: 151.

de Montigny C. (1981) Enhancement of the 5-HT neurotransmission by antidepressant treatments. *J. Physiol.*, 77: 455-461.

de Montigny C. and Aghajanian G. (1978) Tricyclic antidepressants: Long term treatment increases responsivity of rat forebrain neurons to serotonin. *Science*, 202: 1301-1306.

de Montigny C., Chaput Y. and Blier P. (1986) Lithium augmentation of antidepressant treatments: evidence for the involvement of the 5-HT system? In: *New Concepts in Depression* (Eds. Briley M. and Fillon G.), The MacMillan Press, London, pp. 144-160.

de Montigny C., Chaput Y. and Blier P. (1989) Long-term tricyclic and electroconvulsive treatment increases responsiveness of dorsal hippocampus 5-HT_{1A} receptors: an electrophysiological study in the rat. *Soc. Neurosci. Abst.*, 15: 342.21.

de Montigny C., Chaput Y. and Blier P. (1990) Modification of serotonergic neuron properties by long-term treatment with serotonin reuptake blockers. *J. Clin. Psychiatry*, 51:12(suppl B) 4-8.

de Montigny C., Elie R. and Caille G. (1985) Rapid response to the addition of lithium in iprindole-resistant unipolar depression: A pilot study. *Am. J. Psychiat.*, **142**: 220-223.

De Vivo M. and Maayani S. (1986) Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. *J. Pharmacol. Exp. Ther.*, **238**: 248-253.

Deakin J.F.W. (1988) 5-HT$_2$ receptors, depression and anxiety. *Pharmacol. Biochem. Behav.*, **29**: 819-820.

Deakin J.F.W., Guimaraes F.S., Wang M. and Heneman R. (1991) Experimental tests of the 5-HT receptor imbalance theory of affective disturbance. In: *5-Hydroxytryptamine in Psychiatry*, (Eds. Sandler M., Coppen A. and Harnett S.), Oxford University Press, New York, pp. 143-156.

Deakin J.F.W. and Pennel I. (1986) 5-HT receptor subtypes in depression. *Psychopharmacol.*, **89**: 524.

Deakin J.F.W. and Wang M. (1990) Role of 5-HT$_2$ receptors in anxiety and depression. In: *Serotonin: from Cell Biology to Pharmacology and Therapeutics* (Eds. Paulette P. and Vanhoutte P.M.), Kluwer, Dordrecht, pp. 135-153.

Delini-Stula A., Radeke E. and Waldmeier P.C. (1988) Recent studies of monoamine oxidase inhibitors. In: *Current Trends in Psychopharmacology* (Eds. Casey D.E. and Christensen A.V.), Springer-Verlag, Heidelberg, pp. 147-158.

Dewhurst W.G. (1966) New theory of cerebral amine function and its clinical application. *Nature (Lond)*, **210**: 1130-1133.

Dewhurst W.G. and Marley E. (1965) Action of sympathomimetic and allied amines on the central nervous system of the chicken. *Br. J. Pharmacol.*, 25: 705-727.

Dickinson S.L., Kennett G.A. and Curzon G. (1985) Reduced 5-hydroxytryptamine dependent behavior in rats following chronic corticosterone treatment. *Brain Res.*, 345: 10-18.

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

Downs J.M., Downs A.D., Rosenthal T.L., Deal N. and Akiskal H.S. (1989) Increased plasma tricyclic antidepressant concentrations in two patients concurrently treated with fluoxetine. *J. Clin. Psychiat.*, 50: 226-227.

Drebit R., Baker G.B. and Dewhurst W.G. (1988) Determination of maprotiline in plasma and urine by gas-liquid chromatography with nitrogen-phosphorus detection. *J. Chromatogr. Biomed. Appl.*, 432: 334-339.

Dubovsky S.L. (1987) Psychopharmacologic treatment in neuropsychiatry. In: *Textbook of Neuropsychiatry* (Eds. Hales R.E. and Yudofsky S.C.), The American Psychiatric Press Inc., Washington, D.C., pp. 411-438.

Durden D.A., Davis B.A. and Boulton A.A. (1991) Quantification of plasma phenethylamine by electron-capture negative ion gas chromatography-mass spectrometry of the N-acetyl-N-pentafluorobenzoyl derivative. *Biol. Mass Spectrom.*, 20: 375-381.

Earle B.V. (1970) Thyroid hormone and tricyclic antidepressants in resistant depression. *Am. J. Psychiat.*, 126: 1667-1669.

Eichelbaum M., Erbom K., Bertilsson L., Ringberger V. and Rane A. (1975) Plasma kinetics of carbamazepine and its epoxide metabolite in man during single and multiple dosing. *Eur. J. Clin. Pharmacol.*, 8: 337-341.

Elson A. (1989) Fluoxetine and desipramine: A strategy for augmenting antidepressant response. *Pharmacopsychiat.*, 22: 272-273.

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

Feighner J.P. and Boyer W.F. (1991) Selective serotonin re-uptake inhibitors. In: *Perspectives in Psychiatry, vol 1.* (Eds. Feighner J.P. and Boyner W.F.), Wiley, Chichester.

Feighner J.P., Jacobs R.S., Jackson R.E., Hendrickson G., Merideth C.H. and O'Meara P.D. (1983) A double-blind comparative trial with mianserin and amitriptyline in outpatients with major depressive disorders. *Brit. J. Clin. Pharmacol.*, 15: 227s-237s.

Fein S., Paz V., Rao N. and Lagrassa J. (1988) The combination of lithium carbonate and a MAOI in refractory depressions. *Am. J. Psychiat.*, 145: 249-250.

Ferris R.M., Howard J.L. and White H.L. (1975) A relationship between clinical efficacy and various biochemical parameters of monoamine oxidase inhibitors (MAOIs). *Pharmacologist*, 17: 451.

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.

Fuller R.W. and Perry K.W. (1989) Effect of fluoxetine pretreatment on plasma and tissue concentrations of desipramine in rats. *Res. Commun. Chem. Path. Pharmacol.*, 66: 375-384.

Fuxe K., Ogren S., Agnoti L., Benfenati F., Fredholm B., Andersson K., Zini I. and Eneroth P. (1983) Chronic antidepressant treatment and central 5-HT synapses. *Neuropharmacol.*, 22: 399-400.

Gander D.R. and Lond M.B. (1965) Treatment of depressive illness with combined antidepressants. *Lancet*, 2: 107-109.

Giller E.G., Bialos D., Riddle M., Sholomskas A. and Harkness L. (1982) Monoamine oxidase inhibitor-responsive depression. *Psychiat. Res.*, 6: 41-48.

Giller E.G. and Lieb J. (1980) Monoamine oxidase inhibitors and platelet monoamine oxidase inhibition. *Comm. Psychopharmacol.*, 4: 79-82.

Gillette J.R. (1971) Factors affecting drug metabolism. *Ann. N.Y. Acad. Sci.* 179: 43-66.

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

Goldberg D.P., Bridges K., Duncan-Jones P. and Grayson D. (1987) Dimensions of neuroses seen in primary care settings. *Psychological Med.*, 17: 461-470.

Goodnough D.B. and Baker G.B. (1993a) 5-HT_2 binding sites in rat cortex: comparison of the actions of high and low doses of the MAO inhibitor tranylcypromine. *J. Neural Transm.* (in press).

Goodnough D.B. and Baker G.B. (1993b) 5-HT_2 and beta-adrenergic receptor regulation following chronic treatment with desipramine and fluoxetine alone and in combination. *J. Neurochem.* (in press).

Goodnough D.B. and Baker G.B. (1993c) Comparisons of effects of chronic amitriptyline and amitriptyline in combination with tranylcypromine on 5-HT_2 receptors in rat brain. *J. Pharm. Sci.* (in press).

Goodnough D.B., Baker G.B., Mousseau D.D., Greenshaw A.J. and Dewhurst W.G. (1993) Effects of low- and high-dose tranylcypromine on [^{3}H]-tryptamine binding sites in the rat hippocampus and striatum. *Neurochem. Res.* (in press).

Goodwin F.K., Prange A.J., Post R.M., Muscettola G. and Lipton M.A. (1982) Potentiation of antidepressant effects by L-triiodothyronine in tricyclic nonresponders. *Am. J. Psychiat.*, 139:1: 34-38.

Goodwin G.M., De Souza R.J. and Green A.R. (1985) Presynaptic serotonin receptor-mediated response in mice attenuated by antidepressant drugs and electroconvulsive shock. *Nature*, 317: 531-533.

Goodwin G.M., Green A.R. and Johnson P. (1984) 5-HT_2 receptor changes in frontal cortex and 5-HT_2-mediated head twitch behavior following antidepressant treatment to mice. *Br. J. Pharmacol.*, 83: 235-242.

Gram L.F. (1974) Metabolism of the tricyclic antidepressants. *Dan. Med. Bull.*, 21: 218-231.

Gram L.F. (1977) Factors influencing the metabolism of tricyclic antidepressants. *Dan. Med. Bull.*, 24: 81-89.

Gram L.F., Hansen M.G.J., Sindrup S.H., Brosen K., Poulsen J.H., Aase-Jorgensen T. and Overo K.F. (1993) Citalopram: interaction studies with levomepromazine, imipramine and lithium. *Ther. Drug Mon.*, 15: 18-24.

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

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

Gupta R.N., Stefanec M. and Eng F. (1983) Determination of tricyclic antidepressant drugs by gas chromatography with the use of a capillary column. *Clin. Biochem.*, 16: 94-97.

Gutierrez J.L.A. and Lopez-Ibor A.J.J. (1971) Tryptophan and a MAOI (nialamide) in the treatment of depression. *Int. Pharmacopsychiat.*, 6: 92-97.

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

Hall M.D., El Mestikawy S., Emerit M.B., Pichat L., Hamon M. and Gozlan H. (1985) ^{3}H-8-hydroxy-2-(di-n-propylamino)tetralin binding to pre and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. *J. Neurochem.*, 44: 1685-1696.

Hamon M., Gozlan H., El Mestikawy S., Emerit M.B., Bolanos F. and Schechter L. (1990) The central 5-HT$_{1A}$ receptors: Pharmacological, biochemical, functional and regulatory properties. In: *The Neuropharmacology of Serotonin* (Eds. Whitaker-Azmitia P.M. and Peroutka S.J.), Annals of the New York Academy of Sciences, Vol. 600, New York, pp. 114-131.

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: 693-699.

Hampson D.R., Baker G.B. and Coutts R.T. (1988) Neurochemical changes in rat brain amines after short- and long-term inhibition of monoamine oxidase by a low dose of tranylcypromine. *Biol. Psychiat.*, 23: 227-236.

Hare M.C.L. (1926) Tyramine oxidase I. A new enzyme system in liver. *Biochem. J.*, 22: 968-979.

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*, 220: 1330-1331.

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.*, 155:3: 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 R. (1991) Tranylcypromine versus imipramine in anergic bipolar depression. *Am. J. Psychiatry*, 148: 910-916.

Horn A.S. and Snyder S.H. (1972) Steric requirements for catecholamine uptake by rat brain synaptosomes: Studies with rigid analogs of amphetamine. *J. Pharmac. Exp. Ther.*, 180: 523.

Hyttel J. (1982) Citalopram-pharmacological profile of a selective serotonin reuptake inhibitor with antidepressant activity. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, 6: 277-295.

Iber F.L. (1977) Drug metabolism in heavy consumption of ethyl alcohol. *Clin. Pharmacol. Ther.*, 22: 735-742.

Innis R.B., Nestler E.J. and Aghajanian G.K. (1988) Evidence for G protein mediation of serotonin and GABAg-induced hyperpolarization of rat dorsal raphé neurons. *Brain Res.*, 459: 27-36.

Issa A.M., Rowe W., Gauthier S. and Meaney M.J. (1990) Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. *J. Neurosci.*, 10: 3247-3254.

Iversen L.L. (1971) Role of transmitter uptake mechanisms in synaptic transmission. *Br. J. Pharmacol.*, 41: 571-591.

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. (1990) A perspective on the thyroid and depression. *Can. J. Psychiat.*, 35: 754-758.

Joffe R.T., Singer W., Levitt A.J. and MacDonald C. (1993) A placebo-controlled comparison of lithium and triiodothyronine augmentation of tricyclic antidepressants in unipolar refractory depression. *Arch. Gen. Psychiat.*, 50: 387-393.

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

Jones R.S.G. and Boulton A.A. (1981) Tryptamine and 5-hydroxytrypamine's actions and interactions on cortical neurones in the rat. *Life Sci.*, 27:1849-1856.

Jorgensen A. (1975) A gas chromatographic method for the determination of amitriptyline and nortripyline in human serum. *Acta. Pharmacol. Toxicol. (Kbh)*, 36: 79-90.

Joyce P.R., Hewland H.R. and Jones A.V. (1983) Rapid response to lithium in treatment resistant depression. *Br. J. Psychiat.*, 142: 204-214.

Kaulen P., Bruning G., Rommelspacher H. and Baumgarten H. (1986) Characterization and quantitative autoradiography of [^{3}H]tryptamine binding sites in rat brain. *Brain Res.*, 366: 72-86.

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. Psychiatr.*, 52: 250-254.

Keller K.J. and Cascio C.S. (1982) [^{3}H]Tryptamine: high affinity binding sites in rat brain. *Eur. J. Pharmacol.* 86: 475-478.

Keller K.J. and Cascio C.S. (1986) Tryptamine and phenethylamine recognition sites in brain. In: *Neuromethods vol. 4: Receptor Binding* (Eds. Boulton A.A., Baker G.B. and Hrdina P.D.), Humana Press, Clifton, N.J., pp. 119-137.

Keller M.B., Klerman G.L., Lavori P.W., Coryell W., Endicott J. and Taylor J. (1984) Long term outcome of episodes of major depression: Clinical and public health significance. *JAMA*, 252: 788-792.

Keller M.B. (1992) The naturalistic course of anxiety and depressive disorders. *Clin. Neuropharm.*, 15(A): 171A-173A.

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

Kendall D.A. and Nahorski S.R. (1985) 5-Hydroxytryptamine-stimulated inositol phospholipid hydrolysis in rat cerebral cortex slices: pharmacological characterization and effects of antidepressants. *J. Pharmacol. Exp. Ther.*, 233: 473-479.

Khan R.J., McNair D.M., Lipman R.S., Covi L., Rickels R., Downing R., Fisher S. and Frankenthaler L.M. (1986) Imipramine and chlordiazepoxide in depressive and anxiety disorders. *Arch. Gen Psychiat.*, 43: 79-85.

Klein D.F. (1964) Delineation of two drug-responsive anxiety syndromes. *Psychopharmacol.*, 5: 397-408.

Klein D.F., Gittelman R., Quitkin F. and Rifkin A. (1980) Review of the literature on mood stabilizing drugs. In: *Diagnosis and Drug Treatment of Psychiatric Disorders* (Eds. Klein D.F., Gittelman R., Quitkin F., and Rifkin A.), Williams and Wilkins, Baltimore, MD, pp. 288-303.

Klimek V. and Nielsen M. (1987) Chronic treatment with antidepressants decreases the number of ^{3}H-SCH23390 binding sites in the rat striatum and limbic system. *Eur. J. Pharmacol.*, 138: 163-169.

Knoll J., Ecsery Z., Kelemen K., Nievel J. and Knoll B. (1965) Phenylisopropylmethylpropinylamine (E-250), a new spectrum psychic energizer. *Arch. Int. Pharmacol.*, 1: 154-164.

Kraepelin E. (1921) Manic depressive insanity and paranoia. Trans. W. Barclay, Livingstone, Edinburgh.

Kuhn R. (1957) Uber die Behandlund Depressiver Zustande mit einem Iminodibenzylderivat (G22355). *Schweiz. Med. Wochenschr.*, 87: 1135-1140.

Lafaille F., Weiner 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.

Lanfumey L., Haj-Dahmane S., Franc B. and Hamon M. (1989) Potassium channel blockers and 5-HT_{1A} control of serotonergic neuronal activity in the dorsal raphé nucleus. *Soc. Neurosci.*, 15: 66.

Laux G. (1993) Do MAO-B inhibitors have any role in the treatment of depression? In: *Inhibitors of Monoamine Oxidase B: Pharmacology and Clinical Use in Neurodegenerative Disorders* (Ed. Szelenyi I.), Birkhauser Verlag, Basel, Switzerland, pp. 1-38.

Leonard B.E. (1986) Biochemical aspects of refractory depression. *Br. J. Psychiat.*, 152: 453-459.

Leysen J.E., Gommeren W., Van Gompel P., Wynants J., Janssen P.F.M. and Laduron P.M. (1985) Receptor-binding properties *in vitro* and *in vivo* of ritanserin, a very potent and long acting serotonin-S_2 antagonist. *Mol. Pharmacol.*, 27: 600-611.

Leysen J.E., Niemegeers C.J.E., Van Neuten J.M. and Laduron P. (1982) ^{3}H-Ketanserin (R41468), a selective ^{3}H-ligand for serotonin-2 receptor binding sites. *Mol. Pharmacol.*, 21: 301-314.

Leysen J.E. and Pauwels P.J. (1990) 5-HT_2 Receptors, Roles and Regulation. In: *The Neuropharmacology of Serotonin* (Eds. Whitaker-Azmitia P.M. and Peroutka S.J.), Annals of the New York Academy of Sciences, Vol. 600, New York, pp. 183-193.

Lidbrink P., Jonsson G. and Fuxe K. (1971) The effect of imipramine-like drugs and antihistamine drugs on uptake mechanisms in central noradrenaline and 5-hydroxytryptamine neurons. *Neuropharmacol.*, 10: 521-536.

Lingjaerde O., Edmund A.H., Gormsen C.A., Gottfries C.G., Haugstad A., Hermann I.L., Hollnagel P., Makimattila A., Rasmussen K.E., Remvig J. and Robak O.H. (1974) The effect of lithium carbonate in combination with tricyclic antidepressants in endogenous depression. *Acta. Psychiat. Scand.*, 50: 233-242.

Lloyd K.G., Thuret F. and Pilc A. (1985) Upregulation of gamma-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.

Lopez-Ibor A.J.J., Gutierrez J.L.A. and Ingleslas M.L.M. (1973) Tryptophan and amitriptyline in the treatment of depression. *Int. Pharmacopsychiat.*, 8: 145-151.

Louie A.K. and Meltzer H.Y. (1984) Lithium potentiation of antidepressant treatment. *J. Clin. Psychopharmacol.*, 4: 316-321.

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

Madaiksaira S. (1986) Low dose potentiation of lithium in antidepressant augmentation. *Psychiatr. J. Univ. Ottawa*, 11: 107-109.

Maggi A., U'Prichard D.C. and Enna S.J. (1980) Differential effects of antidepressant treatment on brain monoaminergic receptors. *Eur. J. Pharmacol.* 61: 91-98.

Maj J., Przegalinski E. and Moglnicka E. (1984) Hypotheses concerning the mechanism of action of antidepressant drugs. *Rev. Physiol. Biochem. Pharmacol.*, 100: 2-74.

Mallinger A.G., Edwards D.J., Himmelhoch J.M., Knopf S. and Ekler 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 Knapp S. (1990) Plasma tranylcypromine: Relationship to pharmacokinetic variables and clinical antidepressant actions. *J. Clin. Psychopharmacol.*, 10: 176-183.

Mann J.J., Aarons S.F., Wilner P.J., Kelip J.G., Sweeney J.A., Pearlstein T., Frances A.J., Kocsis J.H. and Brown R.P. (1989) A controlled study of the antidepressant efficacy and side effects of (-)-deprenyl. *Arch. Gen. Psychiat.*, 46: 45-50.

Mann J.J., Frances A., Kaplan R.D., Kocsis J., Peselow E.D. and Gershon S. (1982) The relative efficacy of *l*-deprenyl: a selective monoamine oxidase type B inhibitor, in endogenous and nonendogenous depression. *J. Clin. Psychopharmacol.*, 2: 54-57.

Mann J.J. and Gershon S. (1980) *l*-Deprenyl: a selective monoamine oxidase type B inhibitor in endogenous depression. *Life Sci.*, 26: 877-882.

Marcinkiewicz M., Verge D., Gozlan H., Pichat L. and Hamon M. (1984) Autoradiographic evidence for the heterogeneity of 5-HT_1 sites in the rat brain. *Brain Res.*, 291: 159-163.

Martin I.L. and Baker G.B. (1977) A gas-liquid chromatographic method for the estimation of 2-phenylethylamine in rat brain tissue. *Biochem. Pharmacol.*, 26: 11513-1516.

Martin L.L., Neale R.F. and Wood P.L. (1987) Down-regulation of tryptamine receptors following chronic administration of clorgyline. *Brain Res.*, 419: 239-243.

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

McGrath P.J., Stewart J.W., Harrison W., Wager S., Nunes E.N. and Quitkin F.M. (1989) A placebo controlled trial of *l*-deprenyl in atypical depression. *Psychopharmacol. Bull.*, 25: 63-67.

McKeith I.G., Marshall E.F., Ferrier I.N., Armstrong M.M., Kennedy W.N., Perry R.H., Perry E.K. and Eccleston D. (1987) 5-HT receptor binding in postmortem brain from patients with affective disorders. *J. Affec. Dis.* 13: 67-74.

McKenna K.F., Baker G.B. and Coutts R.T. (1992) Urinary excretion of bioactive amines and their metabolites in psychiatric patients receiving phenelzine. *Proc. 4th Int. Trace Amines Conf.*, Galway, Ireland.

McKormack J.K., Bietz A.J. and Larson A.A. (1986) Autoradiographic localization of tryptamine binding sites in the rat and dog central nervous system. *J. Neurosci.*, 6: 94-101.

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

Meltzer H.Y. (1990) Role of Serotonin in Depression. In: *The Neuropharmacology of Serotonin* (Eds. Whitaker-Azmitia P.M. and Peroutka S.J.), Annals of the New York Academy of Sciences, Vol. 600, New York, pp. 486-500.

Mendis N., Pare C.M.B., Sandler M., Glover V. and Stern M. (1981) Is failure of (-)-deprenyl, a selective monoamine oxidase B inhibitor, related to freedom from the cheese effect? *Psychopharmacol.*, 73: 87-90.

Mendlewicz J. and Youdim M.B.H. (1983) *l*-Deprenyl: a selective monoamine oxidase type B inhibitor, in the treatment of depression: a double blind evaluation. *Br. J. Psychiat.*, 142: 508-511.

Miller D.D. and Maklin M. (1984) Cimetidine-imipramine interaction: case report and comments. *Am. J. Psychiat.*, 141: 153.

Mishra R., Janowsky A. and Sulser F. (1979) Subsensitivity of the norepinephrine receptor-coupled adenylate cyclase system in brain: effects of nisoxetine versus fluoxetine. *Eur. J. Pharmacol.* 60: 379-382.

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

Mousseau D.D., McManus D.J., Baker G.B., Juorio A.V., Dewhurst W.G. and Greenshaw A.J. (1992) Effects of age and of chronic antidepressant treatment on ^{3}H-tryptamine binding to rat cortical membranes. *Cell. Mol. Neurobiol.*, 13: 3-13.

Mulder A.H. (1982) An overview of subcellular localization, release, and termination of action of amine, amino acid, and peptide neurotransmitters in the central nervous system. *Prog. Brain Res.*, 55: 135-156.

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* (Ed. Meltzer H.Y.), Raven Press, New York, pp. 545-552.

Murphy D.L., Sunderland T., Campbell I. and Cohen R.M. (1985) Monoamine oxidase inhibitors as antidepressants. In: *Pharmacotherapy of Affective Disorders: Theory and Practice* (Eds. Dewhurst W.G. and Baker G.B.), Croom Helm, London.

Nazarali A.J., Baker G.B., Coutts R.T., Yeung J.M. and Rao T.S. (1987) Rapid analysis of β-phenethylamine in tissues and body fluids utilizing pentafluorobenzoylation followed by electron-capture gas chromatography. *Prog. Neuro-Psychopharmacol. and Biol. Psychiat.* 11: 251-258.

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

Nelson J.C., Mazure C.M., Bower M.B. and Jatlow P.I. (1991) A preliminary, open study of the combination of fluoxetine and desipramine for rapid treatment of major depression. *Arch. Gen. Psychiat.*, 48: 303-307.

Newman M.E. and Lerer B. (1989) Modulation of second messenger function in rat brain by *in vivo* alteration of receptor sensitivity: relevance to the mechanism of action of electroconvulsive shock and antidepressants. *Prog. Neuro-Psychopharmacol. & Biol. Psychiat.*, 13: 2-31.

Newman M.E., Lerer B. and Shapira B. (1993) 5-HT-1A receptor mediated effects of antidepressants. *Prog. Neuro-Psychopharmacol. & Biol. Psychiat.*, 17: 1-19.

Nierenberg A.A. and White K. (1990) What next? a reveiw of pharmacologic strategies for treatment-resistant depression. *Psychopharmacol. Bull.*, 26: 429-460.

Nies A. (1983) Clinical applications of MAOI's. In: *Drugs in Psychiatry*, vol 1 (Eds. Burrows G.D., Norman T.R. and Davies B.), Elsevier, Amsterdam, pp. 229-247.

Nutt D.J. and Glue P. (1989) Monoamine oxidase inhibitors: rehabilitation from recent research. *Br. J. Psychiat.* 154: 287-291.

O'Malley K., Stevenson I.H. and Crooks J. (1972) Impairment of human drug metabolism by oral contraceptive steroids. *Clin. Pharmacol. Ther.*, 13: 552-557.

O'Regan D., Kwok R.P.S., Yu P.H., Bailey B.A., Greenshaw A.J. and Boulton A.A. (1987) A behavioral and neurochemical analysis of chronic and selective monoamine oxidase inhibition. *Psychopharmacol.*, 92: 42-47.

Oldendorf W.H. (1971a) Brain uptake of radiolabeled essential amino acids by brain following arterial injection. *Proc. Soc. Exptl. Biol. Med.*, 136: 385-386.

Oldendorf W.H. (1971b) Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. *Am. J. Physiol.*, 221: 1629-1639.

Ortmann R., Martin S., Radeke E. and Delini-Stula A. (1981) Interaction of β-adrenoceptor agonists with the serotonergic system in rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 316: 225-230.

Otton S.V., Wu D., Joffe R.T., Cheung S.W. and Sellers E.M. (1993) Inhibition by fluoxetine of Cytochrome P4502D6 activity. *Clin Pharmacol. Ther.*, 53: 401-409.

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

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

Pare C.M.B., Kline N., Hellstom C. and Cooper T.B. (1962) Will amitriptyline prevent the "cheese" reaction of monoamine oxidase inhibitors? *Lancet*, ii: 183-186.

Paul S.M., Janowsky A. and Skolnick P. (1985) Monoaminergic neurotransmitters and antidepressant drugs. In: *Psychiatry Update: American Psychiatric Association Annual Review* vol. 4 (Eds. Hales R.E. and Frances A.J.), American Psychiatric Press, Washinton, D.C.

Pazos A. and Palacios J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.*, 346: 205-230.

Pearlman C. (1967) High dose tranylcypromine in refractory depression, *J. Clin. Psychiat.*, 48: 424.

Peroutka S.J. (1993) 5-Hydroxytryptamine receptors. *J. Neurochem.*, 60:2: 408-416.

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

Perry D.C. (1985) [^{3}H]tryptamine autoradiography in rat brain and choroid plexus reveals two distinct sites. *J. Pharmacol. Exp. Ther.* 235: 548-559.

Perry D.C., Manning D.C. and Snyder S.H. (1982) *In vivo* autoradiographic localization of [^{3}H]tryptamine binding sites in rat brain. *Soc. Neurosci. Abstr.* **8**: 783.

Philipe 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.

Philipe S.R. and Boulton A.A. (1979) The effect of monoamine oxidase inhibitors on some arylalkylamines in rat striatum. *J. Neurochem.* **33**: 159-167.

Philipe S.R., Durden D.A. and Boulton A.A. (1974) Identification and distribution of tryptamine in the rat. *Can. J. Biochem.* **52**: 447-451.

Pohl R., Berchov R. and Rainey J.M. (1982) Tricyclic antidepressants and monoamine oxidase inhibitors in the treatment of agoraphobia. *J. Clin. Psychopharmacol.* **2**: 399-407.

Potter W.Z., Calil H.M., Manian A.A., Zavadil A.P. III and Goodwin F.K. (1979) Hydroxylated metabolites of tricyclic antidepressants: preclinical assessment of activity. *Biol. Psychiat.*, **14**: 601-613.

Potter W.Z., Rudorfer M.V. and Lane E.A. (1984) Active metabolites of antidepressants: Pharmacodynamics and relevant pharmacokinetics. In: *Frontiers in Biochemical and Pharmacological Research in Depression* (Eds. Usdin E., Bertilsson L. and Sjoqvist F.), Raven Press, New York, pp. 373-390.

Price L.H., Charney D.S. and Heninger G.R. (1985) Efficacy of lithium - tranylcypromine treatment in refractory depression. *Am. J. Psychiat.*, **142**: 249-250.

Price L.H., Charney D.S. and Heninger G.R. (1986) Variability of response to lithium augmentation in refractory depression. *Am. J. Psychiatry*, **143**: 1387-1392.

Quitkin F.M., Liebowitz M.R., Stewart J.W., McGrath P.J., Harrison W., Rabkin J.G., Markowitz J. and Davies S.O. (1984) *l*-Deprenyl in atypical depression. *Arch. Gen. Psychiat.*, 41: 777-781.

Quitkin F.M., Rabkin J.G., Stewart J.W., McGrath P.J. and Harrison W. (1986) Study duration in antidepressant research: advantages of a 12-week trial. *J. Psychiat. Res.*, 20: 211-216.

Raskin A. (1972) Adverse reactions to phenelzine: results of a nine-hospital depression study. *J. Clin. Pharmacol.*, 12: 22-25.

Razani J., White K.L., White J., Simpson G., Sloane R.B., Rebal R. and Palmer R. (1983) The safety and efficacy of combined amitriptyline and tranylcypromine antidepressant treatment. *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 ^{3}H-norepinephrine metabolism in rat brain. *Psychopharmacol.*, 68: 193-199.

Richelson E. (1978) Tricyclic antidepressants block histamine H1 receptors of mouse neuroblastoma cells. *Nature*, 274: 178.

Riva M.A. and Creese I. (1989) Comparison of two putatively selective radioligands for labelling central non-selective beta-adrenergic receptors: inadequacy of ^{3}H-dihydroalprenolol. *Mol. Pharmacol.*, 36: 201-210.

Robins L.N., Helzer J.E., Weissman M.M., Orvaschel H., Gruenberg E., Burke J.D.Jr. and Regier D.A. (1984) Lifetime prevalence of specific psychiatric disorders in three sites. *Arch. Gen. Psychiat.*, 41: 949-958.

Robinson D.S. (1983) High-dose monoamine oxidase-inhibitor therapy. *J. Am. Med. Assoc.*, 250: 2212.

Robinson D.S., Alms D.R., Shrotriya R.C., Messina M. and Wickramaratne P. (1989) Serotonergic anxiolytics and treatment of depression. *Psychopathol.*, 22(suppl. 1): 27-36.

Robinson D.S., Nies A., Ravaris C.L., Ives J.O. and Bartlett D. (1978) Clinical pharmacology of phenelzine: MAO activity and clinical response. In: *Psychopharmacology: A Generation of Progress* (Eds. Lipton M., Dimascio A. Killam K.F.), Raven Press, New York.

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

Ross S.B., Renyi A.L. and Ogren S.O. (1972) Inhibition of the uptake of noradrenaline and 5-hydroxytryptamine by chlorphentermine and chloroimipramine. *Eur. J. Pharmacol.*, 17: 197-212.

Rowen M.J. and Anwyl R. (1985) The effect of prolonged treatment with tricyclic antidepressants on the actions of 5-hydroxytryptamine in the hippocampal slice of the rat. *Neuropharmacol.*, 24: 131-137.

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.

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* (Eds. Singer T.P., Von Korff R.W. and Murphy D.L.), Academic Press, New York, p. 121.

Sargent W. (1962) The treatment of anxiety states and atypical depressions by the monoamine oxidase inhibitor drugs. *J. Neuropsychiat.* 3: 98-103.

Sargant W. and Dalley P.J. (1962) Treatment of anxiety states by antidepressant drugs. *Br. J. Psychiatry*, 1: 6-9.

Saura Marti 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. (1970) Tranylcypromine: effects on norepinephrine metabolism in rat brain. *Am. J. Psychiat.*, 126: 825-831.

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. and Biol. Psychiat.*, 12: 523-532.

Schmidt B., Bircher J., Preisig R. and Kupfer A. (1985) Polymorphic dextromethorphan metabolism: co-segregation of oxidative O-demethylation with debrisoquine hydroxylation. *Clin. Pharmacol. Ther.*, 38: 618-624.

Schopf J. (1988) Treatment of depressions resistant to tricyclic antidepressants, related drugs or MAO-inhibitors by lithium addition: review of the literature. *Pharmacopsychiat.*, 22: 174-182.

Schotte A., Maloteaux J.M. and Laduron P.M. (1983) Characterization and regional distribution of serotonin S_2-receptors in human brain. *Brain Res.*, 276: 231-235.

Schrami F., Benedetti G., Hoyle K. and Clayton A. (1988) Fluoxetine and nortriptyline combination therapy. *Am. J. Psychiat.*, 145: 1636-1637.

Schweizer E.E., Amsterdam J., Rickels K., Kaplan M. and Droba M. (1988) Open trial of buspirone in the treatment of major depressive disorder. *Psychopharmacol. Bull.*, 22: 183-186.

Selikoff I.J., Robitzek E.H. and Orenstein G.G. (1952) Treatment of pulmonary tuberculosis with hydrazine derivatives of isonicotinic acid. *J. Am. Med Assoc.*, 150: 973-980.

Seth R., Jennings A.L., Bindman J., Phillips J. and Bergmann K. (1992) Combination treatment with noradrenaline and serotonin reuptake inhibitors in resistant depression. *Br. J. Psychiat.*, 161: 562-565.

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

Sharman D.F. (1969) Glycol metabolites of noradrenaline in brain tissue. *Br. J. Pharmacol.*, 36: 523-534.

Shaskan E.G. and Snyder S.H. (1970) Kinetics of serotonin accumulation in slices from rat brain. *J. Pharmacol. Exp. Ther.*, 175: 404-418.

Shaw D.M., Johnson A.L. and MacSweeney D.A. (1972) Tricyclic antidepressants and tryptophan in unipolar affective disorder. *Lancet*, 2: 1245.

Sheehan D.V. (1984) Delineation of anxiety and phobic disorders responsive to monoamine oxidase inhibitors: implications for classification. *J. Clin. Psychiat.*, 45: 29-36.

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.

Shen Y., Monsma F.J., Metcalf M.A., Jose P.A., Hamblin M.W. and Sibley D.R. (1993) Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype. *J. Biol. Chem.*, 268: 18200-18204.

Sherry-McKenna R.L., Baker G.B., Mousseau D.D., Coutts R.T. and Dewhurst W.G. (1992) 4-Methoxytranylcypromine, a monoamine oxidase inhibitor: effects on biogenic amines in rat brain following chronic administration. *Biol. Psychiat.*, 31: 861-868.

Shopsin B., Friedman E. and Gershon S. (1976) *para*-Chlorophenylalanine reversal of tranylcypromine effects in depressed patients. *Arch. Gen. Psychiat.*, 33: 811-819.

Shopsin B., Gershon S., Goldstein F., Friedman F. and Wilk S. (1975) Use of synthesis inhibitors in defining role for biogenic amines during imipramine treatment in depressed patients. *Commun. Psychopharmacol.* 1: 239-249.

Snyder S.H., and Peroutka S.J. (1982) A possible role of serotonin receptors in antidepressant drug action. *Pharmacopsychiat.* 80: 131-134.

Snyder S.H. and Peroutka S.J. (1984) Antidepressants and neurotransmitter receptors. In: *Neurobiology of Mood Disorders* (Eds. Post R.M. and Ballenger J.C.) Williams and Wilkins, Baltimore, MA, pp 696-697.

Snyder S.H. and Yamamura H.T. (1977) Antidepressants and the muscarinic acetylcholine receptor. *Arch. Gen. Psychiat.*, 34: 236-239.

Sovner R.D. (1981) The clinical characteristics and treatment of atypical depression. *J. Clin. Psychiat.*, 42: 295-299.

Squires R.F. (1974) Effects of noradrenaline pump blockers on its uptake by synaptosomes from several brain regions: additional evidence of dopamine terminals in the frontal cortex. *J. Pharm. Pharmacol.*, 26: 384-386.

Stolz J.F., Marsden C.A. and Middlemiss D.N. (1983) Effect of chronic antidepressant treatment and subsequent withdrawal on ^{3}H-spiperone binding in rat frontal cortex and serotonin mediated behaviour. *Psychopharmacol.*, 80: 150-155.

Tariot P.N., Murphy D.L., Sunderland T., Mueller E.A. and Cohen R.M. (1985) Rapid antidepressant effect of addition of lithium to tranylcypromine. *J. Clin. Psychopharmacol.*, 6: 165-167.

Thase M.E., Frank E., Mallinger A.G., Hamon T. and Kupfer D.J. (1992) Treatment of imipramine-resistant depression, III. Efficacy of monoamine oxidase inhibitors. *J. Clin. Psychiat.*, 53: 5-11.

Thomas D.R., Nelson D.R. and Johnson A.M. (1987) Biochemical effects of the antidepressant paroxetine, a specific 5-hydroxytryptamine uptake inhibitor. *Psychopharmacol.*, 93: 193-200.

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 inhuman peripheral tissues and brain. *J. Histochem. Cytochem.*, 35: 23-32.

Todd K.G. and Baker G.B. (1993) Chronic administration of phenelzine, desipramine, clomipramine and maprotiline decreases serotonin type 2 receptor binding without affecting benzodiazepine binding sites in rat brain. *Cell. Mol. Biol.*, (submitted).

Tork I. (1990) Anatomy of the serotonergic system. In: *The Neuropharmacology of Serotonin* (Eds. Whitaker-Azmitia P.M. and Peroutka S.J.), Annals of the New York Academy of Sciences, Vol. 600, New York, pp. 9-35.

Torok-Both G.A., Baker G.B., Coutts R.T., McKenna K.F. and Aspeslet L.J. (1992) Simultaneous determination of fluoxetine and norfluoxetine enantiomers in biological samples by gas chromatography with electron-capture detection. *J. Chromatogr. Biomed. Appl.*, 579: 99-106.

Vahakangas K., Pelkonen O. and Sotaniemi E. (1983) Cigarette smoking and drug metabolism. *Clin. Pharmacol. Ther.*, 33: 375-380.

Van Nguyen T.V. and Juorio A.V. (1989) Down-regulation of tryptamine binding sites following chronic molindone administration. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 340: 366-371.

Van Nguyen T.V., Paterson I.A., Juorio A.V., Greenshaw A.J. and Boulton A.A. (1989) Tryptamine receptors: neurochemical and electrophysiological evidence for postsynaptic and functional binding sites. *Brain Res.*, 476: 85-93.

Vandel S., Bertschy G., Bonin B., Nezelof S., Francois T.H., Vandel B., Sechter D. and Bizouard P. (1992) Tricyclic antidepressant levels after fluoxetine addition. *Neuropsychobiol.*, 25: 202-207.

Vandermeulen C.P. and Aghajanian G.K. (1980) Intracellular studies showing modulation of facial motoneuron excitability by serotonin. *Nature*, 287: 346-347.

Vandermeulen C.P. and Aghajanian G.K. (1982) Serotonin-induced depolarization of rat facial motoneurons *in vivo*: Comparison with amino acid transmitters. *Brain Res.*, 236: 139-152.

Verge D., Daval G., Marcinkiewicz M., Patey A., El Mestikawy S., Gozlan H. and Hamon M. (1986) Quantitative autoradiography of multiple 5-HT_1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. *J. Neurosci.*, 6: 3474-3482.

Verge D., Daval G., Patey A., Gozlan H., El Mestikawy S. and Hamon M. (1985) Presynaptic 5-HT receptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT_{1A} subtype. *Eur. J. Pharmacol.*, 113: 463-464.

Vetulani J., Dingell J.V. and Sulser F. (1976) A possible common mechanism of action of antidepressant treatments. *Nauyn Schmiedeberg's Arch. Pharmacol.*, 293: 109-114.

Vetulani J. and Sulser F. (1975) Actions of various antidepressants treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain. *Nature*, 257: 495-496.

Waldmeier P.C. (1981) Stimulation of central 5-HT turnover by β-adrenoceptor agonists. *Naunym-Schmiedeberg's Arch. Pharmacol.*, 317: 115-119.

Walinder J., Skott A., Carlsson A., Nagy A. and Roos B.-E. (1976) Potentiation of the antidepressant action of clomipramine by tryptophan. *Arch. Gen. Psychiat.*, 33: 1384-1389.

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.

Wamsley J.K., Byerley W.F., McCabe R.T., McConnell E.J., Dawson T.M. and Groseer B.I. (1987) Receptor alterations associated with serotonergic agents: An autoradiographic analysis. *J. Clin. Psychiat.*, 48: 19.

Warrington S.J. (1992) Clinical implications of the pharmacology of serotonin reuptake inhibitors. *Inter. Clin. Psychopharmacol.*, 7 Suppl. 2: 13-19.

Wellburg J.B., Rosenbaum J.F., Biederman J., Sachs G.B., Pollack M.H. and Kelly K. (1988a) Fluoxetine added to non-MAOI antidepressants converts nonresponders to responders: a preliminary report. *J. Clin. Psychiat.*, 88: 447-449.

Wellburg J.B., Rosenbaum J.F., Biederman J., Sachs G.B., Pollack M.H. and Kelly K. (1990b) Tricyclic antidepressant levels after fluoxetine addition. *Neuropsychobiol.*, 23: 202-207.

Weiss S., Sebben M., Kemp D. and Bockaert J. (1986) Serotonin 5-HT_1 receptors mediate inhibition of cyclic AMP production in neurons. *Eur. J. Pharmacol.*, 120: 227-230.

Welsh L.H. (1955) The analysis of solutions of epinephrine and norepinephrine. *J. Am. Pharm. Assoc.*, 44: 507-514.

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.

Wharton R.N., Perel J.M., Dayton P.G. and Malitz S. (1971) A potential clinical use for methylphenidate with tricyclic antidepressants. *Am. J. Psychiat.*, 127: 1619-1625.

White K., Pistole T. and Boyd J. (1990) Combined monoamine-oxidase inhibitor-tricyclic antidepressant treatment: A pilot study. *Am. J. Psychiat.*, 137:11: 1422-1425.

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

Wilens T.E., Biederman J., Baldessarini R.J., McDermott S.P., Puopolo P.R. and Flood J.G. (1992) Fluoxetine inhibits desipramine metabolism. *Arch. Gen. Psychiat.*, 49: 752.

Wilkinson G.R. and Shand D.G. (1975) A physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther.*, 18: 377-390.

Wong D.T., Horrig J.S., Bymaster F.P., Hauser K.L. and Molloy B.B. (1974) A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropyl-amine. *Life Sci.*, 15: 471-479.

Wong D.T., Reid L.R., Bymaster F.P., and Threlkeld P.G. (1985) Chronic effects of fluoxetine, a selective inhibitors of serotonin uptake, on neurotransmitter receptors. *J. Neural Transm.*, 64: 251-269.

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

Yates M., Leake A., Candy J.M., Fairbairn A.F., McKeith I.G. and Ferrier I.N. (1990) 5-HT_2 receptor changes in major depression. *Biol. Psychiat.*, 27: 489-496.

Young J.P.R., Lader M.H. and Hughes W.C. (1979) Controlled trial of trimipramine, monoamine-oxidase inhibitors, and combined treatment in depressed out-patients. *Br. Med. J.*, 4: 1315-1317.

Zall H. (1971) Lithium carbonate and isocarboxazid - an effective drug approach in severe depressions. *Am. J. Psychiat.*, 127:10: 1400-1403.

Zeller E.A. (1951) Oxidation of amines. In: *The Enzymes, 1st Ed., vol. II.* (Eds. Summer J.B. and Myrback K.), Academic Press, New York, pp. 536-538.

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 on bacterial and mammalian enzymes. *Experientia*, 8: 349-350.

END

28-08-96

FIN