NEUROPHARMACOLOGICAL INTERACTIONS IN THE RAT PINEAL GLAND : A STUDY OF ANTIDEPRESSANT DRUGS

DISSERTATION

Submitted in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

of Rhodes University

by

SHABIR BANOO

April 1991

ABSTRACT

The rat pineal gland provides a convenient model for investigating noradrenergic receptor neurotransmission and the effects of various drugs on these processes in health and disease. The effect of a variety of antidepressant drugs on rat pineal gland function following acute and chronic administration is described. Antidepressants from several different classes increase melatonin synthesis in rat pineal gland cultures when administered acutely. This effect appears to be mediated by noradrenaline acting on postsynaptic β-adrenoceptors. Activation of these receptors, in turn, activates the enzyme serotonin N-acetyltransferase via a cyclic adenosine monophosphate (cAMP) second messenger system. Serotonin N-acetyltransferase catalyses the rate-limiting conversion of serotonin to melatonin. Blockade of postsynaptic β-adrenoceptors prevents the antidepressant-induced increase in melatonin synthesis. The possibility that atypical antidepressants as well as those that selectively inhibit serotonin reuptake may increase melatonin synthesis via a β-adrenoceptor mechanism is discussed. In contrast, however, antidepressants from different classes have variable effects on rat pineal gland function when administered repeatedly. Chronic treatment with antidepressants that selectively inhibit noradrenaline reuptake appear to down-regulate the β-adrenoceptor system while, simultaneously, increasing melatonin output. Atypical antidepressants and those that selectively inhibit serotonin reuptake appear to be without these effects when administered repeatedly. The pineal gland of normal rats may therefore not represent a suitable model for evaluating the biochemical effects of chronic antidepressant treatment. In an attempt to investigate pineal gland function in rats with "model depression", antidepressants were administered to chronically reserpinized rats. Treatment with reserpine produced an increase in the density of pineal β-adrenoceptors. In addition, pineal cyclic AMP accumulation and N-acetyltransferase activity were increased in reserpinized rats following exogenous catecholamine stimulation. Reserpine, by depleting intraneuronal catecholamine stores, prevented the nocturnal induction of N-acetyltransferase activity and reduced the synthesis of melatonin in pineal gland cultures. A variety of antidepressants, irrespective of their acute pharmacological actions, reversed these effects when administered chronically to resepinized rats. Acute antidepressant administration was not associated with a reversal of the reserpine-induced effects. These findings provide additional evidence against the hypothesis that antidepressant drugs act by reducing noradrenergic neurotransmission and casts doubt on the importance of β-adrenoceptor down-regulation in the mechanism of antidepressant action. The possibility that the pineal gland of the reserpinized rat may represent an alternative model for evaluating antidepressant therapies is discussed.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to Professor Ben Potgieter and Dr Santy Daya for their expert supervision, guidance and encouragement throughout this study.

I am also particularly grateful to the following organizations and individuals :

The South African Medical Research Council for the generous financial support that made this study possible.

Rhodes University for additional financial assistance towards this study.

Mrs Sally Morley for her expert technical assistance and efficiency in obtaining many of the materials needed for this study.

Mr Patrick January and Mr Wellington Ngandi for the excellent care and maintenance of the animals.

Jensen Electronics (Johannesburg, S.A.) and, in particular, Helen Arsenis for the generous use of their laser printing facilities.

My colleagues and friends for their encouragement and support during the course of this study.

My parents and sisters for their unrelenting patience, support and encouragement, without which this work would not have been possible.

My fiancée Sumaiya for making it all worthwhile.

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
aHT	N-Acetylserotonin
5'-AMP	Adenosine monophosphate
aMT	Melatonin
BBB	Blood-brain barrier
BZP	Benzodiazepine
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
Ci	Curie
CNS	Central nervous system
CSF	Cerebrospinal fluid
CPM	Counts per minute
CRF	Cortisol releasing factor
DA	Dopamine
DD	Constant darkness
DHA	Dihydroalprenolol
DHE	Dihydroergocriptine
DPM	Disintegrations per minute
DST	Dexamethasone suppression test
ECT	Electroconvulsive shock
fmol	Femtomole
GABA	Gamma-amino butyric acid
GH	Growth hormone
G_i	Guanine nucleotide inhibitory protein
G_s	Guanine nucleotide stimulatory protein
h	Hour
H	Histamine
HA	5-Hydroxyindole acetic acid
HCl	Hydrochloric acid
HIOMT	Hydroxyindole-O-methyltransferase
HL	5-Hydroxytryptophol
5-HT	Serotonin
5-HTP	5-Hydroxytryptophan
i.p.	Intraperitoneal
IHYP	Iodohydroxybenzylpindolol
kg	Kilogram
LL	Constant light
LD	Light/dark cycle

MA	5-Methoxyindole acetic acid
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
mg	Milligram
min	Minutes
ml	Millilitre
ML	5-Methoxytryptophol
mm	Millimetre
mM	Millimolar
MOPS	Morpholino-propanesulphonic acid
MT	5-Methoxytryptamine
NA	Noradrenaline
NAT	N-Acetyltransferase
nm	Nanometre
nM	Nanomolar
nmol	Nanomole
PBS	Phosphate buffered saline
PDE	Phosphodiesterase
PI	Phosphoinositide
pmol	Picomole
Px	Pinealectomy
REM	Rapid eye movement
RIA	Radioimmunoassay
SAD	Seasonal affective disorder
SCG	Superior cervical ganglion
SCN	Suprachiasmatic nuclei
sec	Seconds
S.E.M.	Standard error of the mean
TCA	Tricyclic antidepressant
TLC	Thin layer chromatography
μg	Microgram
μl	Microlitre
μM	Micromolar

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

LITERATURE REVIEW

1.1 INTRODUCTION

Depression or affective disorder is a label that is collectively applied to the emotional component of a great variety of human behavioural states related to feelings of sadness, apathy, futility, and despair. The trend of clinical experience and research studies supports the view that clinical depression occurs in relation to the balance between stresses on the person and vulnerability or predisposition. Severe depression is believed to be a genetically linked disorder, or group of disorders, that develops in biologically vulnerable people, sometimes in conjunction with an environmental calamity or major stress, but more often independent of an identifiable precipitating event. Epidemiological studies (*Gershon* et al. 1982, *Weissman* et al. 1984), suggest that 5%-10% of the general population are vulnerable to the development of a depressive disorder. Although there may be behavioural similarities between these genetically vulnerable people and non-vulnerable people unhappy with the course of their lives or with some stressful environmental event, the chronic administration of antidepressants to the former group, but not the latter, generally restores normal emotional tone within 2-6 weeks (*Quitkin* et al. 1984a). Thus, while the accurate identification of those depressed individuals who could benefit from anti-depressant drug therapy remains an important clinical goal, efforts to understand the pathophysiology of depressive illness have come to rely on studying the mechanism of action of the variety of available antidepressant agents.

Investigation into the role of monoamines in affective disorders has suggested that these amines may contribute importantly to the pathogenesis of these disorders. Of particular interest are the noradrenergic and serotonergic systems, in which evidence from a variety of studies suggests differences between depressed patients and normal individuals. The down-regulation of β-adrenoceptors by antidepressants, originally reported by *Vetulani* and *Sulser* (1975), was a germinal finding that led to the discovery of many more time-dependent alterations in the activity of neurotransmitter systems during long-term administration of antidepressant drugs. The basis for the receptor changes, their dependence on changes in other neurotransmitter pathways and their ultimate functional significance remain open areas for investigation. Considerable attention has been directed at the possibility that changes in central neurotransmitter receptors, and perhaps related adaptational events, occur as part of the process of becoming depressed, or are present as vulnerability factors, genetic or otherwise, for affective illness. Such a notion implies that the mechanisms operative in the antidepressant action of drugs in depressed patients may differ from those in normal humans - or normal rats. Thus, the need for developing suitable animal models for studying depression and the effects of antidepressant drug treatment remains an important and challenging goal.

The possibility that the pineal gland, via the rhythmic secretion of the neurohormone melatonin, may be involved in some psychiatric diseases has received special attention in recent years. The mechanisms controlling melatonin production by the pineal gland involve central rhythm-generating and monoamine (noradrenergic) neurotransmitter systems , both of which are of considerable interest to psychiatrists, particularly those concerned with affective disorders. Several studies have demonstrated that pineal melatonin secretion is reduced in depressed patients and increased following treatment with antidepressant drugs. Indeed, these observations have led several investigators to postulate that the beneficial effects of these drugs may relate to their ability to alter pineal gland function and melatonin concentration (see *Srinivasan* 1989). However, studies in laboratory animals, particularly the rat, have provided inconsistent results, which are often at variance with clinical data. The lack of appropriate models to study antidepressant drug-induced effects on pineal function in animals has contributed to this apparent conflict between the laboratory and the clinic. Moreover, the available data from animal studies have focussed mainly on the effects of the older, tricyclic and monoamine oxidase inhibitor antidepressants on pineal gland function. In contrast to these older agents, studies in animals employing the newer, second-generation and atypical antidepressants are required if any interpretation involving neurotransmitter specificity of action is desired. Thus, one of the objectives of the present study was to examine the effects, if any, that the newer antideressants might have on rat pineal function and melatonin production. In addition, an attempt was made, using the pineal gland as a model, to assess the effect of antidepressant administration following disruption of neural function in rats. Such an experimental strategy may be useful to investigate the etiology and neurosubstrates of depression, to elucidate the mechanisms operative in antidepressant therapy, or to identify improved therapies for depression.

In the following sections, the relevant aspects of the vast literature concerning antidepressant drugs, on the one hand, and the pineal gland, on the other, are presented. Clinical data as well as the hypotheses concerning the mechanism of action of antidepressants are briefly reviewed. In addition, the biochemistry of the pineal gland as well as hypotheses concerning its involvement in time-keeping systems, affective disorders and the mechanism of action of antidepressants are dealt with.

1.2 ANTIDEPRESSANT DRUGS

1.2.2 Pharmacology and Clinical Data

Antidepressant drugs may be divided into three major groups : (a) tricyclic antidepressants (TCAs), (b) monoamine oxidase inhibitors (MAOIs) and (c) the newer "second generation" antidepressants. The latter group of antidepressants differ in chemical structure and/or pharmacological activity from the TCAs and MAOIs. They may be further classified on the basis of their selectivity in inhibiting the reuptake of either noradrenaline (NA) or serotonin (5-HT). In addition, some antidepressants in this group have an atypical mechanism of action affecting neither NA nor 5-HT reuptake.

1.2.2.1 The Tricyclic Antidepressants

1.2.2.1.1 General Properties

The serendipitous discovery of the therapeutic action of imipramine (*Kuhn* 1958) initiated the development of a series of tricyclic compounds with antidepressant effects. These include (i) the iminodibenzyls (e.g. desipramine, imipramine, trimipramine, clomipramine) and (ii) the dibenzocycloheptenes (e.g. amitriptyline, nortriptyline, protriptyline, doxepin). Their structural formulae are shown in Fig. 1.1

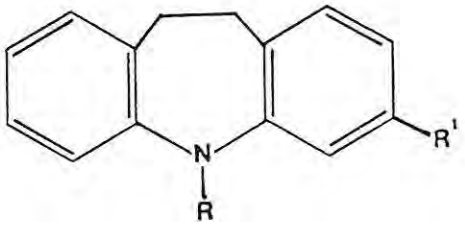
Tricyclic antidepressants inhibit the reuptake of both NA and 5-HT although their relative potencies vary. It is generally accepted that TCAs which are secondary amines (e.g. desipramine, nortriptyline, protriptyline) show some selectivity in inhibiting NA reuptake *in vitro*. By contrast, TCAs with tertiary or quaternary structures in the side chain and/or a halogen in the 3-position of the tricyclic nucleus and a 3-carbon side chain (e.g. imipramine, clomipramine, amitriptyline, butriptyline, trimipramine, doxepin) more selectively inhibit 5-HT reuptake *in vitro*. However, it is well established that many of these drugs produce active metabolites *in vivo* that have selectivity for one or other of the biogenic amines which differs from that of the parent drug. For example, imipramine shows little difference in its inhibitory effect on NA and 5-HT reuptake. However, its N-demethylated metabolite, desipramine, is approximately eight times more active than imipramine in inhibiting NA reuptake (*Nagy* 1977, *Maj* et al. 1982). Conversely, clomipramine is more active in inhibiting 5-HT reuptake than NA, yet its desmethyl metabolite shows selectivity in inhibiting NA reuptake (Hyttel 1982). Thus, the tertiary amine tricyclics not only fail to be selective inhibitors of 5-HT reuptake *in vivo* but the inhibition of NA reuptake seems to prevail.

The TCAs have a wide pharmacological spectrum, displaying anti-α_1-adrenergic, antiserotonergic, antimuscarinic and antihistaminergic receptor properties. The effects of TCAs and other antidepressants on different brain receptors are shown in Table 1.1.

1.2.2.1.2 Clinical Efficacy Data

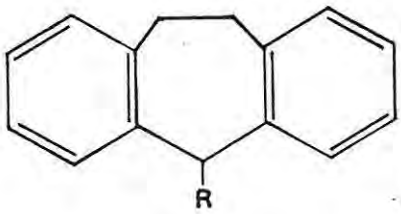
The clinical efficacy of the TCAs has been well investigated. In a comprehensive review, *Morris* and *Beck* (1974) showed that in 85 controlled trials comparing 93 treatment groups, the TCAs were superior to placebo in 60 patients with major affective disorder, indistinguishable in 31 patients but in no case was placebo superior in a controlled study. Although little is known as to which type of depression is most likely to respond to TCAs and even less regarding the choice of TCAs, *Kiloh* et al. (1962) and others have noted that patients with features of endogenous depression, particularly of a hysterical, irritable and hypochondriacal type, respond much less favourably. Amitriptyline is said to be more "sedative" and allegedly also more effective in the treatment of "agitated" depression than imipramine. On the other hand, the "stimulant" tricyclics (imipramine, desipramine, protriptyline) are reported to be more suitable in "retarded" depressions (*Shepard* et al. 1968). However, the judgement on agitation or retardation is

IMINODIBENZYLS

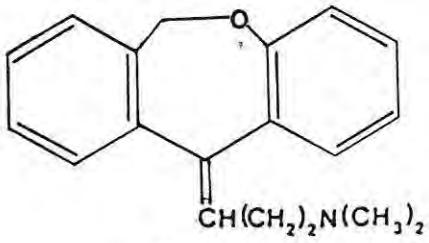


R	R'	
$-(CH_2)_3NHCH_3$	$-H$	**Desipramine**
$-(CH_2)_3N(CH_3)_2$	$-H$	**Imipramine**
$-(CH_2)_3N(CH_3)_2$	$-Cl$	**Chlorimipramine**
$-CH_2CH(CH_3)CH_2N(CH_3)_2$	$-H$	**Trimipramine**

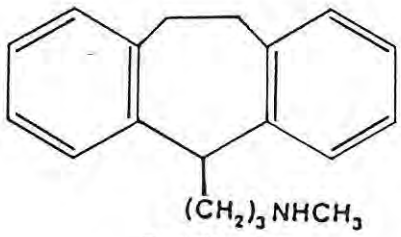
DIBENZOCYCLOHEPTENES



R	
$=CH(CH_2)_2NHCH_3$	**Nortriptyline**
$=CH(CH_2)_2N(CH_3)_2$	**Amitriptyline**
$-CH_2CH(CH_3)CH_2N(CH_3)_2$	**Butriptyline**



Doxepin



Protriptyline

Figure 1.1 Structural formulae of some tricyclic antidepressants

Table 1.1 Effects of antidepressants on various brain receptors (binding studies, IC_{50} in nM)

Drugs	α_1-Adrenoceptor [^{3}H]WB-4101*				α_2-Adrenoceptor [^{3}H]Clonidine		β-Adrenoceptor [^{3}H]DHA**		5-HT_1 receptor [^{3}H]5-HT		5-HT_2 receptor [^{3}H]Spiperone (Cerebral cortex)		Dopamine receptor [^{3}H]Spiperone (striatum)	Muscarinic cholinoceptor [^{3}H]QNB***			Histamine, receptor [^{3}H]Mepyramine	
Tricyclic uptake inhibitors																		
Imipramine	97[a]	58[b]	160[c]	54[e]	4 120[a]	4 930[c]	32 700[a]	13 300[c]	24 600[a]	1 080[c]	10 000[c]	245[c]	1 980[a]	181[a]	89[e]	78[f]	29[a]	26[d]
Desipramine	250[a]	150[b]	440[c]	130[e]	10 600[a]	9 400[c]	17 300[a]	14 200[c]	16 100[a]	3 070[c]	9 500[e]	540[e]	5 030[a]	848[a]	210[e]	170[f]	457[a]	250[d]
Clomipramine	35[a]		130[c]		5 040[a]	7 430[c]	38 900[a]	9 300[c]	21 200[a]	590[c]			268[a]	184[a]			64[a]	
Amitriptyline	22[a]	24[b]	46[c]	22[e]	550[a]	850[c]	21 200[a]	7 000[c]	1 520[a]	240[c]	1 700[e]	13[e]	1 070[a]	69[a]	11[e]	10[f]	6[a]	4.1[d]
Nortriptyline	88[a]	71[b]	130[c]	70[e]	3 870[a]	2 980[c]	15 500[a]	5 400[c]	1 000[a]	380[c]	640[e]	41[e]	2 130[a]	180[a]	81[e]	57[f]	48[a]	46[d]
Protriptyline		280[b]	420[c]			10 360[c]		8 700[c]		1 150[c]								60[d]
Doxepin		23[b]	24[c]			2 000[c]		20 500[c]		240[c]						44[f]		0.7[d]
Tetracyclic uptake inhibitors																		
Maprotiline	350[a]		250[c]		15 500[a]	16 770[c]	25 000[a]	7 900[c]	15 800[a]	220[c]			6 320[a]	650[a]			25[a]	
Other uptake inhibitors																		
Nomifensine	1 270[a]		980[c]		4 560[a]	2 480[c]	94 700[a]	23 300[c]	9 880[a]	1 370[c]			21 300[a]	48 800[a]			8 870[a]	
Nisoxetine		1 000[b]																
Zimelidine	1 180[a]		1 210[c]		3 350[a]	610[c]	186[a]	8 500[c]	33 200[a]	2 500[c]			14 400[a]	33 700[a]			2 900[a]	
Citalopram				500[g]	10 000[g]						20 000[g]	2 000[g]						
Fluoxetine		1 000[b]		8 000[e]							7 400[c]	1 300[c]			13 000[c]			
Atypical antidepressants																		
Mianserin	67[a]	86[b]	56[c]		126[a]	12[c]	21 900[a]	11 200[c]	1 210[a]	90[c]			4 850[a]	566[a]			6[a]	
Trazodone		68[b]																
Irpindole	6 810[a]	1 000[b]	6 150[c]	9 600[e]	6 700[a]	16 000[c]	100[a]	17 000[c]	15 200[a]	6 000[c]	21 000[c]	1 900[c]	18 500[a]	2 370[a]	37 000[c]		250[a]	

[a] *Hall* and *Ögren* (1981); [b] *Maggi* et al. (1980); [c] *Tang* and *Seeman* (1980); [d] *Tran* et al. (1978); [e] *Peroutka* and *Snyder* (1980a); [f] *Snyder* and *Yamamura* (1977); [g] *Hyttel* (1982)

* 2-(2,6-dimethoxyphenoxyethylamino)-methylbenzodioxan

** dihydroalprenolol

*** 3-quinuclidinyl benzilate

Adapted from *Maj* et al. 1984

not based on any objective evidence and furthermore, the superiority of one TCA over another might simply be a function of a greater dosage potency. Clomipramine has been found to be effective in some patients who seem to be refractory to other TCAs (*Rickels* et al. 1974).

1.2.2.1.3 Clinical Safety Data

The TCAs are all accompanied by adverse effects which represent an extension of their pharmacological activity. Foremost among these is the pronounced anticholinergic activity which manifests as blurring of vision, acute glaucoma, dryness of the mouth, sweating, urinary retention and constipation. Consequently, patients on TCAs are notoriously fickle in their compliance (*Johnson* 1981). The most frequent cardiovascular effects consist of postural hypotension, tachycardia, occasional heart block and various arrythmias, particularly in patients with pre-existing cardiac disease. These effects are also prominent in overdosage and the safety of TCAs in depressed patients at high risk of suicide is of particular concern. In the UK, approximately 3.3 % of all overdoses with TCAs have had a fatal outcome (*Crome* and *Newman* 1979). The TCAs have also been associated with such neuroendocrine effects as galactorrhoea and amenorrhoea in women, loss of libido in men, and excessive weight gain in both sexes. The most common central nervous system effects are drowsiness and less frequently, tremors. In addition, the TCAs lower the seizure threshold and could provoke seizures in conventional doses.

1.2.2.2 The Monoamine Oxidase Inhibitors

Although the MAOIs were the first clinically effective antidepressants, they rapidly became second-line treatments. Several factors, described below, contributed to this and for a number of years thereafter prescription of these drugs was limited. More recently, however, the development of newer compounds suggest that the MAOIs may still have a favourable place in the treatment of depressive disorders.

1.2.2.2.1 General Properties

The MAOIs, as a group, are derived from a number of different chemical series and all interact with the active site of monoamine oxidase (MAO) to inactivate this enzyme in brain and peripheral tissues. The older MAOIs, which bind irreversibly with the enzyme, are subdivided according to structure into hydrazine (phenelzine, isocarboxazide, nialamide, iproniazid) and non-hydrazine (tranylcypromine) derivatives.

1.2.2.2.2 Clinical Efficacy Data

The clinical effectiveness of the older MAOIs has been studied extensively and numerous controlled studies (review : *Beck* 1973, review : *Quitkin* et al. 1979, *Nies* and *Robinson* 1982) have noted their effectiveness in treating atypical depression. In addition, a number of placebo-controlled trials comparing phenelzine with imipramine (*Liebowitz* et al. 1985, 1988) and amitriptyline (*Robinson* et al.

1985) showed that patients with atypical depression responded better and more rapidly to phenelzine than the TCAs. The efficacy of the older MAOIs in endogenous depression is less well established, and while a number of patients do respond (*McGrath* et al. 1984, *Vallejo* et al. 1987) further studies are required. In addition, several studies have shown that MAOIs may be of use in the treatment of TCA-resistant endogenous depression (*Pare* 1985, *Nolen* 1989).

1.2.2.2.3 Clinical Safety Data

The major drawback of the older MAOIs relates to their interaction with sympathomimetic drugs and with tyramine, commonly found in ripe cheeses and other foodstuffs. The consequent hypertensive crisis ("cheese reaction") which may occur has limited their usefulness. However, *Raskin* (1972) and others have shown that, following appropriate dietary precautions as well as patient counselling, the incidence of hypertensive crises may be significantly reduced in patients treated with MAOIs. The majority of adverse effects associated with the MAOIs are similar to those associated with the TCAs (Section 1.2.2.1.3) and point to a lack of specificity of these drugs. In addition, many of the older MAOIs have amphetamine-like actions and the hydrazine derivatives, in particular, have been shown to be hepatotoxic (*Tollefson* 1983).

1.2.2.2.4 The Selective MAOIs

A significant development, aimed at reducing some of the adverse effects of the earlier MAOIs, has been the synthesis of inhibitors selective to type A (clorgyline) and type B (deprenyl, pargyline) MAO isoenzymes. Type A MAO preferentially metabolises NA and 5-HT, whereas type B has selectivity towards phenylethylamine and therefore not involved in the "cheese reaction" to dietary tyramine (*Blackwell* 1963). This offered the hope that MAO-B inhibitors would be safer antidepressants (*Quitkin* et al. 1984b), but unfortunately they appear to be effective only at higher doses where it is likely that the specificity to MAO-B would be lost (*Sunderland* et al. 1985). Deprenyl administered in doses selective for MAO-B inhibition, was shown to be ineffective in most patients, while in contrast, 3-6 fold higher doses which inhibited MAO-A as well, had significant antidepressant effects (*Murphy* et al. 1986, *Mann* et al. 1989). These studies strongly suggest that changes in brain functions involving substrates metabolised by MAO-A in humans, specifically NA and 5-HT, are likely to be involved in the mechanism of action of antidepressant drugs. In addition, *Murphy* et al. (1987a) have demonstrated that the MAO-A inhibitor, clorgyline, is an effective antidepressant, comparable with the TCAs. It is unclear whether clorgyline offers any advantage in terms of side-effects or therapeutic responses over the earlier non-selective MAOIs, although it has been shown to be effective even at low doses in patients with bipolar affective disorder (*Robinson* et al. 1985).

1.2.2.2.5 The Newer Reversible, Selective MAOIs

A more recent advance has been the development of short-acting, reversible, selective MAO-A inhibitors (*Youdim* and *Finberg* 1985). These differ from previous MAOIs which bind irreversibly with

the MAO enzyme (*Singer* and *Salach* 1981) and which require synthesis of new enzyme to restore function. They include compounds such as moclobemide, brofaromine, toloxatone, amiflamine and cimoxatone. Their much shorter functional half-lives, compared with conventional MAOIs, make them safer and easier to use (*Schoerlin* et al. 1987). The reversible nature of their inhibition could also minimise reactivity to tyramine, since in theory, this amine could compete with the MAOI and thus some would be metabolised in the periphery (*Youdim* and *Finberg* 1985). Moclobemide and brofaromine have been shown to have antidepressant efficacy comparable to the TCAs (*Larsen* et al. 1984, *Bieck* and *Antonin* 1988, *Schiwy* et al. 1988). Further studies, however, are needed to confirm the evidence that these new drugs have less propensity for potentiation of tyramine.

1.2.2.3 The "Second Generation" Antidepressants

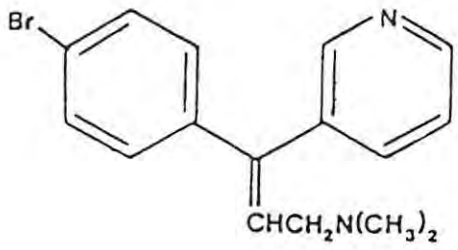
Dissatisfaction with the classical TCAs and MAOIs, described above, as a result of their lack of efficacy, slow onset of action and unacceptable side-effect profile, particularly in overdosage, has led to the development of newer, improved antidepressants. Although there appears to be no clinical evidence to suggest that these drugs are more effective or have a faster onset of action than the older antidepressants, their major advantage appears to be a relative reduction in anticholinergic effects and lack of cardiotoxicity in overdose. As noted earlier, these newer compounds differ in their chemical structure and/or pharmacological properties from the TCAs and MAOIs, and they may be further classified on the basis of their mechanism of action.

1.2.2.3.1 The Selective Serotonin Reuptake Inhibitors

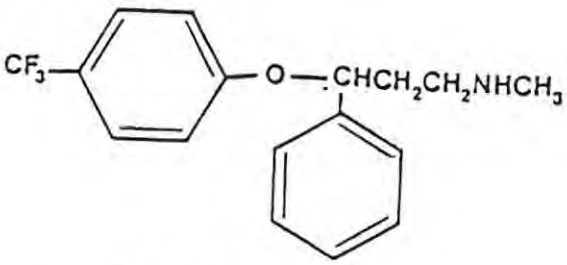
1.2.2.3.1.1 General Properties

The chemical structures of some selective 5-HT reuptake blockers are presented in Fig. 1.2. These drugs have been shown to be potent and highly selective inhibitors of 5-HT reuptake both *in vitro* and *in vivo* (reviews: *Lemberger* et al. 1985, *Fuller* 1987, *Leonard* 1988). Moreover, despite their high affinity for the 5-HT transport site, pre-clinical studies indicate that they have little affinity for any of the neurotransmitter receptors *in vitro*.

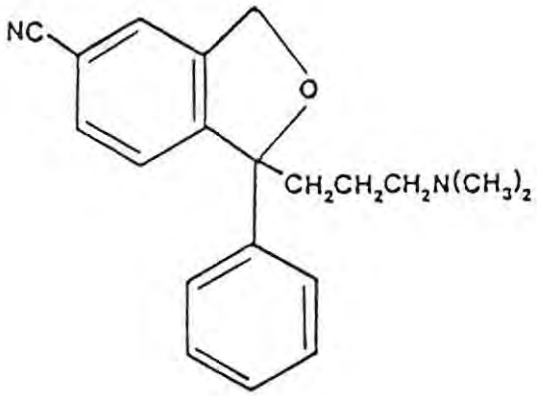
Like the TCAs, many of these newer antidepressants are metabolised by N-demethylation. This metabolism often has marked effects on their potency and specificity as reuptake inhibitors. For example, zimelidine is not so potent as a 5-HT reuptake inhibitor but it is selective, requiring much higher concentrations to inhibit the reuptake of other monoamines (*Hyttel* and *Larsen* 1985). The N-demethylated metabolite norzimelidine is more potent as a 5-HT reuptake inhibitor but is slightly less selective (*Hyttel* and *Larsen* 1985). In addition, norzimelidine appears to predominate in brain at early times following the administration of zimelidine to rats(*Ross* et al. 1981). Thus, it is likely that all of the 5-HT reuptake inhibition of zimelidine is probably due to the metabolite. Indeed, inhibition of metabolic N-demethylation of zimelidine results in a marked loss of potency (*Ross* and *Renyi* 1977).



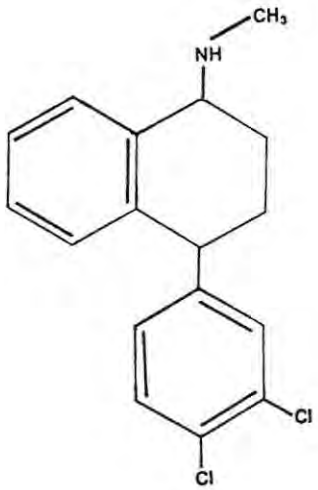
Zimelidine



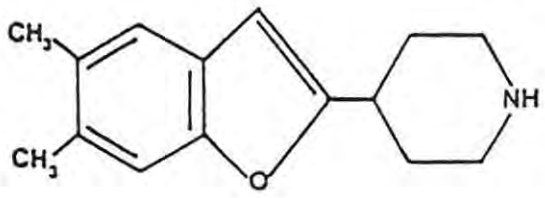
Fluoxetine



Citalopram



Sertraline



CGP-6085A

Figure 1.2 Structural formulae of some selective serotonin reuptake inhibitors

Fluoxetine and its metabolite, norfluoxetine, are both potent and selective inhibitors of 5-HT reuptake *in vitro* and *in vivo* (*Wong* et al. 1975a). In rats, fluoxetine itself seems to account for 5-HT reuptake inhibition at early times after its administration, but norfluoxetine probably accounts for 5-HT reuptake inhibition at later stages (*Schmidt* et al. 1988). Of the newer antidepressants under investigation, sertraline is probably the most potent 5-HT reuptake inhibitor but its desmethyl metabolite is relatively weaker (*Koe* et al. 1983).

1.2.2.3.1.2 Clinical Efficacy Data

The clinical efficacy of this group of compounds was first demonstrated with zimelidine. Subsequently, a number of these antidepressants have been studied in clinical trials. Clinical data suggest that drugs of this class are significantly superior to placebo and comparable with standard antidepressants in relieving symptoms associated with severe, unipolar, major, depressive disorders.

A number of double-blind studies have shown zimelidine to be similar in efficacy to TCAs (*Loudon* et al. 1981, *Montgomery* et al. 1981a, *Aberg* 1981) and maprotiline (*Montgomery* et al. 1981b). In one study (*Merideth* and *Feighner* 1983), zimelidine appeared to be superior to imipramine but placebo was also superior to imipramine. Other studies with imipramine (*Hiramatsu* et al. 1983) have, however, not confirmed this.

Several trials have shown fluvoxamine to be superior to placebo and comparable in overall efficacy with TCAs (*Guelfi* et al. 1983, *Hil* et al. 1983, *Dick* and *Ferrero* 1983).

Fluoxetine has also been shown to be superior to placebo and comparable in overall efficacy with TCAs (*Bremner* 1984, *Cohn* and *Wilcox* 1985, *Levine* et al. 1987, *Feighner* 1985, *Feighner* and *Cohn* 1985, *Tamminen* and *Lehtinen* 1986). In a large multicentre study comparing fluoxetine, imipramine and placebo in 540 patients (*Stark* and *Hardison* 1985), both drugs were significantly better than placebo but not significantly different from each other. Subjective ratings in some studies (*Chouinard* 1985, *Young* et al. 1987) detected some superiority of amitriptyline over fluoxetine. However, this finding was explained primarily as the result of false perception by patients, particularly to the sedative effects of amitriptyline.

More recently, *Doogan* and *Caillard* (1988) demonstrated the superiority of sertraline over placebo in a double-blind study. In a parallel double-blind study, these investigators compared sertraline with amitriptyline and placebo. They showed the two active comparators to be better than placebo but indistinguishable from one another.

Clinical studies using citalopram (*Gottlieb* et al. 1980, *Ofsti* 1982), indalpine (*Damlouji* et al. 1985), paroxetine (*Borup* et al. 1982) and alaproclate (*Frost* et al. 1984) suggest efficacy similar to that of other 5-HT reuptake inhibitors when evaluated in comparative or open studies.

Recently, more potent and highly selective inhibitors of 5-HT reuptake have been developed which include panuramine, CGP-6085A and RU-25591. These drugs have been extensively evaluated in animal studies (review : *Lemberger* et al. 1985). Although their clinical efficacy in depressive disorders is currently under investigation, it has not yet been sufficiently documented.

1.2.2.3.1.3 Clinical Safety Data

To date, all studies of the selective 5-HT reuptake inhibitors (see below) clearly indicate that their major advantage over other currently available antidepressants is a favourable side-effect profile. Several comprehensive reviews (*Lemberger* et al. 1985, *Mendels* 1987, *Burrows* et al. 1988) of the clinical data pertaining to this group of drugs suggest that they have very few, if any, anticholinergic side-effects commonly associated with TCA administration. Thus, they cause relatively little sedation, drowsiness, confusion, constipation, dry mouth, blurred vision or urinary retention compared with TCA compounds. Likewise, postural hypotension and weight gain are unusual occurances in patients recieving 5-HT reuptake inhibitors. In addition, clinical data reveal that these drugs have little effect on cognitive and psychomotor performance, nor do they potentiate the effect of alcohol. On the other hand, these new compounds have been associated with an increased frequency of gastrointestinal side-effects including nausea, vomiting, abdominal cramps and diarrhoea. Insomnia, anxiety, agitation, restlessness and rashes have also been reported. However, these side-effects are relatively mild and appear to decrease in frequency with continued treatment or if an adjustment in dosage is made. Thus, the side-effect profile of these new antidepressants is clearly different from that of the TCAs. Studies to evaluate specifically the cardiovascular effects of these compounds have also been undertaken. In a large study, *Fisch* (1985) examined electrocardiograms in patients being treated with fluoxetine, TCAs or placebo. In contrast to patients treated with TCAs, fluoxetine-treated patients revealed a slightly decreased pulse rate with no significant conduction defects. Similar results have been reported for fluvoxamine (*Roos* 1983) and zimelidine (*Pottage* and *Groschinsky-Grind* 1983). It is unclear whether the diminished pulse rate associated with these drugs reflects a serotonergic effect or lack of anticholinergic effect. The lack of cardiotoxicity of these drugs is further indicated by reports of insignificant changes in electrocardiograms following overdoses with zimelidine (*Georgotas* et al. 1981, *Judd* et al. 1983) and fluoxetine (*Fisch* 1985). Thus, the lack of cardiovascular side-effects seen with these new antidepressants makes them attractive for the treatment of depression in cardiac patients.

Although zimelidine proved to be a useful and an effective antidepressant, it was withdrawn from clinical use in 1983 because of unusual but serious adverse effects. A flu-like illness occurred in approximately 1.5 % of patients receiving the drug (*Nilsson* 1983). In some patients the syndrome progressed to peripheral neuropathy of the Guillain-Barrè type. This side-effect has not been reported with other drugs in this class. A few patients who experienced this hypersensitivity with zimelidine have been successfully treated with fluoxetine (*Chouinard* and *Jones* 1984). These observations suggest that the problem is unique to zimelidine and does not appear to be related to 5-HT reuptake inhibition.

1.2.2.3.2 The Selective Noradrenaline Reuptake Inhibitors

1.2.2.3.2.1 General Properties

The chemical structures of some selective NA reuptake blockers are presented in Fig. 1.3. While NA reuptake inhibition is indeed the outstanding feature of these compounds, many of them are less selective and influence other neurotransmitters and receptors as well.

Nomifensine, a drug with a novel tricyclic structure, has a unique neurochemical profile. Experimental studies have shown it to inhibit the reuptake of both NA and dopamine *in vivo* (*Brogden* et al. 1979, *Leonard* 1980).

Maprotiline, a tetracyclic inhibitor of NA reuptake, exerts, like the TCAs, other effects including histamine (H_1) receptor and α_1-adrenoceptor antagonism (*Maitre* et al. 1971). A structural analogue of maprotiline, viz. (+)-oxaprotiline, has a greater selectivity for NA reuptake and does not appear to influence other neurotransmitter receptors (*Delini-Stula* et al. 1982). The enantiomer, (-)-oxaprotiline (levoprotiline), while retaining antidepressant properties, does not affect monoamine reuptake (*Delini-Stula* et al. 1982). Although (-)-oxaprotiline lacks antimuscarinic and antiserotonergic activity, it retains antihistaminic and α_1-adrenolytic properties (*Delini-Stula* et al. 1982).

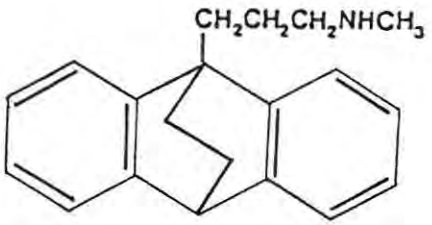
Viloxazine, a potent inhibitor of NA reuptake, also has a 5-HT-mimetic action (*Pinder* et al. 1977a). More recently, compounds that are both potent and selective inhibitors of NA reuptake have been developed. These include nisoxetine (*Wong* et al. 1975b) and tomoxetine (*Wong* et al. 1982) which are bicyclic compounds structurally related to the selective 5-HT reuptake blocker fluoxetine (Section 1.2.2.3.1). In addition, these newer compounds appear to have minimal affinity for neurotransmitter receptors.

1.2.2.3.2.2 Clinical Efficacy Data

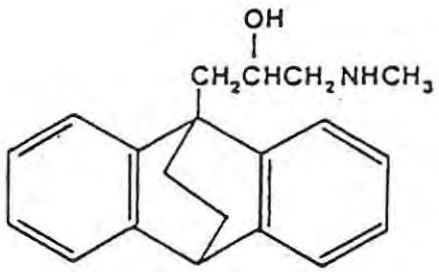
The clinical efficacy of maprotiline in major depression has been extensively investigated and a review of these studies reveals a superiority over placebo and a similar order of efficacy as imipramine and amitriptyline (*Pinder* et al. 1977b).

Several trials have compared nomifensine with either placebo or TCAs (review : *Brogden* et al. 1979, *Fields* 1982). These findings are consistent in demonstrating nomifensine's superiority over placebo and in showing that the drug is at least as effective as imipramine and amitriptyline. In addition, as a result of its effect on the dopaminergic system, clinical studies have shown it to be particularly effective in the treatment of depressed patients with severe motor retardation (*Van Scheyen* et al. 1977).

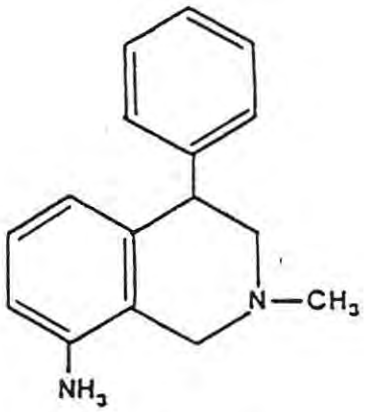
Clinical data also show viloxazine to be similar in efficacy to TCAs (*Pinder* et al. 1977b, *Nair* and *Schwartz* 1982).



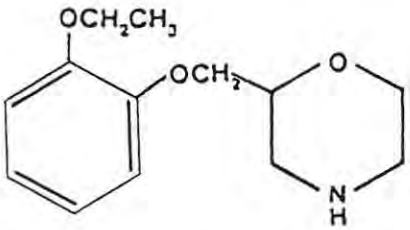
Maprotiline



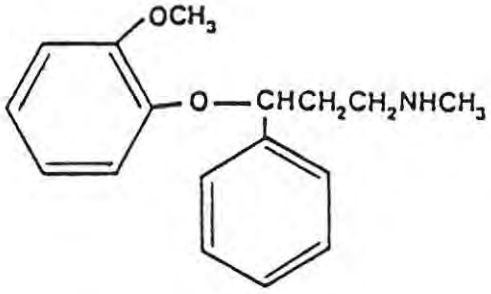
Oxaprotiline



Nomifensine



Viloxazine



Nisoxetine

Figure 1.3 Structural formulae of some selective noradrenaline reuptake inhibitors

Similarly, studies using (+)-oxaprotiline (*Delini-Stula* et al. 1982) and (-)-oxaprotiline (*Wendt* 1988) reveal an antidepressant effect in patients similar to that of reference antidepressants.

Although the more recently introduced selective NA reuptake inhibitors, nisoxetine and tomoxetine, are presently under investigation, their clinical efficacy has yet to be established.

1.2.2.3.2.3 Clinical Safety Data

Generally, the selective NA reuptake inhibitors, like other "second generation" antidepressants, appear to have as their major advantage a more favourable side-effect profile than the classical TCAs and MAOIs. While tricyclic-like adverse events have been reported for some of the compounds in this group, their incidence and severity have been low. Most comparative studies have evaluated these compounds in relation to reference TCAs only, and their efficacy in relation to other antidepressants is still unclear. However, overall clinical studies indicate that NA reuptake inhibitors are a useful addition to the pharmacological treatment of depression.

In a comprehensive review of the clinical data, *Pinder* et al. (1977a) reported a significantly reduced adverse effect profile for maprotiline when compared with the TCAs. The drug does not appear to have the troublesome anticholinergic effects associated with TCA administration. However, it has been shown to have a cardiovascular side-effect profile similar to the TCAs (*Hermann* et al. 1983). In addition, its propensity to cause seizures, particularly at high doses, has limited its usefulness (*Dessain* et al. 1986).

Studies with nomifensine show it to have a low incidence of anticholinergic and cardiovascular side-effects when compared with reference TCAs (review : *Fields* 1982). These studies also reveal a high level of safety in nomifensine overdosage. There were no reports of cardiotoxicity or convulsions and the most common symptoms were drowsiness and tremors. However, since the drug has been shown to cause sleep disturbances and other effects including nausea, dry mouth and hypersensitivity reactions, it has been withdrawn from clinical use.

A review of the clinical data by *Pinder* et al. (1977b) showed viloxazine to cause less sedative, anticholinergic and cardiovascular side-effects although nausea and vomiting were commonly encountered.

While the clinical safety of (+)- and (-)-oxaprotiline has yet to be established, available clinical data indicate that they offer significant advantages over the TCAs (*Delini-Stula* et al. 1982, *Wendt* 1988).

1.2.2.3.3 <u>The Atypical Antidepressants</u>

Despite being clinically effective antidepressants, these compounds have little or no ability to inhibit NA or 5-HT reuptake. Atypical antidepressants constitute a heterogenous group as regards their chemical structure and pharmacological profile. Thus, in the following section, these compounds are considered separately. Their chemical structures are presented in Fig. 1.4.

Mianserin

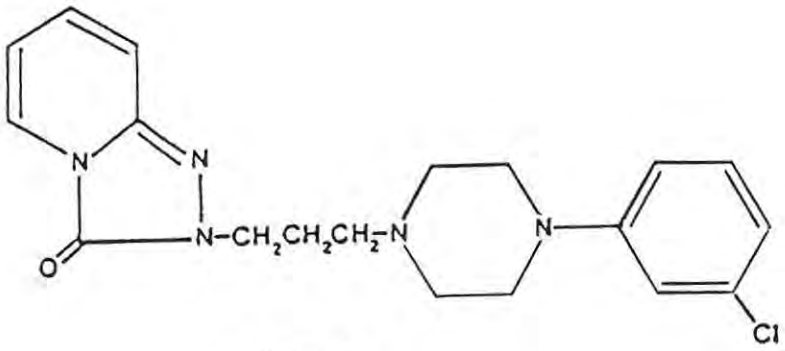
Mianserin, a tetracyclic piperazine derivative, has been the subject of a number of detailed reviews (*Brogden* et al. 1978, *Peet* and *Behagel* 1977, *Leonard* 1978). Although the drug has antiserotonin and antihistamine properties, its pharmacological activity has been attributed to the ability to block presynaptic α_2-adrenoceptors. Numerous clinical studies have shown mianserin to have an antidepressant efficacy superior to placebo (*Smith* et al. 1978) and comparable with TCAs (*Coppen* et al. 1976, review : *Brogden* et al. 1978, *Atamura* et al. 1989). These studies also indicate that mianserin has fewer anticholinergic side-effects and lacks cardiotoxicity. In addition, mianserin overdosage has not been associated with convulsions or electrocardiogram abnormalities, although drowsiness has been reported (*Crome* and *Ali* 1986).

Iprindole

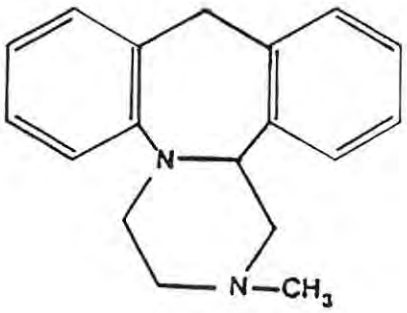
Iprindole, a tricyclic indole, has weak biological activity in acute experiments and produces no characteristic effects (*Rosloff* and *Davis* 1974). The drug appears to have little or no ability to influence NA or 5-HT reuptake but it does seem to selectively inhibit dopamine reuptake *in vitro* (*Randrup* and *Braestrup* 1977). However, chronic experiments with iprindole indicate an effect on noradrenergic receptors (review : *Maj* et al. 1984, Section 1.2.3.2.2). Thus, its mechanism of action remains elusive. Clinical studies have shown iprindole to be an effective antidepressant, comparable with imipramine (*Fann* et al. 1972).

Trazodone

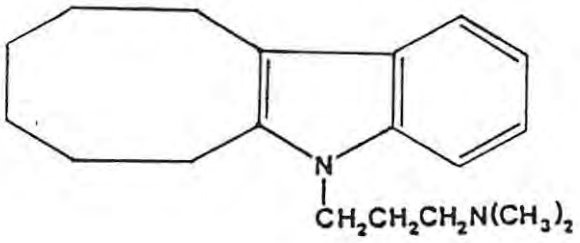
Trazodone is a phenylpiperazine antidepressant with an unusual neurochemical profile. In experimental studies, trazodone has been shown to be a weak inhibitor of NA reuptake and there has also been a suggestion that it may influence dopamine metabolism (see *Leonard* 1984). However, its uniqueness has been shown to be due to a serotonergic action. The drug is a weak inhibitor of 5-HT reuptake and, in small doses, acts as a 5-HT receptor antagonist. Large doses of trazodone produce a profile characteristic of a 5-HT agonist (*Maj* et al. 1979a). This effect has been attributed to the formation of an active metabolite, m-chlorophenylpiperazine (see *Maj* 1981). Several clinical studies have demonstrated that trazodone has an efficacy comparable with other antidepressant drugs (review : *Brogden* et al. 1981, *Atamura* et al. 1989). In addition, studies indicate that trazodone has anxiolytic properties comparable with that of diazepam (review : *Brogden* et al. 1981). Trazodone has little anticholinergic effects and lacks cardiotoxicity at therapeutic doses.



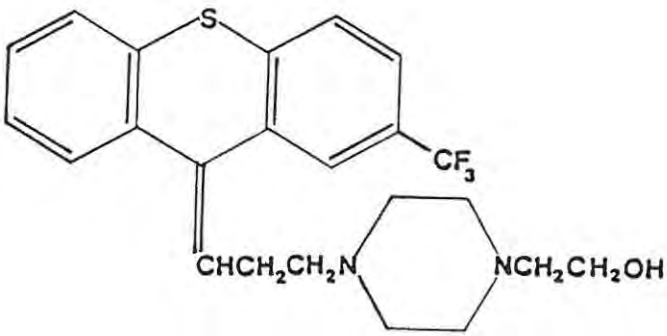
Trazodone



Mianserin



Iprindole



Flupenthixol

Figure 1.4 Structural formulae of some atypical antidepressants

Other Atypical Antidepressants

Numerous other compounds with pharmacological profiles differing from that of the conventional antidepressants have also been investigated. However, more clinical experience is required before a place for them in antidepressant therapy is decided.

Amoxapine, a compound structurally related to the neuroleptic loxapine, has been shown to have an antidepressant efficacy comparable with the TCAs (review : *Jue* et al. 1982). Available data indicate that it has a lower incidence of anticholinergic and cardiovascular side-effects but menstrual disturbances and galactorrhoea have been reported. Amoxapine is also known to cause extrapyramidal side-effects (*Lapierre* and *Anderson* 1983) and to elevate prolactin (*Cooper* et al. 1981).

Buproprion, a phenethylamine derivative, has been shown to possess antidepressant properties comparable to reference antidepressants (*Golden* et al. 1988a, b). The exact mode of action of buproprion is unclear, but it appears to act as a dopamine reuptake inhibitor (*Cooper* et al. 1980). It has little effect on NA reuptake, no anticholinergic actions, and no inhibiting effects on MAO. Seizures have been known to occur following treatment with high doses of the drug (*Hollister* and *Csernansky* 1990).

Flupenthixol, a neuroleptic, has also been evaluated as an antidepressant since its mood elevating effect in schizophrenic patients was noted. Clinical data suggest a comparable efficacy with TCAs (*Johnson* 1979).

Salbutamol, a β_2-adrenoceptor agonist, has been reported to have an antidepressant action following intravenous administration to depressed patients (*Lecrubier* et al. 1981, *Simon* et al. 1984).

Preliminary evidence suggests that another β_2-adrenoceptor agonist, clenbuterol, is also an effective antidepressant particularly in patients with endogenous depression (*Simon* et al. 1984).

1.2.3 Hypothesis Concerning the Mechanism of Action of Antidepressants

1.2.3.1 Studies Based on Acute Antidepressant Administration

1.2.3.1.1 Biogenic Amine Deficiency Hypothesis

The clinical observation that drugs such as reserpine which deplete monoamine stores also produce depression gave rise to the idea that depression may be due to an absolute or relative deficiency of monoamines (*Schildkraut* 1965). The fact that clinically effective antidepressants increase the availability of these monoamines at their postsynaptic receptors, either by blocking reuptake in the case of TCAs or by inhibiting monoamine breakdown in the case of MAOIs, provided further support for this hypothesis. In support of this notion, the existence of two subtypes of depression has been postulated : one with a

deficiency of NA or dopamine and the other with a deficiency of 5-HT (*Bunney* and *Davis* 1965, *Schildkraut* 1965, *Coppen* 1967, *Lapin* and *Oxenkrug* 1969). Numerous clinical studies have been conducted in the hope of detecting the hypothesised deficiencies of these biogenic amines. Measurement of monoamine metabolites in body fluids and post-mortem brains of depressed patients have been performed (see *Bunney* and *Davis* 1965, *Mendels* et al. 1976). In general, studies in depressed patients have failed to identify a robust disorder of monoamine metabolism. In addition, these studies suggest that there is no relationship between changes in monoamine metabolite concentrations and treatment responses (*Charney* et al. 1981). Thus, it remains to be established whether certain subgroups of depressed patients will ultimately prove to have a deficiency in one or other of the biogenic amines.

A number of observations suggest that blockade of monoamine reuptake and inhibition of metabolism may be insufficient to explain the action of antidepressants. The most important of these are :

- Atypical antidepressants such as iprindole, trazodone and mianserin affect neither monoamine reuptake processes nor MAO activity.

- Reuptake blockade and inhibition of monoamine metabolism are seen following acute administration of antidepressants whereas the clinical action of these drugs requires 2-3 weeks to become evident.

- Drugs such as cocaine and amphetamine increase the availability of catecholamines at their receptors but seem not to be effective antidepressants.

1.2.3.1.2 Dopamine Hypothesis

The role of dopamine (DA) has long been emphasized in the aetiology of depression and in the mechanism of action antidepressant drugs (*Randrup* and *Braestrup* 1977, *Waldmeier* 1982) despite the observation that most antidepressants are weak inhibitors of dopamine reuptake. However, there is a growing body of evidence to suggest that a wide range of antidepressants do in fact influence DA receptors and DA-mediated behaviour (see Section 1.2.3.2.6). In addition, newer compounds such as bupropion and amineptine which are potent and relatively selective inhibitors of DA reuptake have been shown to be clinically effective antidepressants (*Ferris* et al. 1982, *Waldmeier* 1982, *Golden* et al. 1988a). Whether these effects are related to their antidepressant action awaits further clarification.

1.2.3.1.3 Acetylcholine Hypothesis

The notion that abnormalities in central cholinergic transmission exist in depression has been given support by evidence suggesting that the antimuscarinic action of antidepressants may be responsible for, or contribute to, their mood-elevating effects (*Biel* et al. 1962, *Janowsky* et al. 1972, 1973). Several binding studies have demonstrated the interaction of many antidepressants with central muscarinic receptors (eg. *Snyder* and *Yamamura* 1977, *Hall* and Ögren 1981). These studies, together with clinical experience, have shown that the TCAs possess more potent anticholinergic properties than the newer

"second generation" antidepressants. However, the lack of correlation between the potency of anticholinergic action and the clinical efficacy of the TCAs, as well as the lack of anticholinergic properties of the MAOIs and of a number of other antidepressants (eg. mianserin, trazodone, selective 5-HT reuptake inhibitors) are the main arguments against the acetylcholine hypothesis. Furthermore, the efficacy of antimuscarinic drugs in the treatment of depression remains to be investigated.

1.2.3.1.4 Histamine Hypothesis

A number of antidepressants are potent inhibitors of histamine (H) receptors. This effect is similar to, or stronger than, that of established antihistamines and neuroleptics. Following the demonstration of the peripheral antihistaminic actions of antidepressant drugs (eg. *Figge* et al. 1979), several workers have described their action on central H-receptors.

The TCAs, mianserin, iprindole and viloxazine have been shown to be potent inhibitors of [^{3}H]mepyramine binding to rat brain H_1-receptors both *in vitro* and *in vivo* (*Tran* et al. 1978, 1981, *Diffley* et al. 1980, *Hall* and Ögren 1981, *Schwartz* et al. 1981). However, their relative potencies in blocking H_1-receptors do not seem to correlate with their clinical efficacy (*Peroutka* and *Snyder* 1980a). Furthermore, the data suggests that their potencies as H_1-receptor antagonists seem to correlate with their ability to induce sedation.

A number of antidepressants have also been shown to antagonize central H_2-receptors. The TCAs, mianserin and iprindole are potent inhibitors of H_2-sensitive adenylate cyclase activity in brain homogenates (*Green* and *Maayani* 1977, *Kanof* and *Greengard* 1978, *Maayani* et al. 1982, *Olianas* et al. 1982). In addition, the depressant effect of histamine, via H_2-receptors, on the firing of cortical neurons is blocked by TCAs (*Haas* 1979). These results have led to the hypothesis that central H_2-receptor blockade by antidepressants may be responsible for their mood-elevating effects. However, H_2-sensitive adenylate cyclase is also inhibited by drugs such as promethazine (*Kanof* and *Greengard* 1978) and cyproheptadine (*Green* et al. 1977) which do not appear to have antidepressant activity. On the other hand, the MAOI antidepressants reveal no H_2-receptor antagonism. Furthermore, antihistaminic effects are seen following acute administration of antidepressants but the therapeutic effect of these drugs requires about 2 weeks of treatment.

1.2.3.2 Studies Based on Chronic Antidepressant Administration

1.2.3.2.1 Effect on Brain Monoamine Metabolism

Antidepressant-induced adaptive changes in monoamine systems have been studied by comparing alterations in neurotransmitter turnover. These changes reflect the complicated interaction between a variety of presynaptic neuronal events, including precursor availability and uptake, neurotransmitter synthesis and metabolism, and feedback mechanisms regulated by both presynaptic and postsynaptic

receptors. The adaptive changes induced by chronic antidepressant treatment on rat brain monoamine turnover have been reviewed by several authors (*Charney* et al. 1981, *Sugrue* 1981a, *Maj* et al. 1984). A summary of the data is presented in Table 1.2.

When given as a single dose, the secondary tricyclics (desipramine, nortriptyline, protriptyline) cause a decrease in brain NA turnover, while the tertiary tricyclics (amitriptyline, clomipramine) do not affect NA turnover (see *Maj* et al. 1984). In contrast to acute treatment, long-term treatment with the secondary tricyclic desipramine was found to increase NA turnover in most studies (see *Maj* et al. 1984). The data also indicate that the tertiary tricyclics (amitriptyline, clomipramine) do not affect NA turnover after chronic treatment although inconsistent results have been reported for imipramine. An increase in NA turnover has also been observed following the chronic administration of nisoxetine, mianserin, trazodone as well as ECT, while chronic treatment with maprotiline, salbutamol or iprindole does not appear to affect NA turnover.

Inconsistent results have also been observed on 5-HT turnover following chronic antidepressant administration (see *Charney* et al. 1981, *Maj* et al. 1984). Long-term treatment with desipramine and mianserin have been shown to have no effect on 5-HT turnover, while zimelidine and fluoxetine appear to decrease it. Chronic treatment with iprindole has been found to increase 5-HT turnover in rat neocortex but not in whole mouse brain. Inconsistent results have been observed following repeated treatment with imipramine, clomipramine as well as ECT.

Thus, the effects of chronic administration of a variety of antidepressants on NA and 5-HT turnover are highly variable with no single common effect discernible. While differing experimental conditions used by different researchers may account for some discrepancies, it is likely that there is no specific effect on NA and 5-HT turnover which may be attributed to the therapeutic effect of all or even most antidepressants.

1.2.3.2.2 Effect on β-Adrenoceptor Sensitivity

Banerjee and co-workers (1977) were the first to report that chronic but not acute treatment with desipramine, doxepin or iprindole reduces the binding of [^{3}H]DHA to β-adrenoceptors in homogenates of whole rat brain. With the aid of Scatchard analysis, these workers showed that this effect was due to a decreased number of binding sites (B_{max}) rather than to a change in the apparent affinity (K_d) of the receptors to the ligand. Subsequently, numerous workers have confirmed these findings in multiple brain areas following chronic treatment with a wide range of antidepressants (reviews : *Charney* et al. 1981, *Sugrue* 1981a, b, *Maj* et al. 1984). The results of these studies are summarised in Table 1.3.

The specificity of this effect on brain β-adrenoceptors for antidepressants was demonstrated by *Sellinger-Barnette* et al. (1980) and *Peroutka* and *Snyder* (1980). These workers showed that chronic

Table 1.2 Effects of chronic antidepressant administration on brain noradrenaline, serotonin and dopamine turnover

Treatment	Noradrenaline turnover	5-HT turnover	Dopamine turnover
Imipramine	↓ ↑	↓ 0 ↑	↓
Desipramine	↓ ↑	0	0
Clomipramine	0	0 ↓	0
Amitriptyline	0		↓
Nortriptyline	0		
Protriptyline	↑		0
Maprotiline	0	0	0
Nisoxetine	↑		
Zimelidine		↓	
Fluoxetine		↓	
Mianserin	↑ ↓	0	0
Trazodone	↑		
Iprindole	0	0 ↑	0
Salbutamol	0	↑	0
MAO inhibitors	0		0
Electroconvulsive shock	↑	0 ↑	0

↑ increased turnover; ↓ decreased turnover; 0 no effect

Adapted from *Maj* et al. 1984

treatment with a variety of other centrally acting drugs including antipsychotics and anxiolytics did not reduce the binding to β-adrenoceptors.

Studies by *Minneman* et al. (1979) have shown that the decrease in binding to β-adrenoceptors in the cerebral cortex following repeated desipramine treatment is related to β_1-adrenoceptors but not to β_2-adrenoceptors. However, β_2-adrenoceptor agonists with reported antidepressant efficacy such as salbutamol, terbutaline and clenbuterol do not affect β_1-adrenoceptor binding in the cerebral cortex (*Hall* et al. 1980, *Frazer* et al. 1986a). Moreover, clenbuterol has been reported to down-regulate β_2-adrenoceptors in the cerebral cortex (*Frazer* et al. 1986a). More recently, using autoradiographic analysis, *Wamsley* et al. (1987) reported that chronic treatment with fluoxetine reduced both β_1- and β_2-adrenoceptors in several brain regions.

The changes in β-adrenoceptor density following repeated antidepressant treatment been shown to correlate with changes previously observed in the adrenoceptor-coupled adenylate cyclase system. Studies by *Sulser* and his associates (*Vetulani* and *Sulser* 1975, *Vetulani* et al. 1976a, b) demonstrated that chronic but not acute treatment with desipramine, iprindole, MAOIs (nialamide, pargyline) or ECT reduced NA-stimulated cAMP accumulation in rat limbic forebrain slices without affecting the basal cAMP level. These findings were confirmed by numerous other workers in which a reduced responsiveness of the adrenoceptor-coupled adenylate cyclase system of the limbic forebrain or cerebral cortex to NA and isoprenaline (*in vitro*) or to electrical stimulation of the locus ceruleus (*in vivo*) was shown after chronic treatment with a variety of antidepressants (reviews : *Charney* et al. 1981, *Sugrue* 1981a, b, *Maj* et al. 1984). A summary of the data is presented in Table 1.3.

The subsensitivity of the adrenoceptor-coupled adenylate cyclase system produced by chronic antidepressants appears to be limited to brain tissue only, since *Wolfe* et al. (1978) and *Frazer* et al. (1978) found no effect on this system in the rat heart following repeated treatment with desipramine. However, the specificity of this effect by antidepressants in the brain was questioned, since such an effect was also found after treatment with the antipsychotic agent chlorpromazine (*Schultz* 1976). It is noteworthy that the latter drug shares some properties with antidepressants (eg. NA reuptake inhibition, presynaptic α-adrenoceptor antagonism).

The development of subsensitivity was found not to be related to the concentration of antidepressant in brain tissue. Studies by *Vetulani* et al. (1976a) showed a decreased responsiveness of the adenylate cyclase system to NA 1 h and 24 h after the last dose of desipramine and iprindole, even though drug levels were considerably higher 1 h after the last dose. Similarly, *Wolfe* et al. (1978) reported that the decreased response to isoprenaline persisted even 3 days after cessation of repeated desipramine treatment and disappeared only 7 days after withdrawal of the drug.

TABLE 1.3 Effect of long-term antidepressant treatment on β-adrenoceptor density and noradrenaline- or isoprenaline-stimulated cyclic AMP accumulation in the rat brain

CATEGORY	DRUGS	REDUCTION IN *BETA*-ADRENOCEPTOR DENSITY	REDUCTION IN cAMP RESPONSE
TCAs	Desipramine	+	+
	Imipramine	+	+
	Amitriptyline	+	+
	Clomipramine	+	+
	Doxepin	+	•••
NA Reuptake Inhibitors	Nisoxetine	0	+
	Maprotiline	+	+
	(±)Oxaprotiline	+	+
	Nomifensine	•••	+
5-HT Reuptake Inhibitors	Zimelidine	+	+
	Fluvoxamine	+	•••
	Fluoxetine	+	0
	Sertraline	+	+
MAOIs	Phenelzine	+	•••
	Pargyline	+	+
	Clorgyline	+	•••
	Nialamide	+	+
	Tranylcypromine	+	+
Atypical Drugs	Iprindole	+	+
	Mianserin	+,0	+
	Trazodone	+	•••
Others	ECT	+	+
	REM Sleep Deprivation	+	+

cAMP = cyclic adenosine 3',5'-monophosphate; TCA = tricyclic antidepressant; MAOI = monoamine oxidase inhibitor; ECT = electroconvulsive therapy; REM = rapid eye movement; ... = data unavailable. Data adapted from *Charney* et al. (1981), *Sugrue* (1981a, b), *Maj* et al. (1984), *Wamsley* et al. (1987) and *Koe* et al. (1987).

The mechanism whereby chronic antidepressant treatments reduce the sensitivity of the β-adrenoceptor-coupled adenylate cyclase system is not fully understood. It is generally accepted that the subsensitivity results from chronic overexposure of postsynaptic adrenoceptors to NA resulting either from inhibition of NA reuptake (tricyclic-like agents) or from inhibition of NA degradation (MAOIs). This hypothesis is strengthened by observations that repeated desipramine or iprindole treatment failed to produce β-adrenoceptor subsensitivity in animals pretreated with 6-hydroxydopamine or concomitantly treated with propranolol (*Wolfe* et al. 1978, *Schweitzer* et al. 1979, *Asakura* et al. 1982). In addition, β-adrenoceptors in the striatum, which lacks noradrenergic input, are resistant to down-regulation by desipramine (*Sulser* 1987). Blockade of α_2-adrenoceptors with yohimbine, which can be expected to elevate synaptic NA, was found to accelerate the reduction in binding to β-adrenoceptors after desipramine (*Johnson* et al. 1980). A similar effect was noted when the nonspecific α-adrenergic antagonist phenoxybenzamine, was combined with desipramine (*Paul* and *Crews* 1980).

However, the hypothesis that a facilitation of NA transmission leads to receptor desensitization cannot account for the down-regulation induced by antidepressants that are without effect on NA reuptake such as iprindole, trazodone and the specific 5-HT reuptake inhibitors (zimelidine, fluoxetine, fluvoxamine, sertraline) (see Table 1.3). Thus, while it appears that synaptic availability of NA is important, it is likely that other factors may be relevant to the regulation of β-adrenoceptors.

A major difficulty in interpreting changes in the adenylate cyclase system arises from the incompletely understood nature of the adrenoceptors involved. It has been established that in addition to β-adrenoceptors, a sub-population of receptors with neither α nor β characteristics, mediates NA-stimulated cAMP accumulation (*Mobley* and *Sulser* 1979). Interestingly, chronic administration of mianserin or zimelidine appears to selectively down-regulate the non-β component (*Mishra* et al. 1980). In the latter studies, the cAMP responses to NA were reduced while those to isoprenaline were not.

The ability of the selective 5-HT reuptake inhibitors to down-regulate the adrenoceptor-coupled adenylate system raises the question of the role of 5-HT in this process. Recently, several studies have shown that an intact serotonergic neuronal input is required for the proper functioning of β-adrenoceptors and for the down-regulation of these receptors by antidepressants. Repeated treatment with desipramine, imipramine, clenbuterol or ECT failed to reduce the density of β-adrenoceptors in rat cortex and limbic forebrain after a selective lesioning of 5-HT neurons with 5,7-dihydroxytryptamine (5,7-DHT) (*Brunello* et al. 1982, *Janowsky* et al. 1982, *Nimgaonkar* et al. 1985). In addition, selective lesions of 5-HT neurons was found to increase the density of β-adrenoceptors in several brain areas (*Stockmeier* et al. 1985). These workers also showed that serotonergic neuronal impairment selectively alters the β-adrenoceptor component of noradrenergic receptor systems without affecting α_1- and α_2-adrenoceptors. Moreover, Administration of *p*-chlorophenylalanine (*p*-CPA), which causes an acute reduction in the synaptic availability of 5-HT, to rats pretreated with desipramine was found to nullify the subsensitivity of β-adrenoceptors within 2 days despite the continuous administration of desipramine (*Manier* et al. 1984).

Thus, on the basis of the experimental evidence cited above, it may be concluded that the down-regulation of central β-adrenoceptors is a consistent feature of chronic, but not of acute, antidepressant treatments. Furthermore, while the mechanisms responsible for this effect are not completely understood, it is clear that NA and 5-HT are *both* required for this process.

1.2.3.2.3 Effect on α_1-Adrenoceptor Sensitivity

The effect of repeated antidepressant treatment on α_1-adrenoceptor sensitivity has been evaluated using several radioactive ligands. Binding studies that have employed [^{3}H]dihydroergocryptine ([^{3}H]DHE) and [^{3}H]WB-4101 as α_1-adrenoceptor ligands have revealed inconsistent results. Thus, chronic administration of TCAs, MAOIs and atypical antidepressants did not affect the binding of [^{3}H]DHE or [^{3}H]WB-4101 to α_1-adrenoceptors in the rat whole brain or cerebral cortex (*Rosenblatt* et al. 1979, *Perouka and Snyder* 1980a, *Tang* et al. 1981, *Snyder and Peroutka* 1982). However, *Rehavi* et al. (1980) showed an increase in binding (B_{max}) of [^{3}H]WB-4101 to mouse brain α_1-adrenoceptors following repeated treatment with amitriptyline. On the other hand, *Cohen* et al. (1982) have reported a decrease in binding of [^{3}H]WB-4101 to rat cortical membranes after chronic treatment with clorgyline. Several factors may account for these apparent discrepancies including that the TCAs (especially tertiary) are themselves potent antagonists of α_1-adrenoceptors (*U`Prichard* et al. 1978) and, furthermore, that the specificity of the ligands employed for these receptors is questionable (*Doxey* et al. 1981, *Massingham* et al. 1981).

More recently, binding studies employing the selective α_1-adrenoceptor ligand, [^{3}H]prazosin, have shown more consistent results. Using this ligand, *Campbell* and *Mckernan* (1982) reported an increase in binding (B_{max}) to rat brain α_1-adrenoceptors after repeated administration of imipramine or clorgyline. Similar results have been obtained by other workers following chronic administration of antidepressants or ECT (*Vetulani* and *Pilc* 1982, *Vetulani* et al. 1986). In these studies, chronic administration of the selective 5-HT reuptake inhibitors, citalopram and zimelidine, were also found to increase α_1-adrenoceptor density although less markedly than imipramine and ECT. In addition, neuroleptics with antidepressant properties such as levomepromazine and chlorprothixene also markedly increased α_1-adrenoceptor density when given repeatedly (*Vetulani* et al. 1986).

Electrophysiological changes, attributable to α_1-adrenoceptors, have also been demonstrated following repeated antidepressant treatment. Single-unit electrophysiological studies have shown that chronically administered TCAs and iprindole but not fluoxetine enhanced the response of postsynaptic α_1-adrenoceptors to microiontophoretically applied NA (*Menkes* et al. 1980, *Menkes* and *Aghajanian* 1981).

Behavioural studies also indicate that the sensitivity of central α_1-adrenoceptors is enhanced by repeated antidepressant treatment. Studies by *Maj* et al. (1979b) have demonstrated a clonidine-induced increase in spontaneous motor activity in rats following chronic, but not acute, treatment with TCAs and mianserin. Furthermore, repeated treatment with imipramine, amitriptyline or zimelidine has been

shown to augment post-decapitation convulsions, mediated by α_1-adrenoceptors, in rats (*Vetulani* and *Pilc* 1982, *Vetulani* et al. 1986). Furthermore, *Plaznik* et al. (1984) reported an enhanced responsiveness to phenylephrine following repeated treatment with desipramine. More recently, (+)- and (-)-oxaprotiline have also been shown to enhance the behavioural responses mediated by α_1-adrenoceptors in rats (*Mogilnicka* et al. 1987).

To summarise, the conflicting findings of early binding studies mentioned above may be attributable to the lack of selectivity of the ligands employed. The data presented suggest that various antidepressants, with different pharmacological profiles, may increase the density of α_1-adrenoceptors as evidenced by the increased binding (B_{max}) of [^{3}H]- prazosin. In addition, these findings are supported by electrophysiological and behavioural data which suggest enhanced responsiveness mediated by these receptors. Thus, it appears that up-regulation of α_1-adrenoceptors may be a heterogenous syndrome; some antidepressants may produce it simply by chronic receptor blockade, while others may rely on different, as yet unestablished, mechanisms.

1.2.3.2.4 Effect on α_2-Adrenoceptor Sensitivity

The results of α_2-adrenoceptor binding studies following repeated antidepressant treatment are controversial. A variety of antidepressants including TCAs, MAOIs and atypical antidepressants given for 4 to 7 days have been shown to increase the binding of [^{3}H]clonidine to α_2-adrenoceptors in the rat cerebral cortex (*Reisine* et al. 1980, 1982, *Asakura* et al. 1982). Different results, however, are seen when these drugs are administered over longer periods. Treatment for 2 to 3 weeks (*Tang* et al. 1981, *Sugrue* 1982a, b) has no effect on the binding of [^{3}H]clonidine, while treatment for 3 to 4 weeks (*Campbell* and *McKernan* 1982, *Cohen* et al. 1982) reduces it. It has been suggested that the increase in [^{3}H]clonidine binding, found after a short period of treatment, may reflect early adaptive changes, which probably are not directly responsible for the antidepressant effect observed only after repeated drug administration. According to *Reisine* et al. (1982), these initial alterations in central α_2-adrenoceptors may be responsible for the down-regulation of β-adrenoceptors in animals treated with antidepressants (see Section 1.2.3.2.2).

Repeated treatment with desipramine, imipramine or ECT has been shown to attenuate the ability of low doses of clonidine to decrease rat brain NA turnover, mediated by α_2-adrenoceptors (*Tang* et al. 1978, *McMillen* et al. 1980, *Sugrue* 1982a, b). However, the repeated administration of amitriptyline, nortriptyline, nisoxetine, trazodone, iprindole, mianserin or salbutamol is without effect on the clonidine-induced decrease in NA turnover (*Tang* et al. 1978, *Sugrue* 1981c, 1982a, b, c). It is likely that the attenuation of the clonidine-induced decrease in NA turnover by repeated desipramine or imipramine treatment may be due to the blockade of α_2-adrenoceptors or to the development of subsensitivity to clonidine. However, since desipramine does not affect central α_2-adrenoceptors (*Sugrue* 1982a, see

Table 1.1) and since attenuation of the activity of clonidine is observed only after chronic treatment of desipramine, it seems that chronic desipramine and imipramine treatment induces subsensitivity to clonidine. Interestingly, however, this effect is not shared by other antidepressants.

Electrophysiological studies also appear to confirm the possibility of a decreased α_2-adrenoceptor sensitivity following repeated treatment with desipramine or imipramine (*Svensson* and *Usdin* 1978, 1979). Moreover, clonidine, which in low doses depresses the firing of locus ceruleus cells in control animals, has no effect in rats chronically treated with imipramine, desipramine or zimelidine, but not clomipramine, mianserin or iprindole (*Svensson* and *Usdin* 1978, *Scuvée-Moreau* and *Svensson* 1982).

A variety of behavioural responses to clonidine are also reduced following chronic antidepressant treatment to laboratory animals. These include clonidine-induced hypothermia, sedation, depression of acoustic startle reflex, hypotension and suppression of hypothalamic self-stimulation (see *Sugrue* 1983, *Charney* et al. 1981, *Maj* et al. 1984).

The data, mentioned above, seem to preclude attempts at correlating the biochemical, physiological and behavioural changes following repeated antidepressant treatment with changes in central α_2-adrenoceptor binding. The existence of α_2-adrenoceptors both presynaptically and postsynaptically may explain the conflicting data on the effect of repeated antidepressant treatments (*U'Pritchard* et al. 1979, see *Green* and *Nutt* 1985). Studies on platelet α_2-adrenoceptor binding in depressives have also been undertaken and, again, the results are contradictory. The number of binding sites have been reported to be reduced (*Wood* and *Coppen* 1981), normal (*Daiguji* et al. 1981) and elevated (*Kafka* et al. 1980). Repeated treatment with TCAs have been reported to decrease the number of α_2-adrenoceptor binding sites on platelets of depressives (*Garcia-Sevilla* et al. 1981). Biochemical and physiological studies in depressives support these findings. The clonidine-induced decrease in both NA turnover and blood pressure is the same in drug-free depressives and normal persons (*Checkley* et al. 1981a) but is attenuated by chronic desipramine treatment (*Checkley* et al. 1981b). Chronically administered clorgyline also attenuates the hypotensive response to clonidine in depressives (*Siever* et al. 1982). Numerous studies have also demonstrated that the growth hormone (GH) response to clonidine is consistently reduced in depressives (review : *Siever* and *Davis* 1985). Desipramine treatment for one week was found to increase the GH response to normal levels in depressives, while treatment for longer periods (1-3 weeks) attenuated it (*Glass* et al. 1982).

Thus, while the data on α_2-adrenoceptors is still incomplete, the whole body of evidence suggests that the ability to decrease α_2-adrenoceptor sensitivity is not shared by many compounds known to be clinically effective antidepressants. However, studies in depressed patients suggest that changes in α_2-adrenoceptors may play an important role in the pathophysiology of this illness.

1.2.3.2.5 Effect on Serotonin Receptor Sensitivity

1.2.3.2.5.1 5-HT_1 Receptor Sensitivity

Numerous studies have examined the effect of chronic antidepressant treatment on the binding of [^{3}H]5-HT to rat brain 5-HT_1 receptors. While there are some reports that antidepressants reduce the binding to cortical 5-HT_1 receptors, most studies reveal that these receptors are not altered by chronic treatment with a variety of antidepressants including clomipramine, amitriptyline, desipramine, mianserin, iprindole and ECT (reviews : *Wilner* 1985, *Zemlan* and *Garver* 1990). A major problem in interpreting 5-HT_1 receptor binding data stems from the fact that little is known of antidepressant effects on 5-HT_1 receptor subtypes. At present, seven different 5-HT_1 receptor subtypes have been identified including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1P} and 5-HT_{1S} receptors (*Zemlan* et al. 1988, *Heuring* and *Peroutka* 1987).

Electrophysiological studies, on the other hand, have consistently shown that neural transmission at central 5-HT_1 receptors is facilitated by chronic antidepressant treatment. Chronic, but not acute treatment with TCAs or ECT enhances the response of postsynaptic 5-HT_1 receptors to iontophoretically applied 5-HT (*de Montigny* and *Aghajanian* 1978, *Wang* and *Aghajanian* 1980, *Menkes* et al. 1980). Additionally, iprindole, which has little or no ability to block 5-HT reuptake, also enhances 5-HT sensitivity (*Hall* and *Ögren* 1981). This latter observation suggests that the enhanced 5-HT response caused by antidepressants may be due an effect on the postsynaptic 5-HT receptor complex. Furthermore, antidepressants such as zimelidine, fluoxetine and indalpine do not directly affect the postsynaptic 5-HT receptor complex (*de Montigny* et al. 1981, *Blier* et al. 1984), but facilitate 5-HT transmission by blocking presynaptic inhibition of 5-HT release (*Blier* et al. 1988). Similarly, it has also been suggested that MAO-A inhibitors such clorgyline, amiflamine and phenelzine may block presynaptic inhibition of 5-HT release with chronic treatment thus facilitating quantal release of 5-HT (*Blier* et al. 1987).

The enhanced 5-HT sensitivity observed following chronic antidepressant treatment is also reflected in behavioural studies. Various behaviours including sleep, the 5-HT behavioural syndrome and locomotor activity, which are regulated by 5-HT, can be reliably elicited by 5-HT agonist administration. Numerous studies indicate that the behavioural responses elicited by 5-HT agonist administration are uniformly enhanced by chronic antidepressant administration (review : *Zemlan* and *Garver* 1990).

1.2.3.2.5.2 5-HT_2 Receptor Sensitivity

Chronic treatment with a variety of antidepressants such as imipramine, desipramine, amitriptyline, zimelidine, mianserin or iprindole was found to decrease the binding of [^{3}H]spiroperidol or [^{3}H]ketanserin to 5-HT_2 receptor sites (reviews : *Maj* et al. 1984, *Wilner* 1985, *Zemlan* and *Garver* 1990). In addition, several studies indicate that that the chronic administration of MAOIs decreases the

number of both 5-HT_1 and 5-HT_2 receptors (*Peroutka* and *Snyder* 1980, *Kellar* et al. 1981, *Lucki* and *Frazer* 1982). Moreover, repeated treatment with other psychotropic drugs including chlorpromazine, haloperidol and methysergide was ineffective in reducing 5-HT_2 receptor number (*Peroutka* and *Snyder* 1980), suggesting that this effect is specific to antidepressants. A problem with interpreting these data as indicating that 5-HT_2 receptor down-regulation is a consistent feature of antidepressant treatment relates to ECT data. Several studies have shown that while chronic antidepressant drug treatment decreased the density of cortical 5-HT_2 receptors, repeated ECT treatment increased the density of these receptors (*Kellar* et al. 1981, *Vetulani* et al. 1981).

Although ligand binding data seem to suggest that chronic antidepressant treatment reduces the responsiveness of 5-HT_2 receptor systems, further studies of 5-HT_2 receptor-coupled function are needed to assess the potential role of these receptors in depression. In this regard, *Kendall* and *Nahorski* (1985) demonstrated that chronic treatment with iprindole and imipramine decreased the 5-HT_2 receptor-coupled turnover of phosphoinositide (PI). Furthermore, *Blackshear* and *Sanders-Bush* (1982) showed that chronic mianserin treatment produced a decrease in the 5-HT_2-mediated head twitch response in mice. The observed decrease in this 5-HT_2 mediated behaviour was paralleled by a decrease in the density of 5-HT_2 receptors.

1.2.3.2.6 Effect on Dopamine Receptor Sensitivity

1.2.3.2.6.1 Postsynaptic Dopamine Receptors

Several investigators have examined the effect of chronic antidepressant administration on radioligand binding to postsynaptic dopamine (DA) receptors. The chronic administration of imipramine, desipramine, amitriptyline, fluoxetine, pargyline, iprindole or ECT was found to be without effect on the binding of [^{3}H]spiroperidol to rat forebrain or striatal homogenates (*Rosenblatt* et al. 1979, *Peroutka* and *Snyder* 1980, *Tang* et al. 1981, *Rehavi* et al. 1980, *Snyder* and *Peroutka* 1982, *Bergstrom* and *Kellar* 1979). In contrast, however, *Koide* and *Matsushita* (1981) observed a decreased (B_{max}) [^{3}H]spiroperidol binding to rat striatal DA receptors following chronic treatment with imipramine or desipramine.

Repeated treatment of rats with imipramine, desipramine, clomipramine, amitriptyline, mianserin or iprindole was found not to affect apomorphine-induced stereotypy (*Maj* et al. 1979b, *Spyraki* and *Fibiger* (1981). However, *Delini-Stula* and *Vassout* (1979) showed that chronic treatment with clomipramine or amitriptyline, but not with imipramine or maprotiline, suppressed the apomorphine-induced stereotypy in rats. On the other hand, the repeated administration of desipramine, imipramine, iprindole or citalopram potentiated the locomotor response to apomorphine or *d*-amphetamine in rats (*Spyraki* and *Fibiger* 1981, see *Maj* et al. 1984).

Thus, the results of locomotor activity studies suggest that chronic antidepressant administration may increase the response of the dopaminergic mesolimbic system to DA agonists. However, since

stereotypy caused by apomorphine or *d*-amphetamine does not appear to be altered by antidepressant treatment, it has been suggested that the involvement of the dopaminergic striatal system may be excluded (see *Maj* et al. 1984). While binding studies of DA receptors in the mesolimbic system have yet to be reported, the negative results of binding studies on striatal DA receptors support this suggestion. It should be noted, however, that noradrenaline (NA) acting via α_1-adrenoceptors can also be involved in the potentiated behavioral responses to apomorphine and *d*-amphetamine (*Maj* et al. 1984). Further studies are therefore necessary before a definite conclusion on the effect of antidepressants on DA receptors can be drawn.

1.2.3.2.6.2 Dopamine Autoreceptors

The effect of chronic antidepressant treatment on the sensitivity of DA autoreceptors has also been investigated. Repeated administration of imipramine, amitriptyline, iprindole or phenelzine antagonized the inhibition of dopaminergic neuronal discharge in the substantia nigra induced by low doses of apomorphine (*Chiodo* and *Antelman* 1980, *Antelman* et al. 1982). Furthermore, the chronic administration of imipramine, amitriptyline, mianserin or ECT was found to reduce the hypomotility induced by low doses of apomorphine in rats (*Serra* et al. 1979, 1981). These findings favour an attenuation of responses mediated by DA autoreceptors. In support of this suggestion, *Lee* and *Tang* (1982) showed that repeated treatment with desipramine or nomifensine reduced the binding of [^{3}H]DA to presynaptic DA receptors in the striatum. Whether this subsensitivity is related to blockade of DA reuptake and subsequent excessive stimulation of the DA autoreceptors is as yet unclear. However, it should be mentioned that *Spyraki* and *Fibiger* (1981) found no effect on the apomorphine-induced hypomotility following chronic treatment with imipramine or desipramine. Thus, further studies are indicated to elucidate the effect of antidepressants on DA autoreceptors

1.2.3.2.7 Antidepressant-Binding Sites

A specific high affinity binding site for [^{3}H]imipramine in the rat brain was first described by *Raisman* et al. (1979). Subsequently, the binding site was also found in the brains of several other species, including man. Clinical studies indicate a decrease in the density of platelet [^{3}H]imipramine-binding sites in untreated severely depressed patients (*Langer* et al. 1984a, *Lewis* and *McChesney* 1985). Similarly, a decrease in the density of [^{3}H]imipramine-binding sites has also been demonstrated in post-mortem brains from depressed patients (*Perry* et al. 1983). These findings suggest that [^{3}H]imipramine binding may be a useful biological marker in depression.

There is now substantial evidence that the [^{3}H]imipramine-binding site is associated with the 5-HT transporter in brain and platelets (see *Langer* et al. 1986). In addition to [^{3}H]imipramine, several non-tricyclic antidepressants including [^{3}H]paroxetine and [^{3}H]indalpine bind with high affinity to the recognition site associated with the 5-HT transporter complex (*Langer* et al. 1986). This recognition site mediates inhibition of the Na^+-dependent reuptake of 5-HT. Dissociation kinetic experiments support

the view that the substrate recognition site for 5-HT within the 5-HT transporter complex is different from the receptor labeled by [^{3}H]imipramine (*Segonzac* et al. 1985). Consequently, the [^{3}H]imipramine-recognition site may represent a novel type of presynaptic receptor whose function is to modulate 5-HT reuptake. Furthermore, [^{3}H]desipramine has been shown to label a specific high affinity site associated with the NA transporter in the periphery and the central nervous system (CNS) (*Raisman* et al. 1982, *Langer* et al. 1981, 1984b), suggesting that NA reuptake may also be modulated in a similar way.

The existence of an endogenous ligand acting on the [^{3}H]imipramine-recognition site to modulate the 5-HT transporter has been proposed by several workers (*Langer* et al. 1983, *Sette* et al. 1983, *Langer* et al. 1986). Among the possible candidates, is 5-methoxytryptoline (6-methoxytetrahydro-β-carboline), a derivative of tryptophan, which inhibits [^{3}H]imipramine binding and [^{3}H]5-HT reuptake in nanomolar concentrations. This compound has been reported to be present in the pineal gland, but probably only in trace amounts (*Langer* et al. 1985). However, the physiological relevance of 5-methoxytryptoline as an endogenous ligand for the [^{3}H]imipramine-recognition site or its role in the pathogenesis of depression remains an open question.

1.2.3.2.8 Other Receptors

In addition to adrenoceptors, 5-HT receptors and DA receptors several other brain receptors have been studied in the search for changes following chronic antidepressant treatment.

Conflicting findings have been reported as regards the effect of chronic antidepressant treatment on the sensitivity of central muscarinic cholinergic receptors. Repeated treatment with amitriptyline increased the density of these receptors in various regions of the mouse brain (*Rehavi* et al. 1980). Similar results were reported in the rat striatum following chronic treatment with desipramine or imipramine (*Koide* and *Matsushita* 1981) and in the rat cerebral cortex following repeated ECT (*Gulati* et al. 1982). However, other workers found no effect of the repeated treatment with desipramine, imipramine, amitriptyline, nisoxetine, fluoxetine, pargyline, iprindole or ECT on the binding to muscarinic cholinergic receptors in the rat cerebral cortex or striatum (*Maggi* et al. 1980, *Peroutka* and *Snyder* 1980, *Deakin* et al. 1981). Moreover, electrophysiological studies revealed no effect of chronic administration of imipramine, desipramine, clomipramine, amitriptyline or iprindole on the responsiveness of hippocampal pyramidal neurons or neurons of the lateral geniculate nucleus to microiontophoretically applied acetylcholine or carbachol (*Gallager* and *Bunney* 1979, *Menkes* and *Aghajanian* 1981). Thus, although antidepressants, particularly the TCAs, have affinity for muscarinic receptors, it is doubtful whether they have a consistent effect on these receptors following repeated administration.

As regards GABA receptors, numerous electrophysiological studies indicate that repeated treatment with a variety of different antidepressants such as imipramine, clomipramine, amitriptyline, maprotiline, zimelidine, clorgyline, tranylcypromine or iprindole does not affect the responsiveness of these receptors

(*Deakin* et al. 1981). Interestingly, *Suranyi-Cadotte* et al. (1984) reported that chronic antidepressant treatment decreased the density of benzodiazepine-binding sites in the rat brain. However, *Vetulani* et al. (1986) were unable to confirm these findings following chronic treatment with a number of different antidepressants.

No consistent effects have also been noted on opiate receptor sensitivity following chronic antidepressant treatment. Repeated desipramine treatment was found to reduce the density of these receptors in the cerebral cortex but not in the striatum and hippocampus (*Reisine* and *Soubrie* 1982). However, repeated ECT did not modify the binding to opiate receptors in the cerebral cortex (*Deakin* et al. 1981). Similarly, behavioural and electrophysiological data reveal that chronic antidepressant administration does not modify the responsiveness of these receptors (*Katz* and *Schmaltz* 1980, *Scuvée-Moreau* and *Svensson* 1982).

1.2.3.3 General Remarks

From the above discussion it is evident that experimental studies of the adaptive effects of chronic treatment with antidepressant drugs and ECT have, as yet, not provided a common mechanism of action which could be specifically and causally related to antidepressant activity. The concept that all forms of antidepressant therapy possess a common mechanism of action may represent an erroneous strategy since depression is not a homogeneous entity and given the immense complexity of brain function. Drugs do not have to possess a common mechanism of action in order to achieve the same final result. Complex interconnections exist among central putative neurotransmitter and modulatory systems. The functioning of one monoaminergic system can be modified by changes in another (see Section 1.2.3.2.2). Moreover, the classical view that each neuron produces, stores and releases only one neurotransmitter is no longer tenable as other neurotransmitters as well as neuropeptides may coexist in the same neuron with a classical neurotransmitter. In the light of the complexity of the brain, the possibility warrants consideration that not only do multiple intervention sites into the central neuronal circuitry exist but also that chronic antidepressant therapies possess different intervention sites. Hence, while the end result may be the same, the initial interaction in the chain leading to this goal may not be identical for all forms of antidepressant therapy. Thus, there can be little doubt that our knowledge of the so much studied monoamine systems as well as the effects of antidepressants on these systems is very far from complete. Finally, it may be said that although the present review has raised a number of important questions, the evidence presented is largely in line with the hypothesis that a dysfunction of central monoaminergic systems is of pathogenetic significance for depression. However, to further assess the validity of this hypothesis more specific pharmacological tools and suitable animal models, among others, are required.

1.3 THE MAMMALIAN PINEAL GLAND

1.3.1 Introduction

The mammalian pineal gland, or epiphysis cerebri, was until recently considered a vestigial organ of little functional importance. Over the last 25 years, however, the burgeoning of neuroscientific research has witnessed the pineal gland attain the status of a *bona fide* participant in the regulation of various physiological and behavioural processes. Indeed, the study of the pineal has developed into an acknowledged area of the neurosciences in which investigators, representing different disciplines, have become involved. Pineal research has proceeded with ever-increasing speed and investigators in this field have the advantage of being able to draw upon a substantial body of information.

The pineal gland of mammals is considered to be an active neuroendocrine transducer (*Wurtman* and *Axelrod* 1965); it converts a neural input, namely a neurotransmitter released at a synapse, to a hormonal output, i.e., methoxyindoles (eg. melatonin) and polypeptides. In addition, it appears that the pineal may itself be subjected to other hormonal signals, suggesting endocrine-endocrine and endocrine-neural transduction mechanisms (*Cardinali* and *Vacas* 1978).

In all mammals, the pineal gland produces melatonin in a rhythmic manner with peak levels occuring at night irrespective of whether the animal is nocturnal or diurnal in its behavioural pattern. The major function of pineal melatonin appears to be to synchronize the seasonal and circadian rhythms of a variety of physiological events (*Armstrong* 1989). These include effects on reproduction, coat growth, temperature regulation and locomotor activity in species whose seasonal cycles depend on photoperiod (*Reiter* 1980, *Tamarkin* et al. 1985, *Armstrong* 1989).

In humans, melatonin may also affect circadian rhythms as evidenced by recent studies on seasonal affective disorder (SAD) (*Rosenthal* et al. 1986), depression (*Arendt* 1989), jet lag (*Arendt* et al. 1987), puberty (*Lang* 1986) and sleep and wakefulness (*Arendt* et al. 1988). In addition, melatonin may be implicated or may have therapeutic value in hormonally dependent tumours (*Blask* 1984), immunocompetence (*Pierpaoli* and *Maestroni* 1987) and stress responses (*Lynch* and *Deng* 1986).

Pineal research is indeed a dynamic field and future endeavours will undoubtedly enhance our understanding of the mechanisms that control the adaptation of organisms to their environment.

The task at hand, namely that of the pineal gland in depression, precludes an exhaustive survey of the vast pineal literature. The following review thus focuses only on relevant aspects of this body of information. In particular, consideration is given to the mechanisms that control melatonin synthesis and release. Evidence of pineal involvement in circadian rhythms, affective disorders and the mechanism of action of antidepressant drugs is also reviewed.

1.3.2 Anatomy

The mammalian pineal organ is a glandular structure derived as an evagination of the neural tube (*Kappers* 1965). In the rat, the pineal appears as a neuroepithelial protrusion from the roof of the diencephalon, the area between the habenular and posterior commissures; its connection with the commissural region is through the pineal stalk. The gland consists of two parenchymal cell types, pinealocytes and interstitial cells (*Wartenberg* and *Gusek* 1965), both of neuroectodermal origin. The pinealocytes are the neurosecretory cells of the gland.

The pinealocytes receive extensive autonomic innervation by way of two nerve bundles (nervi conarii) originating in the superior cervical ganglion (SCG) (*Kappers* 1965). In addition, recent anatomical (*Korf* and *Moller* 1984, *Reuss* and *Moller* 1986) and electrophysiological (*Semm* et al. 1981) studies have provided evidence that a direct innervation from the brain (central innervation) via the pineal stalk is present. The nerve terminals of both the autonomic and central fibres are found located freely in the interstitial or perivascular spaces and not in synaptic contact with pinealocytes (*Korf* and *Moller* 1984).

The rat pineal is highly vascularized, its major blood supply being provided by branches of the posterior choroidal (*Gladstone* and *Wakely* 1940) and posterior cerebral (*Hodde* 1979) arteries. Venous drainage occurs via the distal end of the great cerebral vein into the superior saggital sinus (*Hodde* 1979).

1.3.3 Neural Control of Pineal Function

In the rat pineal gland, noradrenaline (NA) released from sympathetic nerves regulates the daily rhythms in enzyme activity and melatonin synthesis that occur in response to environmental lighting. The neural pathway by which photosensory information reaches the pineal is well established, at least in the rat. The first stage of the pathway is a monosynaptic projection from the retina to the hypothalamic suprachiasmatic nuclei (SCN) (*Moore* 1982). The SCN contain the elements of the circadian timing system which generate pineal and other rhythms. Although the SCN can function in a cyclical manner autonomously, environmental lighting has strong effects on the clock. The neural pathway from the SCN passes first to the paraventricular nuclei and then to the intermediolateral cell collumn of the upper thoracic cord (*Klein* et al. 1983a). The impulses then pass via the SCG to the pineal gland. The link from the SCG to the pineal involves the postganglionic sympathetic system which is inhibited by light and activated by the absence of light.

1.3.3.1 Pineal β-Adrenoceptor-Mediated Function

Noradrenaline released at night acts on the pinealocyte membrane to stimulate β-adrenoceptors. Radioligand binding studies have identified β-adrenoceptors on rat pinealocytes (*Auerbach* et al. 1981) and in sheep and hamster pineal membranes (*Foldes* et al. 1983, *Craft* et al. 1985). In the rat, at least, the β-adrenoceptors resemble the β_1-subtype (*Auerbach* et al. 1981, *Dickinson* et al. 1986).

Stimulation of these receptors results in the activation of the enzyme adenylate cyclase. Noradrenaline-β-adrenoceptor-coupled adenylate cyclase systems consist of at least three separate protein components embedded in the phospholipid bilayer of the membrane. The first component is the receptor with its recognition site for the neurohormone. The second is adenylate cyclase as the effector system. The third component consists of two guanine nucleotide regulatory proteins - one that mediates stimulation of the adenylate cyclase (G_s) and another that mediates inhibition of adenylate cyclase (G_i). Activation of adenylate cyclase results in the synthesis of cAMP. The increase in cAMP mediates the activation of a cAMP-dependent protein kinase, protein kinase A, which initiates the transcription of mRNA required for *de novo* synthesis of enzymes in the melatonin pathway (see *Axelrod* 1983, *Ebadi* 1984). Thus, an increase in cAMP mediates an increase in the activity of serotonin N-acetyltransferase (NAT), the rate limiting enzyme that catalyses the conversion of serotonin to N-acetylserotonin. Similarly, the activity of the enzyme that converts N-acetylserotonin to melatonin, hydroxyindole-O-methyltransferase (HIOMT), is also increased by cAMP.

1.3.3.2 Pineal α-Adrenoceptor-Mediated Function

In addition to β-adrenoceptors, presynaptic α_2-adrenoceptors and postsynaptic α_1-adrenoceptors have been demonstrated in the rat pineal gland (*Pelayo* et al. 1977, *Klein* and *Sugden* 1984). The α_1-adrenoceptors are located on pinealocytes and are present at a density comparable to the β-adrenoceptors.

Recent evidence suggests that NA increases pineal cAMP not only by a β-adrenergic mechanism but also by an α_1-adrenergic mechanism. Activation of pineal α_1-adrenoceptors does not by itself increase cAMP accumulation. However, simultaneous activation of α_1-adrenoceptors markedly potentiates and prolongs β-adrenoceptor stimulation of cAMP accumulation (*Vanecek* et al. 1985) and NAT activity (*Klein* et al. 1983b; *Alphs* and *Lovenberg* 1984).

The mechanism underlying this amplification response appears to involve the phospholipid signaling pathway. Activation of rat pineal α_1-adrenoceptors triggers the hydrolysis of phosphatidylinositol (PI) to yield diacylglycerol and inositol phosphates (mainly inositol monophosphates). In addition, α_1-adrenoceptor stimulation produces an increase in intracellular Ca^{2+}, presumably by opening a ligand-dependent channel (*Sugden* et al.1987). Diacylglycerol is a potent activator of the Ca^{2+}- and phospholipid-dependent enzyme, protein kinase C (PKC). Activation of PKC mediates the amplification of the cAMP and NAT response.

The precise mechanism of this potentiation is not clearly understood. It appears that PKC does not directly affect β-adrenoceptor sensitivity, cAMP efflux from cells or cAMP metabolism (*Sugden* and *Klein* 1988). It has been suggested that PKC probably amplifies β-adrenoceptor stimulation of cAMP by phosphorylating G_s or adenylate cyclase (*Sugden* 1989).

Cyclic GMP, whose concentration is also elevated by NA in pinealocytes, is regulated in a manner somewhat similar to cAMP (*Vanecek* et al. 1985). However, there appears to be an important difference. β-Adrenergic activation alone produces a greater accumulation of cAMP than cGMP whereas α_1-adrenergic potentiation results in more marked increase in the latter. Thus, it appears that stimulation of cGMP production by NA is quantitatively more dependent on α_1-adrenergic stimulation. Cyclic GMP, however, does not appear to play a part in the regulation of NAT induction and its role in the pineal is yet to be established.

1.3.3.3 Regulation of Adrenergic-Mediated Function

The adrenergic regulation of pineal melatonin biosynthesis appears to be modulated by several mechanisms. First, activation of PKC, in addition to amplifying the β-adrenergic stimulation of cAMP, appears to inhibit further α_1-adrenergic stimulation (*Sugden* et al. 1988). Second, pineal β-adrenoceptors exhibit a diurnal rhythm in density such that at the beginning of the dark period, receptor density is greatest (*Romero* et al. 1975). During darkness, adrenergic stimulation reduces the number of these receptors. Third, stimulation of cAMP production induces an increase in cAMP phosphodiesterase (PDE) activity which enhances cAMP metabolism (*Minneman* and *Iversen* 1976). It has also been shown that PDE activity is regulated by a dual (α_1 and β) adrenoceptor mechanism (*Vacas* et al. 1985). Thus, it appears that the adrenergic-mediated cAMP signal is precisely regulated in terms of both its magnitude and its time-course. It is likely that the initial rapid burst of cAMP formation, mediated by α_1- and β-adrenergic stimulation, is necessary to trigger the extent of the induction of NAT activity while the lower concentrations of cAMP seen later are adequate to keep NAT active.

1.3.3.4 The Role of Other Transmitters

Recently, various studies have shown that, in addition to the classical sympathetic innervation, the mammalian pineal gland is directly innervated by central pinealopetal connections (see *Korf* and *Moller* 1984, *Moller* and *Korf* 1987, *Moller* et al. 1987). Complementary to these findings, a number of non-adrenergic neurotransmitter and neuromodulator substances and their acceptor sites have been demonstrated in the mammalian pineal gland (*Shiotani* et al. 1986, *Moller* and *Mikkelsen* 1989). Several of these substances have been shown to be localized in central pinealopetal fibres. While it is clear that many of them are capable of influencing pineal melatonin biosynthesis, their physiological relevance to this process is yet to be elucidated.

The occurrence of gamma-aminobutyric acid (GABA) binding sites has been described in the pineal (*Ebadi* and *Chan* 1980). In bovine and ovine pineals GABA inhibits NA-stimulated melatonin synthesis. Additionally, benzodiazepine (BZP) binding sites, which are allosterically linked to GABA sites, have also been reported in the pineal gland (*Lowenstein* and *Cardinali* 1983). These BZP receptors appear to be under adrenergic control as the binding of BZP is significantly reduced following exposure of rats to constant light or removal of the SCG (*Matthew* et al. 1984, *Weissman* et al. 1984). The administration of

BZP to both rats (*Zatz* and *Brownstein* 1979) and humans (*Kabuto* et al. 1986) was found to decrease the nocturnal elevation of pineal melatonin synthesis.

The pineal gland contains the highest concentration of 5-HT, higher than any other body tissue (*Saavedra* et al. 1973). Recently, the prevailing view that pineal 5-HT serves simply as a precursor of melatonin, has been challenged. The possibility that 5-HT itself might be considered a pineal hormone is suggested by reports that α_1-adrenergic stimulation induces 5-HT release from pinealocytes *per se* of denervated rats (*Aloyo* and *Walker* 1988). Furthermore, the presence of 5-HT binding sites have been reported in bovine pineals (*Ebadi* and *Govitrapong* 1986). It is interesting to speculate that the 5-HT released under adrenergic stimulation may act on pinealocyte 5-HT receptors and, in turn, modulate adrenergic responses. However, such a phenomenon has yet to be reported.

Prostaglandins (PGs), which are produced and released by the pineal gland, have received some attention as possible modulators of melatonin synthesis and release. Stimulation of pineal α_1-adrenoceptors results in the activation of Phospholipase A_2, (*Ho* and *Klein* 1987), which enhances the release of arachidonic acid and PGE_2. PGE_2 added *in vitro* increased rat pineal cAMP levels, NAT activity and melatonin release, while the addition of cyclooxygenase inhibitors decreased NA-induced melatonin release (*Cardinali* and *Ritta* 1983).

Several neuropeptides have also been reported to affect pineal melatonin biosynthesis. One such substance is vasoactive intestinal polypeptide (VIP) which is localized in nerve endings in the pineal (*Moller* and *Mikkelsen* 1989). VIP interacts with specifc receptor sites to induce a rapid increase in pineal cAMP accumulation and NAT activity (*Yuwiler* 1983, *Kaku* et al. 1985, 1986). This effect of VIP has been shown to be potentiated by α_1-adrenoceptor activation, apparently by the same mechanism which potentiates β-adrenergic stimulation (*Chik* et al. 1988).

Another neuropeptide, neuropeptide Y (NPY), co-located with NA in nerve fibres, has been demonstrated in pineal glands (*Shiotani* et al. 1986). In a recent study, *Vacas* et al. (1987) showed that NPY may exert a dual effect in the pineal by enhancing the postsynaptic effects of NA at low concentrations while inhibiting sympathetic neurotransmission at high concentrations. These findings are consistent with a subsequent report that NPY increased daytime NAT activity in rats but decreased the nighttime elevation of NAT (*Reuss* and *Schröder* 1987).

Other candidate neurotransmitters and neuropeptides for which binding sites in the pineal have been reported include glutamate (*Govitrapong* et al. 1986), dopamine (D_2) (*Govitrapong* et al. 1984), acetylcholine (muscarinic) (*Govitrapong* et al. 1989, *Laitinen* et al. 1989), substance P (*Govitrapong* and *Ebadi* 1986) and peptide histidine isoleucine (PHI) (*Tsuchiya* et al. 1987, *Moller* and *Mikkelsen* 1989). However, for many of these, an effect on pineal melatonin synthesis and release is yet to be demonstrated.

A variety of hormones are also believed to affect melatonin synthesis via an action on specific receptors. Receptors for estrogens, progesterone, testosterone, glucocorticoids and melatonin have been identified in pineal cells (see *Cardinali* and *Vacas* 1987). Many of these are under adrenergic control, since pineal denervation produces a depression of binding sites.

Thus, the foregoing discussion raises the possibility that the synthesis and release of pineal melatonin may be under the control of multiple neural signals. Clearly, the major driving force for melatonin output is an intact and functional adrenergic system, without which the pineal lacks the capacity to secrete melatonin in a phasic way. It is conceivable, however, that several of these non-adrenergic transmitter substances, many of them released from central fibres, may play a role in the fine-tuning of melatonin synthesis (Fig. 1.5).

1.3.4 Pineal Indole Biosynthesis and Metabolism

Pineal indole synthesis is initiated by the uptake of the amino acid tryptophan from the circulation. Within the pinealocyte, tryptophan is hydroxylated by the enzyme tryptophan hydroxylase to form 5-hydroxytryptophan (5-HTP). A second enzyme, aromatic amino acid decarboxylase, then decarboxylates 5-HTP to give serotonin (5-HT). The activity of the pineal tryptophan hydroxylase is particularly high and the enzyme does not appear to be saturated, at least in the rat (*Deguchi* and *Barchas* 1972). In this species, tryptophan loading produces a large increase in pineal 5-HT. Once formed, the 5-HT is biotransformed in one of three possible directions : (1) Some of the 5-HT is deaminated by the enzyme monoamine oxidase (MAO) to form 5-hydroxyindole acetic acid and 5-hydroxytryptophol. These hydroxyindole metabolites may then be methylated by the enzyme hydroxyindole-O-methyltransferase (HIOMT). (2) Some of the 5-HT may be directly methylated by HIOMT to form 5-methoxytryptamine. (3) A major route for the metabolism of 5-HT is its conversion to melatonin, via acetylation by the enzyme serotonin N-acetyltransferase (NAT), to yield N-acetyl-serotonin. N-Acetylserotonin is then methylated by HIOMT to form melatonin. The pineal indole biosynthetic pathway is illustrated in Fig. 1.6.

In addition to the pineal, several other organs have the capacity to produce melatonin, including the retina (*Pang* and *Allen* 1986), Harderian gland (*Bubenik* et al. 1976) and lacrimal gland (*Mhatre* et al. 1988). It appears, however, that these other organs lack the ability to secrete melatonin. Thus, under normal circumstances the blood melatonin concentration almost exclusively reflects that of the pineal.

Circadian output from the SCN (Section 1.3.3) generates a marked 24 h rhythm in melatonin as well as in the enzymes and intermediates involved in its biosynthesis. Melatonin synthesis and release is highest at night (*Lynch* 1971), in phase with peaks in NAT (*Klein* and *Weller* 1970) and HIOMT activities (*Axelrod* et al. 1965) and NA release from sympathetic nerve endings (*Wurtman* et al. 1967). The circadian rhythm of pineal 5-HT bears an inverse relationship to the above, with peak levels during the light phase. The oxidation products of 5-HT metabolism, viz., 5-hydroxyindole acetic acid, 5-hydroxy-

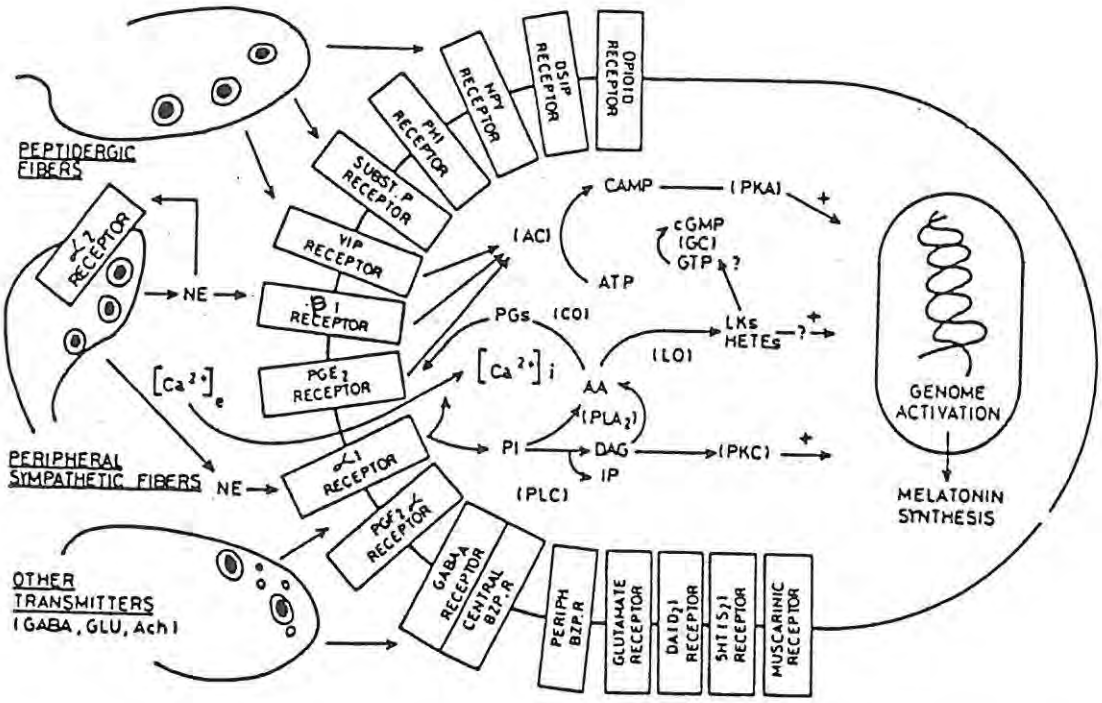


Figure 1.5 Schematic representation of neural control mechanisms in the mammalian pineal gland
Adapted from *Cardinali* and *Vacas* 1987

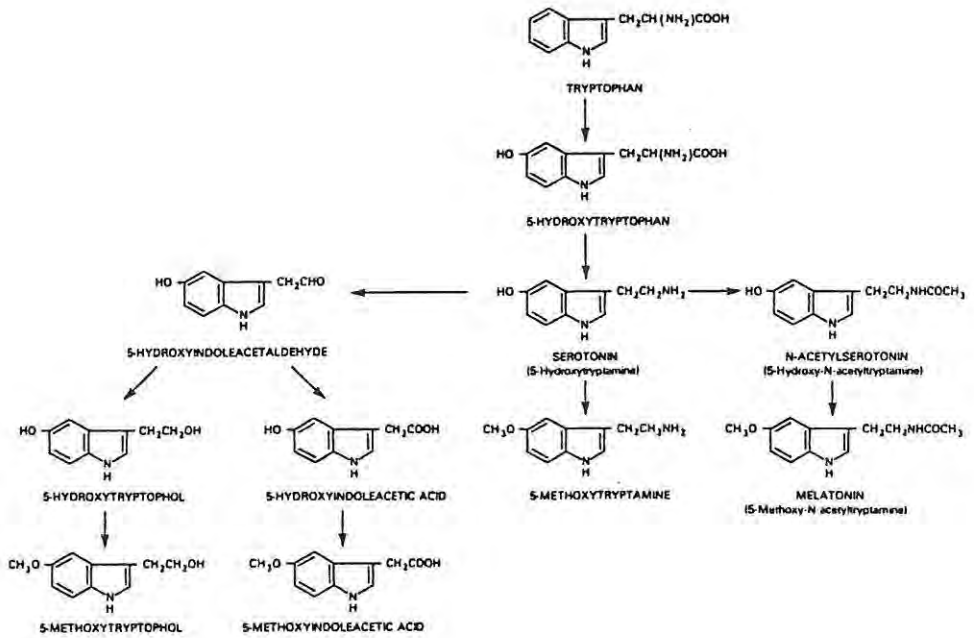


Figure 1.6 Pathway of pineal indole metabolism

tryptophol, 5-methoxyindole acetic acid and 5-methoxytryptophol, also show an inverse circadian pattern to that of melatonin (*Mefford* et al. 1983). Studies by *Balemans* et al. (1983) demonstrated that in addition to a diurnal variation, pineal HIOMT activity and methoxyindole production, show a seasonal variation. The circadian rhythmicity of pineal indole synthesis is a characteristic feature, occuring in all mammalian species so far examined (review : *Binkley* 1981).

In mammals, including humans, melatonin is primarily metabolised in the liver and excreted mainly in the urine (*Kveder* and *McIsaac* 1961, *Jones* et al. 1969). The major metabolite appears to be 6-hydroxymelatonin which is excreted as the sulphate conjugate and, to a lesser extent, as the glucuronide conjugate. A minor route of melatonin metabolism (<1%) involves conversion to 5-methoxytryptamine (*Beck* and *Jonsson* 1981). More recently, N-acetylserotonin has also been identified as a major urinary metabolite (*Young* et al. 1985). The latter finding, that melatonin may be converted to its precursor, suggests the existence of a complex feedback mechanism controlling melatonin synthesis. A second minor route of melatonin metabolism in the brain results in the formation of N-acetyl-5-methoxykynurenamine (*Hirata* et al. 1974). In addition, melatonin and its metabolites in serum and urine show a similar circadian rhythm to that in the pineal.

1.3.5 Melatonin and Circadian Synchronicity in Mammals

Although a variety of indole and peptide substances are synthesized and released by the pineal gland, melatonin has been the most extensively studied substance in relation to its neuroendocrine function. While experimental evidence justifies such an approach, it should be noted that other pineal substances may likewise be important in the neuroendocrine effects of the gland.

The involvement of the pineal gland in rhythm generating systems has received considerable attention. The phasic nature of the melatonin signal directly approximates the circadian output of the SCN located in the central nervous system. The SCN are the major pacemakers that control circadian rhythmicity in mammals and are entrained by environmental lighting. Evidence that the pineal gland, via the chemical signal melatonin, exerts a secondary modulating influence upon circadian rhythms controlled primarily by the SCN is reviewed below.

1.3.5.1 Studies in Laboratory Animals

The zeitgeber properties of melatonin on seasonal reproduction is well established (see *Reiter* 1980) and will not be discussed here. However, a circadian rhythm of sensitivity to the gonadal inhibitory effects of exogenously administered melatonin has been described in hamsters (*Stetson* and *Watson-Whitmyre* 1986).

In laboratory rats housed under conditions of constant darkness (DD) or constant light (LL), the locomotor activity rhythm persists with a period of slightly greater or less than 24 hours. The circadian

pacemaker that generates this rhythm becomes externally desynchronized from the environmental light-dark (LD) cycle and the rhythm is said to free-run. Daily injections of melatonin to rats entrain circadian locomotor activity rhythms, by phase advancement, under conditions of desynchronization (*Armstrong* 1989, *Armstrong* et al. 1989). Likewise, melatonin administration has been shown to phase advance circadian activity rhythms in golden hamsters (*Ellis* et al. 1982) and Djungarian hamsters (*Puchalski* and *Lynch* 1988). Studies have shown that while pinealectomy (Px) does not abolish locomotor activity rhythms in rodents (see *Armstrong* et al. 1989), unlike as in some bird species (*Gaston* and *Menaker* 1968), it does induce a faster re-entrainment to phase shifts of the LD cycle (review : *Armstrong* 1988). Thus, the observation that exogenous melatonin administration can synchronize rat free-running locomotor activity rhythms suggests that it may play a role in synchronizing the pacemaker system to the external LD zeitgeber (*Armstrong* 1988). From these studies, the timing of melatonin administration, i.e., several hours before subjective evening, appears to be critical to its ability to induce synchronization. In general, the data favour the interpretation that a "window" of sensitivity may exist at which the pacemaker becomes responsive to melatonin (see Section 1.3.5.2).

In one study, the timed oral administration of melatonin to adult saddle back tamarins decreased locomotor activity late in the photophase (around melatonin administration time) and increased activity early in the photophase (*Lerchl* and *Küderling* 1989). In this study, however, melatonin did not produce phase shifts in the activity pattern following reversal of the LD cycle. The authors concluded that melatonin may not have the same importance in primate circadian organization as it does in lower mammals.

Hypothalamic implants of melatonin in rats have been shown to accelerate the resynchronization of adrenocorticotropic hormone (ACTH)-cortisol rhythms following reversal of the LD cycle (*Murakami* et al. 1983).

1.3.5.2 Melatonin Target Sites

Recent studies demonstrating radiolabeled melatonin binding sites in several discrete brain regions in a variety of species, have encouraged the view that melatonin may exert its effect through an action on specific receptors. High affinity specific [^{125}I]melatonin binding sites have been demonstrated in the hypothalamus, hippocampus and pons-medulla (*Vanecek* et al.1987, *Williams* and *Morgan* 1988, *Zisapel* et al. 1989). These binding sites exhibit a distinct diurnal variation, with peak binding occurring late in the photophase. In the cerebellum, parietal cortex and striatum, however, no rhythm in binding sites are apparent (*Zisapel* et al. 1989).

The diurnal variation in melatonin binding sites may be a consequence of the daily cyclical changes in endogenous melatonin content. Thus, elevated nocturnal melatonin levels would result in the down-regulation of these binding sites. However, the regional specificity of the rhythm (eg. hypothalamus but not striatum) and the evidence that binding site density declines 5-7 hours before the circulating

melatonin reaches its nocturnal peak, contradicts this possibility (*Zisapel* et al. 1988). It is possible, therefore, that other factors may be involved in regulating the density of melatonin binding sites.

The high density of melatonin binding sites in the SCN (*Vanecek* et al. 1987) suggests that melatonin may affect circadian rhythms via this structure. It has recently been shown that lesions of the SCN disrupt locomotor activity and drinking rhythms and inhibit the effect of administered melatonin (see *Bartness* and *Goldman* 1989). In addition, melatonin interferes with the daily rhythm in metabolic activity of the SCN as evidenced by labeled 2-deoxy-glucose autoradiography (*Cassone* et al. 1988).

The precise mechanism by which melatonin influences circadian rhythms in mammals is unclear. The observation that pinealectomy (Px) does not abolish rodent circadian locomotor activity rhythms, suggests that the melatonin signal may be a passive message. It has been suggested that two distinct cycles may be involved, i.e., a melatonin rhythm and a rhythm of sensitivity ("window") to melatonin by the end organ. Only when these two rhythms overlap, i.e., are coincident, is melatonin capable of exerting its effect. This model, known as the coincidence hypothesis of melatonin action, has been invoked mainly from studies in seasonal reproduction and is discussed in detail elsewhere (*Stetson* and *Watson-Whitmyre* 1986, *Reiter* 1987). Conceivably, therefore, the SCN may have a narrow "window" of sensitivity which allows melatonin to trap circadian rhythms at a certain phase. Recent evidence suggesting that the density of melatonin binding sites in the SCN exhibits a 24-hour rhythm (see *Morgan* and *Williams* 1989) supports such a notion.

1.3.5.3 Studies on Human Subjects

The therapeutic potential of melatonin in jet-lagged travellers, who have traversed eight or more time zones, is well established (*Arendt* et al. 1986, 1987, *Arendt* and *Aldhous* 1988, *Armstrong* and *Chesworth* 1987). The timed administration of melatonin to these individuals improves mood and synchronizes endogenous melatonin and cortisol rhythms. The recent demonstration of melatonin receptors in the SCN of human brains (*Reppert* et al. 1988), encourages the view that exogenous melatonin acts on this structure to synchronize disrupted circadian rhythms.

Preliminary studies in blind patients suggest that melatonin may affect circadian systems. In a controlled study (*Sach* et al. 1987), the timed daily administration of melatonin to two blind subjects was found to phase advance the free-running endogenous melatonin rhythm. In this study, a similar effect was observed with triazolam administration. In another study (*Arendt* et al. 1988) involving a blind subject with a severely disrupted and possibly free-running sleep-wake rhythm, melatonin administration was found to synchronize the endogenous melatonin rhythm and improve synchronization of sleep onset.

In summary, pre-clinical and clincal data suggest that melatonin has zeitgeber properties on a variety of circadian systems. In addition, the recent evidence that hypothalamic melatonin receptors are concentrated in the SCN, suggests the important role of this structure in mediating these phenomena.

Melatonin may thus have important therapeutic value in a variety of disorders characterized by disrupted circadian rhythms. An "internal desynchronization" hypothesis has been advanced whereby two central rhythm-generating systems with slightly different periods might generate a "beat" phenomenon manifested as periodic clinical symptoms. More studies, however, are needed to test the physiological relevance of these findings and to unequivocally demonstrate that entrainment by melatonin does not represent a pharmacological artefact.

1.3.6 Melatonin and Affective Disorders

1.3.6.1 Circadian Rhythm Abnormalities in Depression

Several investigators have proposed that circadian rhythms play a role in the etiology of affective disorders (*Halberg* 1967, *Kripke* et al. 1978, *Wehr* and *Goodwin* 1983). A variety of rhythm abnormalities including phase advances and free-running cycles in temperature (*Kramer* and *Katz* 1978), hormonal secretion (cortisol, prolactin, GH) (*Sachar* 1976), sleep-waking patterns (*Kupfer* 1976) and psychomotor activity (*Kupfer* et al. 1974) have been associated with depression. Depressive symptomology also exhibits a diurnal variation, with exacerbation early in the morning and improvement in the evening.

A non-uniform seasonal variation of depressive symptoms also exists (*Eastwood* and *Peacocke* 1976), with peak episodes occurring in spring and autumn. One possible explanation for this phenomenon relates to the rapid changes in length of day occurring during spring and autumn. As the light-dark (LD) cycle is a major synchronizer of circadian rhythms, it is possible that coupling of the circadian system is less robust at these times (*Papousek* 1975, cited by *Arendt* 1989).

A subgroup of depressed patients typically display a uniform seasonal variation in clinical symptoms. These patients with seasonal affective disorder (SAD) have annually recurring winter depressive episodes (*Rosenthal* et al. 1984). In view of the reported mild spring mania following winter depressive periods, it has been suggested that these patients may well be bipolar (*Checkley* et. al. 1989, cited by *Arendt* 1989).

Furthermore, it is possible that bipolar affective disorders, characterized by periodic episodes of depression and mania may also relate to disorders in the time-keeping systems.

As with other circadian biological rhythms, the melatonin rhythm provides a useful marker in depressive illness. Melatonin synthesis is controlled by noradrenergic fibres which are under the influence of the hypothalamic SCN. Hence, any abnormality of melatonin secretion might reflect a defect in the noradrenergic or circadian rhythm-generating system, both of which have been postulated in depression.

1.3.6.2 Melatonin Secretion in Depressed Patients

The secretion of melatonin in blood and urine follows a 24-hour rhythm. Melatonin levels in blood plasma can reliably be measured using specific and sensitive techniques such as radioimmunoassay (RIA) (eg., *Rollag* and *Niswender* 1976) and gas chromatography/mass spectrometry (GC/MS) (eg., *Lewy* and *Markey* 1978). In addition, the recent introduction of an RIA for the major urinary metabolite of melatonin, 6-hydroxymelatonin sulphate, which correlates closely with plasma melatonin (*Arendt* et al. 1985), provides a less intrusive and more convenient means of estimating total melatonin secretion, although fine bursts may, however, be masked. Several studies have shown that the secretion of melatonin in depressed patients may be significantly altered.

Mendlewicz and co-workers (1979) reported a lowered amplitude of the nocturnal rise in melatonin in three of four severely depressed women. The lowered melatonin levels were still evident four to six weeks later, following recovery, suggesting that low melatonin may be a trait marker (*Branchey* et al. 1982).

Wirz-Justice and *Arendt* (1979) found lower morning plasma melatonin levels in six bipolar depressed patients compared to concentrations found in twelve healthy control subjects. The patients had a low melatonin level during the depressed phase and a normal to high level during the manic phase. *Lewy* and associates (1979) also reported that melatonin levels in serum were higher in bipolar patients in their manic than in their depressed phases.

Wetterberg (1983), summarizing a series of studies by his group, reported lower nocturnal melatonin levels in 17 depressed patients who were non-suppressors of cortisol in response to dexamethasone suppression (DST), as compared to either 22 controls or 15 depressed patients who had a normal suppression of cortisol in response to dexamethasone. Similar results were reported by *Claustrat* et al. (1984) who showed that nine of eleven patients with endogenous depression had low nocturnal melatonin levels associated with raised cortisol secretion. In these studies, remitted, euthymic patients with normalization of cortisol continued to show low melatonin levels six weeks after recovery. *Wetterberg* (1983) has proposed that melatonin may inhibit cortisol releasing factor (CRF) during depression and that low melatonin may be a trait marker of depressive disorders.

Beck-Friis et al. (1984) examined 30 patients with major depressive disorder, 24 during remission and 33 healthy controls. Nocturnal melatonin levels were found to be lower in the depressives than in the controls and did not change with recovery in a small subsample. In a subsequent study (*Beck-Friis* et al. 1985), these workers reported a trend to phase advance in cortisol secretion and the nocturnal melatonin peak in dexamethasone non-suppressors. *Nair* et al. (1984) found nocturnal melatonin secretion to be reduced and phase-advanced in six depressed patients when compared with the control group.

Brown et al. (1985a) reported two separate studies in which they found significantly lower early morning

melatonin levels in seven male patients with endogenous depression compared to five male controls. In the second study of 19 patients in the endogenous subgroup of depression, 9 patients in the non-endogenous subgroup and seven controls, nocturnal melatonin levels were found to be lower only in the endogenous subgroup.

Boyce (1985) measured urinary 6-hydroxymelatonin concentrations in eight patients with endogenous depression. Three of the patients showed no nocturnal increase in 6-hydroxymelatonin.

At least two groups have reported conflicting results. *Jimerson* and co-workers (1977) measured urinary melatonin excretion 8-hourly in six patients with endogenous depression and six healthy controls but were unable to demonstrate a significant difference. However, a relatively insensitive bioassay was used to estimate melatonin in this study. Furthermore, 6-hydroxymelatonin appears to be the major urinary metabolite and not free melatonin. More recently, *Thompson* et al. (1988) examined eleven depressed patients with similarly matched healthy controls and reported no significant differences in plasma melatonin levels over a 24-hour period between the two groups.

Although few studies have reported on melatonin secretion in patients with seasonal affective disorder (SAD), a preliminary report by *Rosenthal* et al. (1986) in four patients suggested a trend towards increased nocturnal secretion. *Lewy* and co-workers (cited by *Rosenthal* et al. 1986) suggested that the onset of melatonin secretion may be delayed in the winter months.

Thus, although conflicting results have been reported, the majority of studies indicate that melatonin secretion is reduced in depressed patients. The mechanism responsible for this phenomenon is still unclear. It has been suggested that low nocturnal melatonin levels seen in these patients might reflect a generalized deficiency in biogenic amine function. A functional deficiency of NA, 5-HT or both has the potential to lower pineal melatonin production (*Arendt* 1989). Studies to test pineal β-adrenoceptor sensitivity in depressives would thus be illuminating. However, treatment with different β-adrenoceptor agonists, including isoprenaline, fails to raise circulating melatonin levels in humans, unlike as in rats (*Vaughan* 1984), at least in doses that are not cardiotoxic. Whether other β-adrenoceptor agonists are capable of stimulating melatonin production in humans in doses that are not cardiotoxic requires further investigation.

The significance of an altered melatonin rhythm and its relationship to that of other rhythm disturbances such as sleep, motor activity and weight loss seen in depressed patients are likewise unclear. While it appears that melatonin rhythms may be phase-advanced in depression (*Nair* et al. 1984, *Beck-Friis* et al. 1985) further studies are needed to confirm this. The nocturnal rise in melatonin does not appear to be reduced in schizophrenia (*Wetterberg* 1982), but whether this occurs in other psychiatric disorders also remains to be established.

1.3.6.3 Antidepressant Treatment and Melatonin Secretion

Studies on the effect of chronic antidepressant treatment in depressed patients have yielded inconsistent results.

Wirz-Justice and *Arendt* (1980) reported on the effect of maprotiline treatment on early morning plasma melatonin levels in three depressed patients. In all three patients melatonin was undetectable before treatment and remained so after two to four weeks of therapy.

In a large collaborative study (*Wetterberg* 1983, *Beck-Friis* 1983) involving 26 patients on TCAs, lithium or neuroleptics, no significant changes in nocturnal melatonin levels were found before or after clinical remission. In this study, however, the depressives were not assessed in a drug-free state, so an effect on melatonin by the medication could not be ruled out.

Thompson et al. (1985) measured nocturnal plasma melatonin concentrations in six depressed patients before and during treatment with desipramine. An increase in melatonin after three weeks of treatment but not after one week was demonstrated. Similarly, *Hariharasubramanian* et al. (1985) reported that four weeks of imipramine treatment increased the 24-hour melatonin secretion in all of the five depressed patients studied.

Sack and *Lewy* (1986) in a study of four depressed patients found increased urinary 6-hydroxymelatonin after one, two and three weeks of desipramine treatment. *Golden* et al. (1988b) also reported an increased 6-hydroxymelatonin excretion following three to six weeks of treatment with desipramine in depressed patients.

However, *Brown* et al. (1985b) and *Frazer* et al. (1986b) found no change in plasma melatonin concentrations after four weeks of desipramine treatment in depressed patients. More recently, *Beam* et al. (1989) in a study of eight depressed patients, measured urinary 6-hydroxymelatonin excretion after one day and one, two and three weeks of desipramine treatment. There was a significant increase in 6-hydroxymelatonin excretion after one week of treatment only.

Murphy et al. (1986b) examined morning plasma melatonin levels in 27 depressed patients before and after three or more weeks of treatment with the MAOIs clorgyline, tranylcypromine and deprenyl. Melatonin levels were increased following treatment with clorgyline (type A MAOI) and tranylcypromine (a non-specific MAOI) but not deprenyl (type B MAOI). Similarly, *Golden* et al. (1988b) demonstrated an increased 24-hour urinary excretion of 6-hydroxymelatonin following treatment of depressed patients with tranylcypromine, clorgyline and bupropion for 3-6 weeks.

Devi et al. (1984) reported that lithium treatment increased urinary melatonin levels in 18 depressed patients. In a clinical trial evaluating the antidepressant efficacy of *S*-adenosylmethionine (*Lipniski* et al.

1984), seven out of nine patients who showed clinical improvement also had higher daily urinary melatonin levels. *Bell* et al. (1988) demonstrated similar findings with *S*-adenosylmethionine in a subsequent clinical trial. The rapid antidepressant effect of destyrosine gamma-endorphin, a naturally occurring peptide, was also found to correlate well with higher urinary melatonin levels (*Chazot* et al. 1985).

In summary, despite some inconsistencies in the results of human studies, no group has reported that antidepressant treatment *reduces* melatonin secretion in depressed patients. These observations are in contrast to animal studies (see Section 1.3.7) which suggest that the overall effect of antidepressant treatment is to reduce pineal noradrenergic transmission. The majority of human studies indicate that melatonin secretion is increased at some stage during treatment, suggesting that noradrenergic transmission may in fact be increased by antidepressants. Interestingly, long-term antidepressant treatment in normal subjects do not increase melatonin secretion when compared with pre-treatment values (*Thompson* et al. 1985). Thus, at least in a subgroup of depressed patients, the increased melatonin secretion seen following chronic antidepressant treatment may represents a "re-regulation" of an apparently dysregulated monoaminergic system. The data are not yet consistent enough to suggest a link between increased melatonin secretion and treatment efficacy, but such a possibility merits further investigation.

The available data also suggest that chronic antidepressant treatment increases the amplitude without affecting the timing mechanisms involved in melatonin secretion in depressed patients. However, considerable inter-individual variation in the melatonin rhythm exists in human subjects (*Arendt* 1988) and any possible phase changes may not be overtly detectable. On the other hand, the rhythm is highly reproducible within the same individual (*Arendt* 1988). Hence, examining possible phase changes in individual depressed patients may be more fruitful.

1.3.7 Antidepressants and Pineal Gland Function

Antidepressant treatment is associated with profound changes in central monoaminergic function. In the rat limbic forebrain and cerebral cortex, changes in neurotransmitter turnover, receptor sensitivity and second messenger systems have been demonstrated (see Section 1.2.3.2). The rat pineal gland has a well defined noradrenergic system that controls melatonin synthesis and release (Section 1.3.3). Several researchers have utilized the rat pineal as a model in which to examine the noradrenergic effects of antidepressant treatment. The rat pineal offers certain advantages for such studies. Firstly, the gland contains a high density of β-adrenoceptors coupled to a catecholamine-sensitive adenylate cyclase system capable of generating cAMP. Secondly, pineal cAMP mediates an increase in NAT, the rate limiting step in the conversion of serotonin to melatonin. Thirdly, pineal β-adrenoceptor responsiveness can be assessed by the daily darkness-induced release of NA from sympathetic nerve endings or from exogenously administered catecholamines.

1.3.7.1 Pineal β-Adrenoceptor Sensitivity

1.3.7.1.1 Effect of Acute Antidepressant Treatment

Few studies have been reported on pineal β-adrenoceptor changes following acute antidepressant treatment. Acute treatment of rats with desipramine (10 mg/kg) or nialamide (40 mg/kg) produced no significant change in the binding of [^{3}H]DHA to pineal homogenates (*Moyer* et al. 1979, 1981). In these studies rats were killed 1 hour after drug administration and binding was assessed at a single concentration of [^{3}H]DHA. A study by *Friedman* et al. (1984) showed that pineal [^{3}H]DHA binding was unaffected in rats fed with an imipramine-containing diet for 3 days.

1.3.7.1.2 Effect of Chronic Antidepressant Treatment

Moyer et al. (1979, 1981) showed that treatment of rats with desipramine (10 mg/kg) or nialamide (40 mg/kg) twice daily for 5 days produced a significant reduction in pineal [^{3}H]DHA binding. Using Scatchard analyses, they demonstrated that the reduced binding was due to a decrease in the number of binding sites (B_{max}) rather than to a change in affinity (K_d) of the receptor to the ligand. A study by *Friedman* et al. (1984) demonstrated similar results following chronic treatment (3 weeks) of rats with an iprindole-supplemented diet.

Skene (1985) measured the binding of a single concentration of [^{3}H]DHA to pineal homogenates following chronic treatment of rats with various antidepressants (10 mg/kg daily for 2 weeks). In this study, only desipramine was found to significantly decrease [^{3}H]DHA binding. Treatment of rats with maprotiline, amitriptyline, clomipramine, fluoxetine and iprindole did not affect pineal [^{3}H]DHA binding. On the other hand, mianserin and trazodone treatment were associated with an increased [^{3}H]DHA binding.

Cowen et al. (1983) evaluated the effect of chronic electroconvulsive therapy (ECT) (125 V, 1 sec; daily for 10 days) and reported no significant effect on pineal [^{3}H]DHA binding.

Friedman and *Yocca* (1981) found that rats fed with a lithium-containing diet for 5 weeks showed a decreased number of pineal [^{3}H]DHA binding sites (B_{max}). More recently, *Wilkinson* et al. (1987), using the ligand [^{3}H]CGP-12177, demonstrated a diurnal rhythm of β-adrenoceptor binding in rat pineal glands. They showed that chronic lithium treatment (6 weeks) produced a significant decrease in [^{3}H]CGP-12177 binding during the dark phase.

1.3.7.2 Pineal Adenylate Cyclase System

1.3.7.2.1 Effect of Acute Antidepressant Treatment

Moyer et al. (1979, 1981) showed that a single dose of desipramine (10 mg/kg) or nialamide (40 mg/kg) did not alter the pineal cAMP response to isoprenaline or NA treatment when compared with controls. However, acute treatment with desipramine, but not with nialamide, produced a small but significant elevation of the basal cAMP level.

1.3.7.2.2 Effect of Chronic Antidepressant Treatment

Moyer et al. (1979, 1981) found that treatment of rats with desipramine (10 mg/kg) or nialamide (40 mg/kg) twice daily for 5 days was associated with a significantly reduced response of cAMP to isoprenaline or NA treatment. In these studies, repeated treatment with desipramine increased basal cAMP levels in one experiment. Nialamide treatment did not affect basal cAMP levels.

Skene (1985) examined basal and isoprenaline-stimulated cAMP levels following repeated treatment of rats with a variety of antidepressants (10 mg/kg daily for 2 weeks). In this study, desipramine and iprindole treatment suppressed the isoprenaline stimulation of cAMP. On the other hand, treatment with maprotiline, amitriptyline, clomipramine, fluoxetine, mianserin and trazodone were without this effect. Curiously, treatment with desipramine, maprotiline, mianserin and trazodone were found to reduce basal cAMP levels in this study.

Yocca et al. (1983) examined the effect of chronic lithium treatment (5 weeks) and found that the isoprenaline-induced increase in pineal cAMP levels was depressed by drug treatment. Basal cAMP levels, however, were unaffected by lithium treatment.

1.3.7.3 Pineal N-Acetyltransferase Activity

1.3.7.3.1 Effect of Acute Antidepressant Treatment

Parfitt and *Klein* (1976) showed that the acute administration of desipramine (10 mg/kg) produced an increase in pineal NAT activity in rats subjected to swimming stress. Desipramine treatment also increased NAT activity in unstressed rats. Treatment of rats with the MAOIs pargyline and harmine (80 mg/kg) was also shown to increase pineal NAT activity (*King* et al. 1982).

1.3.7.3.2 Effect of Chronic Antidepressant Treatment

Bronstein et al. (1984) reported that chronic treatment of rats with desipramine (10 mg/kg daily for 1 week) produced a significantly higher basal level of pineal NAT activity. However, the NAT response to

isoprenaline stimulation was decreased by desipramine treatment. *Friedman* et al. (1984) showed that the nocturnal rise in pineal NAT activity was reduced in rats treated chronically (3 weeks) with an imipramine- or iprindole-containing diet. Similarly, chronic treatment of rats with lithium (5 weeks) was found to reduce the nocturnal rise in pineal NAT acitvity (*Friedman* and *Yocca* 1981, *Yocca* et al. (1983).

1.3.7.4 Pineal Indole Synthesis

1.3.7.4.1 Effect of Acute Antidepressant Treatment

Several studies have demonstrated that desipramine added to rat pineal cultures produces an increase in the conversion of [^{14}C]tryptophan to [^{14}C]N-acetylserotonin and [^{14}C]melatonin (*Parfitt* and *Klein* 1977, *Nir* and *Hirschmann* 1983). In addition, desipramine pretreatment has been shown to decrease the levels of 5-HT and its deaminated products 5-hydroxyindole acetic acid and 5-hydroxytryptophol in rat pineal cultures (*Nir* and *Hirschmann* 1983).

The effect of acute antidepressant treatment on the level of melatonin in rat pineals and plasma has also been investigated. *Wirz-justice* et al. (1980a) reported that acute treatment of rats with a variety of antidepressants (50 mg/kg) including desipramine, maprotiline, imipramine, clomipramine, pargyline and Ro 11-2465 (a specific 5-HT reuptake blocker) elevated pineal and plasma melatonin concentrations. Similar results were reported by *Mouren* et al. (1985) following acute treatment of rats with nomifensine (30 mg/kg) or quinupramine (5 mg/kg).

Friedman et al. (1984) found that rats fed with a diet containing imipramine for 3 days showed a marked increase in pineal 5-HT, N-acetylserotonin and melatonin. Rats fed with an iprindole-containing diet, however, did not show this effect.

Oxenkrug et al. (1985) showed that acute treatment with clorgyline increased rat pineal N-acetylserotonin and melatonin content and decreased 5-hydroxyindole acetic acid content. Treatment with deprenyl did not effect pineal indole levels. The ability of the MAOIs to increase melatonin synthesis appears to be related to a specific inhibition of the MAO-A enzyme subtype (eg. clorgyline), as inhibition of the MAO-B isoenzyme (eg. deprenyl) is without this effect. Since 5-HT is also metabolised by MAO-A, inhibition of this enzyme would be expected to increase the availability of 5-HT as substrate for melatonin synthesis. Moreover, it appears that MAOIs may interact directly with pineal β-adrenoceptors to increase melatonin since superior cervical ganglionectomy diminished but did not abolish the effect of clorgyline (*McIntyre* et al. 1985).

In contrast, *Heydorn* et al. (1982) found that an acute dose of desipramine (10 mg/kg) failed to alter rat pineal or plasma melatonin levels 5 hours later. In this study, the lower dose of desipramine or the longer time interval between drug administration and assay of melatonin could account for the discrepant results.

1.3.7.4.2 Effect of Chronic Antidepressant Treatment

Controversial results regarding the effect of repeated antidepressant treatment on pineal melatonin synthesis has been reported in the literature.

Wirz-Justice et al. (1980) reported that pineal melatonin levels were significantly reduced following chronic (4 weeks) treatment of rats with clomipramine (10 mg/kg or 20 mg/kg), but not with Ro 11-2465 (a 5-HT reuptake inhibitor) (10 mg/kg) or L-5-HTP ester (50 mg/kg).

Heydorn et al. (1982) treated rats with desipramine (10 mg/kg) and nialamide (40 mg/kg) twice daily for 1 week and reported a reduction in isoprenaline- or darkness-induced increase in pineal and serum melatonin content. Daytime melatonin levels were unaffected by this treatment.

Cowen et al. (1983) showed that the repeated administration of desipramine and maprotiline (both 10 mg/kg for 10 days) reduced the isoprenaline-induced increase in rat pineal melatonin content. A similar effect was found following chronic treatment with amitriptyline (20 mg/kg) or the β-adrenoceptor agonist clenbuterol (5 mg/kg). In contrast, the chronic administration of fluoxetine (10 mg/kg), iprindole (20 mg/kg), or mianserin (20 mg/kg) failed to alter pineal melatonin levels following isoprenaline. Chronic ECT was, likewise, without effect. However, chronic desipramine treatment produced no change in the usual dark phase increase in pineal melatonin content.

Friedman et al. (1984) treated rats with a diet containing imipramine for 3 weeks and reported a suppressed nocturnal activation of pineal N-acetylserotonin and melatonin levels. Chronic treatment with iprindole for 4 weeks, but not 3 weeks, had a similar effect.

Ask et al. (1986) found that chronic treatment of rats with amiflamine (a selective MAO-A inhibitor) decreased nocturnal pineal melatonin levels. However, in this study, repeated treatment with imipramine, zimelidine or alaproclate was without this effect.

Seggie et al. (1983, 1984) observed a reduction in nocturnal pineal melatonin content following repeated lithium administration to rats. In addition, these workers found that in albino rats the effect of lithium on melatonin levels occurred later in the dark phase that in pigmented rats.

On the other hand, *Skene* (1985) showed that chronic treatment of rats (10 mg/kg for 2 weeks) with desipramine or maprotiline was associated with an increased conversion of [^{14}C]serotonin to N-acetyl-serotonin and melatonin in cultured pineal glands. Similar results were reported by *Pillay* and *Potgieter* (1984) who showed that [^{14}C]N-acetylserotonin and [^{14}C]-melatonin synthesis progressively increased, for at least 3 weeks, in rat pineal cultures following treatment with desipramine, imipramine, amitriptyline and clomipramine (all 50 mg/kg). In the former study (*Skene* 1985), chronic treatment with

clomipramine, fluoxetine, iprindole, mianserin or trazodone did not affect the synthesis of radiolabeled N-acetylserotonin and melatonin.

Srinivasan (1987) reported that treatment of rats with imipramine or lithium for 2 weeks was associated with increased in pineal melatonin and protein concentrations.

Murphy et al. (1987b) administered clorgyline (type A MAOI) (1 mg/kg for 24 days) to rhesus monkeys and reported increased nocturnal cerebrospinal fluid (CSF) melatonin concentrations. Chronic treatment with deprenyl (type B MAOI) was without this effect.

1.3.7.5 Summary of the Effects of Antidepressant Treatment on Pineal Function

From the above-mentioned studies it appears that acute antidepressant treatment is associated with increased melatonin output. This effect appears to be mediated by an increased synaptic availability of NA as a result of acute reuptake inhibition (TCAs) or inhibition of metabolism (MAOI). Increased synaptic levels of NA enhance cAMP accumulation and NAT activity via activation of pinealocyte β-adrenoceptors. However, the mechanism by which the selective 5-HT reuptake blockers (eg. Ro 11-2465) increase pineal melatonin synthesis following acute treatment is less clear. One possibility is that acute inhibition of 5-HT neuronal reuptake may increase the availability of this amine for entry into pinealocytes and consequent melatonin synthesis. On the other hand, high doses of these drugs may inhibit NA reuptake or stimulate β-adrenoceptors directly, leading to increased melatonin synthesis. Further studies, therefore, are indicated to resolve this question.

Despite some inconsistencies in the available data, chronic antidepressant administration appears to be associated with a reduction in the sensitivity of the pineal β-adrenoceptor/adenylate cyclase system. This effect does not appear to be mediated only by the inhibition of NA reuptake or metabolism since iprindole, which does not have this effect on NA, also reduces pineal β-adrenoceptor density. The limited data available, however, suggest that other atypical antidepressants such as mianserin and trazodone as well as the selective 5-HT reuptake inhibiting antidepressants and ECT are without this effect on pineal β-adrenoceptor density or adenylate cyclase sensitivity. Further studies are therefore indicated to confirm these findings since, in contrast, studies in other brain areas have demonstrated a reduction in β-adrenoceptor density and/or adenylate cyclase sensitivity following treatment with these drugs (Section 1.2.3.2.2).

Several studies indicate that a reduced β-adrenoceptor responsiveness following chronic antidepressant treatment to rats is associated with a reduction in melatonin output (*Wirz-Justice* et al. 1980, *Heydorn* et al. 1982, *Cowen* et al. 1983, *Friedman* et al. 1984, *Ask* et al. 1986, *Seggie* et al. 1983, 1984). Other studies, however, have reported an increased melatonin output following chronic antidepressant administration to rats or primates (*Pillay* and *Potgieter* 1984, *Skene* 1985, *Srinivasan* 1987, *Murphy* et al. 1987b). Further

studies are therefore necessary to resolve this apparent conflict, since an increase or decrease in melatonin synthesis will reflect an overall increase or decrease in noradrenergic neurotransmission. In addition, it appears from studies in animals that increased melatonin levels is associated primarily with antidepressants that inhibit NA reuptake or metabolism. Whether this effect is also observed following chronic treatment with other antidepressants, likewise, remains to be established.

CHAPTER TWO

EXPERIMENTAL PROCEDURE

2.1 ANIMALS

Adult albino rats of the Wistar strain were used throughout this study. All animals were maintained under artificial illumination with a daily photoperiod of 12 hours (lights on at 06:00 h). The light intensity during the light phase was approximately 300 μWatts/cm^2 provided by cool white flourescent tubes. As far as possible, the temperature of the living quarters was kept constant (20-24^{o}C) and an extractor fan ensured the constant removal of stale air. All animals were housed in groups of 3 or 4 in opaque white plastic cages. A metal grid served as a floor in each cage. Animals were maintained, *ad libitum*, on a diet of standard rat pellets and tap water.

Rats were killed swiftly by cervical dislocation and decapitation. Using a pair of scissors, an incision was made through the bone from the foramen magnum to near the orbit. The top of the skull was removed using a clean forceps and the pineal gland was rapidly excised. In many instances, the gland was found to adhere to the inner surface of the skull. With practice, this procedure could easily be accomplished within 30 seconds.

For dark phase experiments, pineal glands were collected with the aid of a dim red, photosafe light (Phillips, 20 W). Previous studies have shown that the activity of pineal enzymes sensitive to white light are not altered by red light (*Cardinali* et al. 1972).

2.2 THE MEASUREMENT OF β-ADRENOCEPTOR BINDING

2.2.1 β-Adrenergic Receptor Binding Studies Employing the Ligand [^{3}H]Dihydroalprenolol

2.2.1.1 Introduction

The study of adrenergic receptors has been greatly facilitated by the ability to measure them directly in the absence of a functionally coupled biological response. This has been accomplished by taking advantage of the specificity of the recognition site of the receptor molecule. Drugs with a very high affinity and specificity for the adrenergic receptor are radioactively labeled, and their interaction with a tissue preparation containing the appropriate receptor molecules is examined. Bound and free radioligand are separated by appropriate techniques such as dialysis, filtration and centrifugation, and the kinetic and pharmacological characteristics of the radioligand bound to the tissue are examined. The binding of the radioligand to receptor and non-receptor sites can be distinguished by examining the saturability and pharmacological specificity of the radioligand binding sites. If the binding sites are

saturable and reversible and display the same stereospecificity and pharmacological characteristics as the receptor-mediated biological response, it can be fairly certain that the radioligand is specifically labeling the recognition site of the receptor. Kinetic and equilibrium methods can then be used to quantify the number of receptors in a given tissue sample and determine their molecular and pharmacological properties.

The initial difficulty of finding β-adrenoceptor radioligands of high specificity and with sufficiently high specific activity to identify the small number of β-adrenoceptors present in tissues, was overcome with the advent of competitive β-adrenergic antagonists such as [^{3}H]dihydroalprenolol (DHA) (*Mukherjee* et al.1975), [^{125}I]iodohydroxybenzylpindolol (IHYP) (*AurbachI* et al. 1974), [^{3}H]propranolol (*Levitzki* et al. 1974), [^{125}H]iodocyanopindolol (*Petrovic* et al. 1983) and [^{3}H]CGP-12177 (*Wilkinson* and *Wilkinson* 1985). These ligands bind with high affinity and specificity to β-adrenoceptors.

Introduced by *Lefkowitz* et al. (1974), [^{3}H]DHA as a radiolabeled antagonist has been highly successful for studying β-adrenoceptors. It has been used to identify β-adrenoceptors in a wide variety of tissues including frog erythrocytes (*Lefkowitz* et al. 1974; *Mukherjee* et al. 1975), rat (*Alexander* et al. 1975) and monkey cerebral cortex (*Bylund* and *Snyder* 1976) and rat pineal glands (*Kebabian* et al. 1975, *Zatz* et al. 1976). These investigators showed the binding of [^{3}H]DHA to β-adrenoceptor sites to be reversible, stereospecific, of high affinity and displaceable by agonist and antagonist drugs. The central binding sites labeled with [^{3}H]DHA have been shown to have similar properties to the adrenoceptors of the β_1 sub-type in the periphery (*Bylund* and *Snyder*, 1976).

In the section that follows, a β-adrenoceptor binding assay employing [^{3}H]DHA as radioligand is described. The methodology of *Alexander* et al. (1975), adapted for the pineal gland by *Greenberg* and *Weiss* (1978) with some minor modifications, was used.

2.2.1.2 Materials and Methods

Animals : Male Wistar rats, weighing 350-380 g were used and were housed under conditions as described previously (Section 2.1). All animals were sacrificed at 11:00 h for use in the experiments.

Chemicals and Reagents : Tritiated DHA (specific activity 60 Ci/mmol) was purchased from Amersham (England); *dl*-propranolol HCl from Sigma Chemical Co (USA) and all other chemicals were obtained from local commercial sources.

[^{3}H]DHA Binding Assay Procedure : Pineal glands were rapidly excised as described previously (Section 2.1). Two similarly treated pineals were placed into a 1.0 ml glass homogeniser containing 1.0 ml of ice cold buffer (50 mM Tris-HCl and 3 mM $MgCl_2$, pH 8.0) and homogenised. The homogenate was centrifuged at 49 000 x g for 15 min at 4^{o}C. The resulting pellet was washed and resuspended in 0.9 ml of the above buffer for use in the binding assay. Six 100 µl aliquots of tissue

suspension were used for [^{3}H]DHA binding while two aliquots of 100 µl were used for the determination of protein. A schematic representation of the assay procedure is presented in Table 2.1. The assay was conducted in a final volume of 450 µl, containing 5 nM [^{3}H]DHA and 100 µl of tissue suspension. Non-specific binding was determined by the addition of 10 µM *dl*-propranolol to half the reaction tubes. Propranolol is assumed to selectively displace [^{3}H]DHA from specific β-adrenoceptor sites. All samples were analysed in triplicate. A blank, in which tissue was omitted, was also incubated in each series of assays. After incubation for 10 min at 37^{o}C, the reaction was terminated by adding 5 ml of ice-cold buffer to each tube. The samples were rapidly filtered under reduced pressure through pre-wetted Whatman GF/C glass fibre filters. Each of the tubes and filters was rapidly rinsed 5 times with 4 ml aliquots of ice-cold buffer. The vacuum pressure was maintained for an additional 2 minutes to allow the filters to dry. These were then transferred to plastic scintillation vials containing 5 ml of Ready-Solv HP scintillation fluid (Beckman, USA) and shaken for 30 minutes. The radioactivity in each sample was quantified using liquid scintillation spectroscopy in a Beckman LS 2500 scintillation counter at an efficiency of 55 to 60%. Counting efficiency, using computer assisted analysis , was determined by the external channel ratio method of quench correction. A correction factor was built into the quench correction programme to allow for tritium decay. This enabled an accurate assessment of the label being quantitated. Radioactivity counts did not appear to fluctuate significantly after storing vials for up to 2 weeks. However, since scintillation fluid does evaporate from plastic vials with time, all samples were quantitated within 24 hours of preparation. Specific binding of [^{3}H]DHA was calculated as total minus non-specific bound and represented 75 to 80% of total bound. Blank values were subtracted from assay values before expressing results.

Protein Determination : The concentration of protein in each batch of tissue suspension was determined by the method of *Lowry* et al. (1951), using bovine serum albumin as the standard. A schematic representation of the method used is illustrated in Table 2.2. A typical standard curve thus obtained is presented in Fig. 2.1.

Saturation Binding Assay Procedure : For the saturation assay, pineal glands from 8 male rats were pooled and homogenised in 1.0 ml of ice-cold buffer containing 50 mM Tris-HCl and 3 mM $MgCl_2$ (pH 8.0). The homogenate was centrifuged and washed as descbribed above, and the pellet was resuspended in 3.6 ml of buffer. The incubation tubes contained 100 µl aliquots of tissue suspension, varying concentrations of [^{3}H]DHA (1-20 mM) and buffer up to a final volume of 450 µl. Non-specific binding was measured by the addition of 10 µM *dl*-propranolol to parallel assay tubes. The samples were incubated, filtered and counted for radioactivity as described above.

2.2.1.3 Results

As shown in Fig. 2.2, the binding of [^{3}H]DHA to pineal membranes was saturable, with half maximal binding occuring at less than 5 nM. Scatchard analysis of the data (Fig. 2.3) demonstrated a single class

TABLE 2.1 Scheme of the [^{3}H]DHA binding assay. (*Greenberg* and *Weiss* 1978)

TUBES / REAGENTS	TOTAL BINDING	NON-SPECIFIC BINDING
Homogenate	100 µl	100 µl
[^{3}H]DHA (5 nM)	27 µl (1:200)	27 µl (1:200)
dl-Propranolol (10 µM)	----	10 µl (450 µM)
Buffer *	323 µl	313 µl
INCUBATE AT 37°C FOR 10 MINUTES		
Buffer * (ice-cold)	5 ml	5 ml
FILTER UNDER REDUCED PRESSURE WASH FILTERS : 5 x 4 ml ICE-COLD BUFFER *		
Scintillation Fluid	5 ml	5 ml
SHAKE FOR 30 MINUTES COUNT RADIOACTIVITY		

Tris HCl buffer (50 mM, pH 8.0) containing 3 mM $MgCl_2$.

TABLE 2.2 Scheme for the determination of protein. (*Lowry* et al. 1951)

TUBES / REAGENTS (ml)	STANDARDS SAMPLES (μg)						
	BLANK	2.5	5.0	10.0	20.0	40.0	UNKNOWN
Standard (0.1 mg/ml)	----	0.025	0.050	0.100	----	----	----
Standard (1.0 mg/ml)	----	----	----	----	0.020	0.040	----
Unknown	----	----	----	----	----	----	0.100
Buffer *	0.400	0.375	0.350	0.300	0.380	0.360	0.300
Reagent C **	2.0	2.0	2.0	2.0	2.0	2.0	2.0
STAND : 15 MINUTES AT ROOM TEMPERATURE							
Reagent E ***	0.2	0.2	0.2	0.2	0.2	0.2	0.2
STAND : 30 MINUTES AT ROOM TEMPERATURE READ AT 750 nm							

* **Tris HCl** buffer (50 mM, pH 8.0) containing 3 mM $MgCl_2$.

** 2% Na_2CO_3 in 0.1 N NaOH : 0.5% $CuSO_4.5H_2O$ in 1% sodium tartrate (50:1).

*** Diluted Folin-Ciocalteu phenol reagent (1:1).

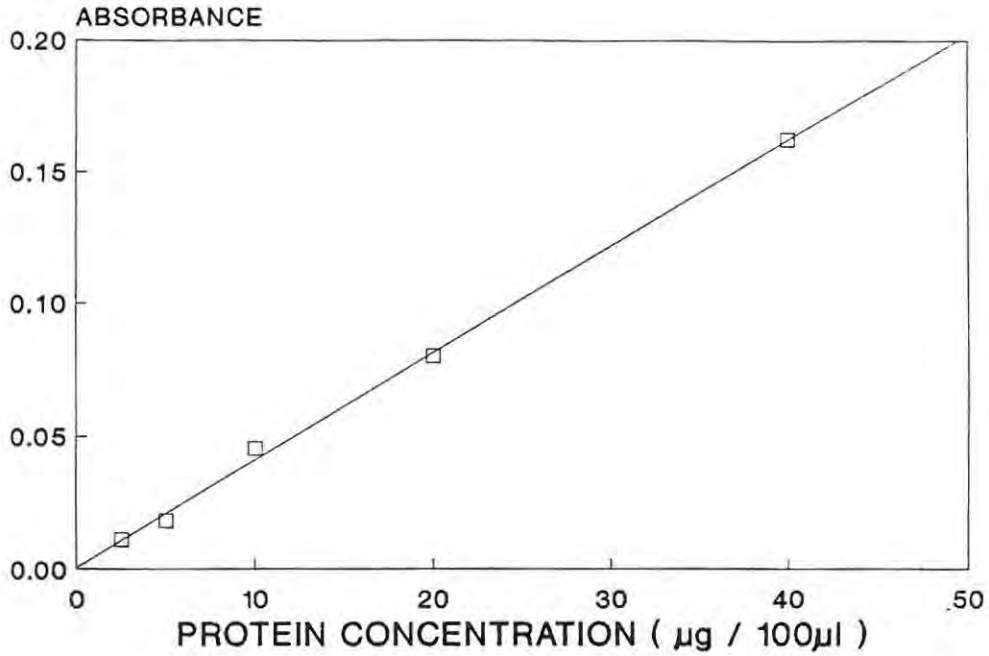


Figure 2.1 A typical protein standard curve using bovine serum albumin as the standard protein.

Each point represents the mean of duplicate determinations.

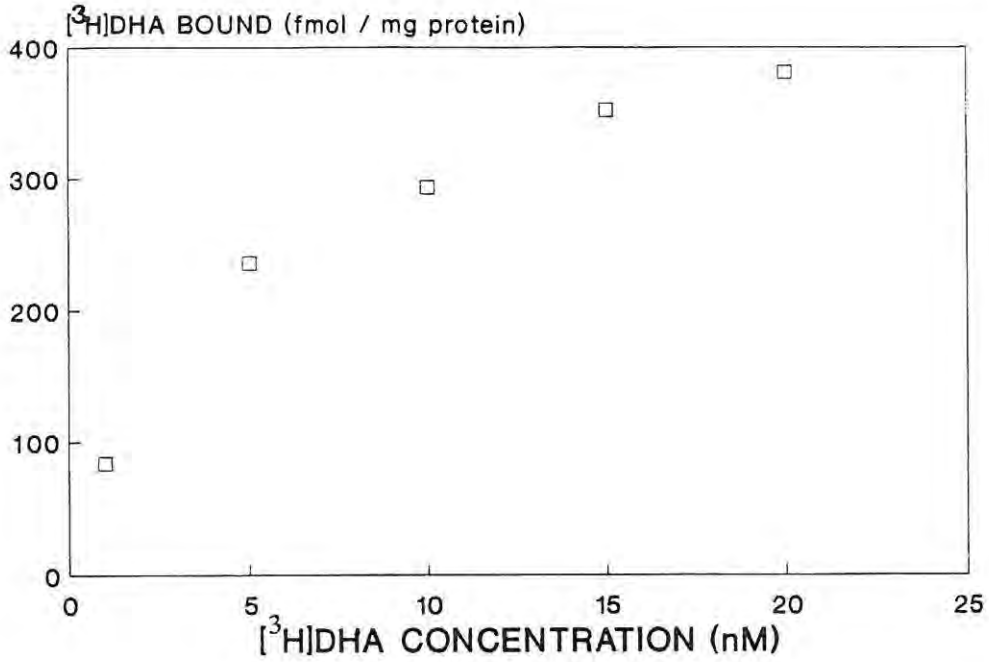


Figure 2.2 Saturation curve of [^{3}H]DHA binding to rat pineal membranes.
Increasing amounts of radiolabeled DHA were added to a fixed amount of pineal homogenate. Specific binding, i.e., the difference between total and non-specific binding, is shown. Each point represents the mean of triplicate determinations.

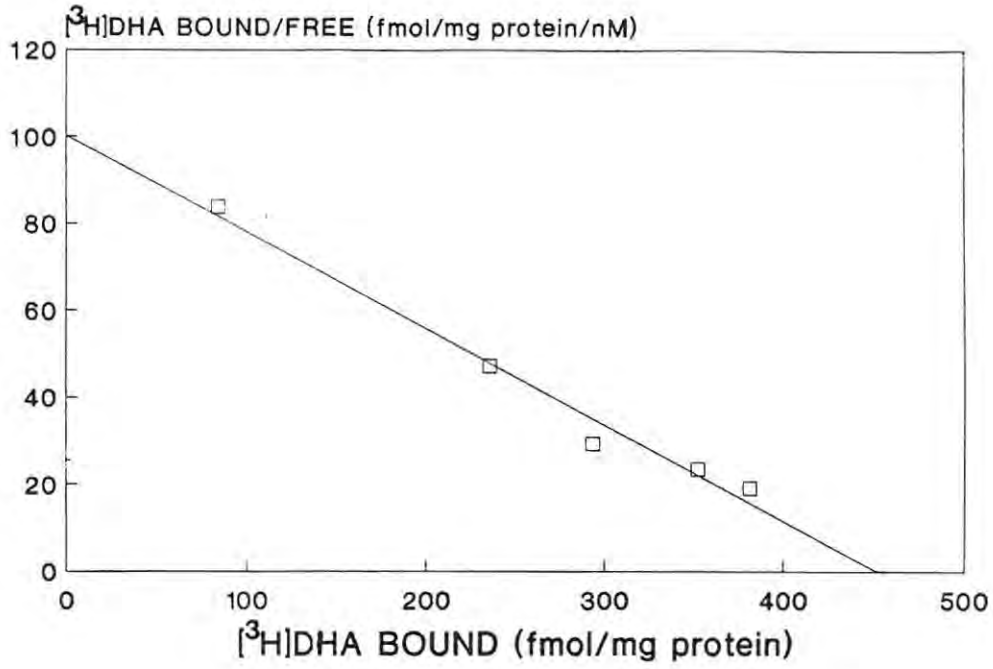


Figure 2.3 Scatchard plot of [^{3}H]DHA binding to rat pineal membranes.

The K_d and B_{max} values for the above plot are 4.5 nM and 451.5 fmol/mg protein, respectively. The correlation coefficient, calculated using Linear Regression Analysis, was found to be 0.991. Each point represents the mean of triplicate determinations.

of receptors with an apparent equilibrium dissociation constant (K_d) of 4.5 nM and a maximal number of binding sites (B_{max}) equivalent to 451.5 fmol/mg of protein.

2.2.1.4 Discussion

The binding of [^{3}H]DHA to rat pineal membranes was saturable at 10-15 nM. This finding is in agreement with previous reports (*Greenberg* and *Weiss* 1978, *Cantor* et al. 1981). At this concentration, specific binding represented 45-50% of total binding. However, in order to maximise the ratio of specific binding to non-specific binding, a concentration of 5 nM [^{3}H]DHA was used in all subsequent assays. At this concentraion, specific binding represented 75-80% of total binding. Since Scatchard analysis of the data revealed linearity with acceptable K_d and B_{max} values (*Greenberg* and *Weiss* 1978, *Cantor* et al. 1981), the developed assay was therefore suitable for use in further studies.

2.2.2 β-Adrenoceptor Binding Studies Employing the Ligand [^{3}H]CGP-12177

2.2.2.1 Introduction

Receptor binding studies have become commonplace among many experimenters, particularly neuroscientists. Indeed, its advent has led to a subsequent exponential growth in the volume of publications, and consequently, in our knowledge of neurotransmission and receptor regulation. The technique most often employed in these studies utilises non-living brain tissue homogenate separated from *in vivo* physiological conditions. However, in order to usefully study the regulatory properties of neurotransmitter receptors under physiological conditions, the necessity for a tissue preparation which retains some degree of cellular integrity is clear. In addition, the retention of tissue structure could conceivably be important for normal neuronal function.

The novel work by *Bhanot* and *Wilkinson* (1983) on opiate receptor binding sites and *Whitaker* et al. (1984) on benzodiazepine receptor binding sites, using [^{3}H]naloxone and [^{3}H]flunitrazepam respectively, demonstrated that the intact brain slice is a valuable alternative to the widely used homogenate method. Using a method that is inexpensive, rapid and involving the minimum of tissue preparation, these investigators demonstrated the binding of ligands to opiate and benzodiazepine receptors to be stereospecific, saturable and of high affinity.

More recently, *Wilkinson* and *Wilkinson* (1985) characterised the binding of the ligand, [^{3}H]CGP-12177, to β-adrenoceptor sites in rat brain slices and intact pineal glands of rats, mice and hamsters. These investigators showed the binding to β adrenoceptor sites to be reversible, stereospecific, of high affinity and displaceable by agonist and antagonist drugs. The ligand, [^{3}H]CGP-12177, reported to bind to the

β-adrenoceptor (*Staehelin* and *Hertel*, 1983) was chosen because of its hydrophilicity and inability to penetrate cell membranes. This ligand can therefore be used to quantify membrane-bound receptor sites.

Using the method of *Wilkinson* and *Wilkinson* (1985), with minor modifications, an attempt was made to evaluate the binding of [^{3}H]CGP-12177 to single, intact pineal glands in the hope of employing the methodology for later work.

2.2.2.2 Materials and Methods

Animals : Female Wistar rats weighing 180-220 g were used and were maintained as previously described (Section 2.1). All animals were sacrificed at 11:00 h for use in the experiments.

Chemicals and Reagents : [^{3}H]CGP-12177 (specific activity 30 Ci/mmol) was purchased from Amersham (England), and *dl*-propranolol HCl from Sigma Chemical Co. (USA). All other chemicals and reagents were obtained from local commercial sources.

[^{3}HCGP-12177 Binding Assay Procedure : Pineal glands were rapidly excised and placed in ice-cold phosphate buffered saline (PBS), pH 7.2. The glands were then individually placed on ice and carefully decapsulated under binocular magnification. Removal of the capsular membrane was achieved with the aid of two plastic syringes equipped with 28 gauge needles. Whenever possible, pineal glands were removed from the buffer for decapsulation within 5 to 10 min. Individual, decapsulated pineals were placed into 12 x 40 mm sterile glass culture tubes containing 4 nM [^{3}H]CGP-12177 in a final volume of 250 µl PBS. Non-specfic binding was measured by the addition of 10 µM *dl*-propranolol to parallel reaction tubes. Samples were incubated at 30^{o}C for 180 min. Following incubation the buffer was carefully removed by suction, with the aid of a Pasteur pipette, and the glands were washed in ice-cold PBS buffer (2 x 250 µl; 2 x 5 minutes). The pineal glands were then removed directly to scintillation vials containing 2 ml Ready-Solv HP (Beckman, USA) scintillation fluid. The vials were capped, vigorously shaken and allowed to stand for 5 hours before counting. Radioactivity counts (effeciency : 48-52%) were normally stable after approximately 3 hours, which suggests that the [^{3}H]-ligands are easily removed from the glands. Specific binding of [^{3}H]CGP-12177 was calculated as total minus non-specific bound and represented approximately 85% of total bound.

Saturation Binding Assay Procedure : In order to reduce costs, the saturation assay was performed using bisected pineal glands since preliminary studies revealed a remarkable correlation in binding between bisected hemispheres and whole pineal glands. Moreover, this procedure proved to be useful since it allowed for the measurement of both total and non-specific binding within the same pineal gland. Pineal glands from 18 female rats (180-220 g) were rapidly removed and prepared as described above. Following removal of the capsular membrane, the glands were carefully bisected under binocular magnification. One hemisphere was utilised for total binding while the other, for non-specific binding.

The incubation tubes contained varying concentrations of [^{3}H]CGP-12177 (1-8 nM) and PBS buffer in a final volume of 250 µl. Non-specific binding was measured by the addition of 10 µM *dl*-propranolol to parallel assay tubes and each assay was done in triplicate. After incubation at 30°C for 180 min, a 50 µl aliquot of the buffer was removed from each of the tubes for the determination of "free" ligand concentration. The rest of the buffer was then removed and the pineals were washed, placed into counting vials and counted for radioactivity as described above.

2.2.2.3 Results

Time-course experiments (Fig. 2.4) revealed that the specific binding of [^{3}H]CGP-12177 reached equilibrium by 90 min and remained stable for at least 180 min (1 nM; 30°C). Half maximal binding occurred around 60 min. In all subsequent assays, a 180 min incubation period was used. As shown in Fig. 2.5, binding of [^{3}H]CGP-12177 to intact pineal glands was saturable with half maximal binding occurring at 2-3 nM. Non-specific binding was remarkably low and varied from less than 5% at 1 nM to 15% at 8 nM. Scatchard analysis of the data (Fig. 2.6), revealed a single class of receptors with an apparent equilibrium dissociation constant (K_d) of 3.5 nM and a maximal number of binding sites (B_{max}) equivalent to 47.3 fmol/gland.

2.2.2.4 Discussion

The results indicate that the binding of [^{3}H]CGP-12177 to intact pineal glands is saturable, displaceable by antagonist drugs and of high affinity. In addition, these determinations are simple to perform when compared with filtration assays (Section 2.2.1) and completely dispenses with the time consuming centrifugation of tissue homogenates. The method also has the crucial advantage of enabling receptor binding to be studied in living tissue. It is interesting to note that the K_d and B_{max} values obtained in this study agree reasonably well with those of *Wilkinson* and *Wilkinson* (1985) considering that rats of a different sex and albino strain were used.

Finally, the method described above should provide new opportunities for the study of neurotransmitter binding sites (receptors) in the field of pineal physiology as other types of receptors could conceivably be examined in this way. In particular, it now becomes possible to examine receptor recycling in intact, fresh tissue as has been described already in tumour cells (*Toews* and *Perkins* 1984, *Hertel* and *Staehelin* 1983).

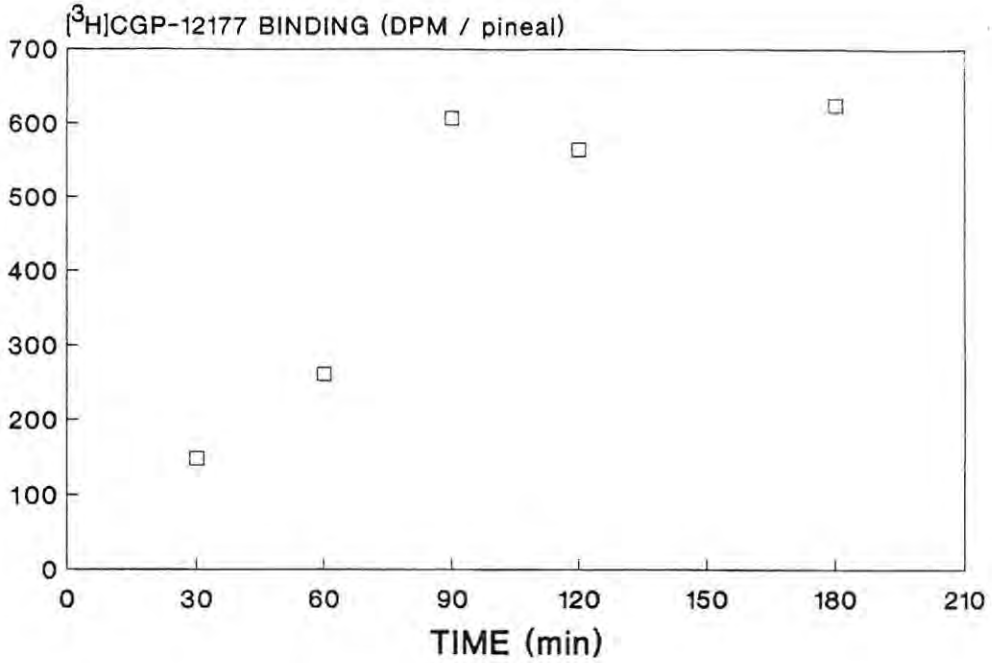


Figure 2.4 Effect of incubation time on the specific binding of [^{3}H] CGP-12177. Each point represents the mean of duplicate determinations. Specific binding of the radioligand is expressed as DPM/pineal gland.

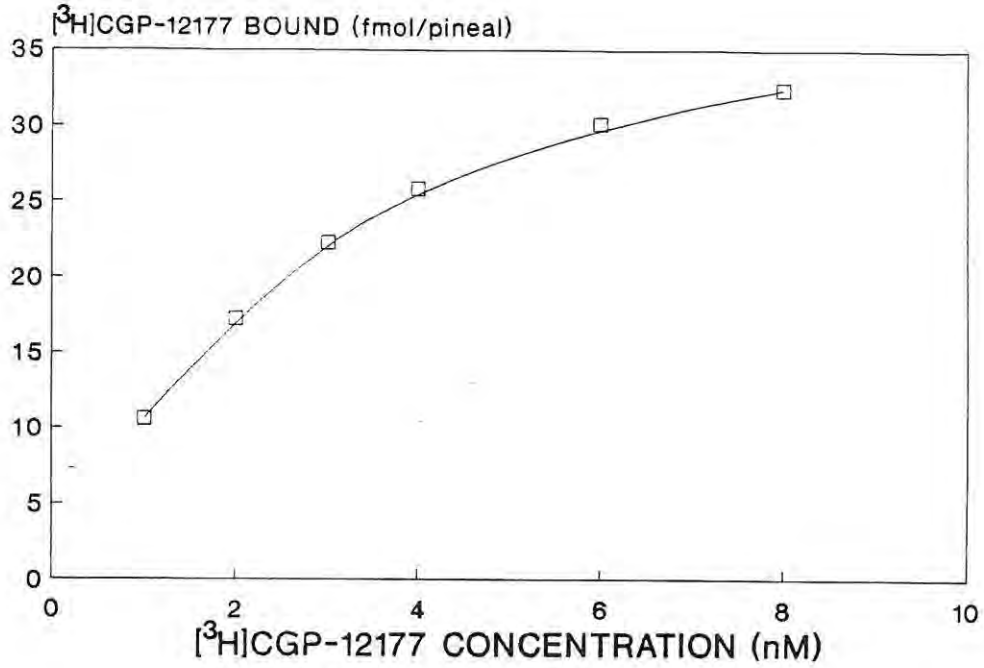


Figure 2.5 Saturation curve of [^{3}H]CGP-12177 binding to rat pineal glands.
Specific binding of the radioligand, i.e., the difference between total and non-specific binding is shown. Each point represents the mean of triplicate determinations.

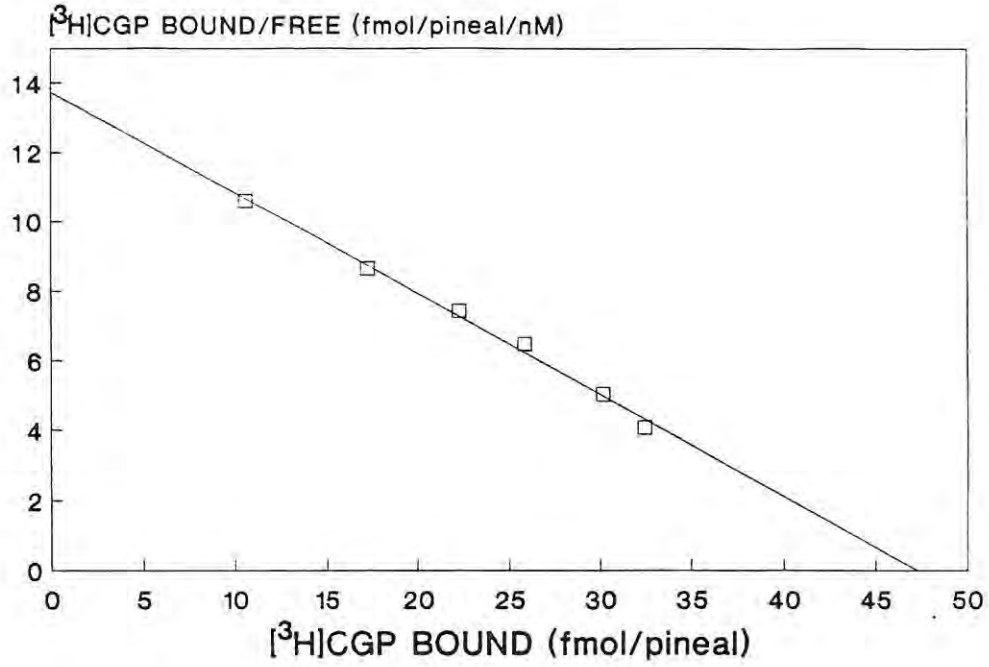


Figure 2.6 Scatchard plot of [^{3}H]CGP-12177 binding to rat pineal glands.

The K_d and B_{max} values for the above plot are 3.5 nM and 47.3 fmol/pineal gland, respectively. The correlation coefficient (Linear Regression Analysis) was found to be 0.997. Each point represents the mean of triplicate determinations.

2.3 THE MEASUREMENT OF PINEAL CYCLIC ADENOSINE MONOPHOSPHATE (cAMP) LEVELS

2.3.1 Introduction

Various researchers have, at different times, employed a variety of techniques to measure cAMP levels. Early attempts, in this regard, were essentially indirect and involved determining the activity of the enzyme adenylate cyclase as an index of cAMP levels (*Krishna* et al. 1968). This method involved measuring the rate of formation of cAMP from its radioactive precursor, [^{14}C]ATP. The cAMP, thus formed, was isolated by ion exchange chromatography followed by precipitation of all the nucleotides and inorganic phosphates. The radioactivity of the cAMP left in the supernatant was then measured.

A direct cAMP assay, using a purified protein from bovine muscle as the specific binding protein, was developed by *Gilman* (1970). Various workers have used this technique to measure rat pineal cAMP levels. Generally, the method involves prior trichloroacetic acid precipitation and ether extraction before measuring cAMP levels. However, *Deguchi* (1973) found no difference in the amount of cAMP between purified and unpurified pineal tissue using the method of *Gilman* (1970).

Brown and co-workers (1971) developed a saturation assay for cAMP using purified bovine adrenal protein as the specific binding protein. The assay was similar to the *Gilman* assay (1970), also involving trichloroacetic acid precipitation and ether extraction prior to measurement of cAMP levels. Although the two assays had similar sensitivity and specificity characteristics, the assay of *Brown* and co-workers (1971) was considerably simpler and less time consuming.

More recently, radioimmunoassays (RIAs) have been developed to measure rat pineal cAMP levels (*Moyer* et al. 1979, *Heydorn* et al. 1980). The cAMP concentration is measured using [^{125}I]antigen and antiserum available commercially. The cAMP RIAs, although expensive, have a high degree of specificity and sensitivity. In addition, these assays are rapid and simple to use.

The assay developed by *Brown* et al. (1971) was chosen to measure pineal cAMP levels for the purposes of this study. The assay is sensitive, specific, easy to use and relatively inexpensive. The following section describes the preparation of the adrenal binding protein as well as the cAMP assay, adapted for the pineal gland.

2.3.2 Materials and Methods

Animals : Female Wistar rats weighing 200-250 g were used in these experiments and were maintained as previously described (Section 2.1).

Chemicals and Reagents : Tritiated cAMP (specific activity 26.1 Ci/mmol) was purchased from

Amersham, (England); theophylline from Sigma Chemical Co (USA); activated charcoal from Merck (West Germany). All other chemicals and reagents were obtained from local commercial sources.

Preparation of cAMP Binding Protein : Bovine adrenal glands were collected from the local abbatoir as soon as possible after slaughter and transported to the laboratory on ice. The cortices were separated, chopped and homogenised in 1.5 volumes of an ice-cold medium comprising 0.25 M sucrose; 50 mM Tris-HCl buffer, pH 7.4; 25 mM potassium chloride and 5 mM magnesium chloride. The homogenate was centrifuged at 2000 x g for 10 min at 4^{o}C. The pellet was discarded and the supernatant respun at 5000 x g for 15 min at 4^{o}C. The resulting supernatant was stored in 0.5 ml aliquots at -20^{o}C. A negligible loss of binding activity was found after at least 3 months of storage. The preparation, which contains a binding protein (protein kinase), was thawed and diluted as required with 50 mM Tris-HCl buffer, pH 7.4 containing 8 mM theophylline and 6 mM 2-mercaptoethanol. This buffer was used for all subsequent procedures.

Preparation of Pineal Tissue and Incubation Procedure : Pineal glands were rapidly removed and immediately placed in ice-cold physiological saline (0.9%). The pineals were then dissected free of adhering connective tissue and carefully bisected, under binocular magnification, with the aid of a scalpel blade. Whenever possible, pineal glands were removed and bisected within 5 to 10 min. The two hemispheres were placed separately in 50 µl of BGJb culture medium (Fitton-Jackson modification, Gibco (Europe); see Section 2.6.2) and pre-incubated at 37^{o}C for 15 min. One hemisphere was then transferred to fresh, pre-warmed incubating medium containing 20 µM isoprenaline while the other hemisphere was placed in fresh medium containing no drug. Both halves were then incubated for a further 10 min at 37^{o}C. Following incubation, the pineal hemispheres were removed from the medium and homogenised in 100 µl of ice-cold distilled water. A 50 µl aliquot of homogenate was used in the cAMP assay.

Cyclic AMP Assay Procedure : A schematic representation of the assay procedure is presented in Table 2.3. **The reaction mixture** contained 50 µl of either a known amount (0-8 pmol) of cAMP standard (**Boehringer Mannheim**) or of an unknown sample; 50 µl of [^{3}H]cAMP (8 nCi); 100 µl of diluted (1:3) **binding protein** and buffer (50 mM Tris-HCl, pH 7.4 containing 8 mM theophylline and 6 mM 2-mercaptoethanol) to a final volume of 350 µl. The reaction tubes were placed on ice and incubated at 4^{o}C for 90 min. Following incubation, 100 µl of a 10% w/v suspension of charcoal in buffer containing 2% w/v of bovine serum albumin, was added to each reaction tube and the tubes were vortexed for 10 sec each. After centrifugation at 1200 x g for 15 min at 4^{o}C, a 100 µl aliquot of the supernatant was added to plastic scintillation vials containing 3 ml Ready-Solv HP (Beckman, USA) scintillation fluid. The vials were briefly agitated and radioactivity quantitated as previously described (Section 2.2.1.2). A calibration curve was plotted in terms of the ratio Co/Cx against the concentration (pmoles/tube)of standard nucleotide. The Co/Cx ratio was obtained by dividing the radioactivity (cpm) of the zero standard (Co) by the radioactivity of the higher standards (Cx) after subtraction of blank values.

TABLE 2.3 Scheme of the cyclic AMP assay. (*Brown* et al. 1971)

	CYCLIC AMP STANDARDS (pmol)								
REAGENTS (µl)	BLANK	0	0.25	0.5	1.0	2.0	4.0	8.0	SAMPLE
Buffer *	250	150	150	150	150	150	150	150	150
Standard	---	---	50	50	50	50	50	50	50
Sample	---	---	---	---	---	---	---	---	50
[^{3}H]cAMP	50	50	50	50	50	50	50	50	50
Diluted protein	---	100	100	100	100	100	100	100	100
INCUBATE ON ICE FOR 90 MINUTES									
Charcoal	100	100	100	100	100	100	100	100	100
CENTRIFUGE AT 1200 x g FOR 15 MINUTES AT 4°C									
Supernatant	100	100	100	100	100	100	100	100	100
ADD TO 3 ml SCINTILLATION FLUID AND COUNT RADIOACTIVITY									

* Tris HCl (50 mM, pH 7.4) containing 8 mM theophylline and 6 mM 2-mercaptoethanol.

2.3.3 Results

Dilution of Binding Protein : In order to determine the optimum dilution of the stock binding protein for the cAMP assay, serial dilutions of binding protein in buffer were assayed. The protein dilution curve (Fig. 2.7), thus obtained, shows that a 1:3 dilution provides optimum binding (85%) and radioactivity counts. This dilution factor was thus used in all subsequent cAMP assays. Each time a new batch of binding protein was prepared, a new dilution curve was determined. However, a 1:3 dilution was found to be appropriate for all batches tested in this way.

Calibration Curve : A typical calibration curve obtained is illustrated in Fig. 2.8. The curve was linear between 0.25 pmol and at least 8.0 pmol of cAMP (linear regression analysis; $r^2 = 0.999$). The amount of cAMP in the samples was determined by extrapolation and the final results expressed as pmol cAMP/pineal.

Estimation of Interference by Non-cyclic AMP Material : Prior incubation of tissue homogenate in the presence of approximately 50 μg of phosphodiesterase (Boehringer Mannheim) for 1 h at 37°C yielded a level of cAMP that was not significantly different from the value obtained for the zero standard. This indicates that the level of interference in the assay by non-cAMP material is negligible.

2.3.4 Discussion

The results presented indicate that the assay procedure described above is sufficiently sensitive and specific to measure cAMP levels in rat pineal tissue. In addition, the assay is considerably simpler than the method of *Brown* et al. (1971) since it allows for the direct quantification of cAMP levels in pineal homogenates without prior trichloroacetic acid precipitation and ether extraction. The assay was therefore suitable for use in subsequent studies.

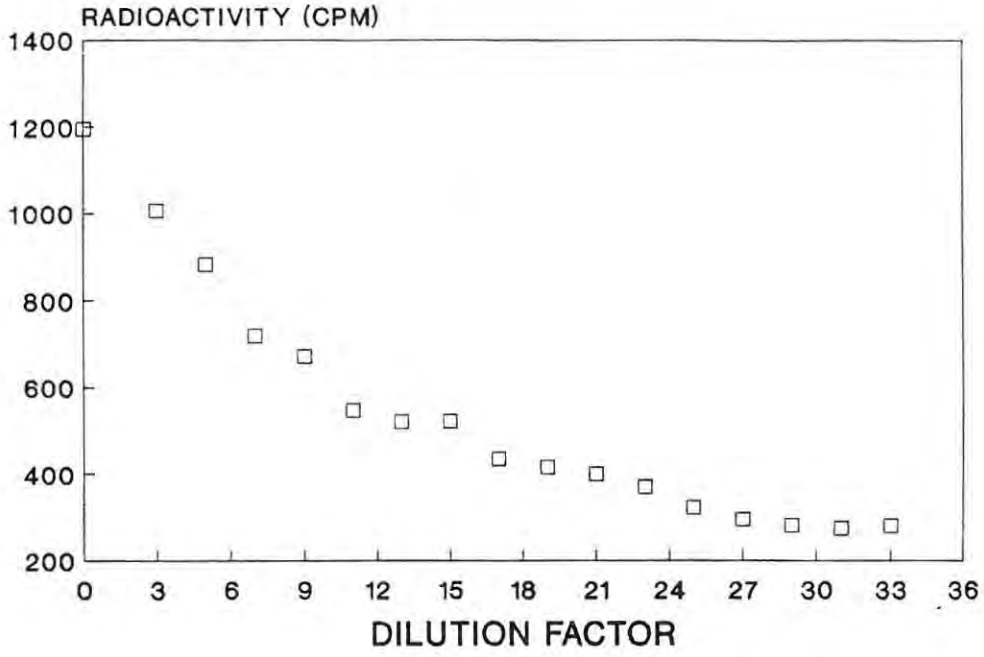


Figure 2.7 Cyclic AMP binding protein dilution curve.

Each point represents the mean of duplicate determinations.

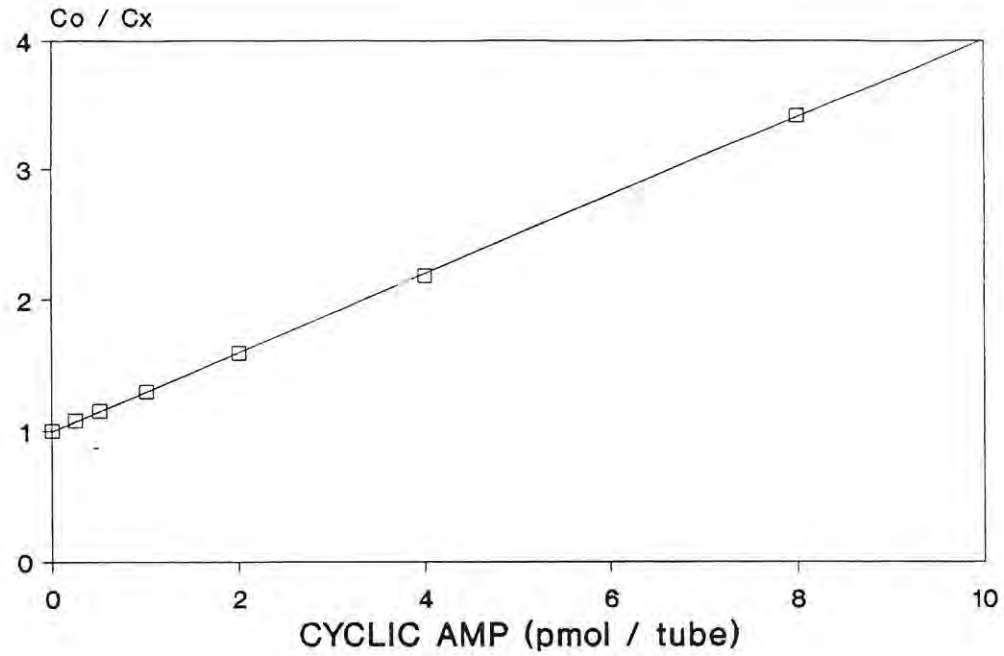


Figure 2.8 A typical cyclic AMP standard curve.

Each point represents the mean of duplicate determinations. The correlation coefficient (Linear Regression Analysis) is 0.999.

2.4 THE DETERMINATION OF PINEAL CYCLIC AMP PHOSPHODIESTERASE ACTIVITY

2.4.1 Introduction

Cyclic nucleotides, which play a primary role in the control of intracellular events, are hydrolysed and inactivated as second messengers by the cyclic nucleotide phosphodiesterases (PDEs). This family of enzymes has been extensively studied in brain and other tissues, and numerous assays have been devised to monitor their activity. The most widely used of these assays are radiometric, and involve the separation of the hydrolysed 5'-nucleotide phosphate product from the substrate. The separation of the hydrolysed radioactive product has been achieved in a number of ways, including batch or column anion-exchange resins (*Thompson* and *Appleman* 1971, *Lynch* and *Cheung* 1975), paper chromatography (*Nakai* and *Brooker* 1975) and precipitation (*LeDonne* and *Coffee* 1979). These assays are all sensitive, relatively inexpensive and easy to use. In the assay, cAMP PDE or cGMP PDE activity can be measured by employing [^{3}H]cAMP or [^{3}H]cGMP, respectively, as substrate.

The activity of cyclic nucleotide PDE in the pineal gland has generally been measured using the radiometric assay of *Thompson* and *Appleman* (1971). In the section that follows, the radiometric assay of *LeDonne* and *Coffee* (1979), adapted to measure cAMP PDE activity in the pineal gland, is described. The assay, which involves separation of the radioactive 5'-nucleotide phosphate product by precipitation, was employed for later work (see Chapter 7).

2.4.2 Materials and Methods

Animals : Male Wistar rats, weighing 200-300 g, were used and were housed under conditions as described previously (Section 2.1). Unless indicated otherwise, animals were sacrificed at 16:00 h or 24:00 h for use in the experiments.

Chemicals and Reagents : Tritiated cAMP (specific activity 26.1 Ci/mmol) was purchased from Amersham (England) and morpholino-propanesulphonic acid (MOPS) from Sigma Chemical Co. (USA). All other chemicals and reagents were obtainable from local commercial sources.

Phosphodiesterase Assay Procedure : Pineal glands were rapidly excised (Section 2.1) and placed individually in glass homogenising tubes containing 0.1 ml of ice-cold distilled water and homogenised for 30 sec (about 15 strokes). An additional 0.4 ml of ice-cold distilled water was added and the homogenization procedure was repeated. The reaction mixture contained 20 µl of homogenate; 1 µM [^{3}H]cAMP (200 000 cpm) and buffer (25 mM MOPS, pH 7.0; 5 mM 2-mercaptoethanol and 3 mM magnesium acetate) in a final volume of 200 µl. Samples were incubated for 20 min at 30^{o}C and the reaction was terminated by boiling for 2 min. A blank, containing no tissue, was also included in each series of incubations. Each assay was done in duplicate. The [^{3}H]5'-AMP formed was precipitated by the addition of 0.1 ml of 0.3 N $Ba(OH)_2$ and 0.1 ml of 5% $ZnSO_4$ to each reaction tube. The precipitate

was filtered under reduced pressure through pre-wetted Whatman GF/C glass fibre filters. Each of the tubes and filters were rapidly rinsed 3 times with 6 ml of 5 mM Tris-HCl, pH 7.5 and twice with 95% ethanol. The vacuum pressure was maintained for an additional 2 min to allow the filters to dry. The filters were then transferred to plastic scintillation vials containing 5 ml of Ready-Solv HP scintillation fluid (Beckman, USA) and shaken for 30 min. The radioactivity in each sample was quantitated as previously described (Section 2.2.1.2) at a counting efficiency of 50%. Blank values were subtracted from assay values before expressing the results.

In Vitro **Studies :** For studies aimed at evaluating the effect of *in vitro* pharmacological manipulation on cAMP PDE activity, the procedure described in Section 2.5.2 was adopted.

2.4.3 Results

The time course for the hydrolysis of cAMP by PDE is shown in Fig. 2.9. Cyclic AMP hydrolysis increased in an almost linear fashion within 20 min. All subsequent assays, therefore, utilised a 20 min incubation period. The hydrolysis of cAMP also increased with increasing concentration of homogenate (Fig. 2.10). A 20 µl aliquot of homogenate (1 pineal : 0.5 ml) was therefore routinely used.

The radioactive product from the enzymatic reaction has the same R_f value as authentic 5'-AMP (Boehringer Mannheim) in the following solvent system : isopropyl alcohol / 25% ammonia / water (7:1:2).

The blank value, obtained by incubation without tissue, was consistently less than 0.5% of the radioactivity of cAMP used.

2.4.4 Discussion

Based on the method of *LeDonne* and *Coffee* (1979), an assay procedure to determine cAMP PDE activity in individual rat pineal glands was developed. The results presented above indicate that the assay procedure is sufficiently sensitive and specific to measure cAMP PDE. In addition, the assay is considerably less costly than that of *Thompson* and *Appleman* (1971) and was therefore suitable for use in subsequent studies.

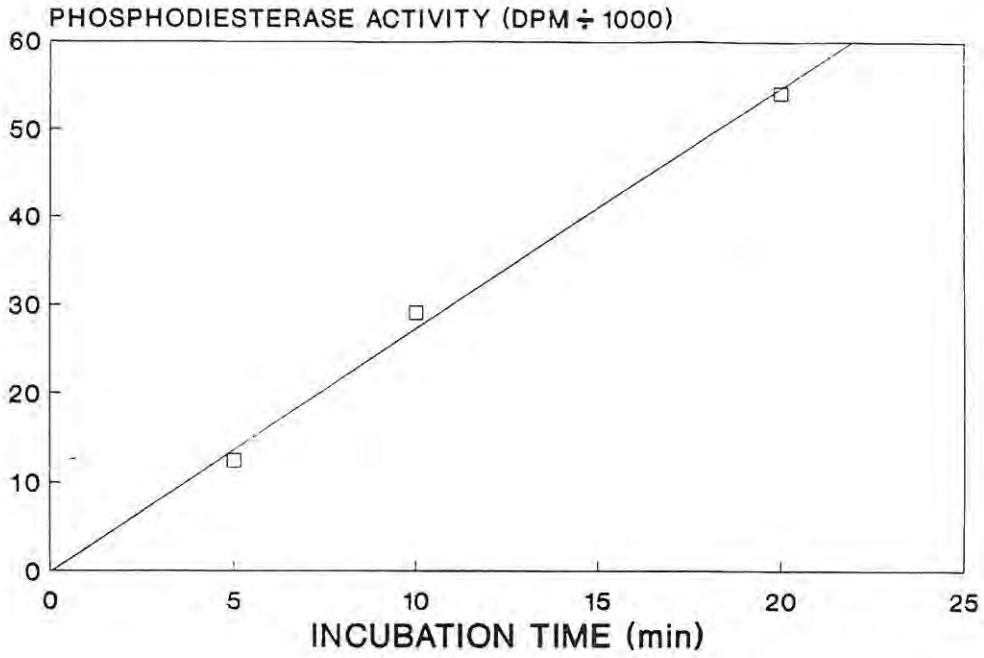


Figure 2.9 Effect of incubation time on phosphodiesterase-mediated hydrolysis of cyclic AMP.

Two pineal glands were collected at midnight and stored at -70°C until assayed. The glands were pooled and homogenised in 1.0 ml of distilled water. A 20 µl aliquot of homogenate was used in the assay in a total reaction volume of 200 µl. Samples were incubated at 30°C for varying time periods. Each point represents the mean of duplicate determinations.

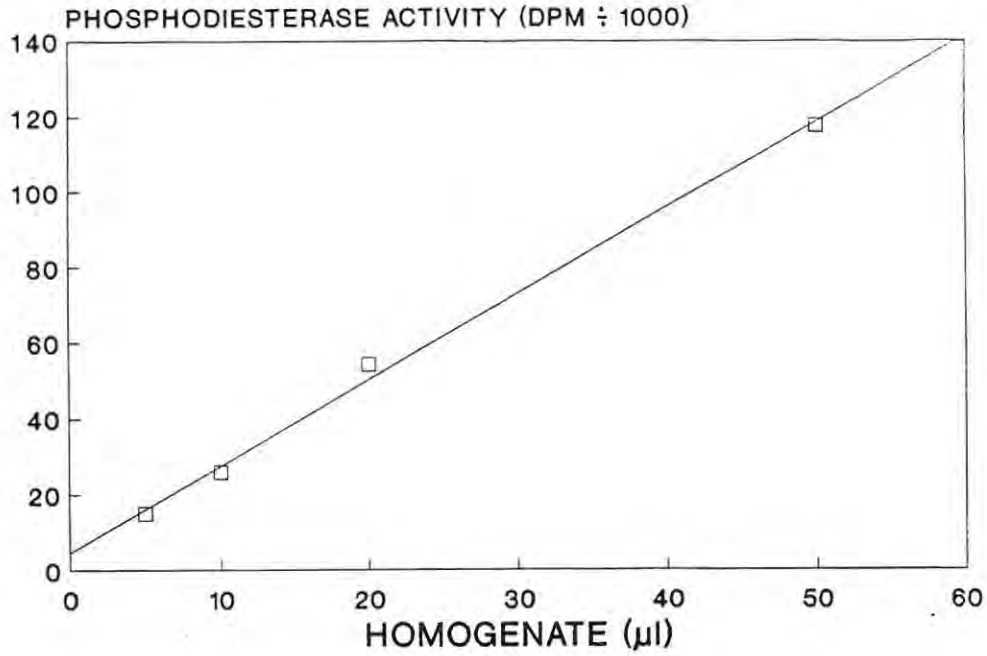


Figure 2.10 Effect of varying homogenate concentration on phosphodiesterase-mediated hydrolysis of cyclic AMP.

Two pineal glands were collected at midnight and stored at -70^{o}C until assayed. The glands were pooled and homogenised in 1.0 ml of distilled water. Varying aliquots of homogenate were used in the assay in a total reaction volume of 200 µl. Samples were incubated at 30^{o}C for 20 min. Each point represents the mean of duplicate determinations.

2.5 THE DETERMINATION OF PINEAL SEROTONIN N-ACETYLTRANSFERASE ACTIVITY

2.5.1 Introduction

Serotonin N-acetyltransferase (NAT) (EC. 2.3.1.5), the enzyme responsible for the conversion of 5-HT to N-acetylserotonin, has been assayed by a number of radiochemical methods. The most attractive of these methods, developed by *Deguchi* and *Axelrod* (1972a), is based on the acetylation of tryptamine with radioactive acetyl coenzyme A by NAT to form radioactive N-acetyltryptamine. Tryptamine is used in place of the natural substrate 5-HT as the enzyme can utilise it far more readily than 5-HT. The radioactive N-acetyltryptamine thus formed can be extracted into an organic solvent and measured by liquid scintillometry. The use of this assay permits a rapid and sensitive evaluation of NAT activity and also enables a more accurate interpretation of any pharmacological effects on the enzyme (*Deguchi* and *Axelrod* 1972a).

In the section that follows, the method of *Deguchi* and *Axelrod* (1972a) with minor modifications, is described.

2.5.2 Materials and Methods

Animals : Adult male Wistar rats (200-250 g) were used. The animals were maintained under normal laboratory conditions as detailed in Section 2.1.

Chemicals and Reagents : Batches of tritiated acetyl coenzyme A (specific activity 4.0-4.3 Ci/mmol) were purchased, as required, from Amersham (England); tryptamine HCl and *l*-isoprenaline HCl from Sigma Chemical Co (USA) and unlabeled acetyl coenzyme A from Merck (West Germany). All other chemicals and reagents were obtained from local commercial sources.

NAT Assay Procedure : Pineal glands were rapidly excised from rats as described previously (Section 2.1). Each pineal was individually placed in a small glass homogeniser containing 70 µl of ice-cold 50 mM phosphate buffer (pH 6.5) and homogenised for 30 sec (about 15 strokes). The reaction mixture contained 50 µl of homogenate; 1.43 mM tryptamine and 0.1 mM [^{3}H]acetyl coenzyme A (250 nCi) in a total volume of 70 µl. All manipulations and additions, up to this stage, were performed on ice. Samples were then incubated for 10 min at 37^{O}C and the reaction was terminated by the addition of 0.5 ml of 0.5 M borate buffer (pH 10.0). A blank, containing no tissue, was also included in each series of incubations. Using a Pasteur pipette, the reaction mixture was then transferred to a glass-stoppered tube containing 3 ml of toluene:isoamyl alcohol (97:3). The tubes were sealed and vortexed on a Rotamixer for 30 sec. The emulsion formed as a result was dispersed by centrifugation at 3500 rpm for 10 min. A two millilitre aliquot of organic phase was transferred to a plastic scintillation vial containing 3 ml scintillation fluid (Ready-Solv HP, Beckman, USA). The radioactivity in each sample was

quantitated as described previously (Section 2.2.1.2) at a counting efficiency of 68%. Blank values were subtracted from assay values before expressing the results.

In Vitro **Studies :** For studies aimed at evaluating the effect of pharmacological manipulation, *in vitro*, on NAT activity, groups of 2 to 3 pineals were incubated in sterile 12 mm diameter glass culture vessels containing 200 µl of culture medium (BGJb Fitton-Jackson modification, Gibco (Europe); see Section 2.6.2). The culture vessels were placed into sterile 20 ml glass vials during incubation. Alternatively, pineals were incubated individually in sterile 10 mm x 40 mm glass culture tubes containing 100 µl of culture medium. In either case, and unless indicated otherwise, drugs were dissolved in distilled water and added to the culture medium as 10 µl aliquots. The atmosphere within the glass vials or culture tubes was saturated with 5% CO_2 : 95% O_2. The vials or tubes were then sealed and incubated at 37^{o}C for a specified time period. Following incubation, the glands were removed, homogenised and enzyme activity determined as described above.

2.5.3 Results

The linear relationship between the amount of [^{3}H]N-acetyltryptamine formed and the amount of homogenate used in each assay is depicted in Fig. 2.11. The time course experiments also showed linearity with the amount of radioactive N-acetyltryptamine formed (Fig. 2.12). In all subsequent assays a 10 min incubation period was used. The blank value, obtained by incubation without enzyme, was approximately 0.2% of the radioactivity of acetyl coenzyme A used. In the assay, duplicate reactions agree within ± 5%.

2.5.4 Discussion

In the assay, the use of tryptamine and radioactive acetyl coenzyme A as substrates makes it possible to extract the enzymically formed N-acetyltryptamine into a nonpolar solvent which does not extract acetyl co-enzyme A. Within the conditions described above, the assay proved to be linear with time and homogenate concentration. In addition, the assay affords the measurement of enzyme activity in a single rat pineal gland.

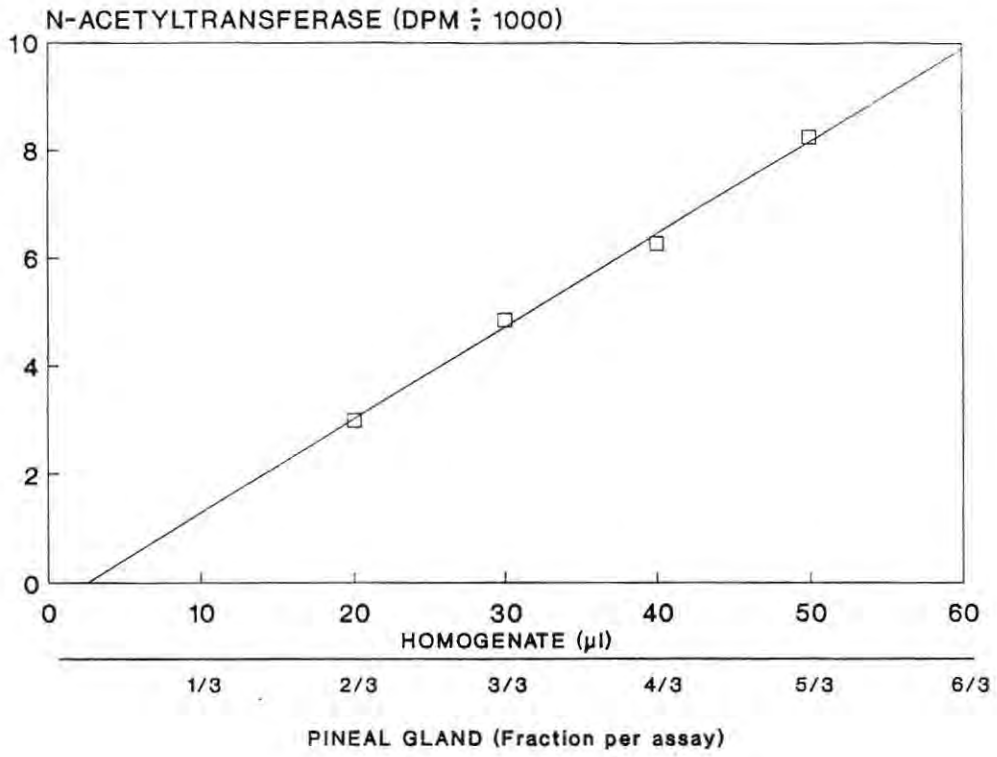


Figure 2.11 Relationship between N-acetyltransferase (NAT) activity and concentration of homogenate.

Ten pineals were collected at midnight and stored at -70^{o}C until assayed. The glands were pooled and homogenised in 0.3 ml of 50 mM phosphate buffer (pH 6.5). Varying aliquots of homogenate were used in the assay in a total reaction volume of 70 µl. Samples were incubated for 10 min at 37^{o}C. Each point represents the mean of duplicate determinations.

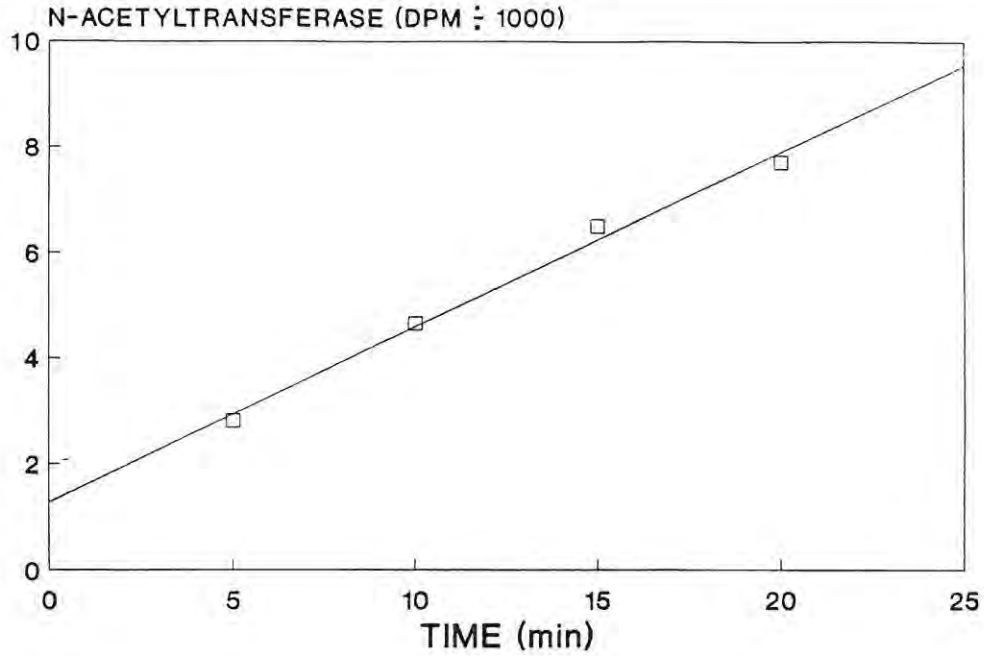


Figure 2.12 Effect of incubation time on N-acetyltransferase (NAT) activity.
Six pineal glands were collected at midnight and stored at -70°C until assayed. The glands were pooled and homogenised in 0.42 ml of 50 mM phosphate buffer (pH 6.5). A 50 µl aliquot of homogenate was used in the assay in a total volume of 70 µl. Samples were incubated at 37°C for varying time periods. Each point represents the mean of duplicate determinations.

2.6 THE MEASUREMENT OF [^{14}C]INDOLE SYNTHESIS BY ORGAN CULTURES OF THE RAT PINEAL GLAND

2.6.1 Introduction

The basic concepts of organ culture are reflected in a statement by *Claude Bernard* (1856) over one hundred years ago :

> "...physiological occurences must, as far as possible, be isolated outside the organism by means of experimental procedures. This isolation can then allow us to see and understand better the deepest associations of the phenomena, so that their vital role may be followed later in the organism".

Strangeways and *Fell* (1926) were amongst the first workers to employ the organ culture technique. At the time, the method was used chiefly for the culture of embryonic rudiments. More recently, refinements in biochemical techniques have led to an increase in the use of organ culture systems for the study of the physiological and biochemical properties of living tissue. *Trowell* (1959) successfully employed a technique that allowed a number of fully differentiated organs, or parts thereof, to be kept viable *in vitro* without either growth or dedifferentiation, thus paving the way for many experimental studies.

The technique of pineal gland organ culture is rendered suitable by virtue of its favourable size and accessibility. Indeed, pineal organ culture systems have been developed and employed by numerous researchers (eg. *Klein* and *Rowe* 1970, *Shein* and *Wurtman* 1969, *Parfitt* et al. 1976). It is also a useful technique since it eliminates the complexities of organ interaction and allows for direct pharmacological manipulation. Rat pineal glands cultured in an adequately aerated medium and incubated at 37^{o}C have been shown to remain viable for at least 6 days (*Klein* 1969).

The pineal gland in culture is able to utilise an exogenous radioactive precursor (tryptophan, 5-HTP or 5-HT) to synthesise various indole metabolites. It has been shown that approximately 95% of the synthesised radioactive indoles are secreted into the culture medium during the incubation period (*Klein* and *Rowe* 1970, *Skene* 1985). The radioactive indoles are then separated, most commonly using bi-dimensional thin layer chromatography (TLC) (*Klein* and *Notides* 1969, *Balemans* et al. 1978, *Daya* and *Potgieter* 1982), and quantitated with the aid of radiospectrometry.

In the following sections, the procedures for pineal organ culture, TLC separation of radioactive indole metabolites and quantification of labeled indoles using radiospectrometry is described.

2.6.2 Materials and Methods

Animals : Adult male Wistar rats (200-250 g) were used and were maintained as described previously (Section 2.1). All animals were sacrificed between 10h30 and 11h30 for use in the experiments.

Chemicals and Reagents : 5-Hydroxy (side-chain-2-^{14}C) tryptamine creatinine sulphate (specific activity 57 mCi/mmol) was purchased from Amersham (England); the synthetic pineal indoles from Sigma Chemical Co (USA); aluminium TLC plates coated with silica gel 60 F_{254} (0.20 mm) from Merck (West Germany) and BGJb culture medium (Fitton-Jackson modification) from Gibco (Europe). All other chemicals and reagents were obtained from local commercial sources.

Culture Medium : The sterile BGJb culture medium was aseptically supplemented with benzyl penicillin sodium 0.06 mg/ml, streptomycin sulphate 0.1 mg/ml and amphotericin B 2.5 µg/ml to prevent the growth of contaminating micro-organisms during the culture period. The culture medium was stored at 4^{o}C, protected from light. The composition of this medium is presented in Table 2.4.

Pineal Gland Culture : The technique employed for pineal culture has been previously described (*Daya* and *Potgieter* 1982, *Daya* and *Fata* 1986). Rat pineal glands were aseptically removed with the minimum of delay and dissected free of adhering tissue. The glands were then individually placed in sterile glass culture tubes, each containing 60 µl of culture medium in which 0.4 µCi of [^{14}C]serotonin was present. The atmosphere within the culture tubes was saturated with 5% CO_2 : 95% O_2. The tubes were immediately sealed and incubated in darkness at 37^{o}C for a specified period of time. A blank containing no tissue was also included in each series of incubations and served to determine background radioactivity for the different indole metabolites. Following the culture period, the glands were removed and the culture medium analysed as described below.

TLC Analysis of Radioactive indoles : The technique employed was a minor modification of the method of *Klein* and *Notides* (1969). To measure the relative amounts of [^{14}C]indoles presented, a 10 µl aliquot of culture medium was spotted on a 10 cm x 10 cm TLC plate. Following this, a solution containing synthetic unlabeled standards of all the pineal indoles to be measured was spotted on top of the culture medium spot. A total of 10 µl of a solution containing 2 µg of each standard was spotted. The indole standard solution was prepared as follows : 1 mg of each standard (listed in Fig. 2.13) was dissolved together in 2.5 ml of 95% ethanol. To this, 2.5 ml of a solution of 1% ascorbic acid in 0.1 N HCl was added. The solution was stored in darkness at -20^{o}C. In all instances the TLC plates were spotted in subdued light and a gentle stream of nitrogen was used to dry the spots. Each spotted plate was then placed in a TLC tank and developed twice in the same direction in a solvent containing chloroform : methanol : acetic acid (93:7:1). The total front movement allowed during each development was 9 cm and the plate was dried under nitrogen between each development. Following this, the plate was developed once in ethyl acetate at right angles to the first direction and with a total front movement of 7 cm. The plate was then dried under nitrogen, sprayed with van Urk's reagent (1 g 4-dimethylamino-

TABLE 2.4 Composition of BGJb culture medium (Fitton-Jackson modification)

CONTENTS	CONCENTRATION (mg/l)
Amino Acids	
L-Alanine	250.00
L-Arginine	175.00
L-Aspartic acid	150.00
L-Cysteine HCl	90.00
L-Glutamine	200.00
Glycine	800.00
L-Histidine	150.00
L-Isoleucine	30.00
L-Leucine	50.00
L-Lysine HCl	240.00
L-Methionine	50.00
L-Phenylalanine	50.00
L-Proline	400.00
L-Serine	200.00
L-Threonine	75.00
L-Tryptophane	40.00
L-Tyrosine	40.00
DL-Valine	65.00
Vitamins	
α-Tocopherol phosphate	1.00
Ascorbic acid	50.00
Biotin	0.20
Calcium pantothenate	0.20
Choline chloride	50.00
Folic acid	0.20
Inositol	0.20
Nicotinamide	20.00
Para aminobenzoic acid	2.00
Pyridoxal phosphate	0.20
Riboflavin	0.20
Thiamine HCl	4.00
Vitamin B_{12}	0.04
Inorganic Salts	
Dihydrogen sodium ortho phosphate	90.00
Magnesium sulphate $7H_2O$	200.00
Potassium chloride	400.00
Potassium dihydrogen phosphate	160.00
Sodium bicarbonate	3 500.00
Sodium chloride	5 300.00
Other Components	
Calcium lactate	555.00
Glucose	10 000.00
Phenol red	20.00
Sodium acetate	50.00

benzaldehyde dissolved in 50 ml of 25% HCl, followed by the addition of 50 ml of 95% ethanol) and dried in an oven at 60°C for 20 min to allow for development of the spots. The spots were then cut out and placed into plastic scintillation vials. One millilitre of absolute ethanol was added to the vials and the vials were left to stand for 20 min. This was done to allow elution of the radioactive metabolites from the cuttings. Thereafter, 3 ml of Ready-Solv HP scintillation fluid (Beckman, USA) was added to each vial and the radioactivity was quantitated as described previously (Section 2.2.1.2) at a counting efficiency of 94%.

2.6.3 Results

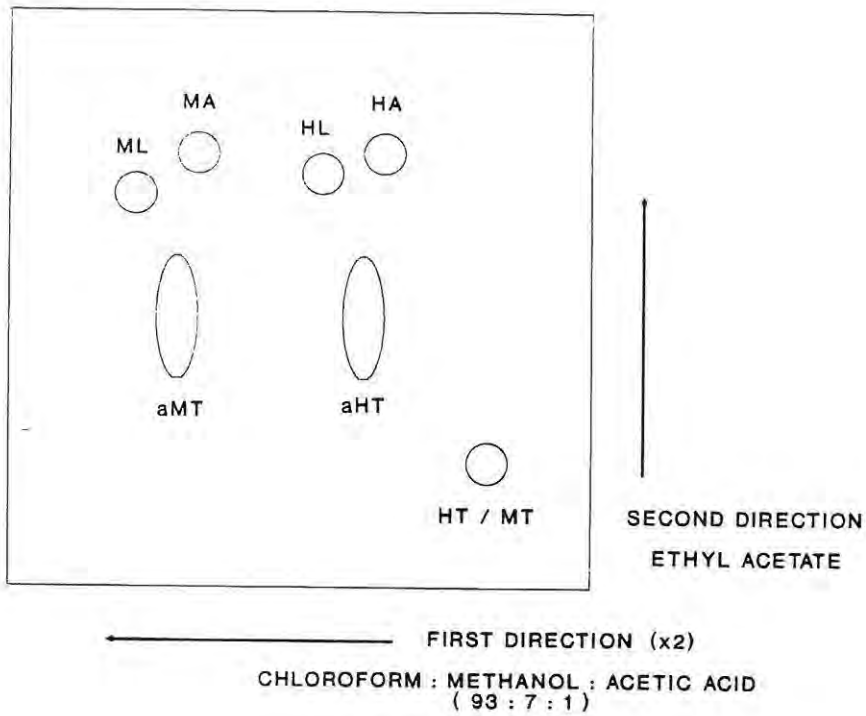
A typical bi-dimensional thin layer chromatogram of the indole metabolites is presented in Fig. 2.13. Adequate separation of all the indoles was achieved and only 5-methoxytryptamine (MT) did not migrate from the starting spot.

Pineal glands from saline treated rats were placed into culture in order to evaluate the relative amounts of [^{14}C]indoles secreted into the culture medium. Aliquots of culture medium were analysed for [^{14}C]indole content after 8 h and 24 h of culture. The relative amount of radioactivity recovered from each indole spot is presented in Table 2.5. The starting spot, containing [^{14}C]serotonin and [^{14}C]MT, represented 79% and 55% of the total counts after 8 h and 24 h of the culture period respectively. The major single metabolite was [^{14}C]5-hydroxyindole acetic acid (HA) representing 15% and 31% of total counts after 8 h and 24 h respectively. Very little radioactivity, less than 5% and 10% respectively, was measured on the other indole metabolites after 8 h and 24 h of culture. The relative amounts of [^{14}C]indoles synthesised from [^{14}C]serotonin is discussed in more detail in Chapter 3.

Evaluation of the level of interference contributed by each of the unlabeled indole standards in the TLC procedure revealed radioactivity counts of 20-50 dpm. These values were not significantly different from blank values obtained by incubation without tissue and represented 0.02-0.05% of the total radioactivity counts.

2.6.4 Discussion

Using TLC, the ability to separate N-acetylserotonin (aHT), melatonin (aMT), 5-hydroxyindole acetic acid (HA), 5-hydroxytryptophol (HL), 5-methoxyindole acetic acid (MA) and 5-methoxytryptophol (ML) was confirmed. The relative amounts of radioactive indoles measured in pineal cultures after 8 h are in agreement with those of reported by *Daya* and *Fata* (1986). Similarly, indole levels measured after 24 h of culture are in accord with previous reports (*Klein* and *Rowe* 1970, *Wainwright* 1977, *Voisin* et al. 1983, *Skene* 1985). The procedure developed above was therefore employed in all subsequent studies.



HT - Serotonin

MT - 5-Methoxytryptamine

aHT - N-Acetylserotonin

aMT - Melatonin

HA - 5-Hydroxyindole acetic acid

HL - 5-Hydroxytryptophol

MA - 5-Methoxyindole acetic acid

ML - 5-Methoxytryptophol

Figure 2.13 A typical bi-dimensional thin layer chromatogram of the pineal indoles tested.

The pineal indoles are abbreviated according to Smith (1982).

TABLE 2.5 Relative amounts of [^{14}C]indoles synthesised in pineal glands of saline-treated rats

[^{14}C]INDOLE METABOLITES	% RADIOACTIVITY ON EACH SPOT (DPM%)	
	8 HOURS	24 HOURS
N-Acetylserotonin	0.34 ± 0.02	1.68 ± 0.19
Melatonin	0.67 ± 0.04	1.92 ± 0.11
5-Hydroxyindole acetic acid	14.95 ± 1.01	31.30 ± 2.22
5-Hydroxytryptophol	4.26 ± 0.63	9.01 ± 1.10
5-Methoxyindole acetic acid	0.20 ± 0.02	0.34 ± 0.03
5-Methoxytryptophol	0.36 ± 0.05	0.49 ± 0.05
Starting spot	79.21 ± 2.69	55.26 ± 1.37

Rats were injected with 0.9% saline (1 ml/kg) once daily for 14 days. Values are expressed as means ± S.E.M. (n = 6).

CHAPTER THREE

INDOLEAMINE METABOLISM BY ORGAN CULTURES OF RAT PINEAL GLANDS

3.1 INTRODUCTION

The pineal gland in culture is able to utilize exogenous radioactive serotonin to produce various indoles including the neurohormone melatonin and its precursor, N-acetylserotonin. The labeled indole metabolites, which are secreted into the culture medium, can be measured using radiospectrometry.

Based on this principle, the method developed by *Klein* and *Notides* (1969) provided the opportunity for many researchers to examine the metabolism of indoles in pineal cultures of many species. *Wainwright* (1977) reported that the major metabolite produced from [^{14}C]serotonin in the chick pineal gland was 5-hydroxyindole acetic acid (HA), followed by 5-methoxyindole acetic acid (MA). Negligible amounts of 5-hydroxytryptophol (HL) were detected. In the pigeon pineal gland, on the other hand, HL was a major metabolite of [^{3}H]serotonin, second only to HA (*Voisin* et al. 1983). In these studies, HA showed the highest level of radioactivity in rat, chick and pigeon pineal organ cultures.

Several researchers who have employed pineal gland organ culture systems usually terminate the culture period after 24 h and measure the levels of radioactive metabolites accumulated in the culture medium (see Section 2.6.1). These studies, however, do not provide information on the progressive formation of the various indole metabolites or of the relative proportion of these metabolites at any particular stage of the culture period. Such information would be useful, since it would also indicate the ideal time to terminate the culture period. It is generally accepted that the rat pineal gland survives well in culture. However, under culture conditions the adrenergic innervation of the gland degenerates (*Minneman* 1977). Such a phenomenon could undermine a meaningful correlation with *in vivo* data. In addition, it could conceivably interfere with the effects of pharmacological manipulation prior to, or during, the culture period.

Thus, it was deemed necessary to monitor the progressive formation of [^{14}C]indoles in the rat pineal over a 24 h culture period. Furthermore, since little is known of the effect that adrenergic drugs have on the pattern of indole formation, the β-adrenoceptor agonist isoprenaline was included for comparison.

3.2 EXPERIMENT 1 : A 24-HOUR PROFILE OF [^{14}C]INDOLE METABOLISM BY ORGAN CULTURES OF RAT PINEAL GLANDS

3.2.1 Materials and Methods

Animals : Naive, untreated male rats of the Wistar strain (200-250 g) were used in the experiment. The

animals were maintained under an automatically regulated lighting cycle of LD 12:12. Their living environment is described in detail in Section 2.1. All animals were sacrificed at 09:00 h.

Chemicals and Reagents : 5-Hydroxy (side-chain-2-^{14}C) tryptamine creatinine sulphate (specific activity 57 mCi/mmol) was purchased from Amersham (England) and *l*-isoprenaline HCl from Sigma Chemical Co. (USA). All other chemicals and reagents were obtained from previously acknowledged sources (Section 2.6.2).

Pineal Organ Culture and Analysis of [^{14}C]Indoles : Briefly, pineals glands (n = 6) were aseptically removed and individually cultured in the presence of 0.4 μCi [^{14}C]serotonin and 10 μM isoprenaline at 37^{o}C, with a relative humidity of 95% and in an atmosphere of 5% CO_2 : 95% O_2 as described previously (Section 2.6.2). In addition, a set of control pineal glands (n = 6) was incubated in the absence of isoprenaline. At two hourly intervals, 5 μl aliquots of culture medium were removed for analysis from each of the tubes and an equivalent volume immediately replenished from an identical set of tubes containing no tissue. The [^{14}C]indoles in the 5 μl samples were isolated using bi-dimensional TLC and quantitated with the aid of liquid scintillometry as described previously (Section 2.6.2).

Analysis of Data : Statistical comparisons were determined using Student's *t*-tests. All data are expressed as DPM/5 μl medium/pineal. Values represent means ± S.E.M. (n = 6).

3.2.2 Results

The production of melatonin (aMT) and its precursor, N-acetylserotonin (aHT), rose sharply in isoprenaline-stimulated pineals and progressively increased, reaching a peak after 16 h of culture (Fig. 3.1). This peak in the levels of aMT and aHT was significantly higher in isoprenaline-stimulated pineals than in unstimulated control pineals ($p < 0.001$). The level of aHT closely followed that of aMT in control pineals up to 16 h, after which aMT decreased to a significantly lower level than that at 16 h of culture ($p < 0.05$). In isoprenaline-stimulated pineals, on the other hand, aMT levels were always significantly higher than aHT levels and remained so even after 24 h of culture. In both stimulated and control pineals, no further increases were observed after 16 h in culture.

The levels of the other indole metabolites, 5-hydroxyindole acetic acid (HA), 5-hydroxytryptophol (HL), 5-methoxyindole acetic acid (MA) and 5-methoxytryptophol (ML), all progressively increased up to 14-16 h in culture with HA > HL > ML > MA (Figs. 3.2-3.5). At this time of the culture period, there were no significant differences in the levels of these metabolites between stimulated and control pineals. However, during the first few hours of the culture period, isoprenaline-stimulated pineal glands showed an increase in HA and HL production ($p < 0.05$) (Figs. 3.2 and 3.3) with a corresponding decrease in MA and ML production ($p < 0.05$) (Figs. 3.4 and 3.5). The increased levels of the 5-hydroxyindoles persisted for the first 4 h of culture, while the decreased levels of MA and ML were sustained for 12 h

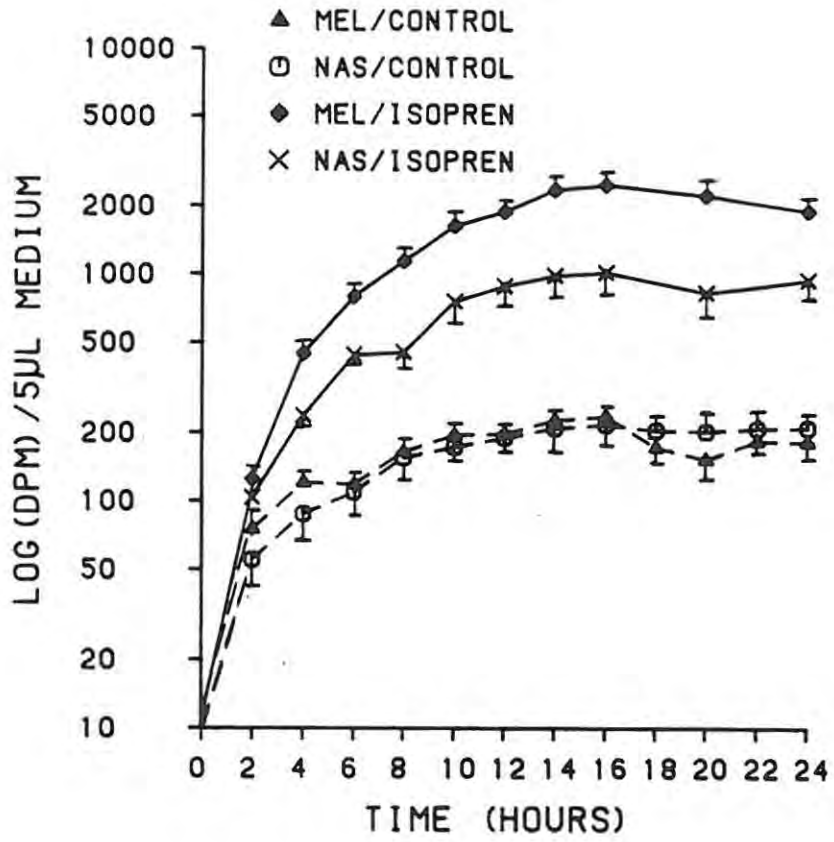


Figure 3.1 A 24-h profile of [^{14}C]serotonin metabolism to N-acetylserotonin (aHT) and melatonin (aMT) in isoprenaline-stimulated and control pineal glands.

Results are expressed as DPM/5 µl medium (mean ± S.E.M.; n=6).

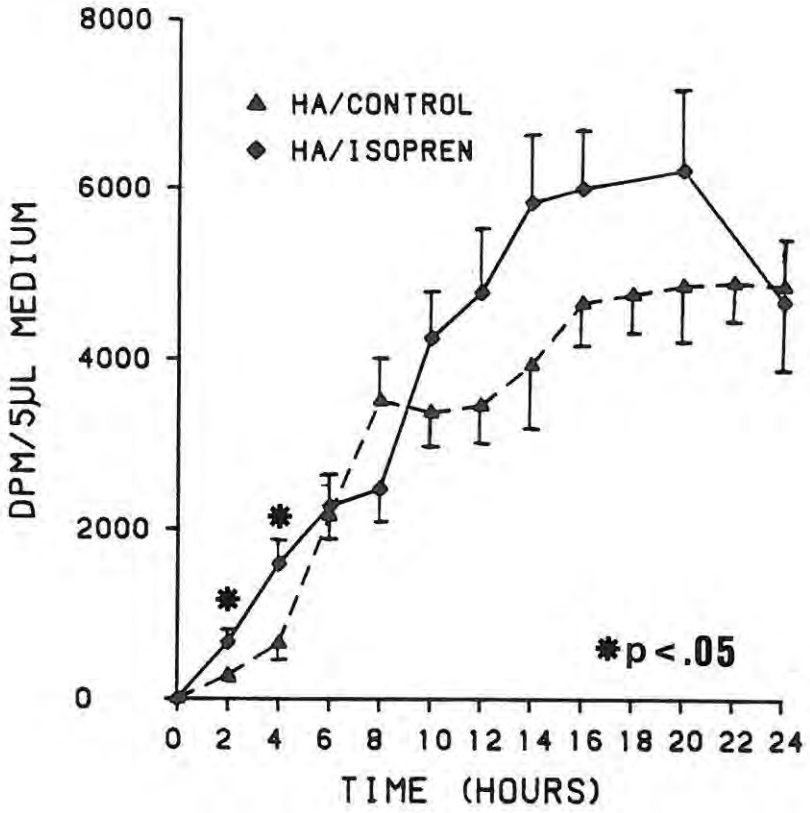


Figure 3.2 A 24-h profile of [^{14}C]serotonin metabolism to 5-hydroxyindole acetic acid (HA) in isoprenaline-stimulated and control pineal glands.
Results are expressed as DPM/5 µl medium (mean ± S.E.M.; n=6).

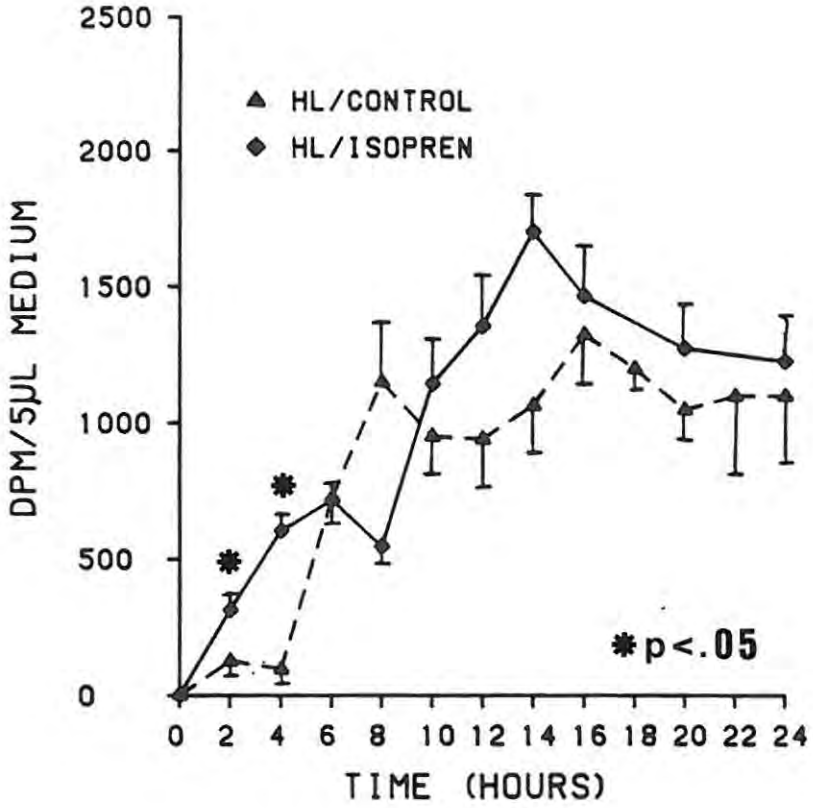


Figure 3.3 A 24-h profile of [^{14}C]serotonin metabolism to 5-hydroxytryptophol (HL) in isoprenaline-stimulated and control pineal glands.

Results are expressed as DPM/5 µl medium (mean ± S.E.M.; n = 6).

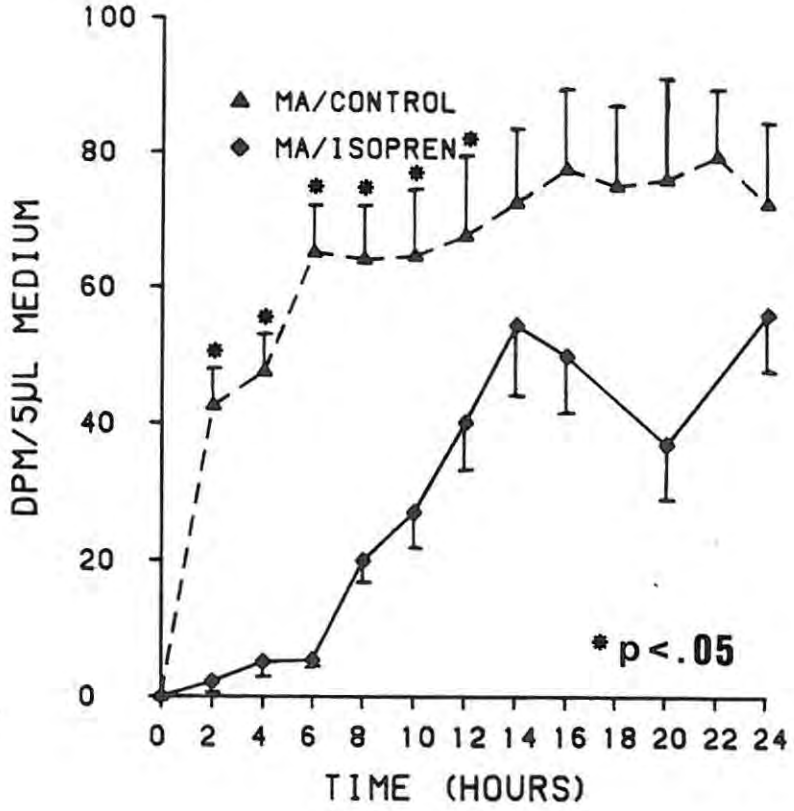


Figure 3.4 A 24-h profile of [^{14}C]serotonin metabolism to 5-methoxyindole acetic acid (MA) in isoprenaline-stimulated and control pineal glands.
Results are expressed as DPM/5 µl medium (mean ± S.E.M.; n=6).

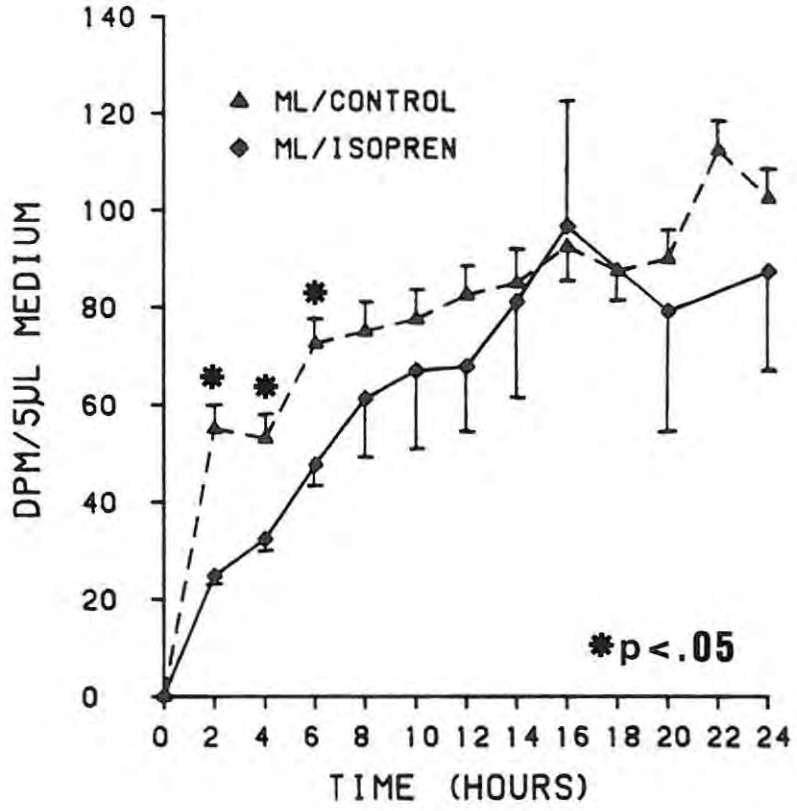


Figure 3.5 A 24-h profile of [^{14}C]serotonin metabolism to 5-methoxytryptophol (ML) in isoprenaline-stimulated and control pineal glands.

Results are expressed as DPM/5 µl medium (mean ± S.E.M.; n = 6).

and 6 h respectively in isoprenaline-stimulated pineals. At 6 h of culture, the levels of the 5-hydroxyindoles were almost superimposable in control and stimulated pineals.

3.2.3 Discussion

As is evident in Fig. 3.1, the rat pineal gland in culture is able to utilize exogenous serotonin to synthesize N-acetylserotonin (aHT) and melatonin (aMT). In addition, the production of these indoles can be sustained for at least 24 h of culture. Stimulation with the β-adrenoceptor agonist isoprenaline causes a marked rise in the synthesis of aHT and aMT when compared with unstimulated control pineal glands. These observations are consistent with those of other workers (eg. *Axelrod* et al. 1969, *Klein* and *Berg* 1970) and demonstrate that the synthesis of aMT is under β-adrenergic control (see Section 1.3.3.1). The marked increase in aHT and aMT levels during the initial culture period can be explained by the observation that the activity of NAT, the enzyme responsible for the synthesis of aHT, increases maximally within 3 h in pineals treated with isoprenaline (*Deguchi* 1973). After 16 h in culture, the aMT level is approximately 10-fold greater and the aHT, 4-fold greater in isoprenaline treated pineals compared to untreated controls.

The methylation of aHT to aMT as well as the other 5-hydroxyindoles to 5-methoxyindoles is controlled by the enzyme HIOMT. Interestingly, isoprenaline significantly reduced the levels of the 5-methoxy-indoles (Figs. 3.2 and 3.3) during the initial culture period, suggesting that substrate competition for HIOMT occurs due to the high aHT levels. On the other hand, it has been shown that aHT elicits the highest substrate specificity for HIOMT compared to other indoles (*Axelrod* and *Weissbach* 1961), suggesting that it is the preferred substrate *in vivo*. Moreover, studies have shown that methoxyindole synthesis occurs during the light phase of the light:dark cycle when a high concentration of aHT is not present (*Carter* et al. 1979, *Pévet* et al. 1980).

Of interest also, is the observation that the levels of the 5-hydroxyindoles are significantly increased by isoprenaline during the initial 4 h of culture (Figs. 3.4 and 3.5). Again, this observation may be explained by the preference of the methylating enzyme for aHT. However, the rather high levels of the 5-hydroxy-indoles do not altogether account for the lack of methylation by HIOMT, since only small quantities of the 5-hydroxyindoles are normally converted to their respective 5-methoxyindoles. This poses the possibility that isoprenaline may influence the synthesis of the 5-hydroxyindoles by another mechanism. It is interesting to speculate that the production of pineal hydroxyindole acetic acid (HA) and hydroxytryptophol (HL) by the enzyme monoamine oxidase (MAO) may be regulated, in part, via a postsynaptic β-adrenoceptor mechanism. Recently, studies by *Olivieri* et al. (1990) provide further support for this notion. These investigators showed the synthesis of MAO products to be significantly increased in pineals cultured at night compared with daytime pineal cultures, suggesting that NA released from pineal nerves at night stimulates MAO activity. However, further research is indicated in this area to confirm these findings.

3.3 CONCLUSION

From these results it appears that 16 h may be the optimal time to terminate the culture period since N-acetylserotonin and melatonin reach maximum levels by this time. However, since isoprenaline appears to influence the levels of the other indoles during the initial period of culture only, it may be more useful to terminate the incubation period much earlier. Moreover, endogenous neurotransmitter substances released by degenerating nerve terminals might influence the levels of the various indoles during the latter part of the culture period. Thus, unless indicated otherwise, an eight-hour incubation period was employed in all subsequent pineal culture studies.

CHAPTER FOUR

STUDIES WITH RESERPINE IN THE RAT PINEAL GLAND

4.1 INTRODUCTION

Reserpine depletes intraneuronal catecholamine and serotonin (5-HT) stores in many organs including the brain and most of its pharmacological effects have been attributed to this action. Originally intended as an antihypertensive agent, its use in this area, however, has largely been superseded by other safer and more specific drugs. The drug has, nonetheless, retained a place as a pharmacological tool, particularly in the field of neuropharmacology, and its application in this regard was the subject of later studies (see Chapter 7).

In a given tissue, the neurotransmitter catecholamines control a variety of metabolic processes by stimulating the formation of cAMP via a β-adrenoceptor. Similarly, in the rat pineal gland β-adrenoceptor stimulation results in the accumulation of cAMP via an increase in adenylate cyclase activity. Cyclic AMP acts to increase the biosynthesis of the enzyme serotonin N-acetyltransferase (NAT), which catalyses the conversion of serotonin to N-acetylserotonin, the precursor of melatonin, a pineal hormone (see Section 1.3.3.1). Thus, the pineal gland is a sensitive and useful model for studying β-adrenoceptors as it provides a system in which the final noradrenergic signal can be assessed.

In the rat pineal gland, the responsiveness of this system to catecholamines may be modified by the prior degree of sympathetic input to the gland. Thus, reducing sympathetic input surgically, chemically, or physiologically increases the density of β-adrenoceptors and produces a supersensitive response of adenylate cyclase to noradrenaline (NA) (*Cantor* et al. 1981). Conversely, chronic overexposure to β-adrenergic agonists decreases the density of β-adrenoceptors and produces a subsensitive response of the enzyme to catecholamines (*Mickey* et al. 1975).

The present study aimed at investigating the functional status of the rat pineal gland following chronic deprivation of sympathetic input as a result of reserpine treatment. It is generally assumed that reducing sympathetic input to the pineal gland in this way would result in a corresponding reduction in N-acetylserotonin and melatonin output. Experimental evidence to confirm this, however, appears to be lacking. Furthermore, whether reserpine treatment produces an enhanced response of these hormones to catecholamines, likewise, requires clarification. Thus, in the sections that follow, the effect of reserpine treatment on rat pineal β-adrenoceptor binding, NAT activity and indoleamine metabolism was investigated.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Adult male rats (200-300 g) of the Wistar strain were used throughout this study. The animals were maintained under an automatically regulated lighting cycle with a photoperiod of 12 h (lights on at 06:00 h). All animals had free access to food and water. Their living environment is described in detail in Section 2.1.

4.2.2 Drugs

Reserpine HCl used in this study, was kindly donated by Lennon (SA). The drug was administered as a solution in a vehicle consisting of the mixture (% by volume), 2% benzyl alcohol + 10% polysorbate + 88% distilled water. The mixture contained 0.25% (w/v) citric acid as antioxidant. Control rats received vehicle only, in a volume of 2 ml/kg. The dose of reserpine, reported as milligrams per kilogram, was based on the weight of the HCl salt supplied.

4.2.3 Statistical Analysis

Statistical comparisons were determined using paired Student's *t*-tests. A *P* value of greater than 0.05 between groups was considered not significantly different.

4.3 EXPERIMENT 1 : EFFECT OF RESERPINE ON PINEAL β-ADRENOCEPTORS

4.3.1 Introduction

The rat pineal gland is a rich source of β-adrenoceptors. These receptors exhibit a diurnal variation in density (*Romero* et al. 1975) and display the pharmacological characteristics typical of the β_1-subtype (*Dickinson* et al. 1986).

The following experiment was conducted to evaluate the effect of reserpine-induced chemical sympathectomy on the binding of [^{3}H]DHA to pineal β-adrenoceptors. In addition, it was of interest to determine the duration of effect that a given dose of reserpine would have on pineal [^{3}H]DHA binding.

4.3.2 Experimental Procedure

Drug Administration: Animals were treated with either reserpine, 5 mg/kg i.p., or vehicle, once daily at 10:00 h for 2 days. Pineals were collected 24 h after the last treatment and assayed. Table 4.1 details the schedule of drug administration for the experiment aimed at determining the duration of effect of a

given dose of reserpine. For example, rats whose pineals were assayed 1 day after the last dose of reserpine received vehicle initially for 6 consecutive days, followed by reserpine for 2 consecutive days. Likewise, rats whose pineals were assayed 7 days after the last dose of reserpine received reserpine initially for 2 consecutive days, followed by vehicle for 6 consecutive days. In this way, animals in the different groups received the same number of injections and were handled for the same number of times on the same days. This treatment regimen ensured that, at the time of sacrifice, all the animals had experienced the same stress induced by the injections. In addition, this regimen allowed for all the animals in the different experimental groups to be killed on the same day, thus avoiding the likelihood of day to day variation.

Assay of β-Adrenoceptors : β-Adrenoceptors were assayed by measuring the binding at a single concentration of [^{3}H]DHA (5 nM) to pineal gland membranes. The assay was performed as outlined in Section 2.2.1.2.

Analysis of Data : Specific [^{3}H]DHA binding is expressed as fmol/mg protein. Values represent means ± S.E.M. of three or four separate experiments.

4.3.3 Results

The effect of reserpine on the binding of [^{3}H]DHA to rat pineal homogenates is shown in Fig. 4.1. Reserpine treatment significantly increased the specific binding of [^{3}H]DHA to β-adrenoceptors ($p < 0.01$).

To determine the duration of this effect, pineal glands were collected at various times following the last dose of reserpine. The results of this experiment are depicted in Fig. 4.2. Treatment of rats with reserpine significantly increased the specific binding of [^{3}H]DHA to pineal homogenates for at least 4 days after the final injection ($p < 0.05$). [^{3}H]DHA binding was also slightly increased in reserpinized pineals one week after cessation of treatment, although this was not significantly different from controls.

4.3.4 Discussion

The results of this experiment are in agreement with those of other workers and demonstrate that chemical sympathectomy increases the density of β-adrenoceptors in the rat pineal gland as measured by [^{3}H]DHA binding (*Cantor* et al. 1981). *Romero* et al. (1975) showed that exposing rats to constant light, which decreases adrenergic input to the pineal, increased the density of β-adrenoceptors. Similarly, surgically decentralizing the superior cervical ganglia increases the density of pineal β-adrenoceptors (*Cantor* et al. 1981). Using Scatchard analysis, these investigators showed that the increase in β-adrenoceptor density following a surgical, physiological or chemical reduction in sympathetic input was due to an increase in B_{max} and not to a change in the affinity of the receptors for the ligand.

TABLE 4.1 Schedule of drug administration to determine the duration of effect of a given dose of reserpine

	NUMBER OF DRUG TREATMENTS							
	1	2	3	4	5	6	7	8
RESERPINE (1 Day)	v	v	v	v	v	v	**R**	**R**
RESERPINE (2 Days)	v	v	v	v	v	**R**	**R**	v
RESERPINE (3 Days)	v	v	v	v	**R**	**R**	v	v
RESERPINE (4 Days)	v	v	v	**R**	**R**	v	v	v
RESERPINE (7 Days)	**R**	**R**	v	v	v	v	v	v
VEHICLE CONTROL	v	v	v	v	v	v	v	v

Rats treated with reserpine (**R**), 5 mg/kg per day, and/or vehicle (v), 2 ml/kg per day, each received a total of 8 injections. Pineal glands from each experimental group were collected on the same day, 24 h after the last dose. Numbers in parentheses refer to the number of days following the last dose of reserpine that pineals were collected. Six rats were used in each experimental group.

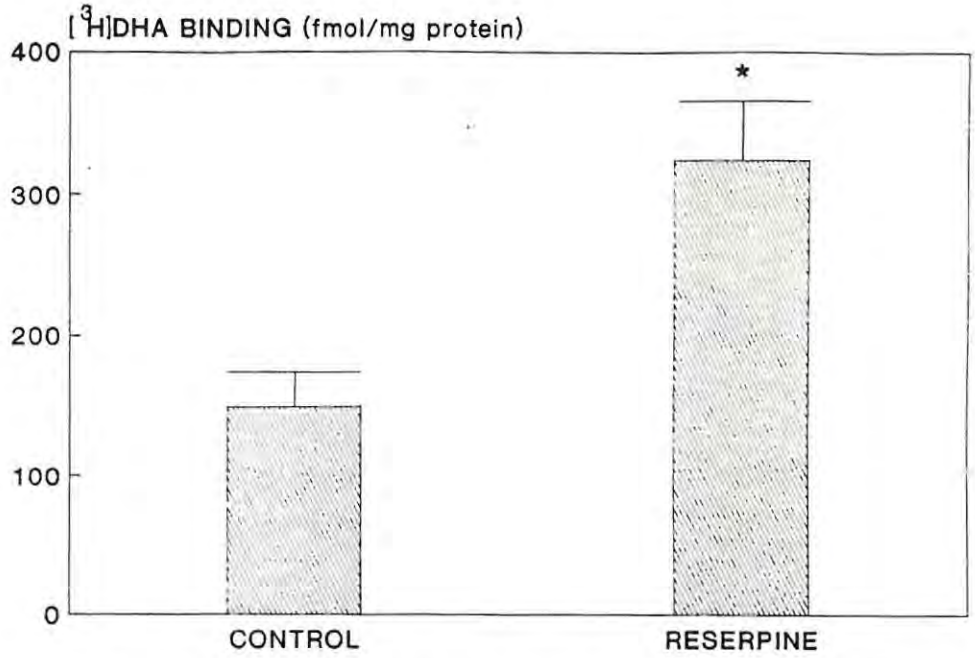


Figure 4.1 Effect of reserpine treatment on the specific binding of [^{3}H]DHA in the rat pineal gland.

Values represent means ± S.E.M. Eight rats were used in each experimental group.

* $p < 0.01$ when compared with controls.

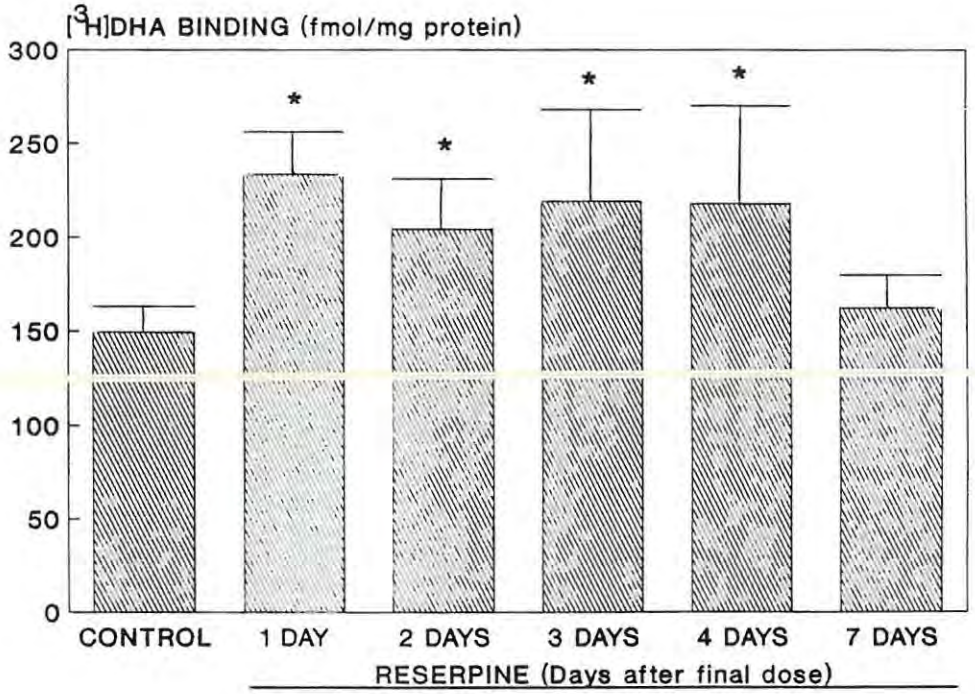


Figure 4.2 Duration of effect of reserpine treatment on the specific binding of [^{3}H]DHA in the rat pineal gland.

Values represent means ± S.E.M. Six rats were used in each experimental group.

* $p < 0.05$ when compared with controls.

Furthermore, the observation that the effect of reserpine on β-adrenoceptor density persists for at least 4 days suggests that the depletion of catecholamines is sustained long after drug administration. These results are consistent with the hypothesis that restoration of normal intraneuronal stores of noradrenaline (NA) is dependent on transport of new storage vesicles down the axon, a process that occurs over a period of days. Furthermore, studies with labeled reserpine suggest that drug remaining in tissues after 24 to 30 h is firmly bound and may persist for many days (*Weiner* 1985). Reserpine also decreases NA synthesis, probably as a result of the inhibition of dopamine uptake into storage granules that contain the enzyme dopamine β-hydroxalase (*Pfeffer* et al. 1975).

4.4 EXPERIMENT 2 : EFFECT OF RESERPINE ON PINEAL SEROTONIN N-ACETYLTRANSFERASE ACTIVITY

4.4.1 Introduction

The activity of serotonin N-acetyltransferase (NAT), the enzyme that catalyses the conversion of serotonin to N-acetylserotonin, exhibits a marked diurnal variation and is regulated by noradrenaline (NA) released from sympathetic nerve endings in the pineal gland. Thus, the measurement of NAT activity offers a productive model for studying the mechanisms underlying altered sensitivity in adrenergically innervated end organs.

To correlate the previously observed changes in pineal β-adrenoceptor density with a functional biochemical parameter, the effect of reserpine on daytime and nocturnal rat pineal NAT activity was investigated.

4.4.2 Experimental Procedure

Drug Administration : Groups of rats were treated either with reserpine, 5 mg/kg i.p., or with vehicle, once daily at 10:00 h for 2 days. For daytime experiments, pineals were collected 24 h after the last dose and assayed. During dark phase experiments, pineals were collected at midnight on the day of the last dose, frozen on solid CO_2 and stored at -70°C prior to analysis.

Assay of Serotonin N-Acetyltransferase : Pineal glands collected during the dark phase and stored at -70°C were allowed to thaw on ice prior to assaying enzyme activity. Pineals collected during the light phase were cultured in the presence or absence of 10 μM *l*-isoprenaline for 3 h prior to the determination of enzyme activity. The technique of pineal culture and assay of enzyme activity were performed as outlined in Section 2.5.2.

Analysis of Data : Enzyme activity is expressed as picomoles of [^{3}H]N-acetyltryptamine formed per pineal per hour (pmol/pineal/h). Values represent means ± S.E.M. of four experiments.

4.4.3 Results

Light Phase Enzyme Activity : Figure 4.3 shows that daytime basal NAT activity was unchanged by reserpine treatment. To determine whether the increased density of β-adrenoceptors in reserpinized pineal glands was associated with a concomitant increase in catecholamine-sensitive NAT activity, pineals were treated with 10 μM isoprenaline *in vitro* (Fig. 4.3). Isoprenaline treatment produced a significant increase in enzyme activity in both reserpinized and control groups when compared with the corresponding unstimulated groups ($p < 0.001$). However, the response to isoprenaline in pineals from reserpinized rats was more than twice that of the control animals ($p < 0.001$).

Dark Phase Enzyme Activity : The effect of reserpine on the nocturnal noradrenaline-induced rise in NAT activity is shown in Fig. 4.4. Reserpine treatment prevented the nocturnal rise in NAT activity that was evident in the control group ($p < 0.001$). Enzyme activity was more than 10-fold higher in control pineals when compared with reserpinized pineals. In addition, the nocturnal release of noradrenaline increased NAT activity by more than 25-fold in control pineals when these were compared with daytime basal enzyme activity (see Figs. 4.3 and 4.4).

4.4.4 Discussion

The present results are in accord with those of other workers (eg. *Klein* et al. 1971) and demonstrate that pineal NAT activity exhibits a marked circadian change, with a 25-fold increase in enzyme activity during darkness, controlled by sympathetic nerves. Activation of pineal β-adrenoceptor-mediated adenylate cyclase enhances cAMP synthesis which elicits the induction of NAT via cAMP-dependent protein kinase (see Section 1.3.3.1). Moreover, these results show that NAT activity is rapidly inducible by exogenous catecholamines during daytime, indicating that the low NAT activity at this time is not due to a lack of responsiveness of β-adrenoceptors, but is due to a lack of or reduced release of neurotransmitter.

The observation that reserpine treatment did not alter daytime basal NAT activity and prevented the darkness-induced increase in enzyme activity, is consistent with the hypothesis that the drug exerts its action by depleting intraneuronal catecholamine stores (*Deguchi* and *Axelrod* 1972b). In addition, the response of the enzyme to exogenous isoprenaline was enhanced by reserpine treatment. These results are in agreement with earlier studies showing an enhanced responsiveness of adenylate cyclase to catecholamines in pineal glands from rats in which sympathetic input to the gland was reduced (*Weiss* 1969, *Cantor* et al. 1981).

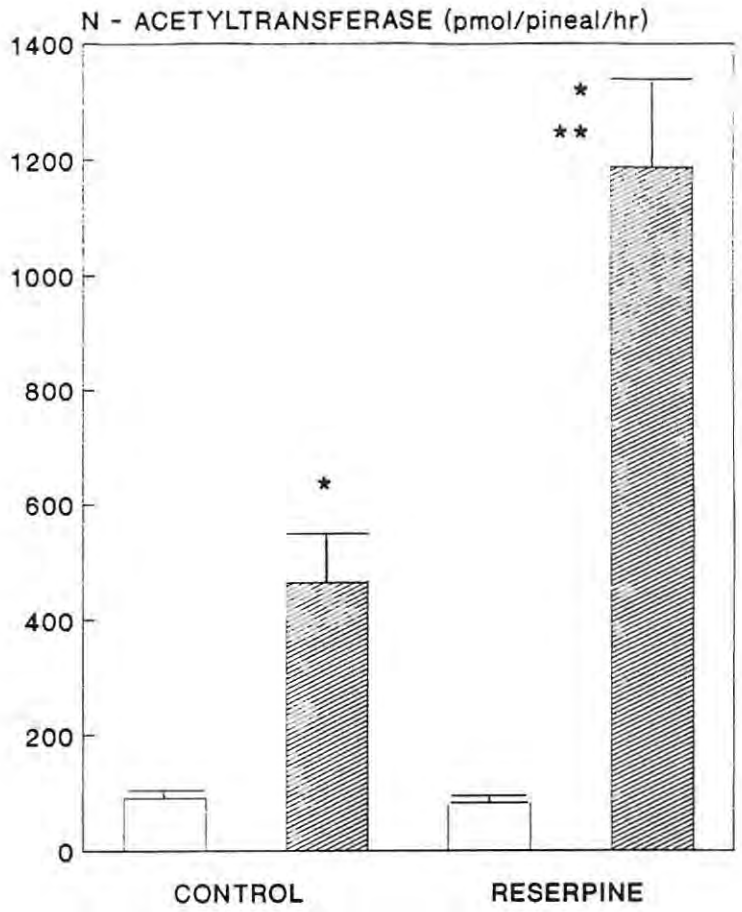


Figure 4.3 Effect of reserpine treatment on daytime basal and isoprenaline-stimulated N-acetyltransferase (NAT) activity.

Plain and striped bars depict basal and isoprenaline-stimulated enzyme levels, respectively. Values represent means ± S.E.M. Four rats were used in each experimental group.

* $p < 0.01$ when compared with unstimulated controls.

** $p < 0.01$ when compared with isoprenaline-stimulated controls.

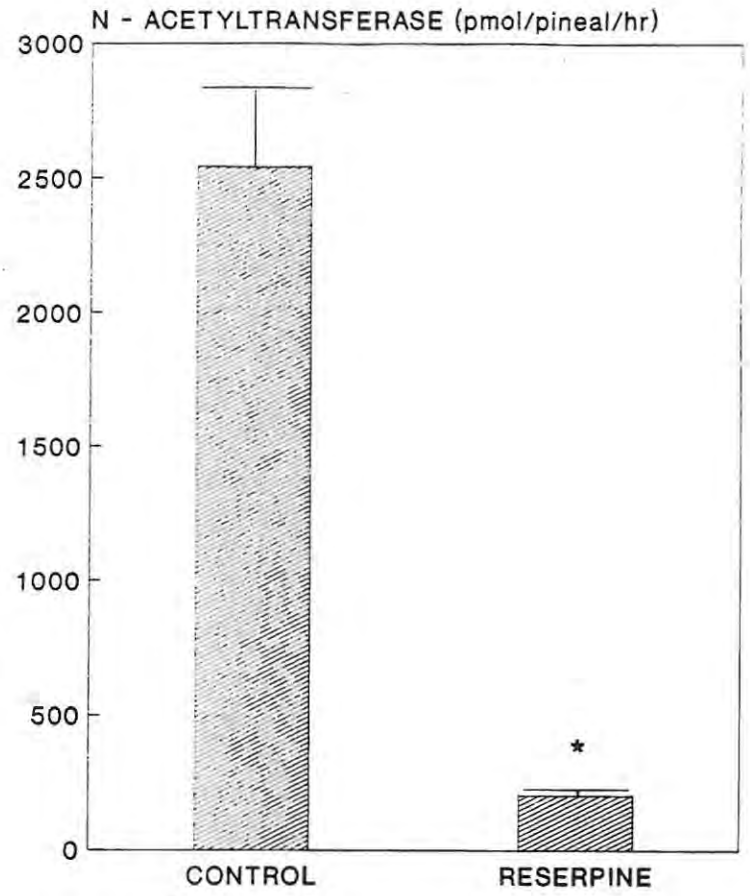


Figure 4.4 Effect of reserpine treatment on the darkness-induced rise in N-acetyltransferase (NAT) activity.

Values represent means ± S.E.M. Four rats were used in each experimental group.

* $p < 0.01$ when compared with controls

4.5 EXPERIMENT 3 : EFFECT OF RESERPINE ON [^{14}C]INDOLE METABOLISM BY ORGAN CULTURES OF RAT PINEAL GLANDS

4.5.1 Introduction

In the rat pineal gland, melatonin synthesis and release is under adrenergic control. Previous studies have shown that the addition of catecholamines to the media of organ cultures of pineal glands results in an increased conversion of [^{14}C]serotonin to [^{14}C]N-acetylserotonin and [^{14}C]melatonin (*Klein* and *Berg* 1970, *Banoo* et al. 1987, see Chapter 3). Thus, the measurement of pineal N-acetylserotonin and melatonin biosynthesis provides yet another convenient model for studying the mechanisms underlying alterations in sympathetic input to end organs.

Previous experiments demonstrated that reserpine treatment increases the density of pineal β-adrenoceptors (Section 4.3) and concomitantly produces a superinduction of catecholamine-sensitive NAT activity (Section 4.4). In the following experiment, the functional significance of these changes on the metabolism of radiolabeled serotonin by organ cultures of rat pineal glands was investigated.

4.5.2 Experimental Procedure

Drug Administration : Groups of rats were treated either with reserpine, 5 mg/kg i.p., or vehicle once daily at 10:00 h for 2 days. Pineal glands were collected 24 h after the last dose for use in the experiment.

Pineal Organ Culture and Analysis of [^{14}C]Indoles : The technique of pineal organ culture and the analysis of labeled indoles was performed as outlined in Section 2.6.2. Briefly, pineal glands were individually cultured in 60 μl of culture medium containing 0.4 μCi of [^{14}C]serotonin and incubated at 37^{o}C under 95% O_2 : 5% CO_2. After 8 h in culture, 10 μM *l*-isoprenaline was added to the culture media and the pineal glands were re-incubated for a further 16 h. Ten microlitre aliquots of culture medium were removed after 8 h and 24 h of incubation. The [^{14}C]indoles in the samples were isolated using bi-dimensional TLC and quantitated with the aid of liquid scintillometry.

Analysis of Data : Results are expressed as the percentage of total radioactivity (DPM%/10 μl medium/pineal). Values represent means ± S.E.M ($n=5$).

4.5.3 Results

4.5.3.1 Effect of Reserpine on the Synthesis of [^{14}C]N-Acetylated Indoles

Reserpine treatment significantly reduced the synthesis of N-acetylserotonin (aHT) and melatonin (aMT) in pineal glands cultured for 8 h ($p<0.05$) (Figs. 4.5 and 4.6). On the other hand, after 24 h of

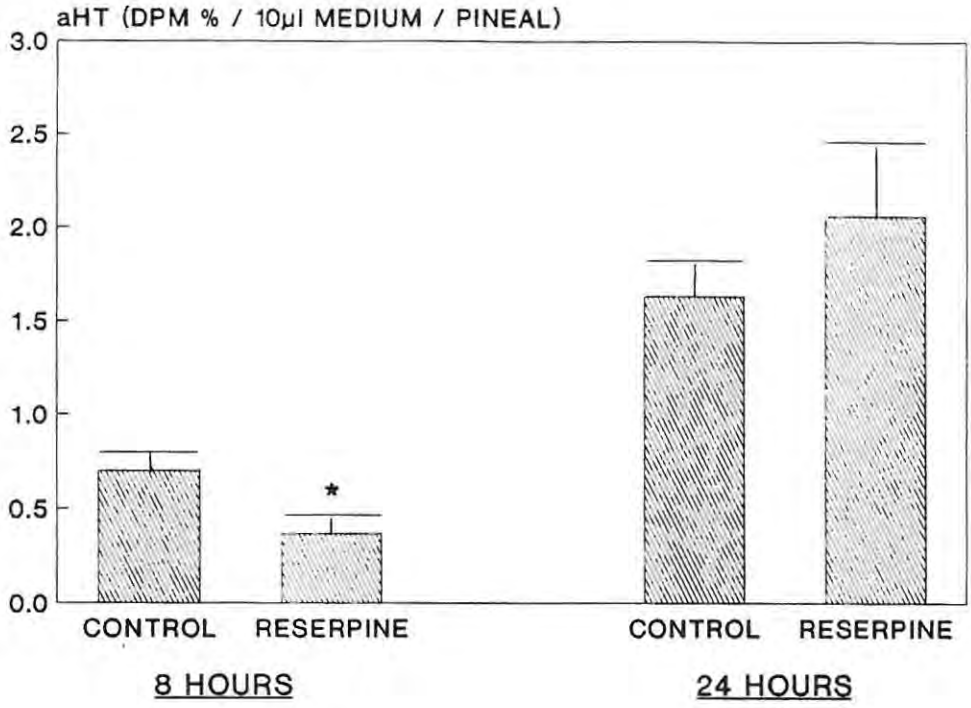


Figure 4.5 Effect of reserpine treatment on [^{14}C]N-acetylserotonin (aHT) synthesis by organ cultures of rat pineal glands.

Isoprenaline stimulation is depicted by 24-h levels. Values represent means ± S.E.M. Five rats were used in each experimental group.

* $p < 0.05$ when compared with controls.

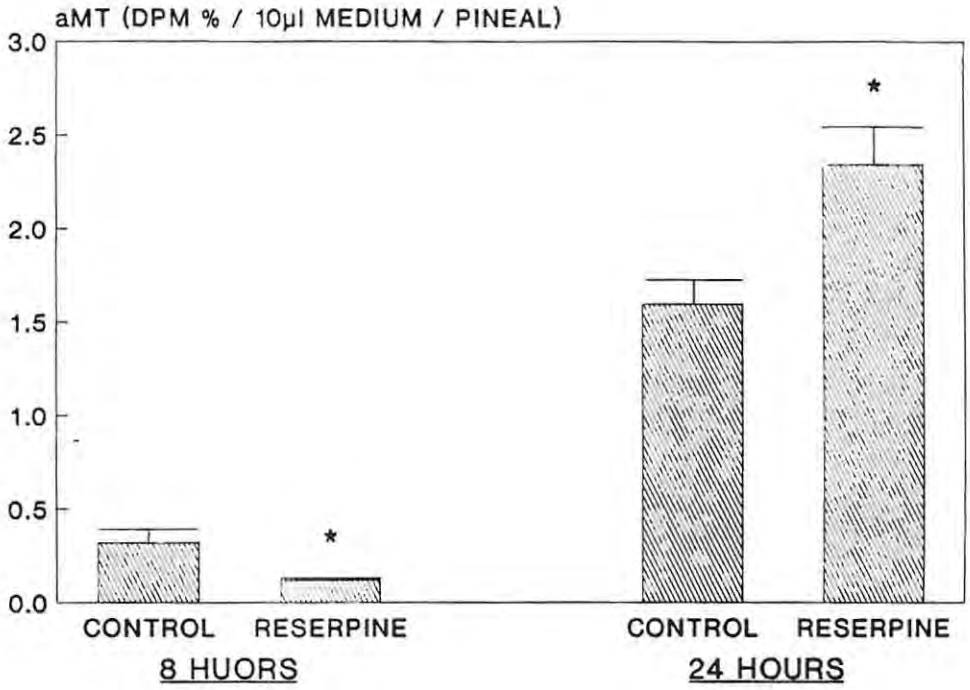


Figure 4.6 Effect of reserpine treatment on [^{14}C]melatonin (aMT) synthesis by organ cultures of rat pineal glands.

Isoprenaline stimulation is depicted by 24-h levels. Values represent means ± S.E.M. Five rats were used in each experimental group.

* $p < 0.05$ when compared with controls.

culture in the presence of isoprenaline, reserpinized pineals showed an increased synthesis of aHT and aMT ($p<0.05$) when compared with controls, although the increase in aHT synthesis was not statistically significant (Figs. 4.5 and 4.6).

When the response to isoprenaline stimulation was calculated as the difference between 24 h and 8 h levels, reserpinized pineals showed a significantly increased level of aHT (1.69 ± 0.30 DPM%) and aMT (2.22 ± 0.19 DPM%) compared to aHT (0.93 ± 0.11 DPM%) and aMT (1.27 ± 0.14 DPM%) in control pineals ($p<0.05$).

The percentage of radioactivity recovered as N-acetylated products, representing the sum of the radioactivity detected on the aHT and aMT spots, was also determined (Fig. 4.7). The synthesis of N-acetylated products in reserpinized pineals was significantly reduced after 8 h of culture ($p<0.05$) and significantly increased after 24 h of culture, in the presence of isoprenaline ($p<0.05$). Calculation of the response to isoprenaline stimulation, revealed significantly increased N-acetylated products in reserpinized pineals (3.92 ± 0.46 DPM%) compared to controls (2.21 ± 0.25 DPM%).

4.5.3.2 Effect of Reserpine on the Synthesis of [^{14}C] Deaminated Indoles

The results are presented in Figs. 4.8 - 4.11. Reserpine treatment did not produce significant changes in the synthesis of 5-hydroxyindole acetic acid (HA) (Fig. 4.8), 5-hydroxytryptophol (HL) (Fig. 4.9) and 5-methoxyindole acetic acid (MA) (Fig. 4.10) after 8 h of culture. However, the synthesis of 5-methoxy-tryptophol (ML) was significantly reduced by reserpine treatment after 8 h of culture ($p<0.05$) (Fig. 4.11). After 24 h of culture, in the presence of isoprenaline, reserpine treatment did not produce any significant changes in the production of these metabolites.

4.5.4 Discussion

The pineal gland in culture is able to synthesize and release radiolabeled aMT into the media continually during a 24-h incubation period as a result of the persistent release of endogenous catecholamines from sympathetic nerve endings (*Minneman* 1977, see Chapter 3). The present results show that reserpine treatment depresses the synthesis of aMT and its precursor, aHT, from radiolabeled serotonin in cultures of rat pineal glands. These findings are consistent with the earlier observation that reserpine treatment prevents the endogenous catecholamine-induced induction of NAT activity (Fig. 4.4) and confirm the hypothesis that the drug induces a marked depletion of intraneuronal catecholamines stores.

Moreover, the observation that reserpine treatment enhanced the isoprenaline-induced stimulation of aHT and aMT synthesis is consistent with earlier observations of increased β-adrenoceptor density and superinduction of catecholamine-sensitive NAT activity.

Reserpine treatment was without effect on the synthesis of HA and HL, suggesting that their synthesis

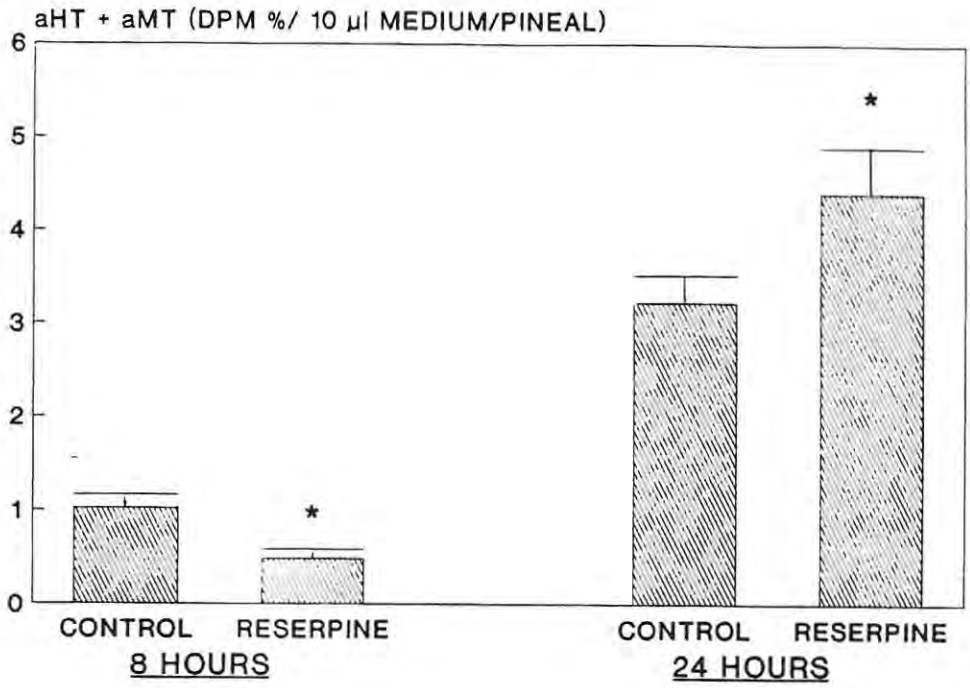


Figure 4.7 Effect of reserpine treatment on the synthesis of [^{14}C]N-acetylated products (aHT + aMT) by organ cultures of rat pineal glands. Isoprenaline stimulation is depicted by 24-h levels. Values represent means ± S.E.M. Five rats were used in each experimental group.

* $p < 0.05$ when compared with controls.

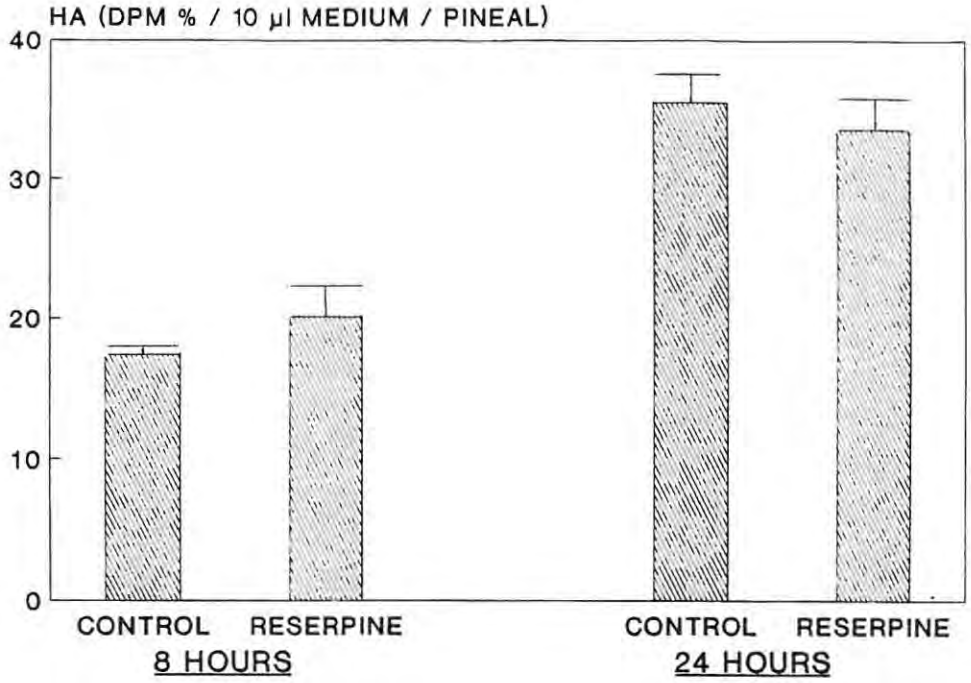


Figure 4.8 Effect of reserpine treatment on [^{14}C]5-hydroxyindole acetic acid (HA) synthesis by organ cultures of rat pineal glands.

Isoprenaline stimulation is depicted by 24-h levels. Values represent means ± S.E.M. Five rats were used in each experimental group.

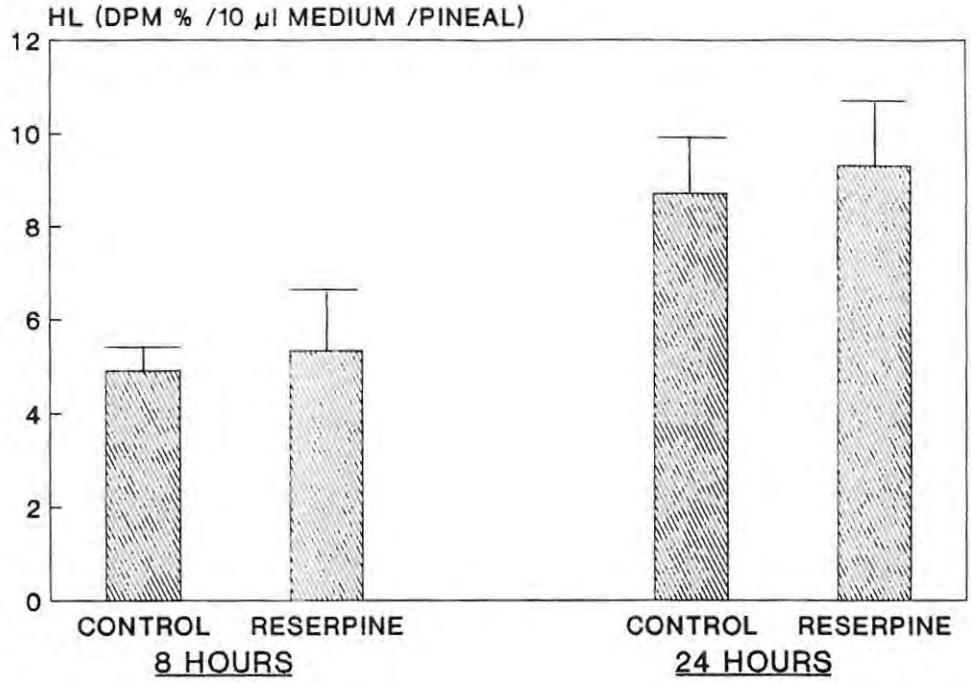


Figure 4.9 Effect of reserpine treatment on [^{14}C]5-hydroxytryptophol (HL) synthesis by organ cultures of rat pineal glands.

Isoprenaline stimulation is depicted by 24-h levels. Values represent means ± S.E.M. Five rats were used in each experimental group.

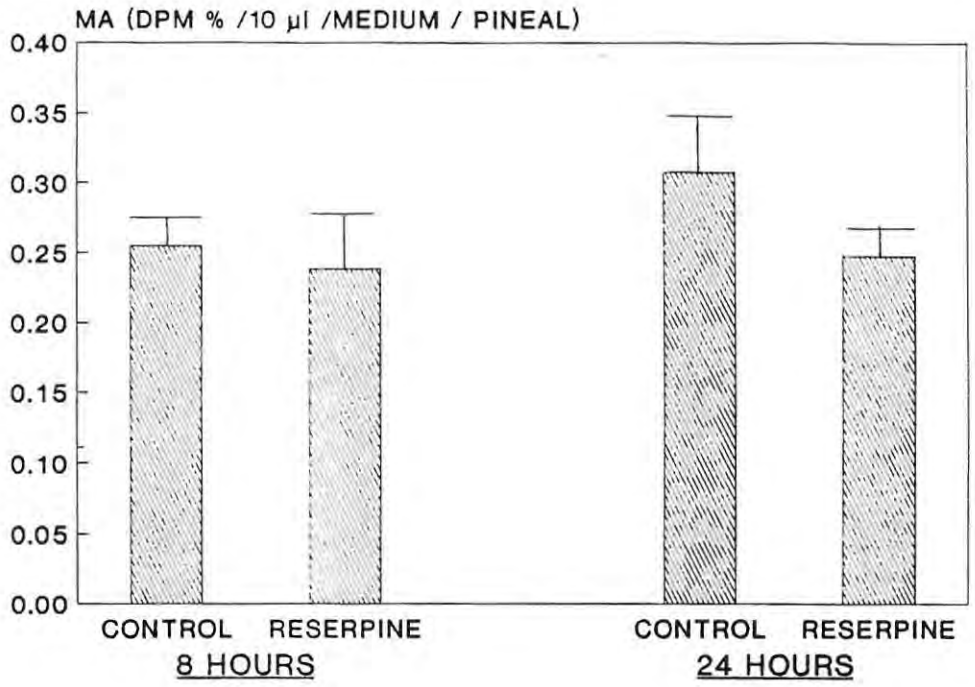


Figure 4.10 Effect of reserpine treatment on [^{14}C]5-methoxyindole acetic acid (MA) synthesis by organ cultures of rat pineal glands.
Isoprenaline stimulation is depicted by 24-h levels. Values represent means ± S.E.M. Five rats were used in each experimental group.

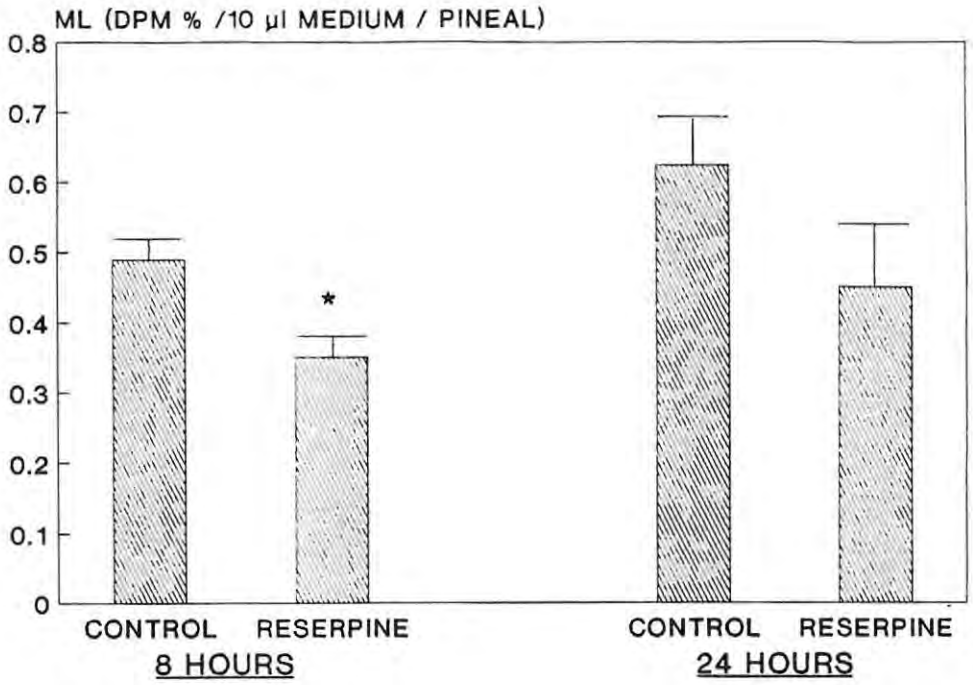


Figure 4.11 Effect of reserpine treatment on [^{14}C]5-methoxytryptophol (ML) synthesis by organ cultures of rat pineal glands.

Isoprenaline stimulation is depicted by 24-h levels. Values represent means ± S.E.M. Five rats were used in each experimental group.

* $p < 0.05$ when compared with controls.

by the MAO enzyme is unlikely to be altered by changes in adrenergic input to the gland. Interestingly, reserpine treatment decreased the methylation of HL to ML by the enzyme HIOMT, suggesting that chemical sympathectomy may alter HIOMT activity. *Sugden* and *Klein* (1983) demonstrated that HIOMT activity is regulated by β-adrenoceptors. These workers showed that exposure of rats to constant light or removal of the superior cervical ganglia reduced pineal HIOMT activity by 70% after 3 weeks. Moreover, they showed that daily administration of isoprenaline or NA can prevent or reverse this decline in activity.

4.6 CONCLUSION

The experiments described in this study lend further support for the hypothesis that long-term alterations in sympathetic activity can modify the density of β-adrenoceptors and the responsiveness of the adenylate cyclase system to catecholamines in the target tissue (*Weiss* 1969). Using the pineal gland as a model, chemical sympathectomy with reserpine was found to induce a marked increase in the density of β-adrenoceptors. The resulting deficiency of endogenous catecholamines also prevented the darkness-induced rise in NAT activity and reduced the conversion of serotonin to N-acetylserotonin and melatonin. The increased number of β-adrenoceptors was associated functionally with a markedly greater induction of NAT activity when stimulated with a catecholamine such as isoprenaline. Superinduction of NAT activity was also correlated with an enhanced conversion of serotonin to N-acetylserotonin and melatonin.

CHAPTER FIVE

EFFECT OF ACUTE ANTIDEPRESSANT TREATMENT ON RAT PINEAL GLAND FUNCTION

5.1 INTRODUCTION

The presence of sympathetic nerve endings in the pineal permits the use of the gland to study noradrenaline (NA) synthesis, release and reuptake. In addition, a large proportion of the postsynaptically synthesized serotonin (5-HT) is co-localized with NA in these nerve endings. The pineal may therefore be useful for studying the comparative pharmacology of NA and 5-HT reuptake. The release of NA mediates the synthesis of melatonin via postsynaptic β-adrenoceptors. The physiological significance of 5-HT released from sympathetic nerves, however, is not clearly understood. Whether this pool of 5-HT is made available for the synthesis of melatonin in the pinealocyte is yet to be established.

Several studies have shown that antidepressant drugs which increase the availability of synaptic NA, either as a result of inhibition of reuptake (TCAs) or inhibition of catabolism (MAOIs), increase pineal melatonin synthesis following acute administration (Section 1.3.7). It is less clear, however, whether antidepressant drugs which do not primarily affect NA reuptake or metabolism also have this effect. The selective 5-HT reuptake inhibitor Ro 11-2465 has been reported to increase rat pineal melatonin synthesis following acute administration (*Wirz-Justice* et al. 1980a). More recently, acute treatment with fluvoxamine, another 5-HT reuptake inhibitor, was shown to increase melatonin synthesis in healthy human subjects (*Skene* et al. 1989, cited by *Arendt* 1989). Further studies are needed to confirm these preliminary findings and to establish the mechanism(s) involved in this phenomenon. On the other hand, acute treatment of rats with iprindole, a drug which affects neither NA nor 5-HT reuptake, was found not to increase melatonin synthesis (*Friedmen* et al. 1984). It remains to be seen, therefore, whether this effect on melatonin synthesis is restricted to antidepressants which affect monoamine reuptake or catabolism.

The present study thus sought to further characterize the possible changes that may occur in rat pineal function as a result of acute antidepressant treatment. Antidepressants with specific actions on noradrenergic (desipramine and maprotiline) or serotonergic (trazodone) function were employed in this study. The newer selective 5-HT reuptake inhibiting antidepressants, sertraline and RU-25591 were also employed in some experiments. In addition to β-adrenoceptor binding studies, NAT activity and pineal indole levels were measured as functional correlates of noradrenergic function.

5.2 MATERIALS AND METHODS

5.2.1 Animals

Adult male rats (200-250 g) of the Wistar strain were used throughout this study. The animals were maintained under an automatically regulated lighting cycle with a daily photoperiod of 12 h (lights on at 06:00 h). All animals had free access to food and water. Their living environment is described in detail in Section 2.1.

5.2.2 Drugs

The following antidepressant drugs, used in this study, were kindly donated by the companies concerned : Desipramine HCl and maprotiline HCl (Ciba-Geigy), trazodone HCl and RU-25591 (Roussel), and sertraline HCl (Pfizer).

Desipramine and trazodone were administered as solutions in 0.9% saline. Maprotiline was administered as a solution in distilled water. Control rats received 0.9% saline in a volume of 1 ml/kg. When the experimental antidepressant compounds, sertraline and RU-25591 were used, these were administered as solutions in a vehicle consisting of the mixture (% by volume), 1.5% benzyl alcohol + 5% polysorbate + 93.5% distilled water. Under these circumstances, a separate group of control rats receiving vehicle, 2 ml/kg, were used. In all experiments, antidepressant doses reported as milligrams per kilogram were based on the weight of the HCl salt supplied.

5.2.3 Statistical Analysis

Statistical analyses were performed using Student's *t*-test. A P value of greater than 0.05 between groups was considered not significantly different. Where applicable, the data were analysed using one-way analysis of variance followed by Scheffe's post hoc tests for multiple range comparisons.

5.3 EXPERIMENT 1 : EFFECT OF ACUTE ANTIDEPRESSANT TREATMENT ON PINEAL β-ADRENOCEPTORS

5.3.1 Introduction

The aim of the following experiment was to investigate the effect of acute antidepressant treatment on the binding of [^{3}H]dihydroalprenolol (DHA) to rat pineal β-adrenoceptors. Antidepressants with different pharmacological profiles were included in the experiment, in order to examine whether these may exert different effects on [^{3}H]DHA binding.

5.3.2 Experimental Procedure

Drug Administration : Rats were treated with a single dose of antidepressant, 10 mg/kg i.p., at 10:00 h. The antidepressants evaluated in this experiment were desipramine, trazodone and maprotiline. Control rats received 0.9% saline, 1 ml/kg. Pineal glands were collected 3 h after drug administration for use in the experiment.

Assay of β-Adrenoceptors : β-Adrenoceptors were assayed by measuring the binding at a single concentration of [^{3}H]DHA (5 nM) to pineal gland membranes. The assay was performed as outlined in Section 2.2.1.2.

Analysis of Data : Specific [^{3}H]DHA binding is expressed as fmol/mg protein. Values represent means ± S.E.M. Eight rats were used in each experimental group.

5.3.3 Results

The effect of acute antidepressant administration on pineal β-adrenoceptors is presented in Fig. 5.1. The specific binding of [^{3}H]DHA to rat pineal gland membranes was not significantly altered by acute treatment with desipramine, trazodone or maprotiline when compared with saline-treated controls.

5.3.4 Discussion

Moyer et al. (1979, 1981) showed that acute treatment with desipramine or nialamide did not affect the binding of [^{3}H]DHA to pineal β-adrenoceptors in rats maintained in constant light. In support of these findings, these workers showed that the cAMP response to isoprenaline or NA was not altered following acute treatment with desipramine or nialamide. The lack of effect observed with acute desipramine treatment in the present study, therefore supports these previous findings. In addition, the present study demonstrates that acute treatment with maprotiline or trazodone, likewise, does not affect pineal β-adrenoceptors. It is well established that long-term β-adrenoceptor stimulation results in desensitization (subsensitivity) and conversely, long-term receptor blockade results in supersensitization (supersensitivity). Presumably, therefore, the increased availability of synaptic NA resulting from inhibition of reuptake by desipramine or maprotiline is insufficient to produce changes in pineal β-adrenoceptors in the short term. On the other hand, the lack of effect of trazodone may be attributable to the relatively selective action of this drug on the serotonergic system instead (Section 1.2.2.3.3).

Consistent with studies in the pineal, β-adrenoceptors in several other brain areas, including the limbic forebrain and cerebral cortex, likewise appear to be unaffected by acute antidepressant treatment (see Section 1.2.3.2.2).

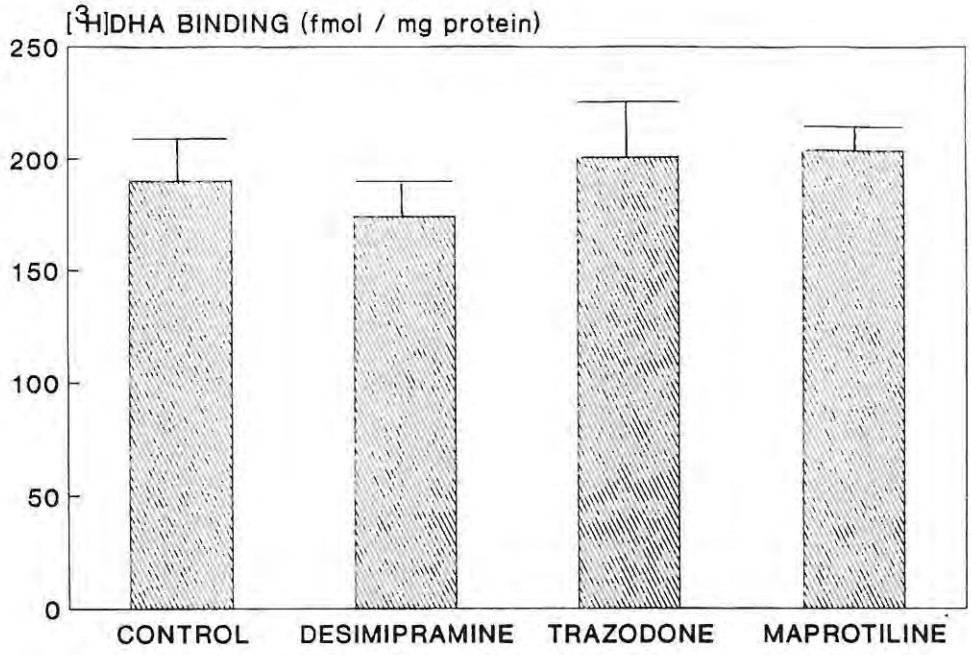


Figure 5.1 Effect of acute antidepressant treatment on the specific binding of [^{3}H]DHA in the rat pineal gland.

Values represent means ± S.E.M. Eight rats were used in each experimental group.

5.4 EXPERIMENT 2 : EFFECT OF ACUTE ANTIDEPRESSANT TREATMENT ON PINEAL SEROTONIN N-ACETYLTRANSFERASE ACTIVITY

5.4.1 Introduction

Noradrenergic stimulation of pinealocyte β-adrenoceptors causes a marked increase in N-acetyltransferase (NAT) activity via a cAMP dependent mechanism which induces synthesis of new enzyme protein, or activates existing NAT (Section 1.3.3.1). This enzyme catalyses the conversion of 5-HT to N-acetylserotonin, the rate-limiting step in the synthesis of melatonin. Thus, the measurement of NAT activity provides a functional biochemical index of noradrenergic neurotransmitter mechanisms.

The following experiment was designed to investigate the effect of acute antidepressant administration on NAT activity in the rat pineal gland. Furthermore, since experimental data regarding the effects of acute antidepressant treatment on catecholamine-induced NAT activity appears to be lacking, daytime isoprenaline-stimulated and nocturnal NAT activity was also measured following such treatment.

5.4.2 Experimental Procedure

Drug Administration : Groups of rats received a single dose of desipramine, trazodone or maprotiline, 10 mg/kg i.p. Control rats were treated with 0.9% saline, 1 ml/kg i.p. For daytime experiments, animals were injected at 10:00 h and pineals were collected 3 h later for use in the assay. During dark phase experiments, rats were injected 15 min before lights off (18:00 h) and were sacrificed 3 h later. Pineal glands collected during the dark phase were frozen on solid CO_2 and stored at -70^{o}C prior to analysis.

Assay of Serotonin N-Acetyltransferase : Pineals collected during the dark phase and stored at -70^{o}C were allowed to thaw on ice prior to assaying enzyme activity. Pineals collected during the light phase were cultured in the presence or absence of 10 μM *l*-isoprenaline for 3 h prior to the determination of enzyme activity. The technique of organ culture and assay of enzyme activity were performed as outlined in Section 2.5.2.

Analysis of Data : Enzyme activity is expressed as picomoles of [^{3}H]N-acetyltryptamine formed per pineal per hour. Values represent means ± S.E.M. of four experiments.

5.4.3 Results

Light Phase Enzyme Activity : The effect of acute antidepressant treatment on daytime basal NAT activity is shown in Fig. 5.2. Only desipramine significantly increased basal NAT activity when compared with controls ($p<0.05$). To determine whether acutely administered antidepressants would influence the ability of exogenously administered catecholamines to stimulate NAT activity, pineals were stimulated with 10 μM isoprenaline *in vitro*. The results of this experiment are presented in Fig. 5.3.

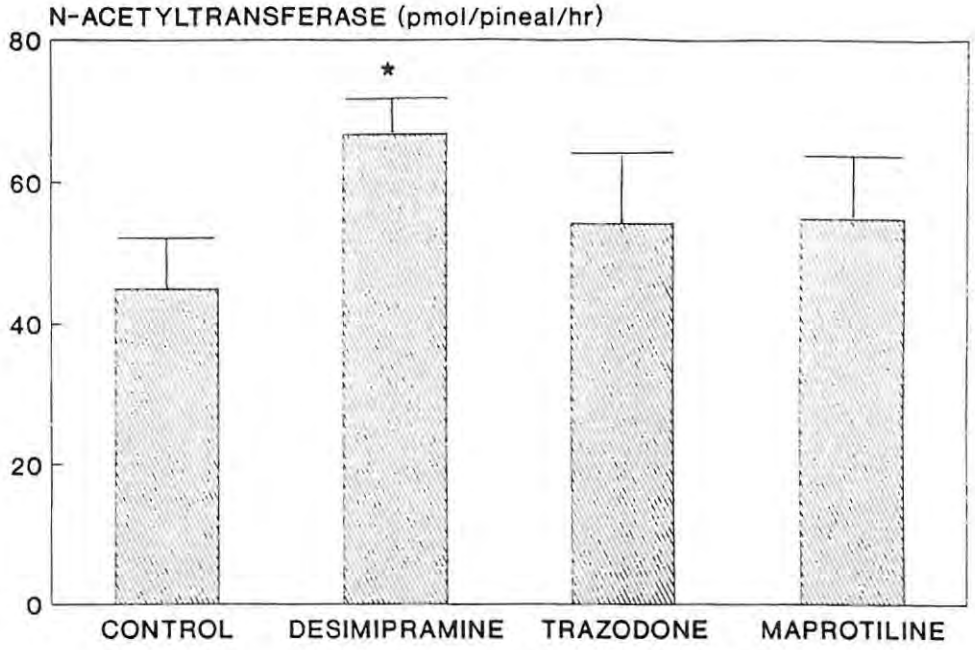


Figure 5.2 Effect of acute antidepressant treatment on daytime basal N-acetyltransferase (NAT) activity.

Values represent means ± S.E.M. Four rats were used in each experimental group.

* $p < 0.05$ when compared with controls.

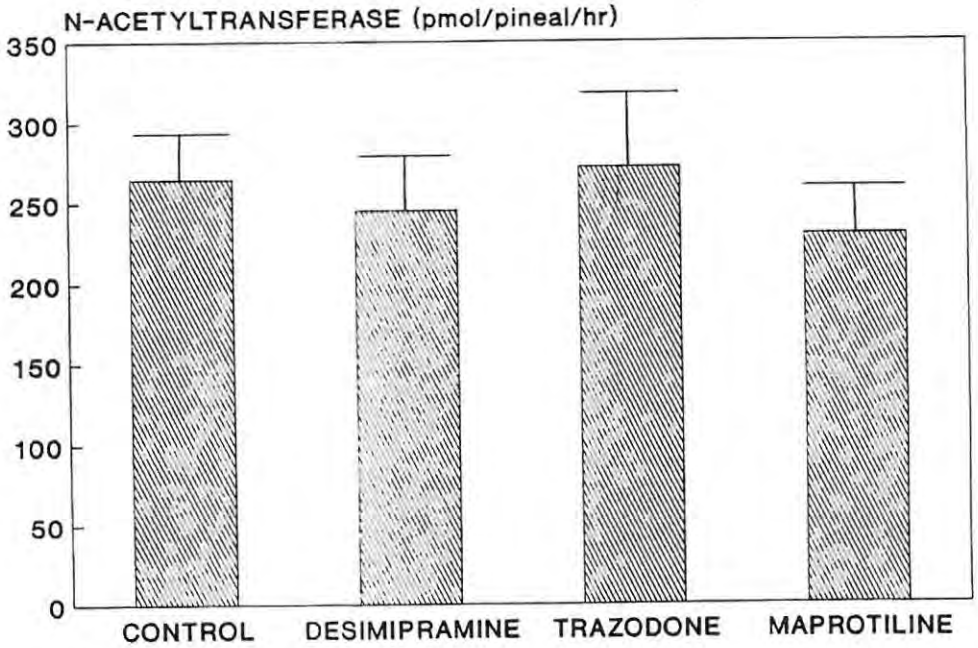


Figure 5.3 Effect of acute antidepressant treatment on *in vitro* isoprenaline-stimulated N-acetyltransferase (NAT) activity.

Values represent means ± S.E.M. Four rats were used in each experimental group.

Isoprenaline produced a significant increase in NAT activity in both antidepressant-treated and control groups when compared with basal enzyme activity ($p < 0.01$) (Figs. 5.2 and 5.3). However, the response to isoprenaline was not significantly altered in antidepressant-treated pineals when compared with controls.

Dark Phase Enzyme Activity : The effect of acute antidepressant administration on the night-time rise in NAT activity is shown in Fig. 5.4. Three hours into the dark phase of the lighting cycle, saline treated rats showed the usual marked rise in pineal NAT activity (*Klein* and *Weller* 1970). Acute treatment of rats with desipramine significantly enhanced the nocturnal rise in NAT activity when compared with saline treatment ($p < 0.01$). Trazodone also caused a small, but significant, enhancement of NAT activity ($p < 0.05$). Maprotiline, on the other hand, was without this effect.

5.4.4 Discussion

The results presented show that acute desipramine treatment produced a small increase in daytime basal NAT activity, consistent with previous observations (*Parfitt* and *Klein* 1976). In contrast, however, a markedly enhanced nocturnal rise in NAT activity was noted following desipramine treatment. These findings are consistent with the observation that pineal NA concentrations are low during the day and high at night (*Brownstein* and *Axelrod* 1974). Taken together, these results suggest that the desipramine-induced increase in NAT activity derives from increased NA availability at pinealocyte β-adrenoceptors resulting from reuptake inhibition. Activation of pineal β-adrenoceptors produces an increase in NAT activity via a cAMP-dependent mechanism.

Interestingly, maprotiline, a selective NA reuptake inhibitor, did not affect daytime or nocturnal NAT activity. These observations contradict those of *Wirz-Justice* and co-workers (1980a), who demonstrated a marked increase in pineal melatonin synthesis 2 h after the administration of a single 50 mg/kg dose of maprotiline to rats. However, the lower dose (10 mg/kg) or longer time interval (3 h) may account for the lack of effect observed with maprotiline in the present study. Furthermore, it is possible that the high dose of maprotiline used by *Wirz-Justice* et al. (1980a) may be less selective and may displace NA from presynaptic nerve terminals.

Of interest also, is the observation that trazodone produced a small but significant enhancement of nocturnal NAT activity. The mechanism by which trazodone produces this effect, however, is unclear. It is unlikely that this effect is due to a direct interaction with pineal β-adrenoceptors, since daytime enzyme levels appeared to be unaffected by trazodone treatment. A possible explanation may relate to the ability of this drug to weakly inhibit NA reuptake (*Leonard* 1984) especially during darkness when pineal NA levels are high. Trazodone may also increase synaptic NA levels as a result of its ability to antagonize presynaptic α_2-adrenoceptors (*Richelson* and *Nelson* 1984). Alternatively, it is interesting to speculate that pineal NA release and thus NAT activity may be influenced by trazodone acting via a serotonergic receptor mechanism. Trazodone enhances serotonergic neurotransmission by inhibiting

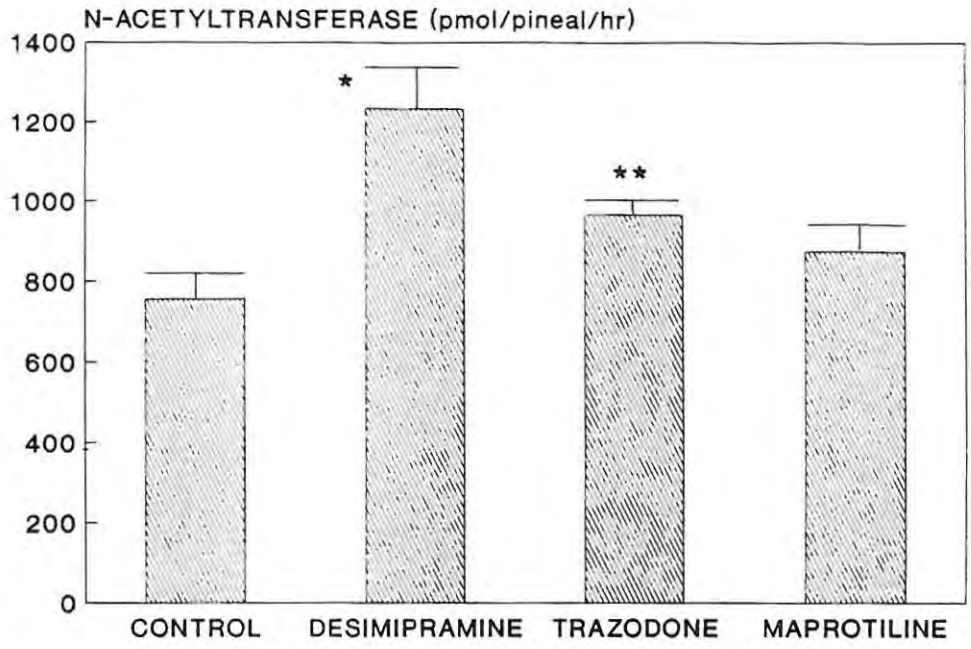


Figure 5.4 Effect of acute antidepressant treatment on the darkness-induced rise in N-acetyltransferase (NAT) activity.

Values represent means ± S.E.M. Four rats were used in each experimental group.

* $p < 0.01$ when compared with controls.

** $p < 0.05$ when compared with controls.

presynaptic 5-HT autoreceptors in low concentrations and by stimulating postsynaptic 5-HT receptors in high concentrations (Section 1.2.2.3.3). In addition, the drug is a weak inhibitor of 5-HT reuptake. However, a sertonergic receptor-mediated effect on pineal NAT activity has yet to be demonstrated.

Furthermore, the finding that acute treatment with desipramine, maprotiline or trazodone did not alter the NAT response to isoprenaline stimulation, confirms the previous observation that pineal β-adrenoceptor sensitivity is not altered by such treatment.

5.5 EXPERIMENT 3 : EFFECT OF ACUTE ANTIDEPRESSANT TREATMENT ON [^{14}C]INDOLE METABOLISM BY ORGAN CULTURES OF RAT PINEAL GLANDS

5.5.1 Introduction

Radiolabeled 5-HT, when added to pineal gland cultures, is metabolised in the pinealocyte to yield a number of indoles including melatonin and its precursor, N-acetylserotonin. Previous studies have demonstrated that the synthesis of melatonin is dependent on the degree of noradrenergic input to the gland (Chapters 3 and 4).

The present experiment was aimed at investigating the effect of acute antidepressant treatment on the metabolism of radiolabeled 5-HT in organ cultures of rat pineal glands. In addition to desipramine, trazodone and maprotiline, used in previous experiments, the selective 5-HT reuptake inhibitors, sertraline and RU-25591, were also employed.

5.5.2 Experimental Procedure

Drug Administration : Rats received a single dose of antidepressant, 10 mg/kg i.p., at 10:00 h. In one experiment, desipramine, trazodone or maprotiline was administered and controls received 0.9% saline. In another experiment, sertraline or RU-25591 was administered and controls received vehicle (see Section 5.2.2). In both experiments, pineal glands were collected 3 h after drug administration.

Pineal Organ Culture and Analysis of [^{14}C]Indoles : The technique of pineal organ culture and the analysis of radiolabeled indoles are outlined in Section 2.6.2. Briefly, pineals were cultured individually in the presence of [^{14}C]serotonin and incubated at 37^{o}C under 95% O_2 : 5% CO_2. After 8 h of culture, a 10 µl aliquot of culture medium was removed and analysed for its content of radiolabeled indoles.

Analysis of Data : Results are expressed as the percentage of total radioactivity (DPM%/10 µl medium/pineal). Values represent means ± S.E.M. Four or eight rats were used in the experimental groups.

5.5.3 Results

5.5.3.1 Effect on the Synthesis of [^{14}C]N-Acetylated Indoles

5.5.3.1.1 [^{14}C]N-Acetylserotonin

The effect of acute antidepressant treatment on the synthesis of labeled N-acetylserotonin (aHT) is presented in Fig. 5.5. The synthesis of this metabolite was significantly increased by treatment with desipramine, trazodone or maprotiline when compared with saline-treated controls ($p < 0.01$) (Fig. 5.5a). Treatment of rats with the experimental antidepressants, sertraline or RU-25591, did not alter aHT synthesis when compared with their respective vehicle-treated controls (Fig. 5.5b).

5.5.3.1.2 [^{14}C]Melatonin

The effect of acute antidepressant treatment on melatonin (aMT) synthesis is shown in Fig. 5.6. When compared with saline treatment, desipramine significantly increased aMT synthesis ($p < 0.01$) (Fig. 5.6a). Treatment with trazodone or maprotiline, on the other hand, did not significantly affect aMT production. When compared with vehicle-treated controls, sertraline but not RU-25591 was found to increase aMT synthesis ($p < 0.01$) (Fig. 5.6b).

5.5.3.1.3 [^{14}C]N-Acetylated Products

The radioactivity recovered as N-acetylated products represents the sum of the radioactivity detected on the aHT and aMT spots. Figure 5.7 depicts the effect of acute antidepressant treatment on the synthesis of N-acetylated products. Treatment with desipramine ($p < 0.01$), trazodone ($p < 0.05$) or maprotiline ($p < 0.05$), raised the level of N-acetylated products above that of the saline-treated group (Fig. 5.7a). On the other hand, sertraline and RU-25591 were without effect on the levels of N-acetylated products when compared with vehicle treatment (Fig. 5.7b).

5.5.3.2 Effect on the Synthesis of [^{14}C]Deaminated Indoles

5.5.3.2.1 [^{14}C]5-Hydroxyindole Acetic Acid

The effect of acute antidepressant treatment on the synthesis of 5-hydroxyindole acetic acid (HA) is presented in Fig. 5.8. Compared with saline treatment, desipramine and maprotiline produced a significant reduction in the synthesis of HA ($p < 0.05$) (Fig. 5.8a). Treatment of rats with trazodone did not alter the synthesis of HA relative to saline treatment. Similarly, sertraline or RU-25591 treatment did not alter HA synthesis when compared with the respective vehicle-treated controls (Fig. 5.8b).

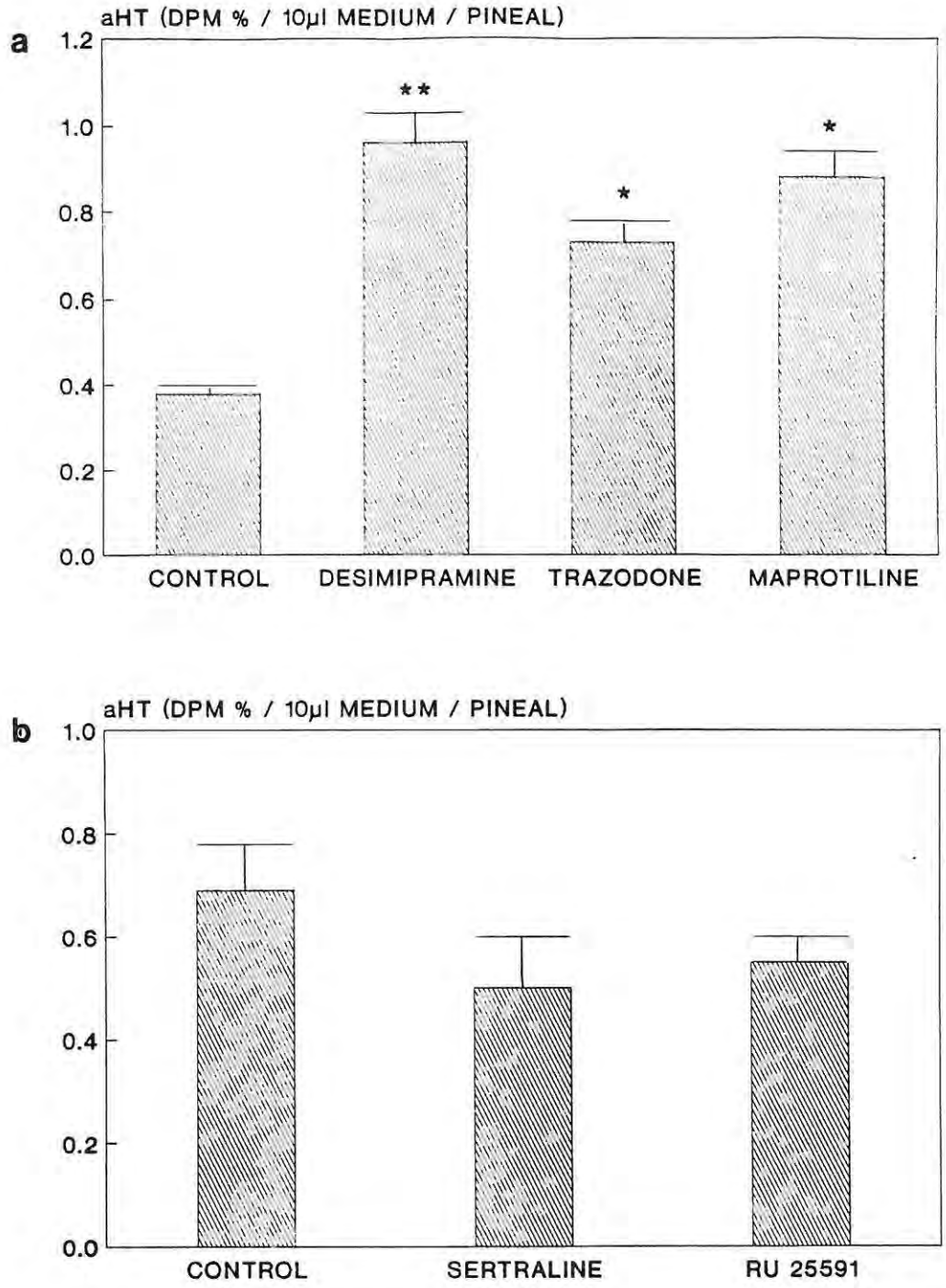


Figure 5.5 Effect of acute antidepressant treatment on the synthesis of [^{14}C]N-acetylserotonin (aHT) by organ cultures of rat pineal glands.

Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 5.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

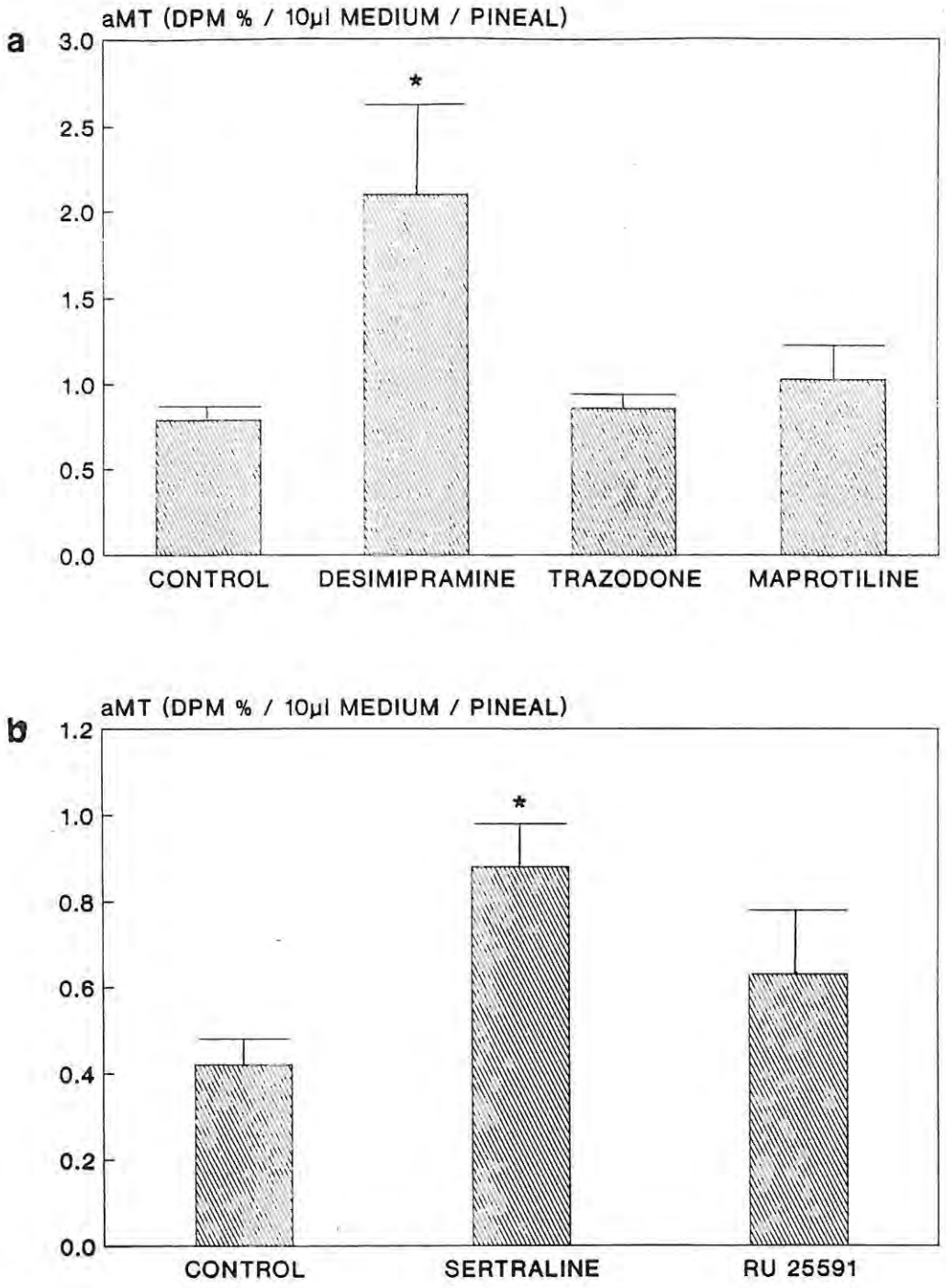


Figure 5.6 Effect of acute antidepressant treatment on the synthesis of [^{14}C]melatonin (aMT) by organ cultures of rat pineal glands.

Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 5.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

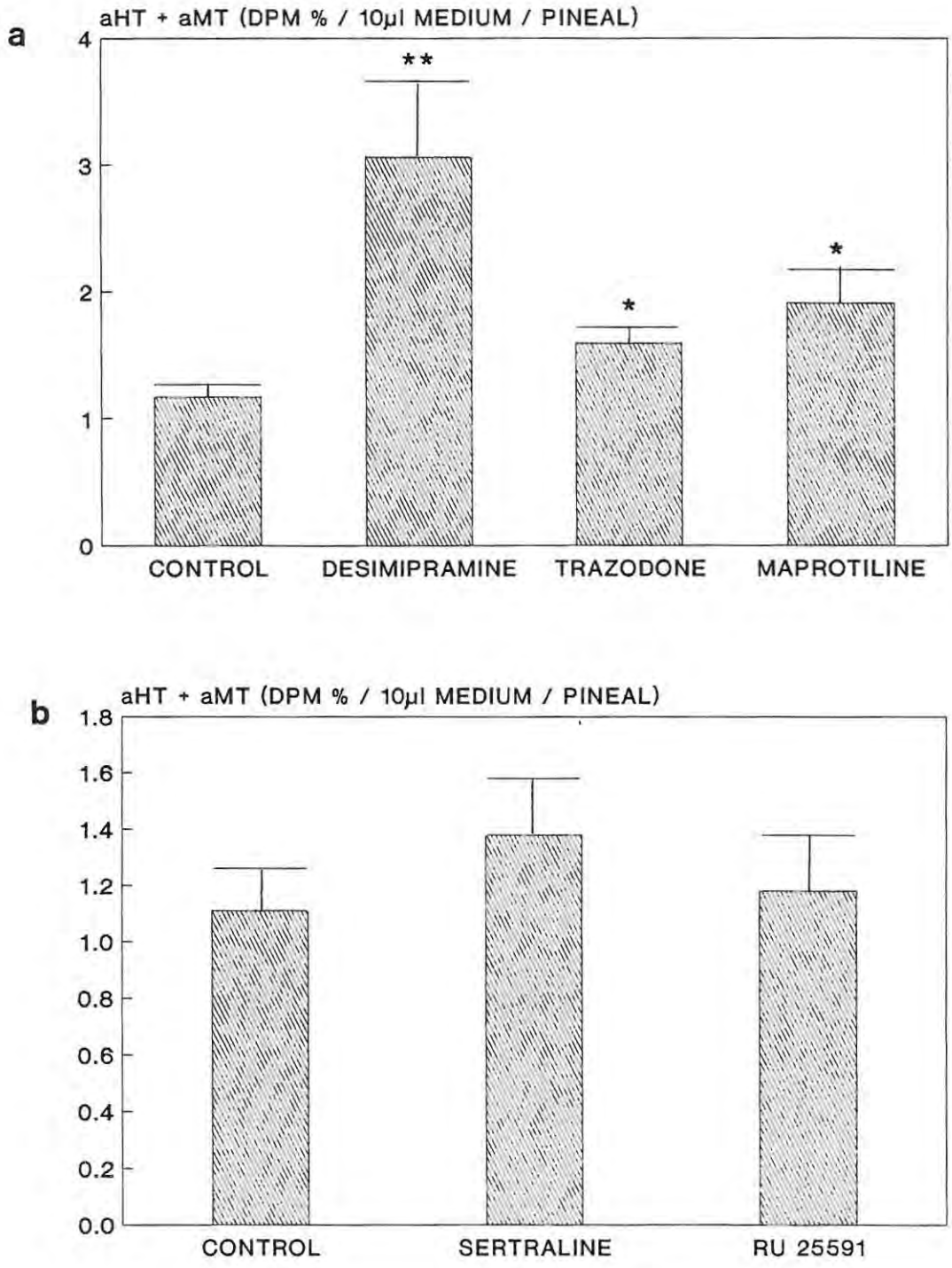


Figure 5.7 Effect of acute antidepressant treatment on the synthesis of [^{14}C]N-acetylated products (aHT + aMT) by organ cultures of rat pineal glands. Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 5.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

** $p < 0.01$ when compared with controls.

* $p < 0.05$ when compared with controls.

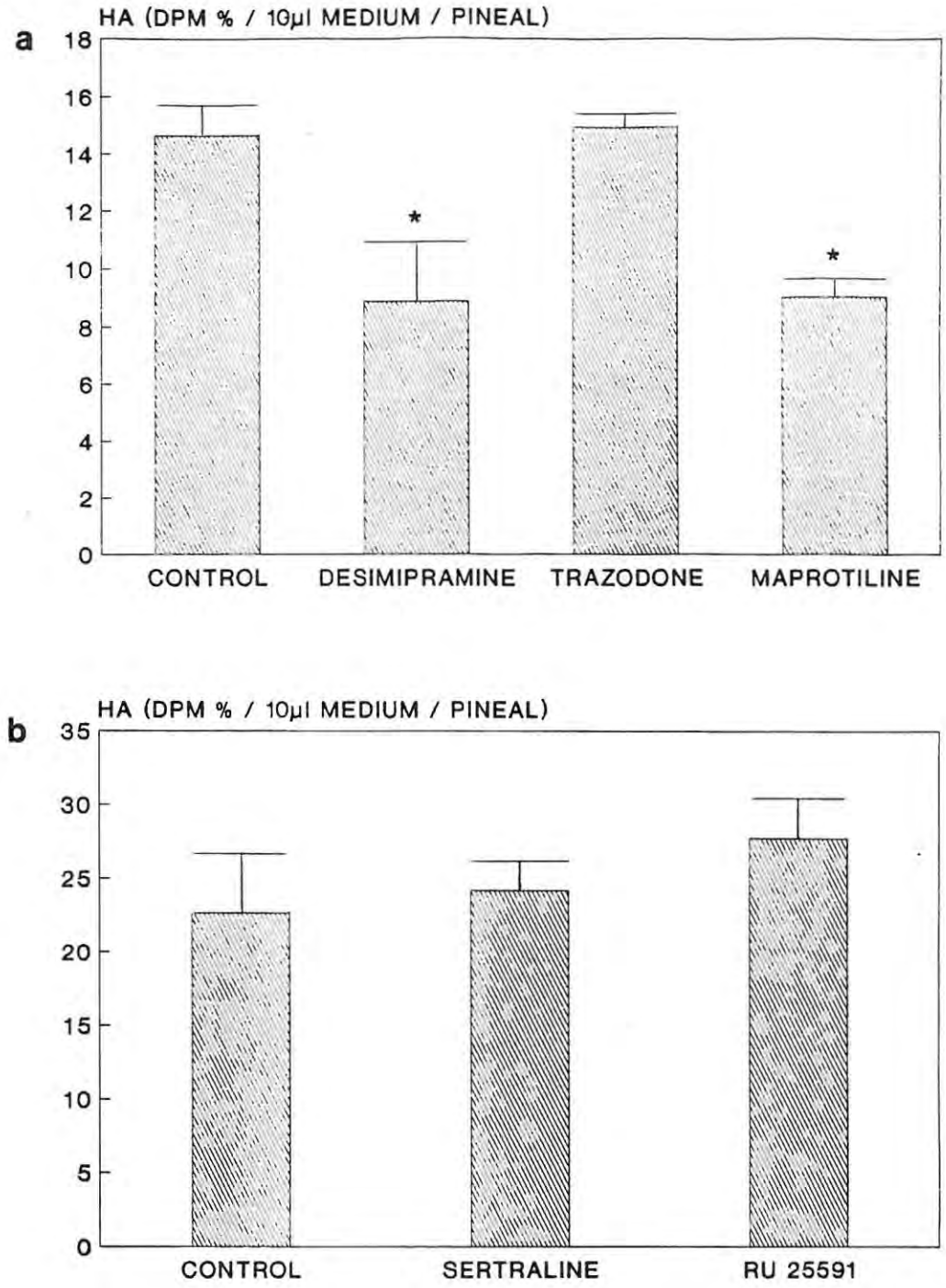


Figure 5.8 Effect of acute antidepressant treatment on the synthesis of [^{14}C]5-hydroxyindole acetic acid (HA) by organ cultures of rat pineal glands. Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 5.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.05$ when compared with controls.

5.5.3.2.2 [^{14}C]5-Hydroxytryptophol

Figure 5.9 shows the effect of acute antidepressant treatment on 5-hydroxytryptophol (HL) synthesis. Maprotiline treatment produced a significant reduction in HL synthesis ($p<0.01$) (Fig. 5.9a) when compared with saline treatment. The other antidepressants showed no significant alteration in HL synthesis when compared with the respective control groups (Figs. 5.9a and b).

5.5.3.2.3 [^{14}C]5-Methoxyindole Acetic Acid

The effect of acute antidepressant treatment on 5-methoxyindole acetic acid (MA) production is shown in Fig. 5.10. Acute treatment with desipramine significantly decreased MA synthesis when compared with controls ($p<0.05$) (Fig. 5.10a). None of the other antidepressants tested produced any significant change in the synthesis of MA when compared with the respective control groups.

5.5.3.2.4 [^{14}C]5-Methoxytryptophol

The effect of acute antidepressant administration on the synthesis of 5-methoxytryptophol (ML) is presented in Fig. 5.11. Desipramine treatment produced a significant decrease in ML synthesis relative to saline treatment ($p<0.01$) (Fig. 5.11a). The other antidepressants produced no significant alteration in ML synthesis when compared with the respective control groups (Figs. 5.11a and b).

5.5.3.2.5 [^{14}C]Monoamine Oxidase Products

The monoamine oxidase (MAO) products represent the sum of those indoles formed by the oxidative deamination of radiolabeled serotonin via the enzyme MAO; i.e., HA, HL, MA and ML. Figure 5.12 represents the effect of acute antidepressant treatment on the synthesis of MAO products. Treatment with desipramine or maprotiline produced a significant decrease in the level of MAO products when compared with saline treatment ($p<0.01$) (Fig. 5.12a). On the other hand, treatment with trazodone, sertraline or RU-25591 produced no significant change in the level of MAO products when compared with the respective controls (Figs. 5.12a and b).

5.5.4 Discussion

The increased synthesis of N-acetylserotonin and/or melatonin observed following acute treatment with desipramine and maprotiline is in accord with the findings of others (eg. *Wirz-Justice* et al. 1980a). Taken together with the previously noted changes in pineal NAT activity (Section 5.4.3), the results suggest that the effect of these antidepressants derives from the presynaptic inhibition of NA reuptake. Desipramine treatment produced a greater increase in N-acetylated products than treatment with

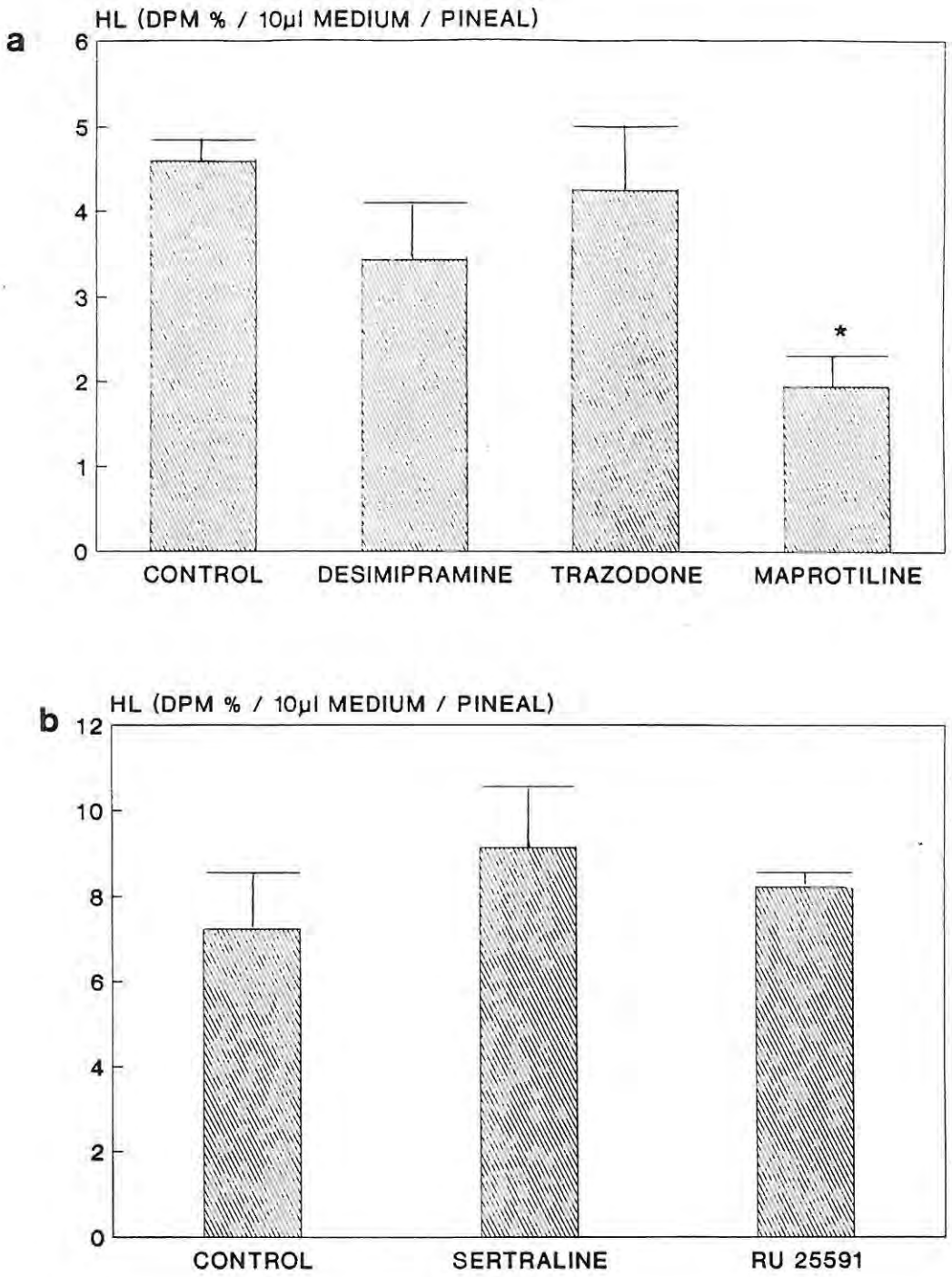


Figure 5.9 Effect of acute antidepressant treatment on the synthesis of [^{14}C]5-hydroxytryptophol (HL) by organ cultures of rat pineal glands.

Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 5.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

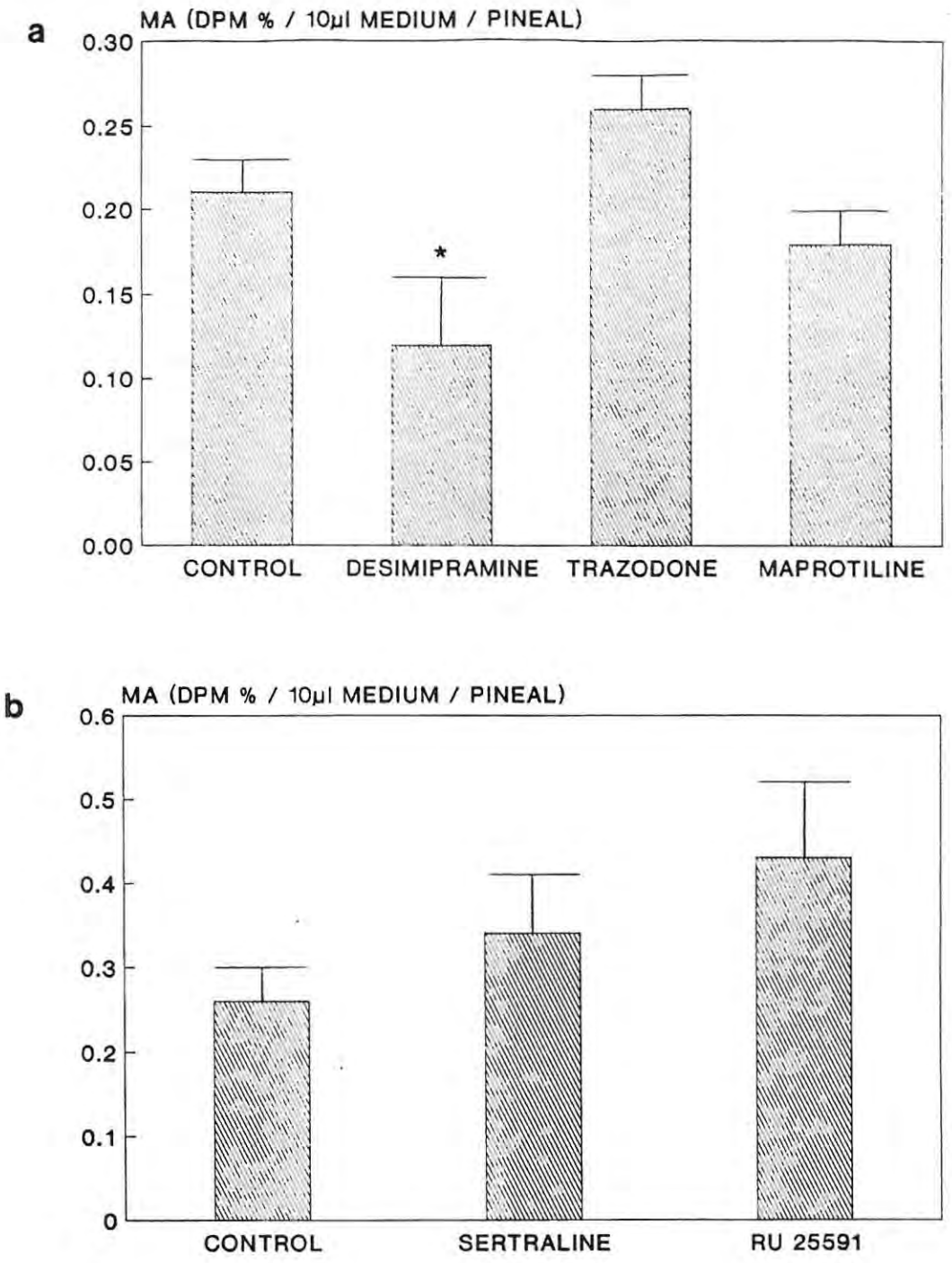


Figure 5.10 Effect of acute antidepressant treatment on the synthesis of [^{14}C]5-methoxyindole acetic acid (MA) by organ cultures of rat pineal glands. Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 5.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.05$ when compared with controls.

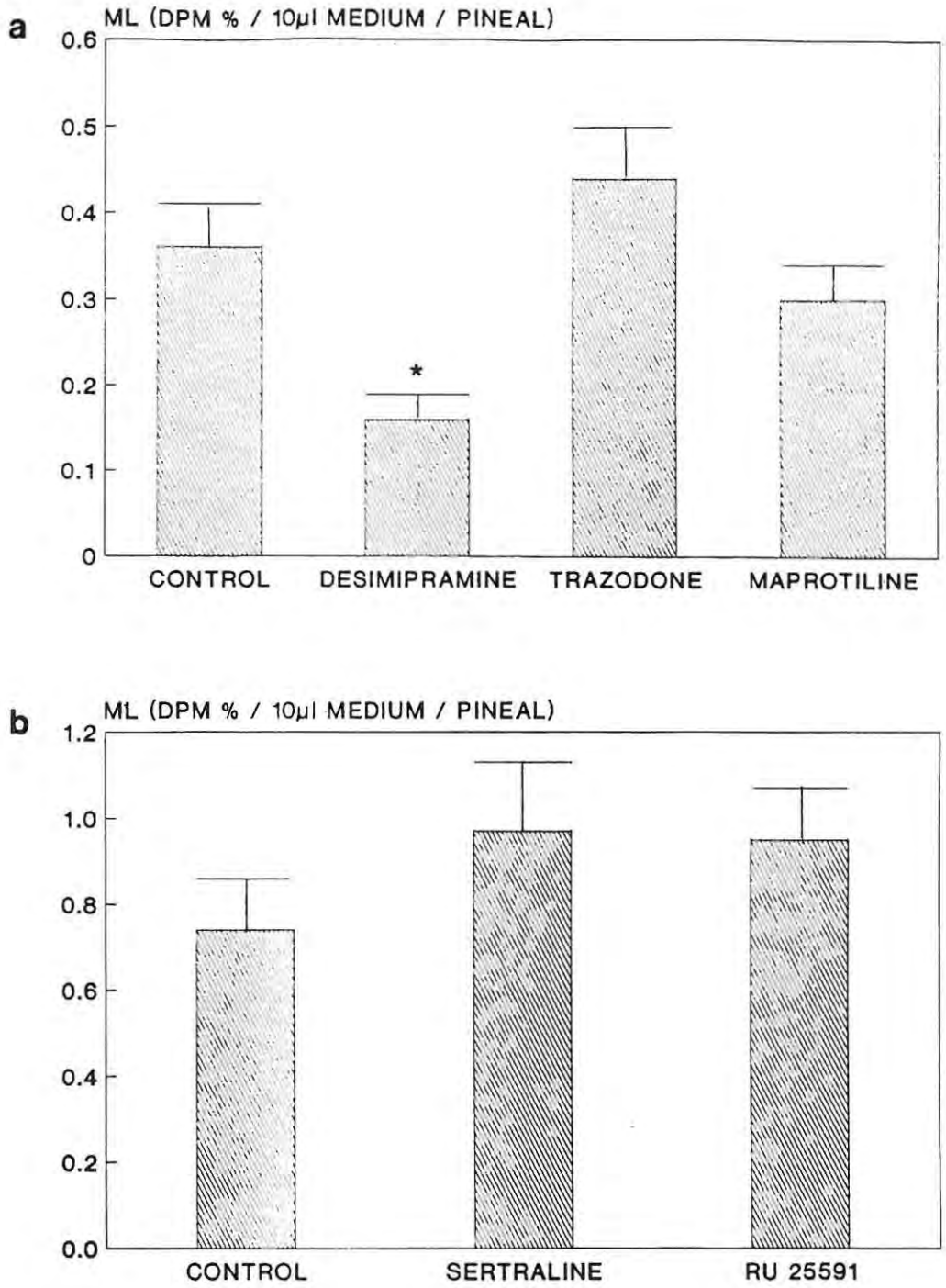


Figure 5.11 Effect of acute antidepressant treatment on the synthesis of [^{14}C]5-methoxytryptophol (ML) by organ cultures of rat pineal glands.
Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 5.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

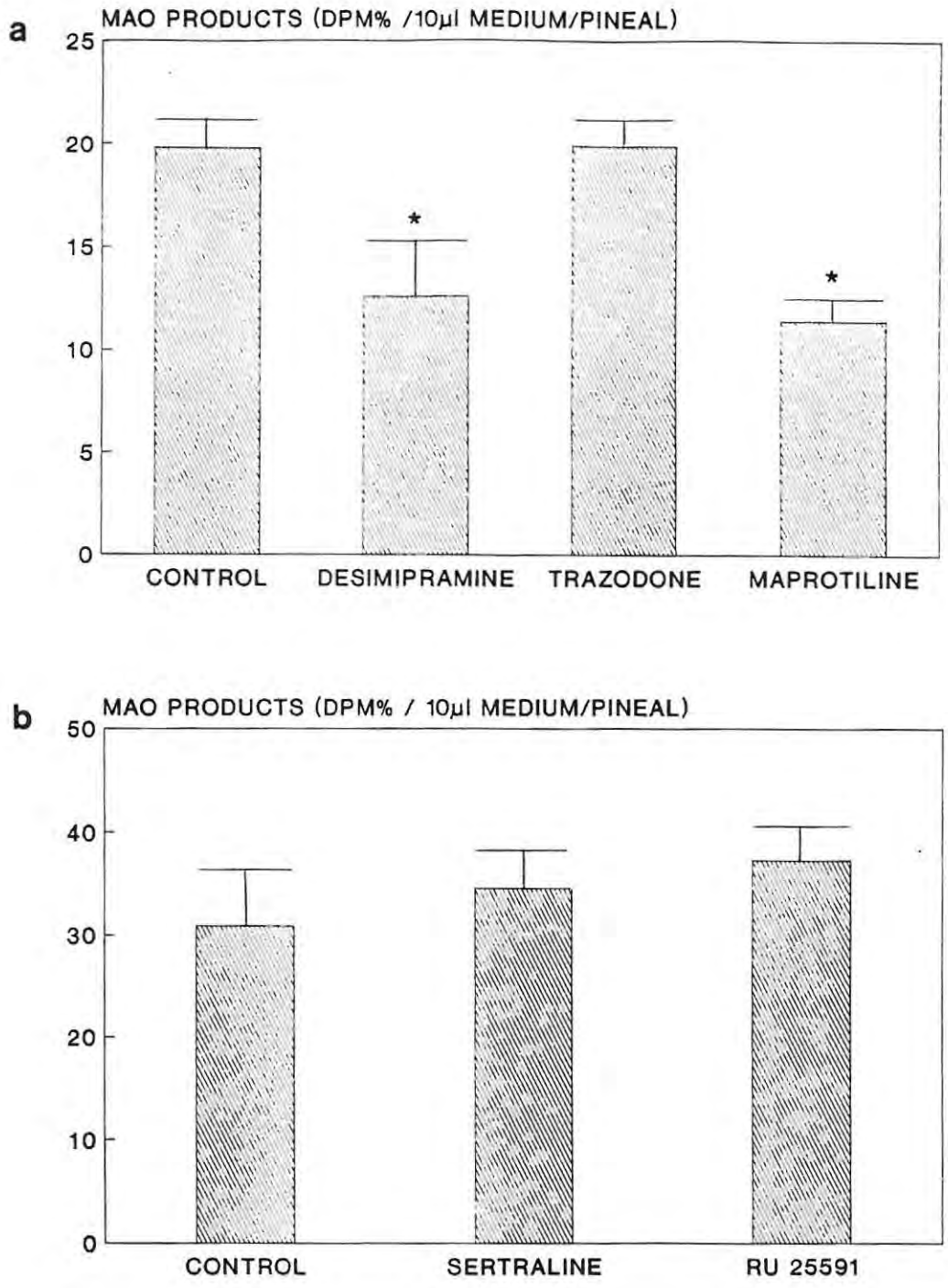


Figure 5.12 Effect of acute antidepressant treatment on the synthesis of [^{14}C]monoamine oxidase (MAO) products by organ cultures of rat pineal glands.

Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 5.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.05$ when compared with controls.

maprotiline, thus possibly reflecting the respective potencies of these drugs to inhibit NA reuptake *in vivo*. This interpretation is supported by the observation that NAT activity was not significantly increased by maprotiline treatment.

Interestingly, trazodone and sertraline were also found to increase the synthesis of N-acetylserotonin and/or melatonin. The mechanism(s) involved in this effect, however, is unclear since the acute effects of these drugs relate primarily to the serotonergic system. However, as regards trazodone, the weak inhibition of NA reuptake or antagonism of presynaptic α_2-adrenoceptors mentioned previously (Section 5.4.4), may account for the observed effect. Sertraline, on the other hand, is a selective and potent inhibitor of 5-HT reuptake, with no appreciable effect on NA reuptake (*Heym* and *Koe* 1988). At least two other studies have demonstrated that acute treatment with selective 5-HT reuptake inhibitors increased melatonin synthesis in rats and healthy human volunteers (*Wirz-Justice* et al. 1980a, *Skene* et al. 1989 cited by *Arendt* 1989). One possibility is that the presynaptic inhibition of 5-HT reuptake may increase the availability of 5-HT within the pinealocyte as substrate for conversion to melatonin. This effect would be equivalent to precursor loading. However, RU-25591, another potent and selective inhibitor of 5-HT reuptake (*Dumont* et al. 1981), failed to increase N-acetylserotonin or melatonin synthesis in the present study. Several investigators have demonstrated that exogenously administered 5-HT or L-5-HTP does not result in increased pineal melatonin synthesis (*Klein* and *Weller* 1973, *Wirz-Justice* et al. 1980a), at least in the rat. Moreover, since the conversion of 5-HT to melatonin is rate limited by the enzyme NAT, which is under adrenergic control, it is unlikely that precursor loading alone would increase melatonin synthesis. Whether NAT activity may also be regulated via a serotonergic mechanism as discussed previously (Section 5.4.4), however, remains to be seen. Likewise, whether sertraline increases melatonin synthesis by a mechanism that is not related to 5-HT reuptake inhibition, perhaps one involving β-adrenoceptors, requires further clarification.

However, since desipramine was found to produce the greatest increase in melatonin synthesis, the potency of the antidepressants to elicit this effect appears to depend on their ability to primarily inhibit the reuptake of NA. Thus, the rank order of potency of the different antidepressants to increase N-acetylated product synthesis was found to be : desipramine > maprotiline > trazodone ≥ sertraline > RU-25591.

Of interest also, is the observation that desipramine and maprotiline treatment depressed the oxidative deamination of 5-HT as evidenced by reduced synthesis of monoamine oxidase (MAO) products. A possible explanation for this effect by desipramine and maprotiline may be that 5-HT is preferentially used in the melatonin biosynthetic pathway, thus reducing the amount available for oxidative deamination by MAO. However, since both 5-HT concentrations and MAO activity in the pineal are exceptionally high (*Snyder* et al. 1965, *Saavedra* et al. 1973), it is unlikely that the reduction in MAO products is due merely to increased N-acetylation of 5-HT by NAT. An alternative explanation may be that both desipramine and maprotiline inhibit pineal MAO activity. This suggestion is consistent with

that of *Nir* and *Hirschmann* (1983) who showed that desipramine reduced the synthesis of MAO products when added to pineal glands *in vitro*.

Trazodone and sertraline did not alter the synthesis of MAO products, suggesting that these drugs do not affect pineal MAO activity. In addition, this finding argues against the possibility that they increase N-acetylserotonin and/or melatonin levels as a result of increased 5-HT utilization. Since, if this had occurred, the levels of all the 5-HT products would have been increased.

Desipramine treatment also reduced the levels of 5-methoxyindole acetic acid and 5-methoxytryptophol, suggesting that the methylating enzyme HIOMT preferentially utilizes the increased N-acetylserotonin as substrate rather than the 5-hydroxyindoles. The results of previous studies also suggest that pineal HIOMT exhibits substrate selectivity (see Chapter 3).

5.6 EXPERIMENT 4 : EFFECT OF β-ADRENOCEPTOR BLOCKADE ON THE ACUTE ANTIDEPRESSANT-INDUCED INCREASE IN [^{14}C]MELATONIN SYNTHESIS

5.6.1 Introduction

The previous experiment demonstrated that antidepressant drugs with different pharmacological profiles increase pineal N-acetylserotonin or melatonin synthesis following acute administration. This effect is mediated by inhibition of NA reuptake and consequent β-adrenoceptor activation in the case of antidepressants such as desipramine and maprotiline. As discussed previously, the mechanism by which some selective 5-HT reuptake inhibiting and atypical antidepressants achieve this, is speculative at the present time.

Thus, in an attempt to address this question, the present experiment investigated whether the non-NA reuptake inhibiting antidepressants, trazodone and sertraline, increase melatonin synthesis via a β-adrenoceptor mechanism. The antidepressant desipramine was included in the experiment for comparison.

5.6.2 Experimental Procedure

Drug Administration : Rats were given a single dose of atenolol (Tenormin), 2.5 mg/kg, or saline, 5 ml/kg, i.p. Fifteen minutes later, the same group of rats received a single dose of antidepressant, 10 mg/kg, or vehicle, 2 ml/kg, i.p. All animals were sacrificed 3 h after the final injection. The antidepressants evaluated in this experiment were desipramine, trazodone and sertraline.

Pineal Organ Culture and Analysis of [^{14}C]Indoles : The procedure employed is outlined in Section 2.6.2. Briefly, pineal glands were individually cultured in the presence of [^{14}C]serotonin and incubated at 37^{o}C under 95% O_2 : 5% CO_2. After 8 h of culture, a 10 µl aliquot of culture medium was removed and analysed for its content of radioactive indoles.

Analysis of Data : Results are expressed as the percentage of total radioactivity (DPM%/10 µl medium/pineal). Values represent means ± S.E.M. Four rats were used in each treatment group.

5.6.3 Results

5.6.3.1 [^{14}C]N-Acetylserotonin

Results are presented in Fig. 5.13. The production of N-acetylserotonin (aHT) was increased in pineal cultures of rats treated with desipramine when compared with vehicle-treated controls ($p<0.01$). Sertraline and, surprisingly, trazodone did not affect aHT synthesis relative to controls. Pretreatment of rats with atenolol did not alter the levels of aHT in pineals of vehicle-treated rats. Atenolol did, however, prevent the increase in aHT synthesis caused by desipramine ($p<0.01$).

5.6.3.2 [^{14}C]Melatonin

Results are shown in Fig. 5.14. When compared with controls, the synthesis of melatonin (aMT) was increased in pineals of rats treated with desipramine ($p<0.01$), trazodone ($p<0.05$) and sertraline ($p<0.05$). Pretreatment with atenolol, again, did not alter the levels of aMT in pineals of vehicle-treated rats. However, atenolol prevented the increase in aMT synthesis caused by desipramine ($p<0.01$), trazodone ($p<0.05$) and sertraline ($p<0.05$).

5.6.3.3 [^{14}C]N-Acetylated Products

Results are shown in Fig. 5.15. The level of N-acetylated products (i.e., aHT + aMT) showed a significant increase only in pineals of rats treated with desipramine ($p<0.01$). Pretreatment with atenolol did not alter the levels of N-acetylated products in pineals of vehicle-treated rats, but prevented the increase caused by desipramine ($p<0.01$).

5.6.4 Discussion

Atenolol, a β_1-adrenoceptor antagonist, crosses the blood-brain barrier (BBB) poorly (*Day* et al. 1977). Thus, its ability to antagonize the desipramine-induced increase in melatonin synthesis, following *in vivo* administration, is consistent with evidence suggesting that the pineal gland lies outside the BBB (*Arendt* et al. 1981). Moreover, this action of atenolol supports the evidence suggesting that the postsynaptic

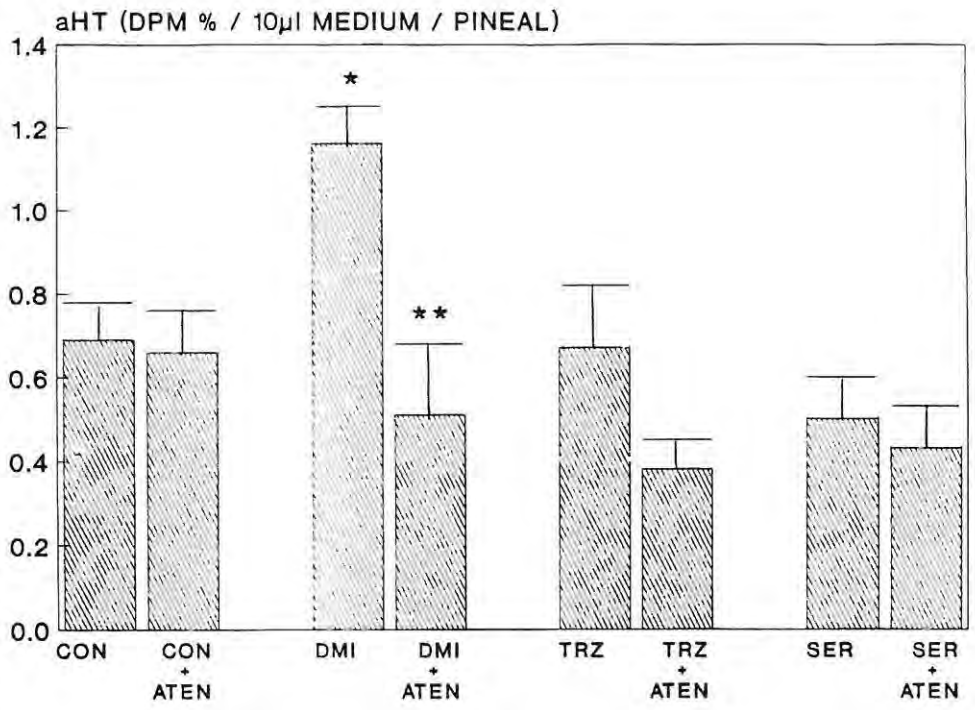


Figure 5.13 Effect of atenolol on the acute antidepressant-induced increase in [^{14}C]N-acetylserotonin (aHT) synthesis.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; DMI = desipramine; TRZ = trazodone; SER = sertraline; ATEN = atenolol.

* $p < 0.01$ when compared with controls.

** $p < 0.01$ when compared with desipramine.

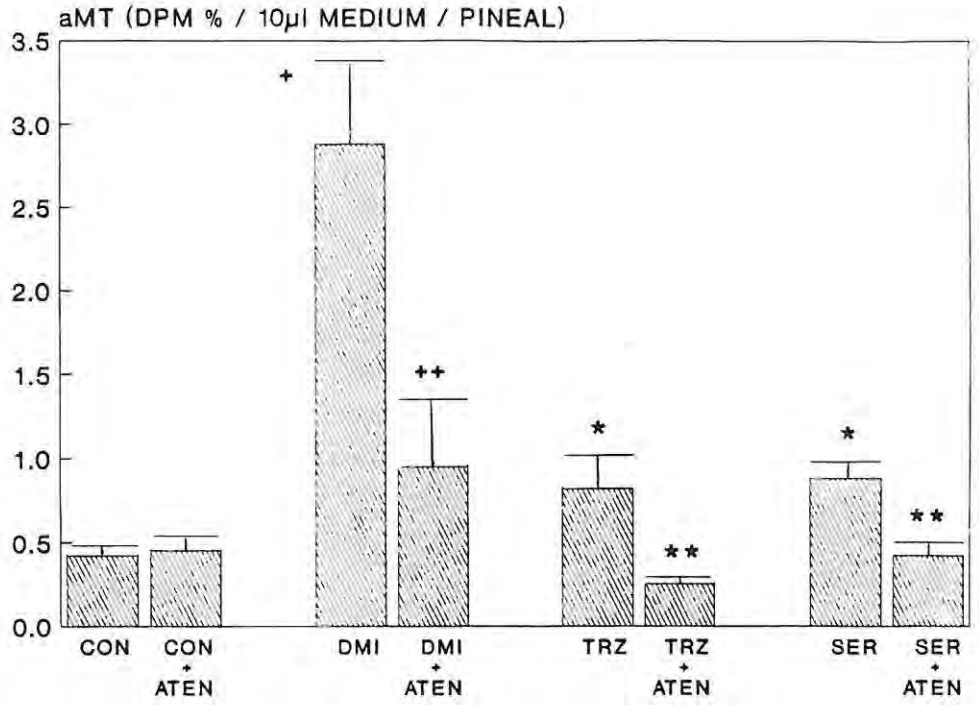


Figure 5.14 Effect of atenolol on the acute antidepressant-induced increase in [^{14}C]melatonin (aMT) synthesis.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; DMI = desipramine; TRZ = trazodone; SER = sertraline; ATEN = atenolol.

\+ $p < 0.01$ when compared with controls.

\+ + $p < 0.01$ when compared with desipramine.

* $p < 0.05$ when compared with controls.

** $p < 0.05$ when compared with respective antidepressant treatment alone.

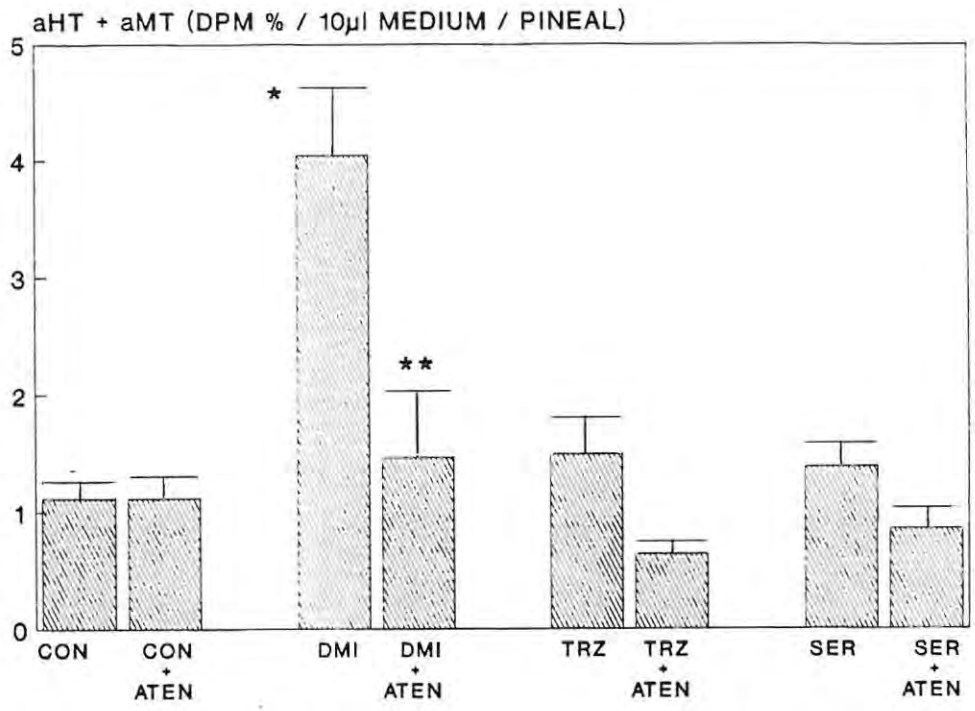


Figure 5.15 Effect of atenolol on the acute antidepressant-induced increase in [^{14}C]N-acetylated product (aHT + aMT) synthesis.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; DMI = desipramine; TRZ = trazodone; SER = sertraline; ATEN = atenolol.

* $p < 0.01$ when compared with controls.

** $p < 0.01$ when compared with desipramine.

β-adrenoceptors mediating the synthesis of melatonin are of the β_1-subtype (*Auerbach* et al. 1981, *Dickenson* et al. 1986).

More importantly, however, the present results demonstrate that atenolol pretreatment prevented the increase in melatonin synthesis caused by the acute administration of all the antidepressants examined. Further evidence is thus provided that desipramine increases melatonin synthesis via a β-adrenoceptor mechanism. For trazodone and sertraline, however, these results are unique in demonstrating such an effect. The experiment was not designed to distinguish between the effects of reuptake inhibition and β-adrenoceptor stimulation although the increased melatonin synthesis caused by these drugs does suggest a common β-adrenoceptor mechanism independent of differences in presynaptic pharmacology. The increase in melatonin synthesis observed with trazodone treatment may relate to its ability, although weak, to increase synaptic levels of NA as discussed previously (Section 5.4.4). Although the contribution to increased melatonin synthesis from a possible blockade of NA reuptake cannot be entirely excluded in the case of sertraline, the drug appears to be about 200 times less active than desipramine in inhibiting NA reuptake (*Koe* et al. 1983). Whether this effect of sertraline is due to a direct interaction with pinealocyte β-adrenoceptors, merits further investigation. It is interesting to speculate, however, that NA release and thus β-adrenoceptor activation may be modulated by trazodone and sertraline acting on specific serotonergic receptors. Although the existence of 5-HT binding sites has been shown in the pineal, such an interaction, however, is yet to be demonstrated.

5.7 CONCLUSION

The present study thus demonstrates that antidepressants with different pharmacological profiles increase pineal melatonin synthesis when administered acutely. This effect appears to be mediated by increased synaptic levels of NA as a result of reuptake inhibition, at least in the case of desipramine and maprotiline. The increased levels of NA mediate an increase in cAMP production via a β-adrenoceptor/adenylate cyclase system. An increase in cAMP levels promotes the induction of NAT activity which catalyses the conversion of 5-HT to melatonin. From the results it appears that trazodone and sertraline, drugs which primarily affect serotonergic neurotransmitter systems, also increase melatonin synthesis presumably via a β-adrenoceptor mechanism since treatment with the β-adrenoceptor antagonist, atenolol, prevented this effect. Whether this effect by trazodone and sertraline is mediated by increased synaptic NA levels or by direct β-adrenoceptor stimulation requires further clarification. It is unlikely, however, that trazodone interacts directly with β-adrenoceptors since daytime basal NAT activity remained unaffected by such treatment. These results thus suggest the intriguing possibility that trazodone and sertraline may increase the release of NA from pineal noradrenergic nerve terminals as a result of an interaction with the serotonergic neurotransmitter system. Although an interaction between these two neurotransmitter systems is yet to be demonstrated in the pineal gland, such a possibility merits further investigation.

CHAPTER SIX

EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON RAT PINEAL GLAND FUNCTION

6.1 INTRODUCTION

The essentially immediate reduction in monoamine reuptake and monoamine oxidase (MAO) activity produced by antidepressants do not coincide with their therapeutic actions, which appear only after 2-4 weeks of treatment. Current interest has therefore focussed on the longer-term adaptational alterations in monoamine feedback regulation and neurotransmitter receptor sensitivity that occur as a result of chronic antidepressant administration. Among the effects observed following chronic antidepressant administration, the most significant and constant effect appears to be the down-regulation of the adenylate cyclase system by noradrenaline (NA) in brain slices (see Section 1.2.3.2.2). This subsensitivity is generally but not always linked to a reduction in the density of β-adrenoceptor recognition sites.

The rat pineal gland offers a convenient model for studying such adaptational phenomena. It is innervated by noradrenergic fibres and has a high density of β-adrenoceptors (Section 1.3.3.1). Moreover, the synthesis of the pineal hormone melatonin is regulated by NA acting via these β-adrenoceptors. Several researchers have investigated the effect of chronic antidepressant administration on rat pineal function (Section 1.3.7). Despite some inconsistencies in the data, chronic treatment with tricyclic antidepressants (TCAs) or MAO inhibitors (MAOIs) appears to reduce both the density of pineal β-adrenoceptors (Section 1.3.7.1.2) and the sensitivity of adenylate cyclase (Section 1.3.7.2.2). This effect does not appear to be correlated only with inhibition of NA reuptake or metabolism since iprindole and lithium, which affect neither monoamine reuptake nor metabolism, also reduce pineal β-adrenoceptor density. The limited data available, however, indicate that other atypical antidepressants such as mianserin and trazodone as well as the selective serotonin (5-HT) reuptake inhibiting antidepressants and electroconvulsive shock therapy (ECT) do not reduce pineal β-adrenoceptor density or adenylate cyclase sensitivity. Further studies are therefore necessary to confirm these findings since, in contrast, studies in the limbic forebrain and cerebral cortex indicate that these antidepressants as well as ECT cause a reduction in β-adrenoceptor density and/or adenylate cyclase sensitivity (Section 1.2.3.2.2).

Moreover, the available data are inconclusive as to whether chronic antidepressant administration enhances or reduces noradrenergic neurotransmission as measured by melatonin output. Several studies indicate that a reduced β-adrenoceptor responsiveness following chronic antidepressant treatment to rats is associated with a reduction in melatonin output (Section 1.3.7.4.2). These findings have been used in support of the hypothesis that antidepressant drugs act by reducing noradrenergic neurotransmission (*Sulser* et al. 1978, *Sulser* 1984). In contrast, however, studies in depressed patients indicate that antidepressant drugs may enhance noradrenergic neurotransmission, as evidenced by

increased melatonin synthesis (Section 1.3.6.3). In support of these findings, on the other hand, a few studies have shown that chronic antidepressant treatment to rats is associated with an increased melatonin output (Section 1.3.7.4.2). Moreover, studies in primates also show that chronic antidepressant administration results in increased melatonin concentrations (*Murphy* et al. 1987b). These observations cast doubt on the importance of β-adrenoceptor down-regulation as a mechanism of antidepressant action. Thus, together with the data on melatonin alterations in depression, these findings suggest that changes in melatonin synthesis may be involved in the mechanism of action of antidepressant drugs. However, it appears from studies in animals that increased melatonin levels are associated primarily with antidepressants that inhibit NA reuptake or metabolism. Whether this effect is also observed following chronic treatment with other antidepressants remains to be established.

Thus, the present study sought to investigate the effect of chronic treatment with different antidepressants on rat pineal gland function. Antidepressants with relatively selective actions on noradrenergic (desipramine and maprotiline) or serotonergic (trazodone) function were employed in this study. In addition to β-adrenoceptor binding studies, NAT activity and pineal indole synthesis were also evaluated. Furthermore, some newer, selective 5-HT reuptake inhibitors (sertraline, panuramine, CGP-6085A and RU-25591) were investigated for their effects on pineal indole synthesis.

Other aspects of pineal function, including cAMP accumulation and phosphodiesterase activity, were also evaluated following chronic antidepressant administration but since these formed part of another study employing additional antidepressants, they are discussed separately (see Chapter 7).

6.2 MATERIALS AND METHODS

6.2.1 Animals

Adult male rats (200-300 g) of the Wistar strain were used throughout this study. The animals were subjected to a daily photoperiod of 12 h (lights on at 06:00 h). All animals had free access to food and water. Their living environment is described in detail in Section 2.1.

6.2.2 Drugs

The antidepressants used in this study and were kindly donated by the Companies indicated as follows : Desipramine HCl, maprotiline HCl and CGP-6085A (Ciba-Geigy); trazodone HCl and RU-25591 (Rousell); panuramine HCl (Wyeth) and sertraline HCl (Pfizer).

Desipramine and trazodone were administered as solutions in 0.9% saline. Maprotiline was administered as a solution in distilled water. Control rats in these experiments received 0.9% saline in a volume of 1 ml/kg. When the experimental antidepressants sertraline, panuramine, CGP-6085A and

RU-25591 were used, these were administered as solutions in a vehicle consisting of the mixture (% by volume), 1.5% benzyl alcohol + 5% polysorbate + 93.5% distilled water. The control group in this case received vehicle, 2 ml/kg.

6.2.3 Statistical Analysis

Statistical analyses were performed using Student's *t*-test. A *P* value of greater than 0.05 between groups was considered not significantly different. Where applicable, the data were analysed using one-way analysis of variance followed by Scheffe's post hoc tests for multiple range comparisons.

6.3 EXPERIMENT 1 : EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON PINEAL β-ADRENOCEPTORS

6.3.1 Introduction

The present experiment sought to investigate the effect of chronic treatment with different antidepressants on the binding of [^{3}H]DHA to pineal β-adrenoceptors. The antidepressants that were evaluated in this experiment included desipramine, trazodone and maprotiline.

6.3.2 Experimental Procedure

Drug Administration : Groups of rats were treated chronically with antidepressant, 10 mg/kg i.p., once daily at 10:00 h for 2 weeks. Control rats received saline, 1 ml/kg, chronically. All animals were sacrificed 24 h after final drug administration for use in the experiment.

Assay of β-Adrenoceptors : β-Adrenoceptors were assayed by measuring the specific binding of a single concentration of [^{3}H]DHA (5 nM) to pineal gland membranes. The assay was performed as outlined in Section 2.2.1.2.

Analysis of Data : Specific binding of [^{3}H]DHA is expressed as fmol/mg protein. Values represent means ± S.E.M. Eight rats were used in each treatment group.

6.3.3 Results

The effect of chronic antidepressant treatment on pineal β-adrenoceptors is presented in Fig. 6.1. Chronic treatment of rats with desipramine significantly reduced the specific binding of [^{3}H]DHA to pineal gland membranes by more than 40% ($p<0.05$). On the other hand, the specific binding of [^{3}H]DHA to pineal membranes was not significantly altered following chronic treatment of rats with either trazodone or maprotiline.

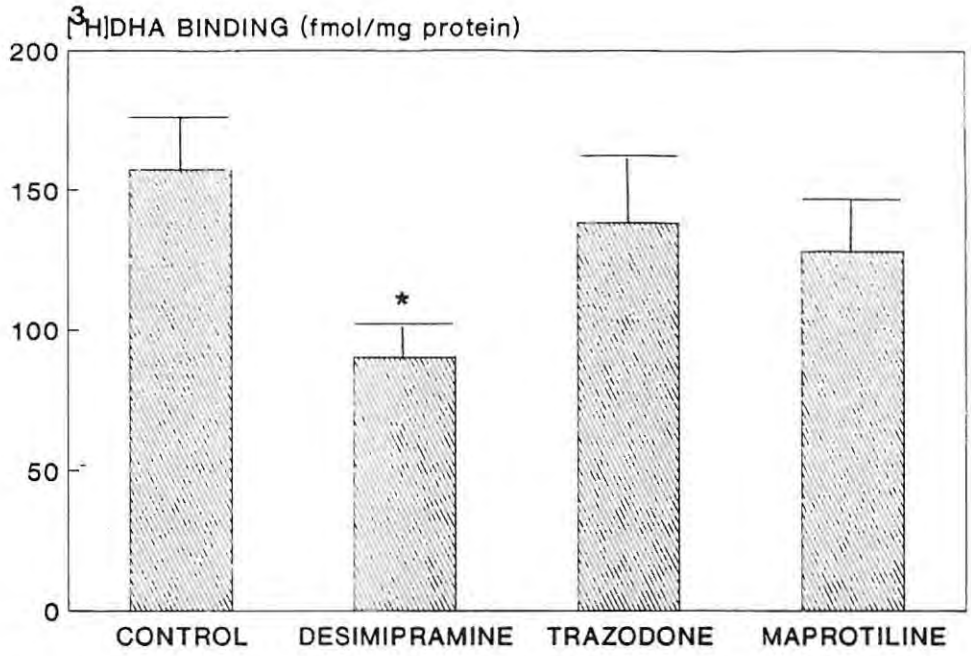


Figure 6.1 Effect of chronic antidepressant treatment on the specific binding of [^{3}H]DHA in the rat pineal gland.

Values represent means ± S.E.M. Eight rats were used in each experimental group.

* $p < 0.01$ when compared with controls.

6.3.4 Discussion

The reduced binding of [^{3}H]DHA to pineal membranes following chronic desipramine treatment observed in the present study is consistent with previous findings suggesting a reduction in β-adrenoceptor density. *Moyer* et al. (1981) demonstrated that the changes in β-adrenoceptor density produced by chronic desipramine treatment were due to a reduced number (B_{max}) of receptors and not to alterations in their affinity (K_d). This effect of desipramine is presumably related to chronic overexposure of the β-adrenoceptors to NA consequent to reuptake inhibition. This suggestion is supported by the finding that the desipramine-induced down-regulation of pineal β-adrenoceptors was prevented by superior cervical ganglionectomy, a procedure that reduces sympathetic input to the gland (*Moyer* et al. 1981). However, the observation that another NA reuptake inhibitor, maprotiline, did not reduce β-adrenoceptor density suggests that other mechanisms may also be involved in this process. Furthermore, *Friedman* et al. (1984) showed that iprindole, which has no appreciable effect on monoamine reuptake, release or turnover, also reduced β-adrenoceptor density following chronic administration. *Skene* (1985), also found maprotiline to be without effect on pineal β-adrenoceptors. Suprisingly, in the latter study, chronic trazodone administration was associated with an increased binding to pineal β-adrenoceptors. In the present study, however, trazodone did not alter β-adrenoceptor binding. In contrast, trazodone has been shown to reduce β-adrenoceptor density in rat cerebral cortex (see *Maj* et al. 1984). This poses the possibility that there may be differences between pineal and cortical β-adrenoceptors. Alternatively, the apparent lack of effect of trazodone and maprotiline may relate to the disadvantage of assessing receptor binding at a fixed time of day only. The diurnal variation in pineal β-adrenoceptor density is well documented (*Romero* et al. 1975) and, furthermore, chronic antidepressant treatment has been shown to alter the circadian rhythm of β-adrenoceptor density in rat brain (*Wirz-Justice* et al. 1980b). It is possible, therefore, that changes in β-adrenoceptor density, caused by chronic treatment with different antidepressants, may not be apparent at the same time of day.

From the present results, however, it appears that antidepressants with different pharmacological profiles do not have a common effect on β-adrenoceptors in the rat pineal gland, unlike that suggested for other areas of the brain.

6.4 EXPERIMENT 2 : EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON PINEAL SEROTONIN N-ACETYLTRANSFERASE ACTIVITY

6.4.1 Introduction

Serotonin N-acetyltransferase (NAT) activity in the pineal gland is regulated primarily via a β-adrenoceptor-mediated mechanism. The measurement of NAT activity thus affords a useful model for assessing the functional significance of altered β-adrenoceptor sensitivity. Few researchers, however, have utilized this model to evaluate the effect of chronic antidepressant administration.

Thus, in order to correlate the results of the previous β-adrenoceptor binding experiment with a functional biochemical parameter, pineal NAT activity was assayed following chronic treatment of rats with desipramine, trazodone or maprotiline.

6.4.2 Experimental Procedure

Drug Administration : Rats were treated chronically with antidepressant, 10 mg/kg i.p., once daily at 10:00 h for 2 weeks. Control rats received saline, 1 ml/kg, chronically. All animals were sacrificed 24 h after final drug administration for use in the experiment.

Assay of Serotonin N-Acetyltransferase : Rat pineal glands were rapidly excised and incubated in the presence or absence of 10 μM *l*-isoprenaline for 3 h prior to the determination of enzyme activity. The technique of pineal culture and the assay of enzyme activity were performed as outlined in Section 2.5.2.

Analysis of Data : Results are expressed as picomoles of [^{3}H]N-acetyltryptamine formed per pineal per hour. Values represent means ± S.E.M. Four rats were used in each experimental group.

6.4.3 Results

Basal Enzyme Activity : The effect of chronic antidepressant administration on basal NAT activity is shown in Fig. 6.2a. None of the antidepressants tested produced any significant alteration of basal NAT activity when compared with saline treatment.

In Vitro **Isoprenaline-Stimulated Enzyme Activity :** To determine whether chronically administered antidepressants would influence the ability of exogenous catecholamines to stimulate NAT activity, pineals were incubated with 10 μM isoprenaline. The results of this experiment are presented in Fig. 6.2b. Isoprenaline produced a significant increase in NAT activity in both antidepressant- and saline-treated groups when compared with basal enzyme activity ($p < 0.01$). However, the response to isoprenaline following chronic desipramine treatment was significantly decreased when compared with that following chronic saline treatment ($p < 0.01$). Repeated treatment with trazodone or maprotiline did not alter the response to isoprenaline relative to saline-treated controls.

6.4.4 Discussion

The observation that chronic desipramine treatment decreased the isoprenaline-induced elevation in pineal NAT activity is consistent with the previously noted down-regulation of β-adrenoceptors following such treatment (Section 6.3.3). This effect of desipramine is presumed to be due to excessive noradrenergic input, consequent to chronic reuptake inhibition. *Moyer* et al. (1981) demonstrated that desipramine decreased the catecholamine-induced stimulation of adenylate cyclase activity and cAMP accumulation following chronic administration. *Bronstein* et al. (1984) found that while desipramine

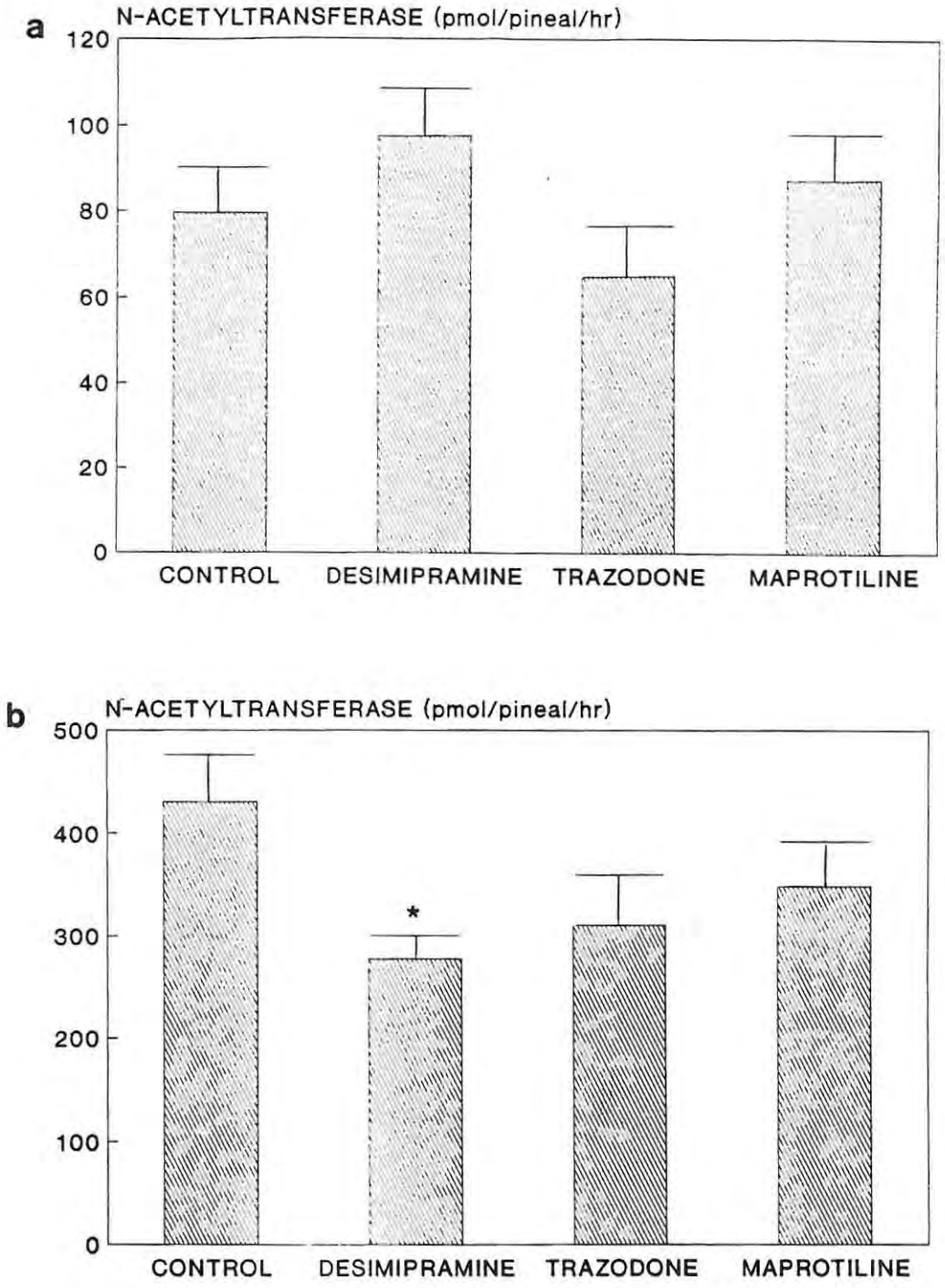


Figure 6.2 Effect of chronic antidepressant treatment on daytime basal (a) and *in vitro* isoprenaline-stimulated (b) N-acetyltransferase (NAT) activity.
Values represent means S.E.M. Four rats were used in each experimental group.

* $p < 0.01$ when compared with controls.

treatment reduced the NAT response to isoprenaline, it also produced a significant increase in daytime basal NAT activity. In the present study, however, although basal NAT levels were slightly increased by desipramine, these were not statistically significant. Differences in experimental design may account for these conflicting results. Basal NAT activity could conceivably be elevated by desipramine as a result of the increased NA levels at the synapse.

Furthermore, the observation that chronic trazodone or maprotiline administration did not affect basal or isoprenaline-stimulated NAT activity is consistent with the results of the previous experiment (Section 6.3.3) showing that β-adrenoceptor binding was not affected by such treatment.

6.5 EXPERIMENT 3 : EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON [^{14}C]INDOLE METABOLISM BY ORGAN CULTURES OF RAT PINEAL GLANDS

6.5.1 Introduction

In the present experiment, the effect of chronic treatment with desipramine, trazodone or maprotiline on pineal indole metabolism was evaluated in an attempt to correlate the results of the previous β-adrenoceptor binding (Section 6.3) and NAT activity (Section 6.4) experiments following such treatment. In addition, several newer, selective 5-HT reuptake inhibitors were investigated for their effect on indole metabolism following chronic administration.

6.5.2 Experimental Procedure

Drug Administration : Rats were treated chronically with antidepressant, 10 mg/kg i.p., once daily at 10:00 h for 2 weeks. In one experiment, desipramine, trazodone and maprotiline were administered and controls received 0.9% saline. In another experiment, sertraline, panuramine, CGP-6085A and RU-25591 were administered and control rats received vehicle (see Section 6.2.2). In both experiments, pineals were collected 24 h after final drug administration.

Pineal Organ Culture and Analysis of [^{14}C]Indoles : The technique of organ culture and the analysis of radiolabeled indoles were performed as outlined in Section 2.6.2. Briefly, pineal glands were individually cultured in the presence of [^{14}C]serotonin and incubated at 37^{o}C under 95% O_2 : 5% CO_2. After 8 h of culture, a 10 µl aliquot of culture medium was removed and analysed for its content of radioactive indoles.

Analysis of Data : Results are expressed as the percentage of total radioactivity (DPM%/10 µl medium/pineal). Values represent means ± S.E.M for four or eight rats.

6.5.3 Results

6.5.3.1 Effect on the Synthesis of [^{14}C]N-Acetylated Indoles

6.5.3.1.1 [^{14}C]N-Acetylserotonin

The effect of chronic antidepressant treatment on the synthesis of N-acetylserotonin (aHT) is presented in Fig. 6.3. When compared with chronic saline treatment, desipramine produced a significant increase in aHT synthesis ($p < 0.01$) (Fig. 6.3a). Trazodone and maprotiline did not significantly alter aHT levels relative to saline treatment.

In a separate experiment, chronic treatment with RU-25591 produced a significant increase in aHT synthesis when compared with vehicle treatment ($p < 0.01$) (Fig. 6.3b). On the other hand, panuramine produced a significant decrease in aHT synthesis ($p < 0.05$). Sertraline and CGP-6085A did not significantly alter aHT synthesis relative to controls.

6.5.3.1.2 [^{14}C]Melatonin

The influence of chronic antidepressant treatment on melatonin (aMT) synthesis is shown in Fig. 6.4. Treatment with desipramine significantly increased aMT synthesis when compared with saline-treated controls ($p < 0.01$) (Fig. 6.4a). Treatment with trazodone or maprotiline was without any significant effect on aMT synthesis.

Relative to chronic vehicle treatment, none of the experimental antidepressants significantly altered aMT synthesis (Fig.6.4b).

6.5.3.1.3 [^{14}C]N-Acetylated Products

The radioactivity recovered as the N-acetylated products (i.e. aHT + aMT) following chronic antidepressant treatment is presented in Fig. 6.5. These levels were raised following chronic treatment of rats with desipramine ($p < 0.01$) when compared with saline-treated controls (Fig. 6.5a). Trazodone or maprotiline treatment, on the other hand, produced no significant change in the level of these metabolites when compared with saline treatment.

Chronic treatment with RU-25591 also raised the level of N-acetylated products when compared with vehicle-treatment ($p < 0.05$) (Fig. 6.5b). While sertraline and CGP-6085A did not alter these levels following repeated treatment, panuramine was found to produced a significant decrease ($p < 0.05$).

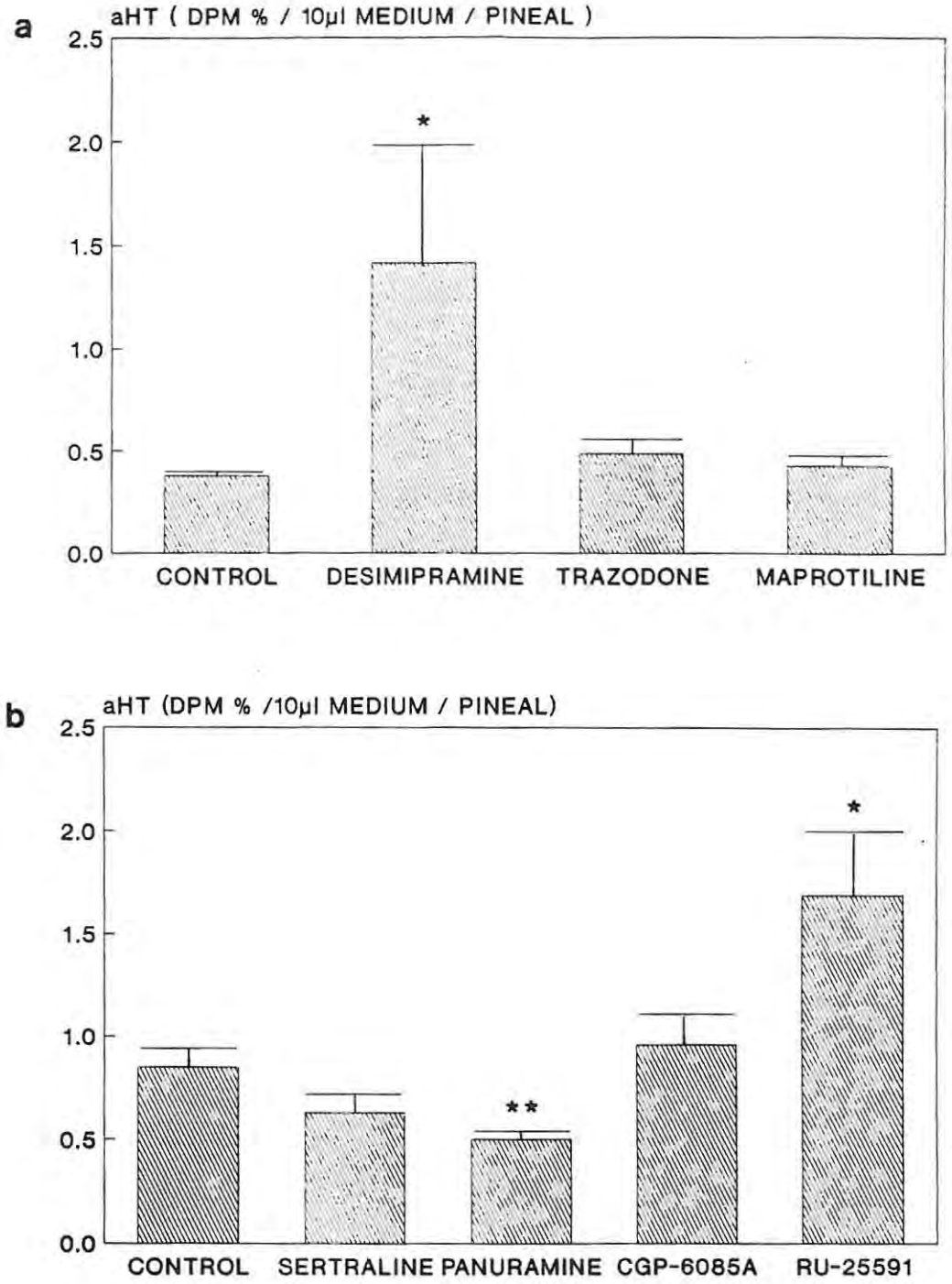


Figure 6.3 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]N-acetylserotonin (aHT) by organ cultures of rat pineal glands.

Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 6.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

** $p < 0.05$ when compared with controls.

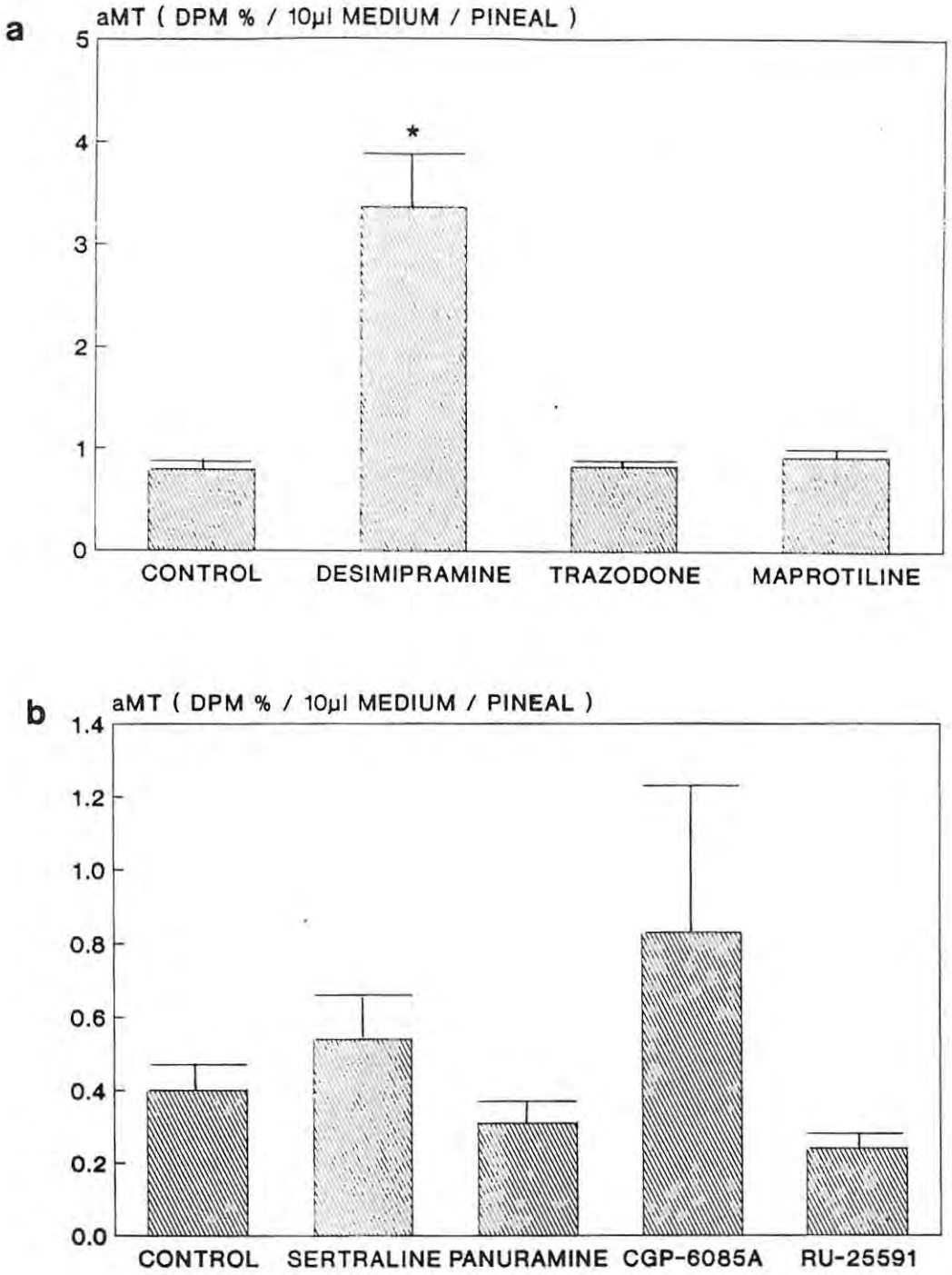


Figure 6.4 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]melatonin (aMT) by organ cultures of rat pineal glands.

Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 6.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

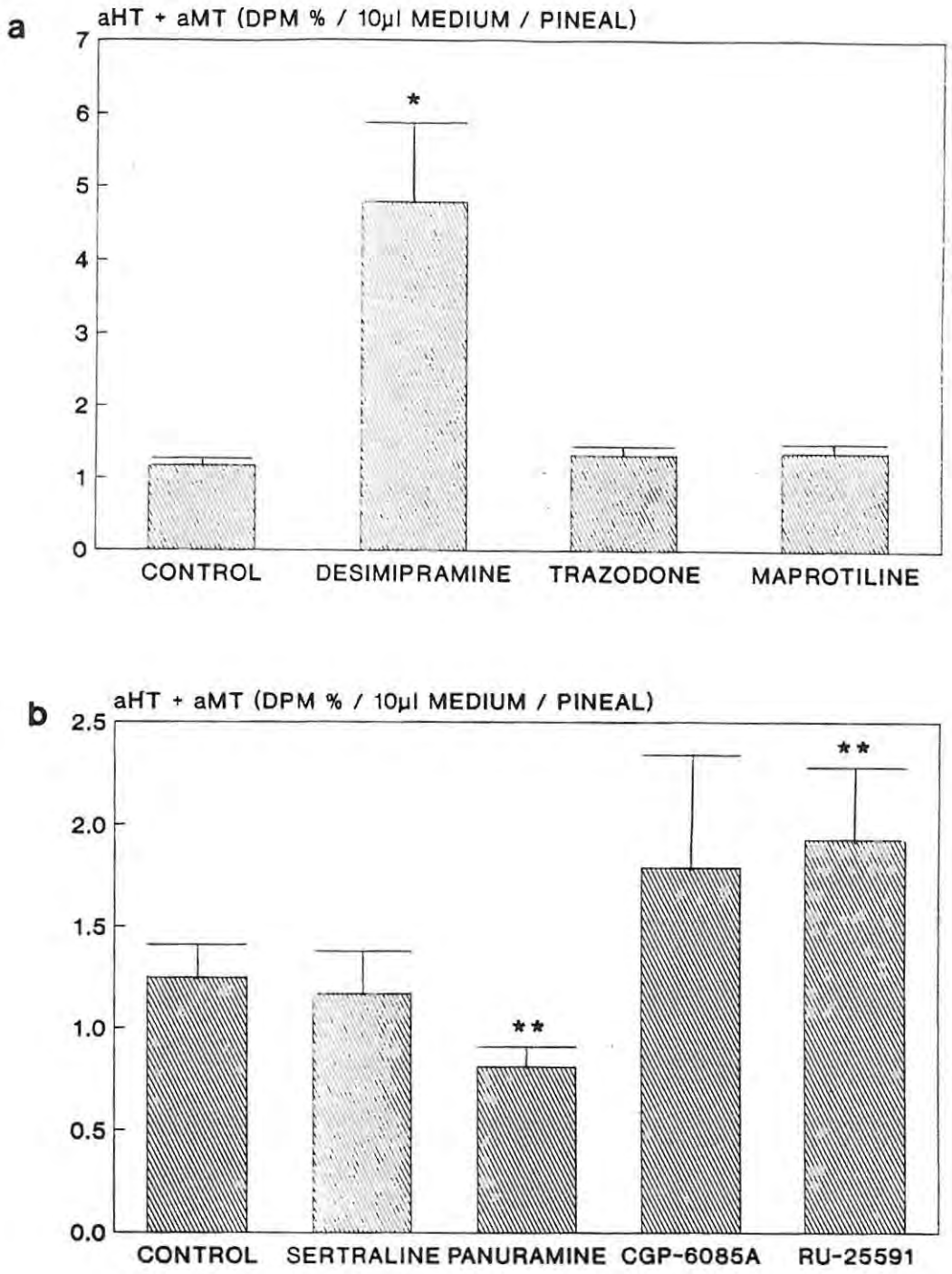


Figure 6.5 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]N-acetylated products (aHT + aMT) by organ cultures of rat pineal glands. Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 6.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

** $p < 0.05$ when compared with controls.

6.5.3.2 Effect on the Synthesis of [^{14}C]Deaminated Indoles

6.5.3.2.1 [^{14}C]5-Hydroxyindole Acetic Acid

The effect of chronic antidepressant treatment on 5-hydroxyindole acetic acid (HA) synthesis is shown in Fig. 6.6. The synthesis of HA was significantly decreased in pineals of rats treated repeatedly with desipramine when compared with saline treatment ($p<0.01$) (Fig. 6.6a). The other antidepressants showed no significant alteration in HA synthesis when compared with their respective controls (Figs. 6.6a and b).

6.5.3.2.2 [^{14}C]5-Hydroxytryptophol

The effect of chronic antidepressant treatment on 5-hydroxytryptophol (HL) synthesis is shown in Fig. 6.7. When compared with saline treatment, desipramine and maprotiline significantly decreased the synthesis of HL ($p<0.01$) (Fig. 6.7a). Treatment of rats with the other antidepressants did not significantly alter HL synthesis (Figs. 6.7a and b).

6.5.3.2.3 [^{14}C]5-Methoxyindole Acetic Acid

The effect of repeated antidepressant treatment on 5-methoxyindole acetic acid (MA) synthesis is shown in Fig. 6.8. Desipramine treatment significantly decreased MA synthesis when compared with saline treatment ($p<0.01$) (Fig. 6.8a). None of the other antidepressants tested produced any significant change in the level of MA when compared with their respective controls (Fig. 6.8b).

6.5.3.2.4 [^{14}C]5-Methoxytryptophol

The effect of chronic antidepressant treatment on 5-methoxytryptophol (ML) synthesis is shown in Fig. 6.9. Treatment with desipramine was found to significantly decrease ML synthesis relative to saline-treated controls ($p<0.01$) (Fig. 6.9a). None of the other antidepressants appeared to alter MA levels when compared with their respective controls (Figs. 6.9a and b).

6.5.3.2.5 [^{14}C]Monoamine Oxidase Products

The monoamine oxidase (MAO) products represent the sum of those indoles formed by the oxidative deamination of radiolabeled serotonin via the enzyme MAO; i.e., HA, HL, MA, ML. Figure 6.10 represents the effect of chronic antidepressants on the synthesis of MAO products. Of the antidepressants tested, only desipramine produced a significant decrease in the level of MAO products when compared to controls ($p<0.01$) (Fig. 6.10a). The other antidepressants did not appear to alter these levels relative to their respective controls (Figs. 6.10a and b).

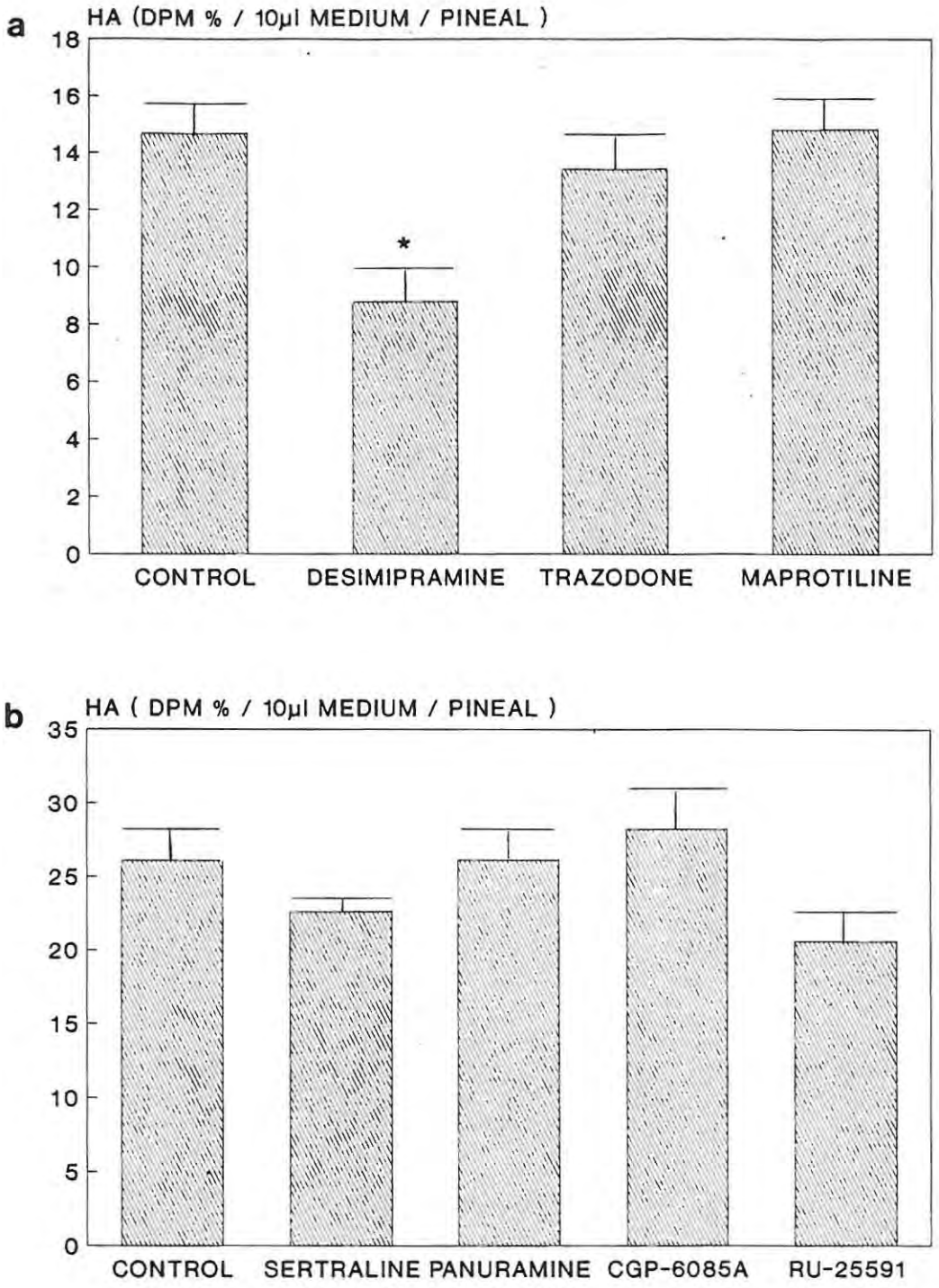


Figure 6.6 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]5-hydroxyindole acetic acid (HA) by organ cultures of rat pineal glands. Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 6.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

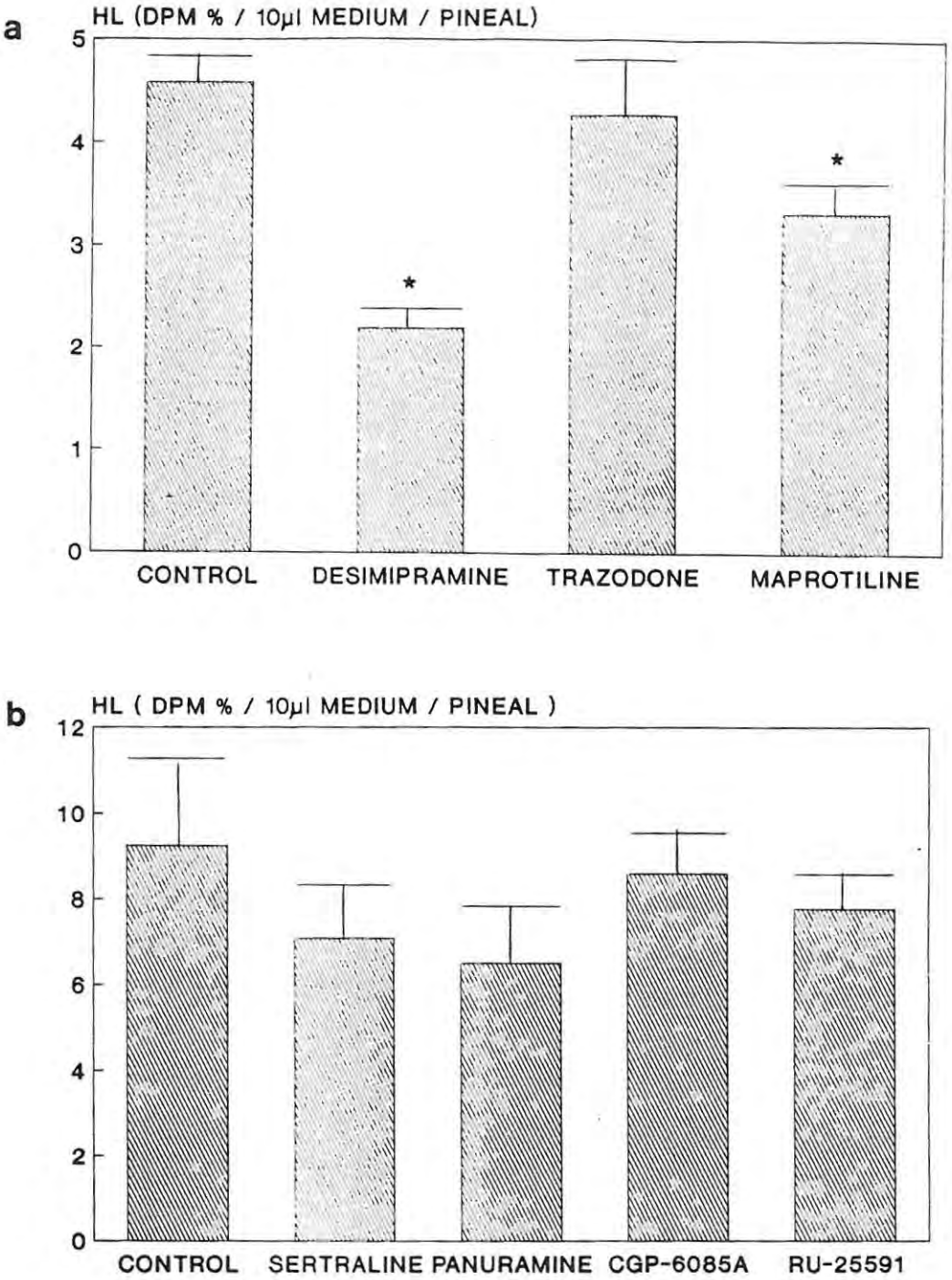


Figure 6.7 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]5-hydroxytryptophol (HL) by organ cultures of rat pineal glands.

Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 6.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

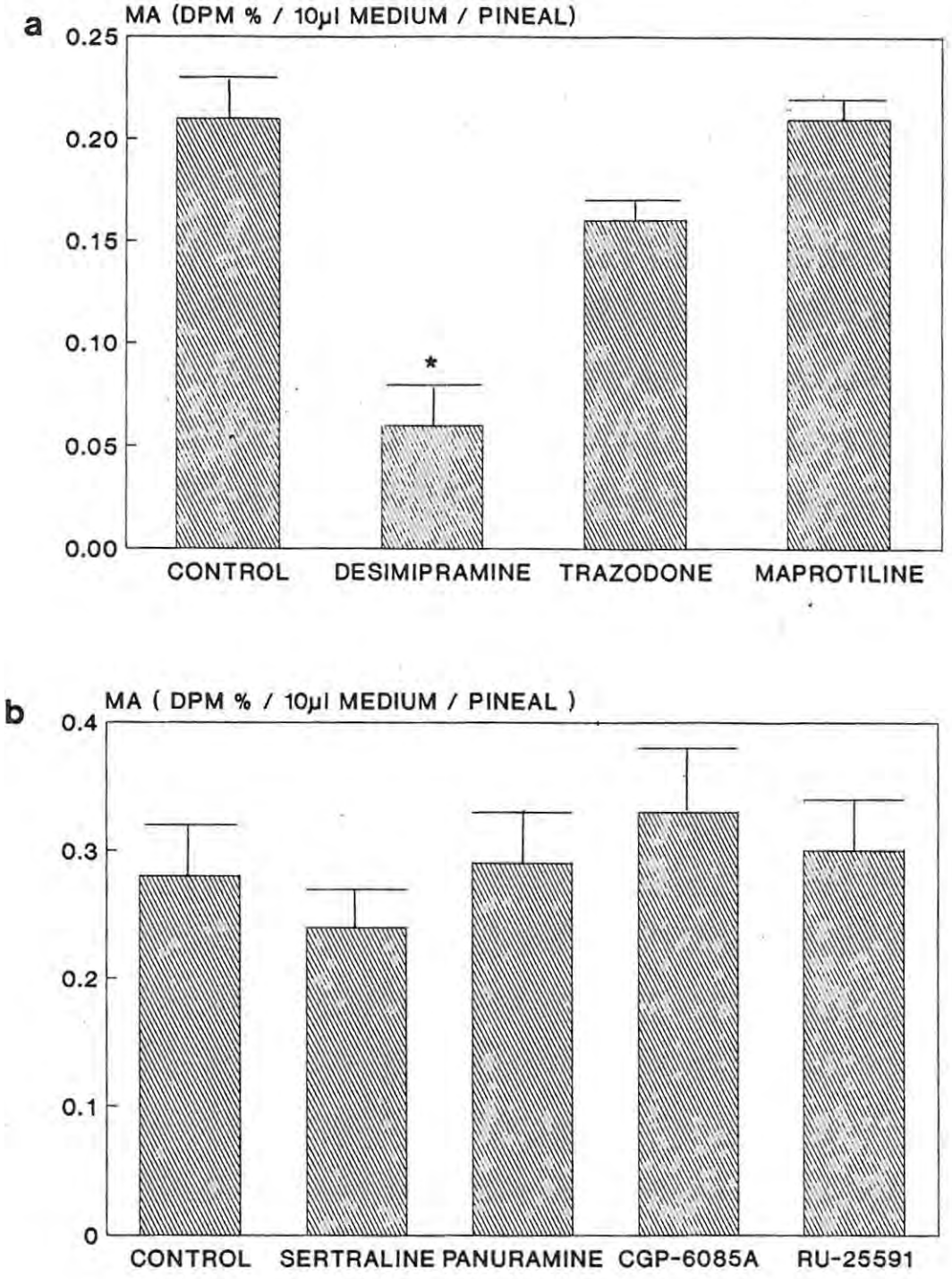


Figure 6.8 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]5-methoxyindole acetic acid (MA) by organ cultures of rat pineal glands. Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 6.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

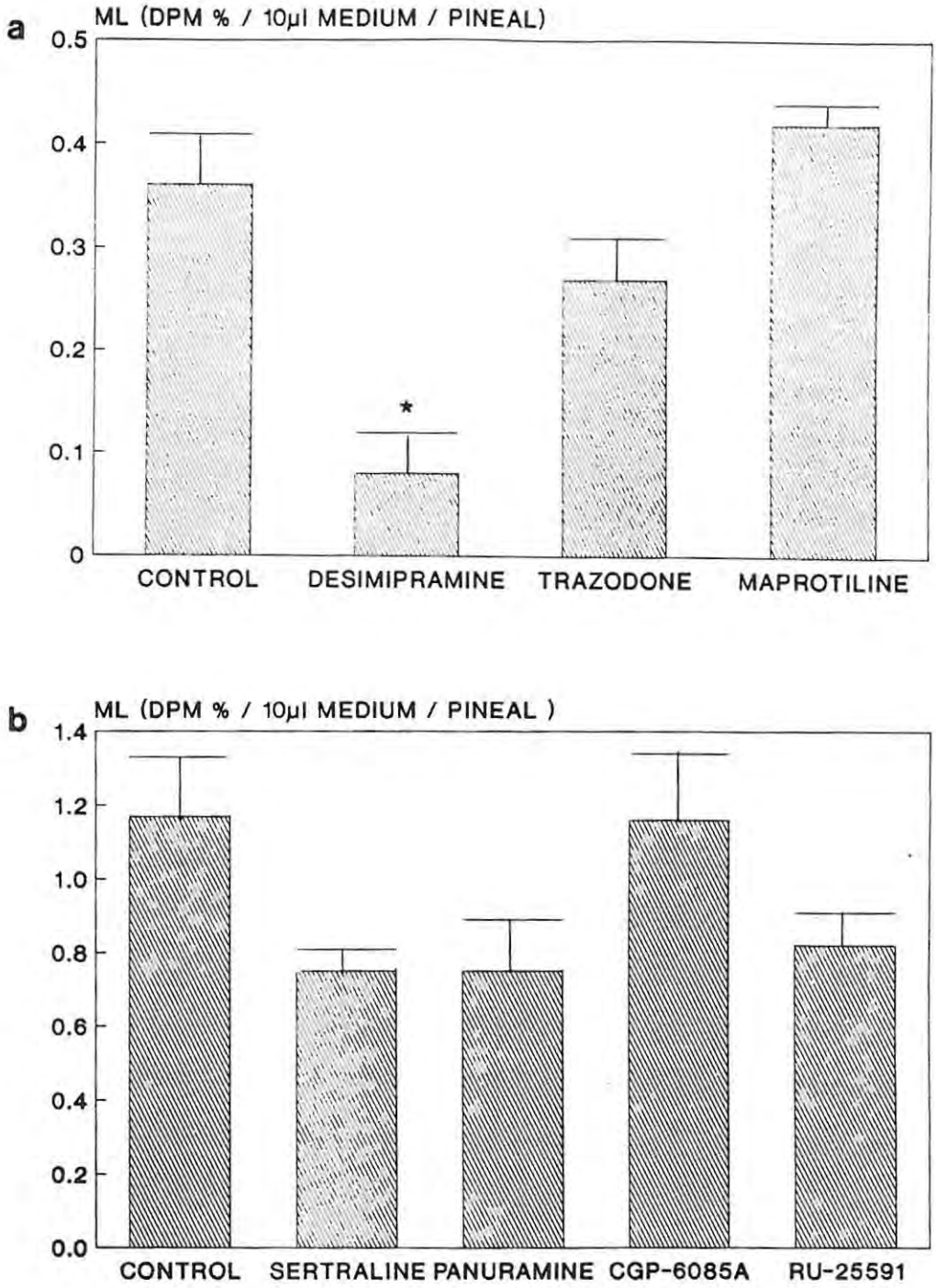


Figure 6.9 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]5-methoxytryptophol (ML) by organ cultures of rat pineal glands.
Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 6.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

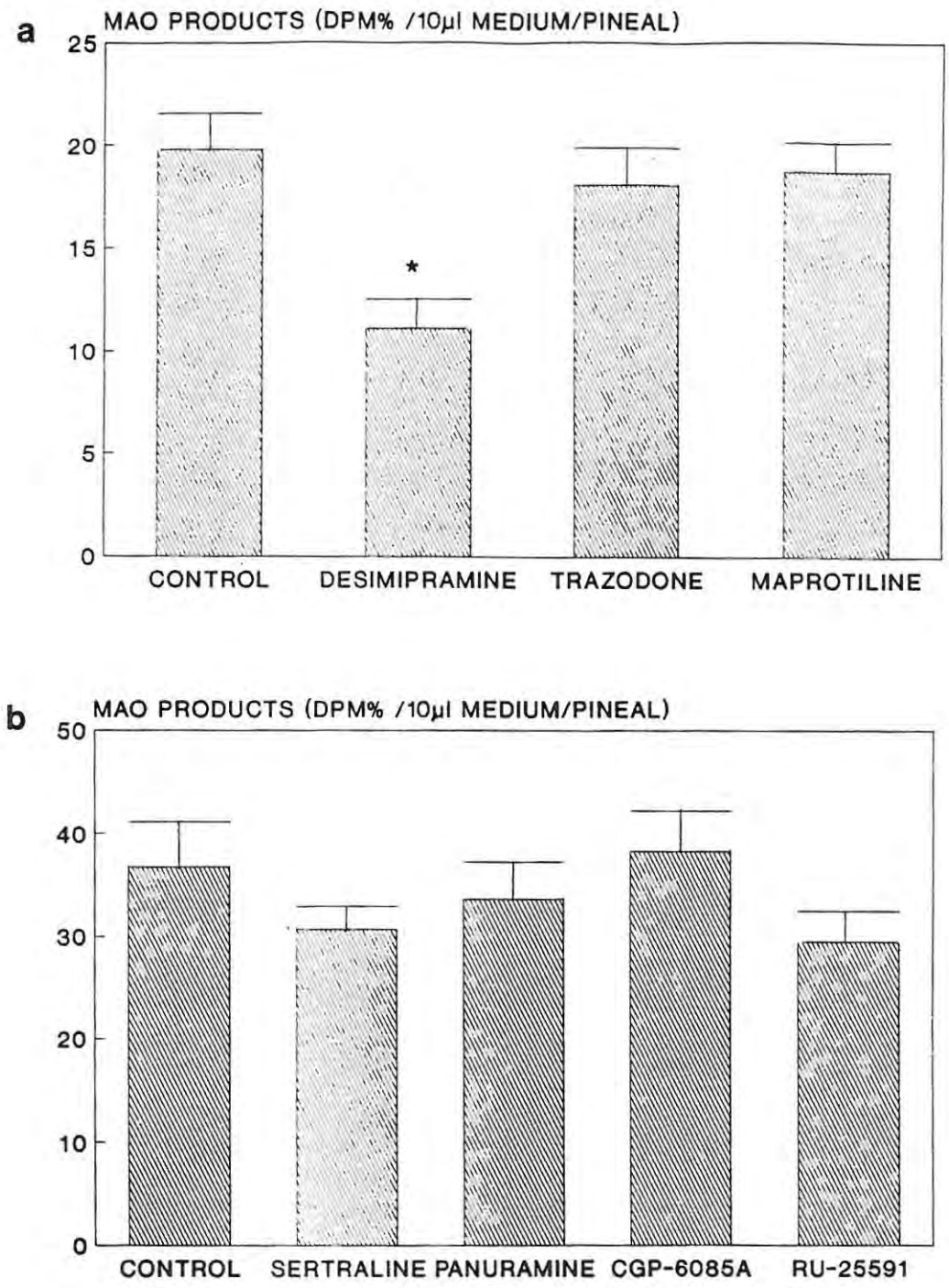


Figure 6.10 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]monoamine oxidase (MAO) products by organ cultures of rat pineal glands.

Antidepressants were administered as solutions in either 0.9% saline (a) or vehicle (b) (see Section 6.2.2). Values represent means ± S.E.M. Four and eight rats were used in the antidepressant-treated and control groups respectively.

* $p < 0.01$ when compared with controls.

6.5.4 Discussion

The present results indicate that chronic desipramine treatment produced a large increase in the synthesis of N-acetylserotonin and melatonin in rat pineal gland cultures. This finding is consistent with previous studies (*Pillay* and *Potgieter* 1984, *Skene* 1985). On the other hand, maprotiline was found to be without effect on the synthesis of N-acetylated products. *Skene* (1985) demonstrated a similar lack of effect on melatonin synthesis following chronic maprotiline treatment, although N-acetylserotonin levels were slightly increased.

In the light of previous findings showing that chronic desipramine treatment produced subsensitivity of the pineal β-adrenoceptor/adenylate cyclase system, the present findings may seem paradoxical. However, it is possible that the elevated synaptic NA levels caused by desipramine administration may be sufficient to overcome the apparent reduction in β-adrenoceptor sensitivity. This suggestion is supported by the work of *Moyer* et al. (1979) showing that 24 h following chronic desipramine treatment, there were appreciable amounts of drug remaining in the pineal to inhibit NA reuptake. Thus, the increased NA levels may be sufficient to maintain high basal levels of NAT activity which, in turn, would increase the conversion of 5-HT to N-acetylserotonin and melatonin. The observation by *Bronstein* et al. (1984) that basal NAT activity is elevated following chronic desipramine treatment, is consistent with this hypothesis. It is likely, therefore, that the differences in effects between desipramine and maprotiline noted in the present study may relate to differences in their respective potencies as NA reuptake inhibitors.

These observations therefore suggest that the net effect of chronic desipramine treatment is to increase noradrenergic neurotransmission. It has previously been shown that desipramine and other TCAs reduced the melatonin response to β-adrenoceptor stimulation following their chronic administration (eg. *Cowen* et al. 1983, *Friedman* et al. 1984). These findings, however, do not indicate a reduction in noradrenergic neurotransmission, as suggested by the authors, since pineal melatonin synthesis was used in these studies as an index of β-adrenoceptor sensitivity. The present study demonstrates that in unstimulated pineal glands, despite β-adrenoceptor subsensitivity (Section 6.3), melatonin synthesis is significantly increased by chronic desipramine treatment.

However, Trazodone and the selective 5-HT reuptake inhibitors, sertraline, panuramine and CGP-6085A failed to increase the synthesis of N-acetylated products following chronic administration. *Skene* (1985) showed a similar lack of effect of trazodone and fluoxetine (another selective 5-HT reuptake inhibitor) on N-acetylated product synthesis. Interestingly, in a previous experiment (Section 5.5) trazodone and sertraline were found to increase N-acetylserotonin and/or melatonin synthesis following their acute administration. The reason for this apparent inconsistency is not known, but it is clear that acute trazodone or sertraline treatment did not increase N-acetylated product synthesis by increasing the amount of 5-HT available in the pinealocyte, since, if this had occurred, a similar effect would have been noted following their chronic administration. The inability of panuramine and CGP-6085A to increase

N-acetylated product synthesis in the present experiment supports this suggestion. Moreover, panuramine decreased the synthesis of N-acetylated products, suggesting that it may reduce the uptake of exogenous 5-HT into the pinealocyte for conversion to melatonin. On the other hand, the selective 5-HT reuptake inhibitor, RU-25591, increased N-acetylated product synthesis following chronic, but not acute (Section 5.5), administration. Taken together, the results suggest that 5-HT reuptake inhibiting antidepressants do not have a common effect on melatonin synthesis.

Several factors may account for the disparate effects observed following chronic treatment with the selective 5-HT reuptake inhibitors. The protocol employed in the present study, involving a once daily dosing regimen, may have been inadequate to elicit increases in melatonin synthesis with most of these drugs. Studies that have demonstrated β-adrenoceptor subsensitivity effects with these drugs in other brain areas, have mostly employed twice daily dosing regimens. On the other hand, the increased synthesis of melatonin noted with RU-25591 may be due to the formation of an active metabolite with a greater selectivity for inhibiting NA reuptake than the parent compound.

Also of interest, is the observation that chronic desipramine treatment reduced the synthesis of monoamine oxidase (MAO) products. This finding is consistent with previous experiments showing that desipramine reduced the synthesis of MAO products when administered acutely (Section 5.5.3.2.5) or when added to pineal cultures (*Nir* and *Hirschmann* 1983). As discussed previously (Section 5.5.4), this finding may reflect the ability of desipramine to inhibit pineal MAO activity. Alternatively, since the amount of 5-HT utilized for N-acetylation by NAT is increased by desipramine treatment, the amount of 5-HT available for oxidative deamination may be reduced. However, *Skene* (1985) was unable to demonstrate a change in the level of MAO products following chronic desipramine treatment. Furthermore, the present finding that desipramine reduced the methylation of the 5-hydroxyindoles to their respective 5-methoxyindoles, suggests that the enzyme HIOMT preferentially utilizes the increased levels of N-acetylserotonin as substrate. In contrast to acute treatment, chronic treatment with maprotiline did not reduce the level of total MAO products, although the level of 5-hydroxytryptophol was found to be decreased. As observed with acute treatment, chronic treatment with trazodone was found to be without effect on the level of MAO products.

6.6 CONCLUSION

The results of the present study indicate that antidepressants with different pharmacological profiles have variable effects, when administered repeatedly, on rat pineal gland function. Chronic desipramine treatment was found to significantly reduce the specific binding of [^{3}H]DHA to pineal β-adrenoceptors. This reduction in binding following desipramine treatment was accompanied by a corresponding reduction in the responsiveness of adenylate cyclase activity as evidenced by a reduction in the NAT response to *in vitro* isoprenaline stimulation. The results also show that while chronic desipramine treatment produced subsensitivity of the β-adrenoceptor/adenylate cyclase system, the overall function of the noradrenergic synapse did not appear to be reduced. The increased synaptic concentrations of

NA produced by desipramine appears to have been sufficient to overcome the apparent β-adrenoceptor subsensitivity, leading to an enhancement of NAT activity and consequently, increased melatonin synthesis. Melatonin synthesis by the pineal gland has been proposed as a model of the overall effect of antidepressant drugs on noradrenergic neurotransmission (*Checkley* et al. 1986). The gland is innervated by noradrenergic fibres which have presynaptic α_2-adrenoceptors and postsynaptic α_1- and β_1-adrenoceptors, at least in the rat (Section 1.3.3). Thus, desipramine may increase noradrenergic neurotransmission as a result of inhibition of NA reuptake in combination with down-regulation of presynaptic α_2-adrenoceptors (Section 1.2.3.2.4) and up-regulation of postsynaptic α_1-adrenoceptors (Section 1.2.3.2.3). The present findings therefore bring into question the importance of β-adrenoceptor down-regulation as a mechanism of antidepressant action.

However, it appears from this study that the ability to increase melatonin synthesis is a property not shared by all antidepressant drugs when administered chronically, at least in the rat. The results show that trazodone and maprotiline as well as the selective 5-HT reuptake inhibitors sertraline, panuramine and CGP-6085A failed to increase melatonin synthesis. Taken together, these results suggest that the ability to increase melatonin synthesis is dependent on the ability of the drug to inhibit NA reuptake. Furthermore, the present findings are inconclusive with regard to the possibility that increased melatonin synthesis may be involved in the mechanism of action of antidepressant drugs. However, since the selective 5-HT reuptake inhibitor RU-25591 was found to increase melatonin synthesis, the possibility that the apparent lack of effect of the other antidepressant drugs may have been due to pharmacokinetic factors requires further investigation.

CHAPTER SEVEN

RESERPINE-ANTIDEPRESSANT INTERACTIONS IN THE RAT PINEAL GLAND

7.1 INTRODUCTION

A major limitation in attempting to elucidate the mechanism(s) of action of antidepressant therapies has been, and still is, the lack of appropriate models for monitoring central neurotransmitter function. The vast majority of pre-clinical studies involving the biochemical effects of chronic antidepressant treatment have employed normal laboratory animals, in particular, the rat. Considerable evidence, however, suggests that severe depression is a biochemical disorder that develops in those individuals with some predisposing neurochemical vulnerability (*Siever* and *Davis* 1985). Although the predisposing biochemical abnormality has not yet been identified, it may be related to the neurochemical mechanisms that regulate impulse traffic in various neural systems and maintain the homeostatic balance of neural activity within the brain. It seems relevant, therefore, that an animal model of this disease should include animals with some primary defect in neuronal function. Furthermore, this defect should be sensitive to chronic antidepressant treatment.

The most widely used models for studying antidepressant actions are those that are pharmacological in nature. Amongst these, the syndrome induced by reserpine and related compounds has received the most attention. High doses of these drugs, by depleting peripheral and central intraneuronal stores of biogenic amines, induce a variety of symptoms including sedation, hypothermia, ptosis, miosis, diarrhoea, hypersalivation, gastric hypersecretion, and bradycardia when administered acutely (*Garattini* and *Jori* 1967). Interest in the psychotropic effects of these drugs arose from the clinical observation that some patients treated with reserpine developed clear signs of clinical depression. It is not surprising, therefore, that the ability of drugs to antagonize reserpine-induced states has been employed as a test of antidepressant activity. In this test, the TCAs and MAOIs appear to reverse the reserpine-induced symptoms by elevating the synaptic concentrations of monoamines due to reuptake inhibition and blockade of catabolism respectively. However, newer antidepressants such as iprindole, mianserin, salbutamol and flupenthixol have screened as false negatives in this test. In addition, many false positives including psychostimulants, anticholinergics, antihistamines and analgesics have been found when screened in this test. Moreover, a major drawback of this model is that it is based on the acute effects of antidepressants whereas clinically these drugs are effective only after chronic administration. Thus the usefulness and selectivity of the acute reserpine antagonism test has been seriously questioned and the need to develop new animals models that would select a compound for its potential antidepressant activity irrespective of its acute mechanism of action remains an important goal.

Jancsar and *Leonard* (1983) showed that chronic low dose reserpine treatment produced hyperactivity in rats when assessed in the "open field". This behavioural alteration was normalized by chronic but not

acute treatment with two pharmacologically different antidepressants. Furthermore, these workers showed that the decrease in the concentration of monoamines in the amygdaloid cortex and midbrain caused by chronic reserpine treatment could be reversed by chronic but not acute antidepressant treatment. From these findings it may be argued that an animal model that can preferentially select antidepressants following their chronic administration are more useful in assessing the potential therapeutic activity of a new drug than those that can detect the acute effects of antidepressants. The chronic reserpinized rat model may therefore provide an alternative experimental basis for evaluating drugs with antidepressant activity. Moreover, such an approach may be of value in elucidating the subcellular and biochemical basis of antidepressant action.

Previous studies showed that reserpine treatment causes an increase in the sensitivity of the pineal β-adrenoceptor-coupled adenylate cyclase system and produces a concomitant reduction in pineal melatonin output (Chapter 4). As there is experimental evidence to show that chronic antidepressant treatment reduces the sensitivity of the pineal β-adrenoceptor-coupled adenylate cyclase system (Chapter 6), it is not unreasonable to postulate that such drugs may reverse the neurochemical effects seen following chronic reserpine administration. The application of such an approach to the pineal gland would not only enable the detection of putative antidepressant compounds but also provide useful information on the net effect of antidepressant treatment on noradrenergic neurotransmission - an, as yet, unresolved question. Moreover, the validity of this experimental approach is given support by the clinical observation that pineal melatonin output is reduced in depressed patients (see Section 1.3.6.2). Thus, the aim of the present series of experiments was to investigate what effects various antidepressants with differing pharmacological profiles had on pineal gland function of rats treated chronically with low doses of reserpine. By using this approach, it was hoped to deplete pineal biogenic amines without causing the severe debilitating effects normally seen after the acute administration of high doses of reserpine. The biochemical parameters assessed in this study included β-adrenoceptor binding, cAMP accumulation, NAT activity, and pineal indole synthesis. The antidepressants employed in this study were also assessed for their effect on rat pineal phosphodiesterase activity and are discussed simultaneously.

7.2 MATERIALS AND METHODS

7.2.1 Animals

Adult male (200-250 g) or female (180-220 g) rats of the Wistar strain were used in this study. The animals were maintained under an automatically regulated lighting cycle with a daily photoperiod of 12 h (lights on at 06:00 h) and were given food and water *ad libitum*. Their living environment is described in detail in Section 2.1.

7.2.2 Drugs

The drugs employed in this study were : Reserpine HCl, desipramine HCl, trazodone HCl, mianserin HCl and fluoxetine HCl. Mianserin HCl was donated by Organon and fluoxetine HCl by Eli Lilly. The other drugs were donated by previously acknowledged sources (Sections 4.2.2 and 6.2.2). The antidepressants were administered as solutions in 0.9% saline. Reserpine was prepared as described in Section 4.2.2, except that the final solution was diluted 1:10 with 0.9% saline to a concentration of 0.25 mg/ml.

7.2.3 Statistical Analysis

Statistical analyses were performed using Student's *t*-tests. A *P* value of greater than 0.05 between groups was considered not significantly different. Where applicable, the data were analysed using one-way analysis of variance followed by Scheffe's post hoc tests for multiple range comparisons.

7.3 EXPERIMENT 1 : EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON THE RESERPINE-INDUCED ALTERATION IN PINEAL β-ADRENOCEPTOR SENSITIVITY

7.3.1 Introduction

The present experiment describes the effect of chronic antidepressant treatment on the reserpine-induced increase in [^{3}H]CGP-12177 binding to intact rat pineal glands.

7.3.2 Experimental Procedure

Drug Administration : Groups of female rats were treated with reserpine (0.2 mg/kg), antidepressant (10 mg/kg), reserpine (0.2 mg/kg) plus antidepressant (10 mg/kg), or 0.9% saline (1 ml/kg), administered i.p., once daily at 10:00 h for 2 weeks. Pineal glands were collected 24 h after final drug administration for use in the experiment.

Assay of β-Adrenoceptors : β-Adrenoceptors were estimated by measuring the specific binding at a single concentration of [^{3}H]CGP-12177 (4 nM) to intact whole pineal glands. The assay was performed as outlined in Section 2.2.2.2. In each treatment group, at least four pineals were used for the determination of total binding and two pineals for non-specific binding (*Wilkinson* et al. 1987).

Analysis of Data : Specific [^{3}H]CGP-12177 binding is expressed as fmol/pineal. Values represent means ± S.E.M.; n = 4 or 8 per point.

7.3.3 Results

The results of this experiment are presented in Fig. 7.1. Repeated treatment of rats with low doses of reserpine significantly increased the binding of [^{3}H]CGP-12177 to pineal β-adrenoceptors when compared with chronic saline treatment ($p < 0.05$).

Chronic treatment of rats with desipramine alone produced a significant reduction of ligand binding to pineal β-adrenoceptors when compared with saline treatment ($p < 0.05$). On the other hand, chronic treatment with trazodone, mianserin or fluoxetine did not appear to reduce the binding to β-adrenoceptors when compared with saline-treated controls.

However, the reserpine-induced increase in binding to β-adrenoceptors was reversed by the concomitant administration of desipramine, trazodone or mianserin ($p < 0.05$) but not fluoxetine. Conversely, the desipramine-induced reduction in binding to β-adrenoceptors was prevented by chronic treatment with reserpine ($p < 0.05$).

7.3.4 Discussion

The changes observed in the binding of [^{3}H]CGP-12177 to pineal β-adrenoceptors following chronic treatment of rats with low doses of reserpine are consistent with previous findings that reserpine increases the density of pineal β-adrenoceptors as a result of the depletion of intraneuronal NA stores (see Chapter 4).

On the other hand, chronic desipramine treatment was associated with a marked decrease in the binding of [^{3}H]CGP-12177 to pineal β-adrenoceptors. This finding is in agreement with previous observations suggesting that desipramine down-regulates β-adrenoceptors as a result of chronic NA reuptake inhibition (see Section 6.3.3). In support of this suggestion, the present results show that concomitant reserpine administration prevented the down-regulation of β-adrenoceptors caused by chronic desipramine treatment.

Consistent with previous observations (Section 6.3.3), chronic treatment with trazodone did not alter pineal β-adrenoceptor density. A similar lack of effect on pineal β-adrenoceptors was noted following chronic treatment with mianserin and fluoxetine. In view of the fact that neither mianserin nor fluoxetine inhibit NA reuptake, these findings may be anticipated. In support of this, both mianserin and fluoxetine were found to be without effect on cortical β-adrenoceptors (*Mishra* et al. 1980, 1981). More recently, however, *Wamsley* et al. (1987) using quantitative autoradiography, showed that β-adrenoceptor populations in discrete areas of the cerebral cortex were down-regulated following chronic treatment with high doses (30 mg/kg q.i.d) of fluoxetine.

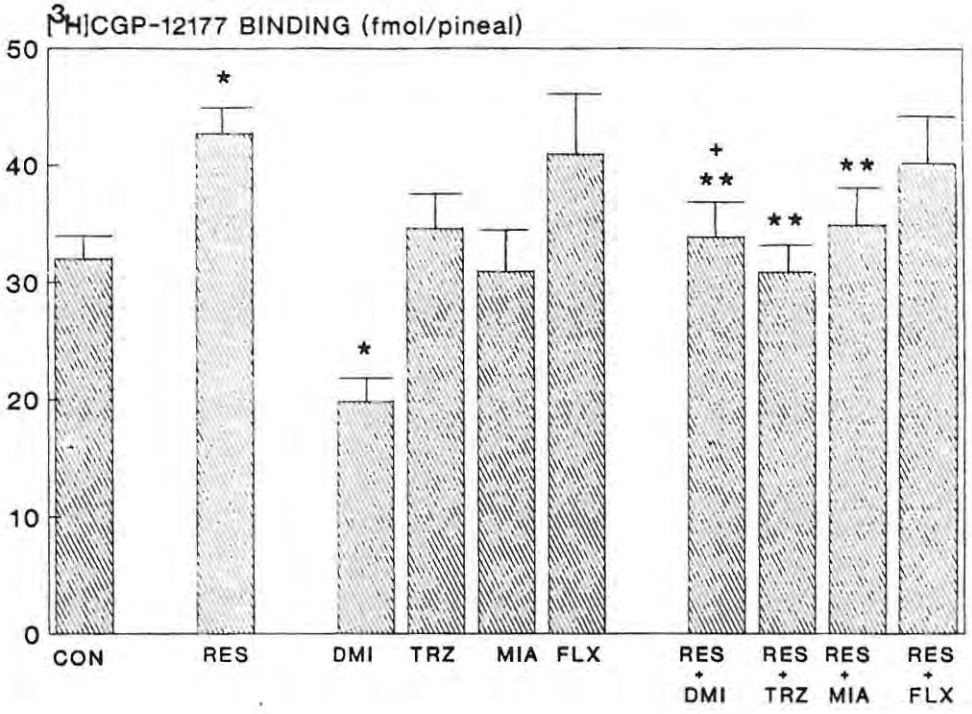


Figure 7.1 Effect of chronic antidepressant treatment on the specific binding of [^{3}H]CGP-12177 to intact pineal glands of chronically reserpinized rats. Values represent means ± S.E.M.; n = 4 or 8 per point. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.05$ when compared with controls.

** $p < 0.05$ when compared with reserpine treatment.

\+ $p < 0.05$ when compared with desipramine treatment.

Interestingly, the present results also indicate that chronic treatment with desipramine, trazodone or mianserin reversed the increase in pineal β-adrenoceptor density produced by the chronic administration of reserpine. These findings suggest that the synaptic concentrations of NA are increased by antidepressant treatment in order to offset the reserpine-induced increase in β-adrenoceptor density. Moreover, these findings are consistent with previous observations that chronic treatment with low doses of reserpine decreased but did not completely deplete intraneuronal NA stores (*Jancsar* and *Leonard* 1983). The reversal of this effect of reserpine by desipramine and mianserin may be anticipated from the effects of these drugs on NA metabolism. Thus, desipramine inhibits the reuptake of NA whereas mianserin increases NA release as a result of presynaptic α_2-adrenoceptor antagonism. In support of this, *Jancsar* and *Leonard* (1983) showed that chronic treatment with mianserin reversed the reduction in NA levels in rat amygdaloid cortex and midbrain caused by chronic low-dose reserpine treatment.

However, the explanation for the ability of trazodone to antagonize the reserpine-induced increase in β-adrenoceptor density is less clear. The possibility that trazodone may increase NA levels as a result of its weak NA reuptake inhibiting (Section 1.2.2.3.3) and presynaptic α_2-adrenoceptor blocking (*Richelsen* and *Nelsen* 1984) properties, cannot entirely be excluded. However, since this drug primarily affects serotonergic function (Section 1.2.2.3.3), it is not unreasonable to speculate that NA levels may be increased as a result of an interaction with this system. The fact that the pineal gland receives a direct central neuronal input from other brain areas (Section 1.3.3.4) including the raphe nuclei (*Aghajanian* al. 1975), in addition to its peripheral sympathetic innervation, makes such a possibility attractive. Moreover, 5-HT binding sites have also been demonstrated in the pineal gland. Several investigators have demonstrated the existence of a functional linkage between the noradrenergic and serotonergic neuronal systems in other brain areas (Section 1.2.3.2.2). The nature of the interaction between these two systems is still unclear, but it appears that an intact serotonergic neuronal input is necessary for the proper functioning of β-adrenoceptors and for their down-regulation by antidepressant treatments (Section 1.2.3.2.2). The simplest model of this "5-HT/NA link" hypothesis posits that serotonergic neurons may project onto noradrenergic neurons and that stimulation of 5-HT receptors may induce the release of NA from noradrenergic nerve terminals which produces β-adrenoceptor down-regulation later on. This hypothesis has been invoked to explain the ability of selective 5-HT reuptake inhibitors to induce β-adrenoceptor subsensitivity. Furthermore, it has been suggested that this effect of the selective 5-HT reuptake inhibitors may reflect their efficacy as antidepressants (*Koe* et al. 1983). A more complex interpretation of this 5-HT/NA link relates to the possibility that 5-HT may also directly interfere with the process of "coupling" of β-adrenoceptors, as evidenced by a decreased β-adrenoceptor agonist affinity but an unchanged antagonist affinity in 5-HT-lesioned rats (*Sulser* 1987). In view of this hypothesis, the apparent inability of fluoxetine to counteract the reserpine-induced increase in β-adrenoceptor density, in the present study, may be explained by inadequate dosing or duration of treatment. As mentioned earlier, a rather high dose of fluoxetine was found to be necessary to down-regulate cortical β-adrenoceptors (*Wamsley* et al. 1987). Furthermore, *Jancsar* and *Leonard* (1983) showed that chronic treatment with the selective 5-HT reuptake inhibitor, ORG-6582, reversed the reduction of NA levels in the amygdaloid cortex of rats treated chronically with low-dose reserpine.

Thus, from the present results it appears that antidepressants with different pharmacological profiles do not have a common effect on pineal β-adrenoceptor density in normal rats and the ability to reduce β-adrenoceptor density appears to correlate with the ability of the drug to inhibit NA reuptake. However, the increased β-adrenoceptor density observed in reserpinized rats was reversed by chronic treatment with antidepressants, irrespective of their acute pharmacological actions. Furthermore, desipramine, trazodone and mianserin appeared to be equipotent in their ability to antagonize the effects of reserpine.

7.4 EXPERIMENT 2 : EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON THE RESERPINE-INDUCED ALTERATION IN PINEAL CYCLIC AMP ACCUMULATION

7.4.1 Introduction

In an attempt to correlate the findings of the previous ligand-binding experiments (Section 7.3) with a functional biochemical parameter, the effect of chronic antidepressant treatment on the chronic reserpine-induced alteration in pineal cAMP accumulation was assessed.

7.4.2 Experimental Procedure

Drug Administration : Groups of female rats were treated with reserpine (0.2 mg/kg), antidepressant (10 mg/kg), reserpine (0.2 mg/kg) plus antidepressant (10 mg/kg), or saline (1 ml/kg), administered i.p., once daily at 10:00 h for two weeks. For use in the experiment, pineal glands were collected 24 h after final drug administration.

Cyclic AMP Stimulation *In Vitro* : The preparation of pineal tissue and incubation procedure were performed as outlined in Section 2.3.2. Briefly, pineal glands were rapidly excised, cleaned of adhering connective tissue and bisected under binocular magnification. Following a 15 min pre-incubation period at 37°C, the pineal hemispheres were incubated for a further 10 min in the presence or absence of 20 μM *l*-isoprenaline.

Cyclic AMP Assay : Cyclic AMP levels in the pineal hemispheres were determined as outlined in Section 2.3.2.

Analysis of Data : Results are expressed as picomoles of cAMP formed per pineal. Values represent means ± S.E.M. Four rats were used in each experimental group.

7.4.3 Results

7.4.3.1 Basal Cyclic AMP Levels

The results of this experiment are shown in Table 7.1. Low doses of reserpine, administered chronically to rats, did not significantly alter basal cAMP levels when compared with chronic saline treatment.

Similarly, repeated treatment with desipramine, trazodone, mianserin or fluoxetine was without effect on basal cAMP levels when compared with chronic saline-treated controls.

Interestingly, however, combined reserpine and antidepressant administration produced an increase in basal cAMP levels when compared with either reserpine or antidepressant treatment alone ($p < 0.05$), although this effect was not observed with desipramine.

7.4.3.1 Isoprenaline-Stimulated Cyclic AMP Levels

As shown in Table 7.1, stimulation with isoprenaline *in vitro* produced a significant increase in cAMP levels in all the treatment groups. However, in order to accurately reflect the cAMP response to isoprenaline stimulation by the various treatment groups, the difference in cAMP levels between the halves exposed to isoprenaline and that of the control halves was determined. Figure 7.2 depicts the response to isoprenaline stimulation by the various treatment groups.

Repeated treatment of rats with low-dose reserpine significantly increased the cAMP response to isoprenaline when compared with saline treatment ($p < 0.01$).

Conversely, repeated treatment with desipramine significantly decreased the cAMP response to isoprenaline when compared with saline treatment ($p < 0.01$). Repeated treatment with trazodone, mianserin or fluoxetine was without this effect.

However, the reserpine-induced increase in cAMP levels following isoprenaline stimulation was reversed by the concomitant treatment with desipramine ($p < 0.01$), trazodone ($p < 0.05$) or mianserin ($p < 0.01$) but not fluoxetine. Conversely, the desipramine-induced decrease in cAMP levels following isoprenaline stimulation was prevented by simultaneous reserpine administration.

7.4.4 Discussion

The observation that chronic treatment with low doses of reserpine increased the cAMP response to isoprenaline stimulation is consistent with the previous finding that such treatment markedly increased the density of pineal β-adrenoceptors (Section 7.3.3), presumably as a result of reduced synaptic NA levels (see Chapter 4).

TABLE 7.1 Effect of chronic antidepressant treatment on the reserpine-induced alteration in pineal cyclic AMP accumulation

	Cyclic AMP : pmol / pineal		
DRUG TREATMENT	BASAL	ISOPRENALINE	SIGNIFICANCE*
Saline (Control)	2.40 ± 0.10	4.08 ± 0.20	p<0.01
Reserpine	2.31 ± 0.25	5.86 ± 0.65	p<0.01
Desipramine	1.93 ± 0.27	2.90 ± 0.45	p<0.05
Trazodone	2.13 ± 0.17	4.14 ± 0.57	p<0.01
Mianserin	2.82 ± 0.34	6.18 ± 0.97	p<0.01
Fluoxetine	2.55 ± 0.37	4.44 ± 0.29	p<0.01
Reserpine + Desipramine	2.58 ± 0.43	3.74 ± 0.62	p<0.05
Reserpine + Trazodone	4.43 ± 0.30 **	6.78 ± 0.30	p<0.01
Reserpine + Mianserin	4.48 ± 0.20 **	5.67 ± 0.23	p<0.05
Reserpine + Fluoxetine	3.74 ± 0.39 **	7.32 ± 0.60	p<0.01

* Level of significance between basal and isoprenaline-stimulated groups

** p<0.05 when compared with saline-treated controls

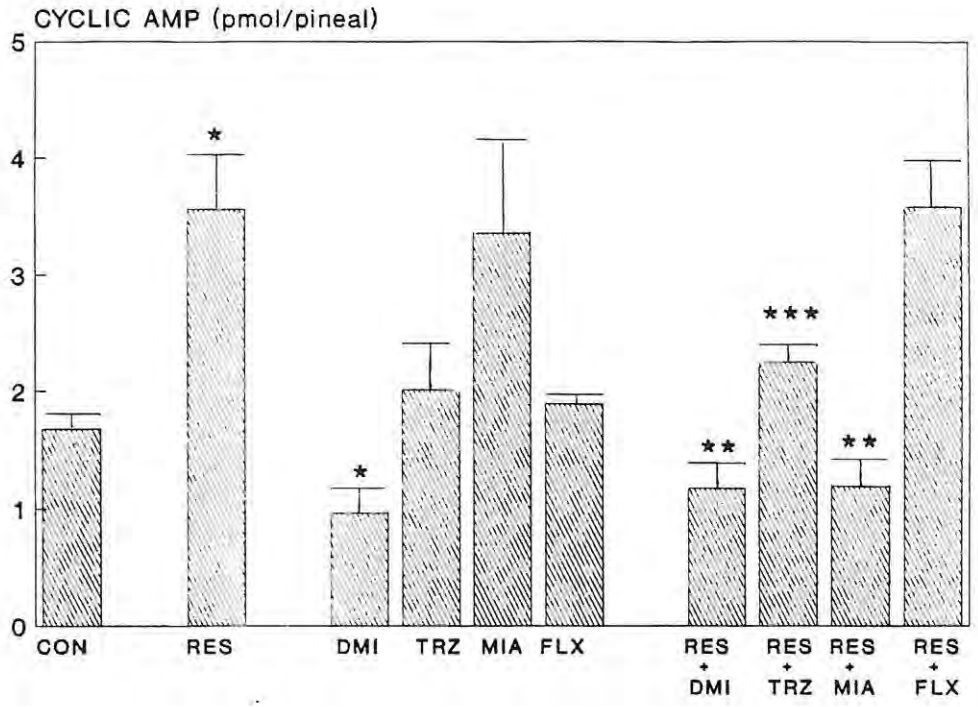


Figure 7.2 Pineal cyclic AMP levels showing the response to *in vitro* isoprenaline stimulation following chronic antidepressant treatment to chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.01$ when compared with controls.

** $p < 0.01$ when compared with reserpine treatment.

*** $p < 0.05$ when compared with reserpine treatment.

\+ $p < 0.05$ when compared with desipramine treatment.

Chronic desipramine treatment, on the other hand, reduced the cAMP response to isoprenaline stimulation. This finding supports that of other workers (*Moyer* et al. 1979, 1981, *Skene* 1985) and is consistent with the previously noted down-regulation of β-adrenoceptors following such treatment (Sections 6.3.3 and 7.3.3). Furthermore, the observation that this effect was prevented by concomitant reserpine treatment supports the hypothesis that chronic desipramine treatment down-regulates β-adrenoceptors by increasing synaptic concentrations of NA. In the present study, desipramine treatment did not alter basal cAMP levels. In contrast, *Skene* (1985) found that such treatment was associated with reduced basal cAMP levels. The reason for this discrepancy is unclear, since cAMP levels were assayed by the same method (*Brown* et al. 1971) in the two studies. *Skene* (1985), however, reported markedly higher basal cAMP levels in the saline-treated control group than that found in the present experiment (10.2 ± 1.2 pmol/pineal compared with 2.4 ± 0.1 pmol/pineal). The higher basal levels of cAMP found in the former experiment (*Skene* 1985) may have been due to increased levels of circulating catecholamines, suggesting that the animals were subjected to higher levels of stress, possibly as a result of excessive handling. Consistent with the present results, *Moyer* et al. (1979, 1981) found that basal cAMP levels remained unchanged, or were even slightly raised, following chronic desipramine treatment.

The lack of effect of trazodone, mianserin and fluoxetine on either basal or isoprenaline-stimulated cAMP levels is consistent with the observation that these antidepressants were unable to modify the density of pineal β-adrenoceptors (Section 7.3.3). Similarly, *Skene* (1985) demonstrated that chronic treatment with trazodone, mianserin or fluoxetine did not alter the responsiveness of pineal cAMP to isoprenaline stimulation. In the latter study, however, trazodone and mianserin treatment were found to decrease basal cAMP levels. As mentioned earlier, the rather high basal cAMP levels in the control group may account for this discrepancy.

The present results also demonstrate that concomitant chronic treatment with desipramine, trazodone or mianserin reversed the reserpine-induced increase in isoprenaline-stimulated cAMP levels. These findings support the previous observation that such treatment reversed the increase in density of pineal β-adrenoceptors caused by reserpine treatment (Section 7.3.3), presumably as a result of increased synaptic NA levels. The observation that concomitant treatment with trazodone, mianserin or fluoxetine increased basal cAMP levels when compared with reserpine treatment alone, provides further support for the suggestion that antidepressant drugs, irrespective of their acute pharmacological actions, increase pineal synaptic NA levels when administered chronically (see Section 7.3.4). Moreover, the finding that these antidepressants were unable to modify cAMP levels in normal unreserpinized rats, emphasizes the importance of evaluating antidepressant treatments in animals with impaired central noradrenergic transmission. Interestingly, the selective 5-HT reuptake inhibitor, fluoxetine, increased basal cAMP levels in reserpinized rats without affecting isoprenaline-stimulated cAMP levels. This finding is consistent with the previous observation that fluoxetine was unable to reverse the increase in density of pineal β-adrenoceptors in reserpinized rats (Section 7.3.3). Taken together, these results suggest that while fluoxetine treatment may increase synaptic NA levels in reserpinized rats, the reversal of the

reserpine-induced β-adrenoceptor supersensitivity may be a time-dependent phenomenon that occurs with longer-term fluoxetine treatment. As discussed previously (Section 7.3.4), the mechanism(s) by which fluoxetine and trazodone increase pineal NA levels is speculative but an interaction between the serotonergic and noradrenergic neurotransmitter systems which may facilitate NA release cannot be excluded.

Thus, the present experiment indicates that in normal rats the ability of antidepressants to alter the cAMP response to exogenous catecholamine stimulation appears to depend on their ability to inhibit NA reuptake. However, it appears from these results that antidepressants with different pharmacological profiles do have the ability to alter pineal cAMP production in chronically reserpinized rats. The potency of the antidepressants in reducing the cAMP response to isoprenaline stimulation in reserpinized rats, however, appears to depend on their ability to directly influence NA metabolism. Thus, the rank order of potency was found to be : desipramine ≥ mianserin > trazodone > fluoxetine.

7.5 EXPERIMENT 3 : EFFECT OF ANTIDEPRESSANT TREATMENT ON PINEAL CYCLIC AMP PHOSPHODIESTERASE ACTIVITY

7.5.1 Introduction

The intracellular concentration of cAMP is regulated in part by cAMP phosphodiesterase (PDE). As in many other tissues, pineal PDE activity is characterized as having both a high and a low K_m (*Oleshansky* and *Neff*, 1975). It appears, however, that the low K_m enzyme is probably more important for the inactivation of cAMP *in vivo*, although the high K_m form of the enzyme may be important under certain conditions (*Oleshansky* and *Neff*, 1975). The activity of pineal PDE changes as a function of adrenergic stimulation, with the highest activity occurring during darkness (*Minneman* and *Iversen*, 1976).

Some studies have shown that basal cAMP levels in the pineal may be altered by chronic treatment with some antidepressants (*Moyer* et al. 1979, 1981, *Skene* 1985). These observations raise the possibility that such treatment may alter basal cAMP levels as a result of an effect on PDE activity. Since changes in cAMP content mediate changes in the activity of pineal NAT activity, it is conceivable that alterations in PDE activity can lead to alterations in melatonin biosynthesis. Few studies, however, have evaluated the effect of antidepressant treatment on pineal PDE activity.

Thus, the present experiment sought to investigate the effect of chronic treatment with different antidepressant drugs on the nocturnal increase in pineal PDE activity. Additionally, pineals were treated *in vitro* with varying concentrations of these drugs and PDE activity was measured. Pineal PDE activity was assayed using 1 μM cAMP, which primarily measures the low K_m enzyme activity (*Oleshansky* and *Neff*, 1975).

7.5.2 Experimental Procedure

Diurnal Variation in Enzyme Activity : To examine the diurnal variation in PDE activity, pineals from groups of naive, untreated male rats were collected at 12:00 h, 16:00 h and 24:00 h, frozen on solid CO_2, and stored at -70^{o}C prior to analysis.

Chronic Antidepressant Administration : Groups of male rats were treated with either saline (1 ml/kg) or antidepressant (10 mg/kg) i.p., once daily at 10:00 h for 2 weeks. For use in the experiment, pineals were collected at 24:00 h on the day of final drug administration, frozen on solid CO_2 and stored at -70^{o}C prior to analysis.

In Vitro **Antidepressant Treatment :** Pineal glands from naive, untreated male rats were collected at 16:00 h and each pineal was carefully bisected under binocular magnification. Each hemisphere was incubated at 37^{o}C for 2 h in the presence of either 10 μM or 100 μM antidepressant (see Section 2.5.2 for further details of the technique involved). Pineal hemispheres which were incubated in the absence of antidepressant, served as controls. Following incubation, the glands were removed from the medium, frozen on solid CO_2 and stored at -70^{o}C prior to analysis.

Assay of Cyclic AMP Phosphodiesterase : The frozen pineals were allowed to thaw on ice prior to the determination of enzyme activity. The assays were performed as outlined in Section 2.4.2 with the following modifications. In chronic antidepressant studies, each pineal was homogenised in 75 μl of 50 mM phosphate buffer (pH 6.5) and duplicate 3 μl aliquots of homogenate were assayed in a final reaction volume of 200 μl. This procedure was adopted since the remaining homogenate was used in another experiment (Section 7.6). In *in vitro* studies, a pineal hemisphere was homogenised in 250 μl of distilled water and 20 μl aliquots of homogenate were assayed in duplicate.

Analysis of Data : Enzyme activity is expressed as nanomoles of cAMP hydrolysed per pineal per hour. Each value represents the mean ± S.E.M. of four separate experiments done in duplicate.

7.5.3 Results

7.5.3.1 Diurnal Variation of Pineal Cyclic AMP Phosphodiesterase Acitvity

Figure 7.3 depicts the diurnal variation in pineal cAMP PDE activity. From the results, it is apparent that the enzyme exhibits a marked diurnal variation in the hydrolysis of cAMP, with the highest values occurring during the dark phase. Pineal PDE activity at midnight was found to be about 3-fold higher than that observed at noon ($p < 0.001$). Enzyme activity at 16:00 h was not significantly different from that at noon.

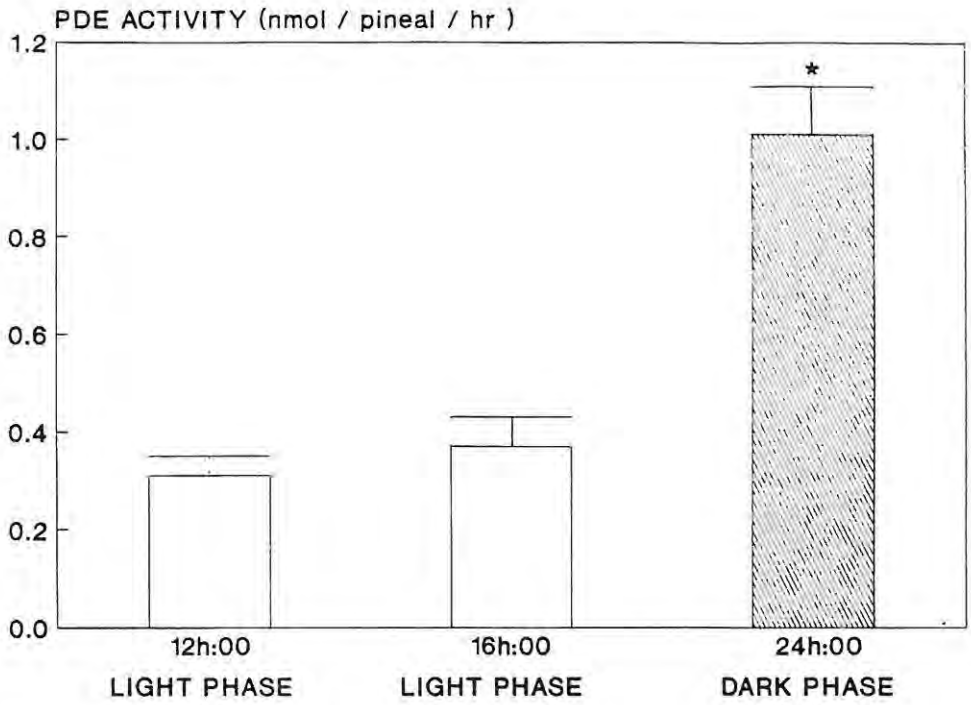


Figure 7.3 Diurnal variation of pineal cAMP phosphodiesterase activity.
Values represent means ± S.E.M. Four rats were used in each experimental group.

* $p < 0.001$ when compared with light-phase enzyme activity.

7.5.3.2 Effect of Chronic Antidepressant Administration on Nocturnal Pineal Cyclic AMP Phosphodiesterase Activity

The results are shown in Fig. 7.4. Chronic treatment of rats with desipramine produced a significant decrease in the nocturnal rise in PDE activity when compared with saline-treated controls ($p < 0.01$). Treatment with trazodone, mianserin or fluoxetine did not appear to alter night-time PDE activity when compared with saline treatment.

7.5.3.3 Effect of *In Vitro* Antidepressant Treatment on Pineal Cyclic AMP Phosphodiesterase Activity

The results of this experiment are presented in Fig. 7.5. The activity of daytime basal PDE was significantly increased in pineals incubated with 10 μM desipramine when compared with controls ($p < 0.01$). At this concentration, none of the other antidepressants significantly altered enzyme activity relative to controls. However, at a concentration of 100 μM, desipramine, trazodone and mianserin produced a significant increase in pineal PDE activity ($p < 0.01$). Fluoxetine, at this concentration, did not significantly affect enzyme activity when compared with controls.

7.5.4 Discussion

The observation that pineal cAMP PDE activity exhibits a marked diurnal variation with the highest activity occurring during the dark phase, supports the findings of *Minneman* and *Iversen* (1976). These investigators also showed that removal of the superior cervical ganglia (SCG) abolished the diurnal rhythm, suggesting that the activity of this enzyme is physiologically under neural control. More recently, *Vacas* et al. (1985) showed that PDE activity is regulated by NA acting via a dual α_1- and β-adrenoceptor mechanism.

Chronic treatment of rats with desipramine was found to supress the nocturnal NA-induced elevation in PDE activity. This finding is in agreement with the previously observed subsensitivity of the β-adrenoceptor/adenylate cyclase system caused by chronic desipramine treatment (Sections 7.3.3, 7.4.3) and supports the suggestion that pineal PDE activity is under β-adrenergic control (*Oleshansky* and *Neff* 1975). Moreover, the observation that *in vitro* desipramine treatment produced an increase in daytime basal enzyme activity provides further support for the notion that PDE activity mirrors pineal sympathetic activity. Treatment with desipramine *in vitro* increases the synaptic availability of NA as a result of reuptake inhibition. Taken together, the present results suggest that desipramine itself does not have a direct effect on PDE activity. In support of this suggestion, *Moyer* et al. (1981) showed that *in vivo* or *in vitro* desipramine treatment did not affect PDE activity when sympathetic input to the pineal was reduced. Furthermore, the present results indicate that treatment with trazodone, mianserin or fluoxetine did not affect nocturnal PDE activity, suggesting that these drugs, likewise, do not directly

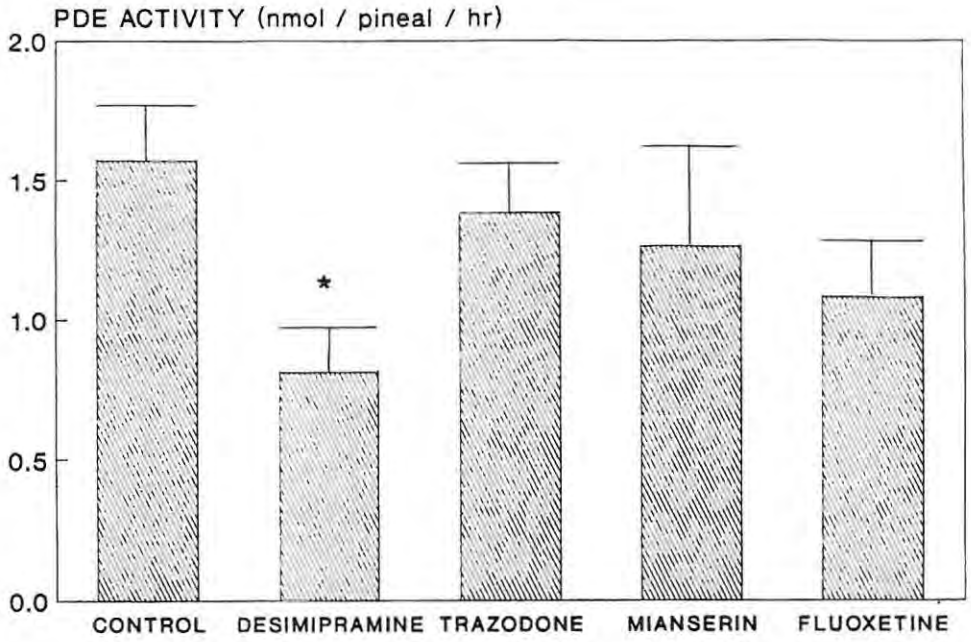


Figure 7.4 Effect of chronic antidepressant treatment on nocturnal pineal cAMP phosphodiesterase activity.

Values represent means ± S.E.M. Four rats were used in each experimental group.

* $p < 0.01$ when compared with controls.

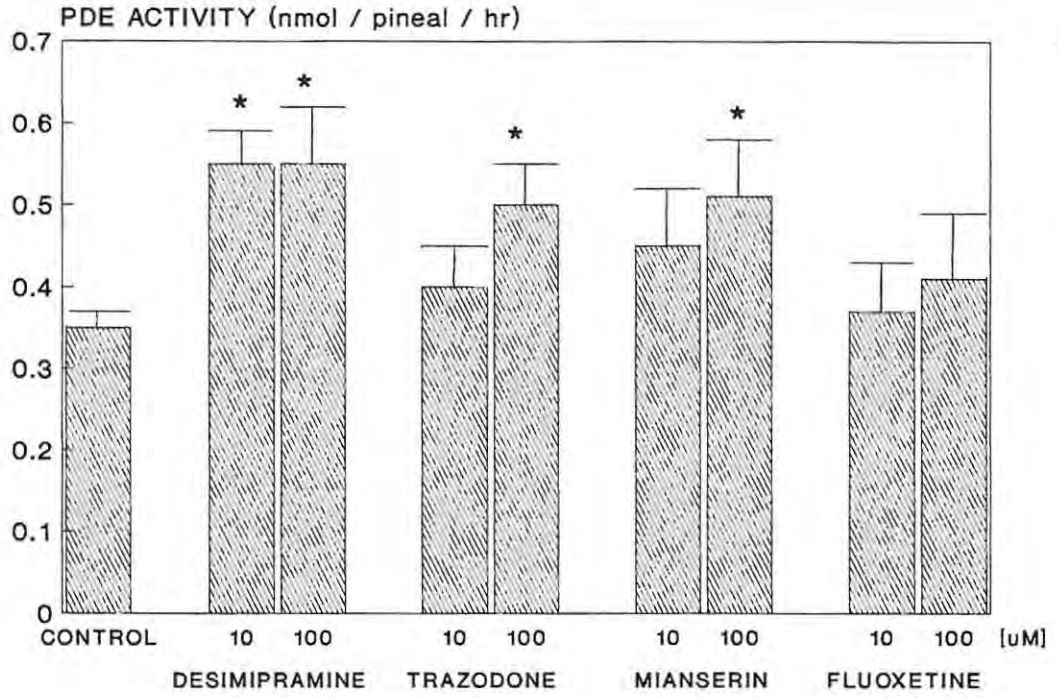


Figure 7.5 Effect of *in vitro* antidepressant treatment on pineal cAMP phosphodiesterase activity.

Values represent means ± S.E.M. Four rats were used in each experimental group.

* $p < 0.01$ when compared with controls.

affect enzyme activity. On the other hand, the finding that high concentrations of trazodone and mianserin increased daytime basal PDE activity *in vitro*, suggests that these drugs increase synaptic levels of NA.

7.6 EXPERIMENT 4 : EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON THE RESERPINE-INDUCED ALTERATION IN PINEAL SEROTONIN N-ACETYLTRANSFERASE ACTIVITY

7.6.1 Introduction

In the present experiment, nocturnal serotonin N-acetyltransferase (NAT) activity was evaluated following chronic antidepressant treatment to chronically reserpinized rats.

7.6.2 Experimental Procedure

Drug Administration : Groups of male rats were treated with reserpine (0.2 mg/kg), antidepressant (10 mg/kg), reserpine (0.2 mg/kg) plus antidepressant (10 mg/kg), or saline (1 ml/kg), administered i.p., once daily at 10:00 h for two weeks. For use in the experiment, pineal glands were collected at midnight on the day of final drug administration, frozen on solid CO_2 and stored at -70^{o}C prior to analysis.

Assay of Serotonin N-Acetyltransferase : Frozen pineals were allowed to thaw on ice prior to the determination of NAT activity. The assay was performed as outlined in Section 2.5.2 except that each pineal was homogenised in 75 µl, rather than 70 µl, of buffer.

Analysis of Data : Enzyme activity is expressed as picomoles of [^{3}H]N-acetyltryptamine formed per pineal per hour. Values represent means ± S.E.M. Four to eight rats were used in each experimental group.

7.6.3 Results

The results of this experiment are presented in Fig. 7.6. Repeated treatment of rats with low doses of reserpine significantly reduced the nocturnal NA-induced rise in pineal NAT activity that was evident in rats treated chronically with saline ($p < 0.01$).

Chronic treatment of rats with desipramine ($p < 0.01$) or trazodone ($p < 0.05$) was also found to depress the nocturnal elevation in pineal NAT activity when compared with saline-treated controls. Treatment of rats with mianserin or fluoxetine, however, was without this effect.

Furthermore, the reserpine-induced reduction in nocturnal NAT activity was reversed by the simultaneous chronic administration of desipramine, trazodone, mianserin or fluoxetine ($p < 0.01$).

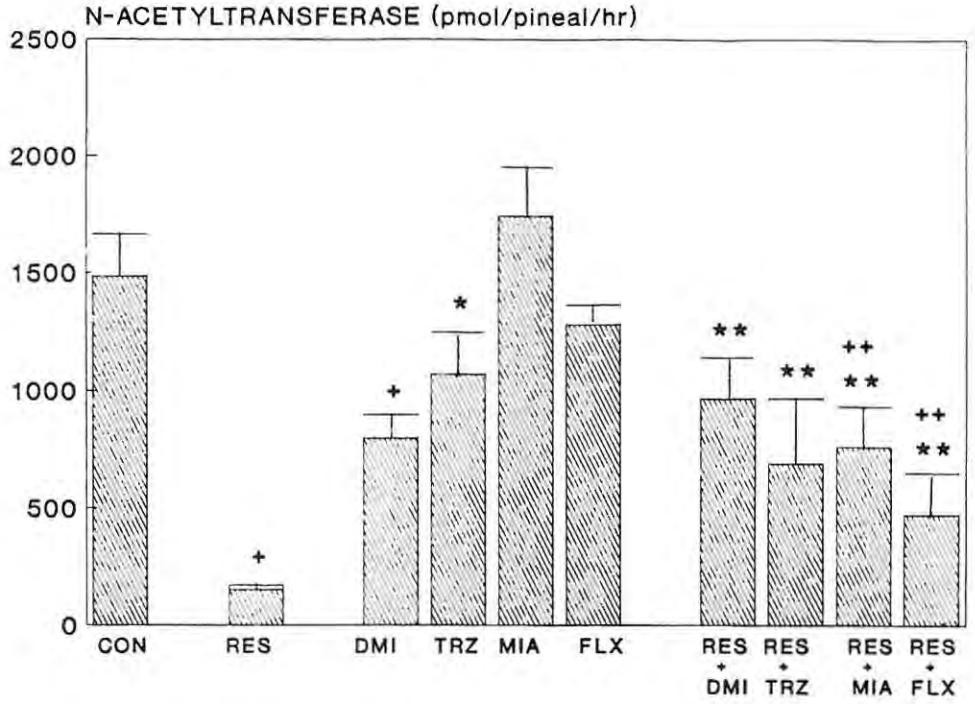


Figure 7.6 Effect of chronic antidepressant treatment on nocturnal pineal N-acetyltransferase (NAT) activity in chronically reserpinized rats.

Values represent means ± S.E.M. of four to eight rats. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.05$ when compared with controls.

** $p < 0.01$ when compared with reserpine treatment.

\+ $p < 0.01$ when compared with controls.

\+ + $p < 0.05$ when compared with the respective antidepressant treatment alone.

Moreover, combined reserpine treatment with either mianserin or fluoxetine produced a significant depression of NAT activity when compared with the respective antidepressants alone ($p < 0.05$).

7.6.4 Discussion

The results of the present experiment indicate that chronic low-dose reserpine treatment reduced the usual nocturnal rise in pineal NAT activity. This finding is consistent with previous studies (Section 4.4.4) and suggests that reserpine treatment prevented the nocturnal induction of NAT activity by depleting intraneuronal NA stores.

Furthermore, chronic treatment with desipramine was also observed to reduce the nocturnal elevation of NAT activity and is in agreement with the previously noted reduction in both β-adrenoceptor density (Sections 6.3.3 and 7.3.3) and catecholamine-stimulated cAMP production (Section 7.4.3) following such treatment. This effect of desipramine is presumed to be due to the chronic overexposure of the β-adrenoceptors to NA as a result of reuptake inhibition, since concomitant reserpine treatment prevented the desipramine-induced reduction in nocturnal NAT activity. In support of these findings, *Friedman* et al. (1984) showed that chronic treatment with imipramine likewise depressed the nocturnal rise in pineal NAT activity. The results of the present study are unique in demonstrating that chronic treatment with trazodone reduced nocturnal NAT activity, suggesting that such treatment may likewise reduce the sensitivity of pineal β-adrenoceptors. Previous studies with trazodone failed to demonstrate changes in daytime pineal β-adrenoceptor density (see Sections 6.3.3 and 7.3.3, *Skene* 1985) or in the response of pineal cAMP (Section 7.4.3) and NAT (Section 6.4.3) to exogenous catecholamine stimulation. However, acute treatment with trazodone was shown to increase nocturnal NAT activity in a previous study (Section 5.4.3), suggesting that it increases synaptic levels of NA. Taken together, these results emphasize the importance of evaluating antidepressant treatments on both daytime and nocturnal pineal function. However, consistent with daytime studies, chronic treatment with mianserin or fluoxetine was found to be without effect on nocturnal NAT activity. Whether this apparent lack of effect of mianserin or fluoxetine relates to inadequate dosing, requires further investigation.

However, the present experiment also demonstrates that chronic treatment with desipramine, trazodone, mianserin or fluoxetine reversed the reserpine-induced reduction in nocturnal NAT activity. These findings are consistent with previous experiments suggesting that antidepressant treatment antagonizes the effect of reserpine on β-adrenoceptor density and on cAMP production by increasing the synaptic availability of NA (see Sections 7.3.4 and 7.4.4). As discussed previously, desipramine increases NA levels as a result of reuptake inhibition, whereas mianserin achieves this effect by blocking presynaptic α_2-adrenoceptors thus increasing the release of NA from noradrenergic nerve terminals (Section 7.3.4). However, since trazodone and fluoxetine have a relatively selective action on 5-HT neurotransmitter systems, the mechanism(s) by which they antagonize the effect of reserpine on NAT activity is speculative. While an interaction between noradrenergic and serotonergic neurotransmitter systems

which may facilitate the release of NA from noradrenergic nerve terminals has yet to be demonstrated in the pineal gland, such a possibility may explain the effects observed with trazodone and fluoxetine in this study.

Thus, the present experiment indicates that antidepressants with different pharmacological profiles were able to reverse the reduction in nocturnal NAT activity seen in chronically reserpinized rats. Furthermore, the results indicate that the different antidepressants were more or less equipotent in their ability to antagonize the effects of reserpine on NAT activity. In normal rats, however, antidepressant treatment had a variable effect on nocturnal NAT activity.

7.7 EXPERIMENT 5 : EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON THE RESERPINE-INDUCED ALTERATION IN PINEAL [^{14}C]INDOLE METABOLISM

7.7.1 Introduction

In the present experiment, pineal indole biosynthesis and metabolism was studied following chronic antidepressant treatment to chronically reserpinized rats.

7.7.2 Experimental Procedure

Drug Administration : Groups of male rats were treated with reserpine (0.2 mg/kg), antidepressant (10 mg/kg), reserpine (0.2 mg/kg) plus antidepressant (10 mg/kg), or saline (1 ml/kg), administered i.p., once daily at 10:00 h for 2 weeks. Pineal glands were collected 24 h after final drug administration for use in the experiment.

Pineal Organ Culture and Analysis of [^{14}C]Indoles : The technique of pineal organ culture and the analysis of radiolabeled indoles were performed as outlined in Section 2.6.2. Briefly, pineal glands were cultured individually in the presence of [^{14}C]serotonin and incubated at 37^{o}C under 95% O_2 : 5% CO_2. Following 8 h of culture, a 10 µl aliquot of culture medium was removed and analysed for its content of radioactive indoles.

Analysis of Data : Results are expressed as the percentage of total radioactivity (DPM%/10 µl medium/pineal). Values represent means ± S.E.M. Four to six rats were used in each experimental group.

7.7.3 Results

7.7.3.1 Effect on the Synthesis of [^{14}C]N-Acetylated Indoles

7.7.3.1.1 [^{14}C]N-Acetylserotonin

The results of this experiment are shown in Fig. 7.7. The chronic administration of low-dose reserpine to rats significantly reduced the synthesis of N-acetylserotonin (aHT) when compared with chronic saline treatment ($p < 0.05$).

The chronic administration of desipramine ($p < 0.01$) or trazodone ($p < 0.05$) produced a significant increase in aHT synthesis when compared with chronic saline-treated controls. Treatment with mianserin was without this effect. On the other hand, repeated treatment with fluoxetine produced a significant decrease in aHT synthesis relative to saline treatment ($p < 0.05$).

Furthermore, the chronic administration of all the antidepressants tested reversed the reduction in aHT synthesis caused by reserpine treatment ($p < 0.05$). Interestingly, reserpine treatment prevented the increase in aHT synthesis caused by desipramine ($p < 0.01$), but had no effect on the increased aHT synthesis produced by trazodone. Moreover, the combined administration of reserpine and fluoxetine prevented the reduction in aHT synthesis seen with fluoxetine treatment alone ($p < 0.05$).

7.7.3.1.2. [^{14}C]Melatonin

The results of this experiment are shown in Fig. 7.8. The chronic administration of reserpine produced a significant decrease in melatonin (aMT) synthesis when compared with saline-treated controls ($p < 0.01$).

Repeated administration of desipramine significantly increased the synthesis of aMT when compared with saline treatment ($p < 0.01$). On the other hand, treatment with trazodone, mianserin or fluoxetine produced a significant decrease in aMT synthesis relative to saline treatment ($p < 0.01$).

The chronic administration of desipramine ($p < 0.01$), trazodone ($p < 0.01$), mianserin ($p < 0.05$) or fluoxetine ($p < 0.05$) reversed the reduction in aMT synthesis caused by reserpine treatment. Interestingly, reserpine co-administration prevented the reduction in aMT synthesis seen with trazodone, mianserin or fluoxetine treatment alone ($p < 0.01$).

7.7.3.1.3 [^{14}C]N-Acetylated Products

The results are presented in Fig. 7.9. Chronic treatment of rats with reserpine produced a significant reduction in the level of N-acetylated products (i.e. aHT + aMT) when compared with that of saline-treated controls ($p < 0.01$).

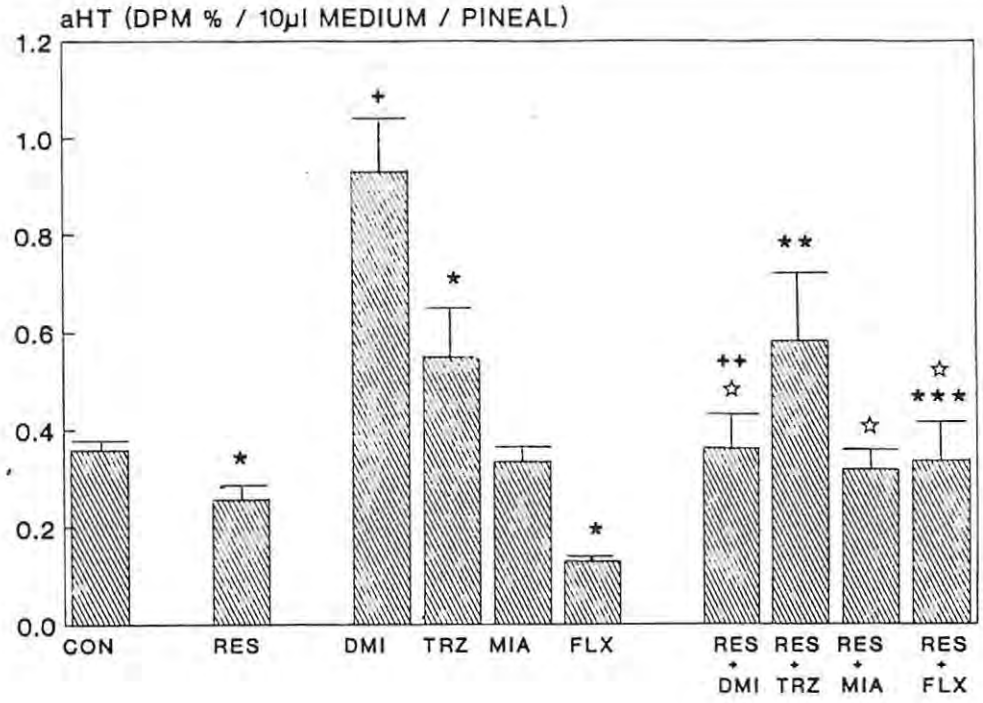


Figure 7.7 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]N-acetylserotonin (aHT) in pineal cultures of chronically reserpinized rats. Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.05$ when compared with controls.

** $p < 0.05$ when compared with reserpine treatment.

*** $p < 0.05$ when compared with fluoxetine treatment.

\+ $p < 0.01$ when compared with controls.

\+ + $p < 0.01$ when compared with desipramine treatment.

☆ not significantly different to reserpine treatment or controls.

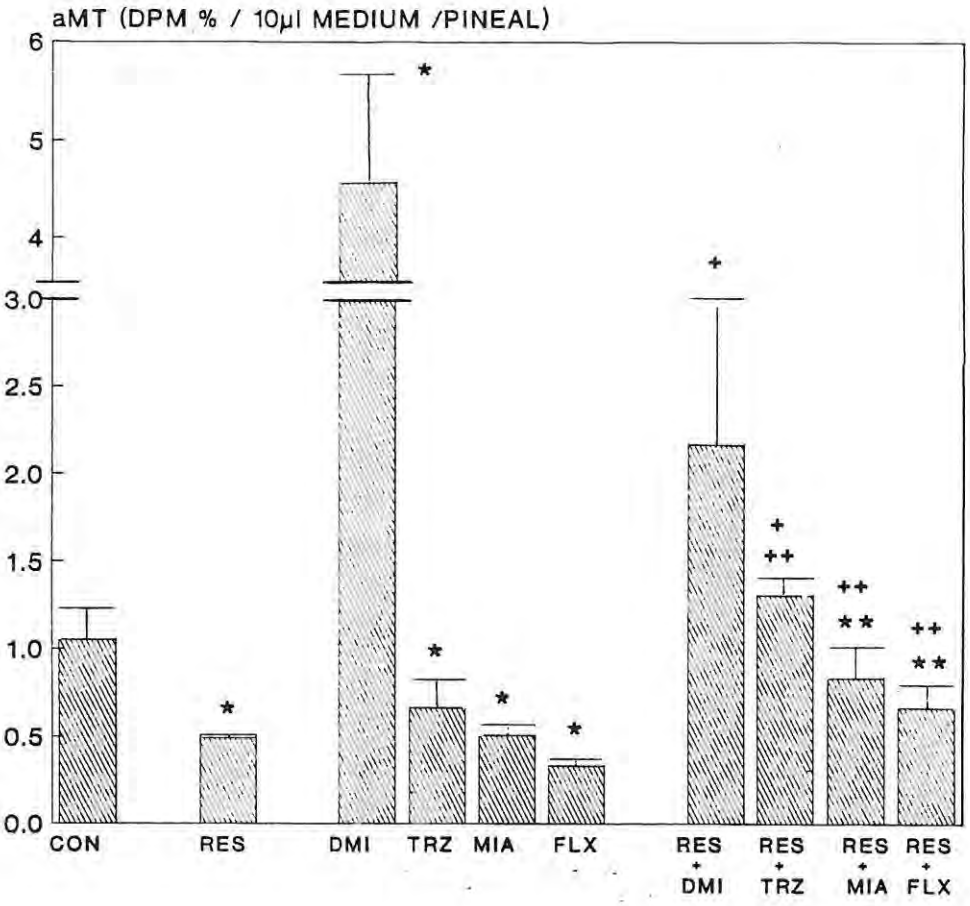


Figure 7.8 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]melatonin (aMT) in pineal cultures of chronically reserpinized rats. Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.01$ when compared with controls.

** $p < 0.05$ when compared with reserpine treatment.

\+ $p < 0.01$ when compared with reserpine treatment.

\+ + $p < 0.01$ when compared with the respective antidepressant treatment alone.

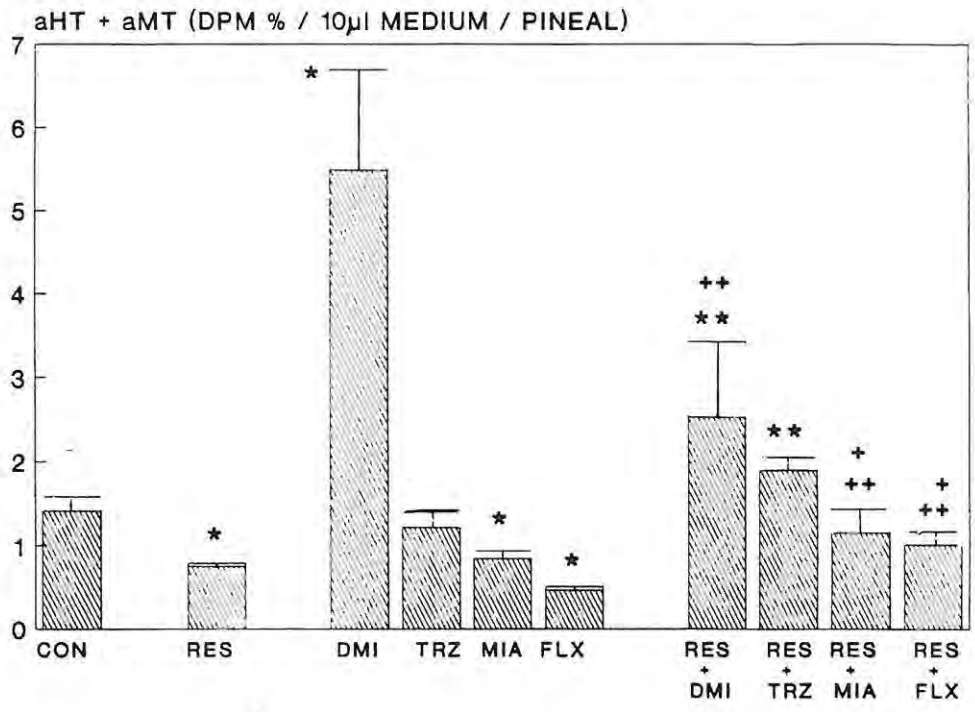


Figure 7.9 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]N-acetylated products (aHT + aMT) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.01$ when compared with controls.

** $p < 0.01$ when compared with reserpine treatment.

\+ $p < 0.05$ when compared with reserpine treatment.

\+ + $p < 0.05$ when compared with the respective antidepressant treatment alone.

Repeated treatment of rats with desipramine significantly increased the level of N-acetylated products when compared with saline treatment ($p < 0.01$). While trazodone treatment did not significantly alter the level of N-acetylated products, mianserin or fluoxetine treatment significantly decreased it ($p < 0.01$).

The chronic administration of desipramine ($p < 0.01$), trazodone ($p < 0.01$), mianserin ($p < 0.05$) or fluoxetine ($p < 0.05$) reversed the reduction in N-acetylated products caused by reserpine treatment. Conversely, reserpine co-administration prevented the reduction in N-acetylated products seen with mianserin or fluoxetine treatment alone ($p < 0.05$), although the effect on mianserin treatment was not statistically significant.

7.7.3.2 Effect on the Synthesis of [^{14}C] Deaminated Indoles

The effect of combined reserpine and antidepressant treatment on the synthesis of deaminated indoles is presented in Figs. 7.10 - 7.13. The chronic administration of reserpine to rats did not alter the levels of 5-hydroxyindole acetic acid (HA) and 5-hydroxytryptophol (HL) when compared with chronic saline treatment (Figs. 7.10 and 7.11). However, chronic reserpine administration did produce a significant reduction in the levels of 5-methoxyindole acetic acid (MA) and 5-methoxytryptophol (ML) ($p < 0.05$) (Figs. 7.12 and 7.13).

The chronic administration of desipramine produced a significant reduction in the levels of HA, HL, MA and ML when compared with saline treatment ($p < 0.01$). Treatment of rats with trazodone or fluoxetine did not alter the levels of deaminated indoles when compared with saline-treated controls. Treatment with mianserin, however, significantly increased the level of HL ($p < 0.01$), while not altering the levels of the other deaminated indoles (Fig. 7.11).

Combined chronic treatment of rats with reserpine and desipramine or trazodone was found to reduce the level of HL when compared with reserpine treatment alone ($p < 0.01$) (Fig. 7.11). The levels of the other deaminated indoles did not appear to be affected by the combination of reserpine and antidepressants.

7.7.4 Discussion

The present results indicating that chronic low-dose reserpine treatment reduced the synthesis of N-acetylserotonin and melatonin by rat pineal cultures are consistent with the previous observation that such treatment reduced the NA-induced rise in NAT activity (Section 7.6.3) and supports the hypothesis that reserpine depletes intraneuronal catecholamine stores.

Chronic desipramine treatment, on the other hand, was observed to markedly increase the synthesis of N-acetylserotonin and melatonin, consistent with previous findings (see Section 6.5.3). As discussed previously, the chronic inhibition of NA reuptake caused by desipramine may be sufficient to overcome

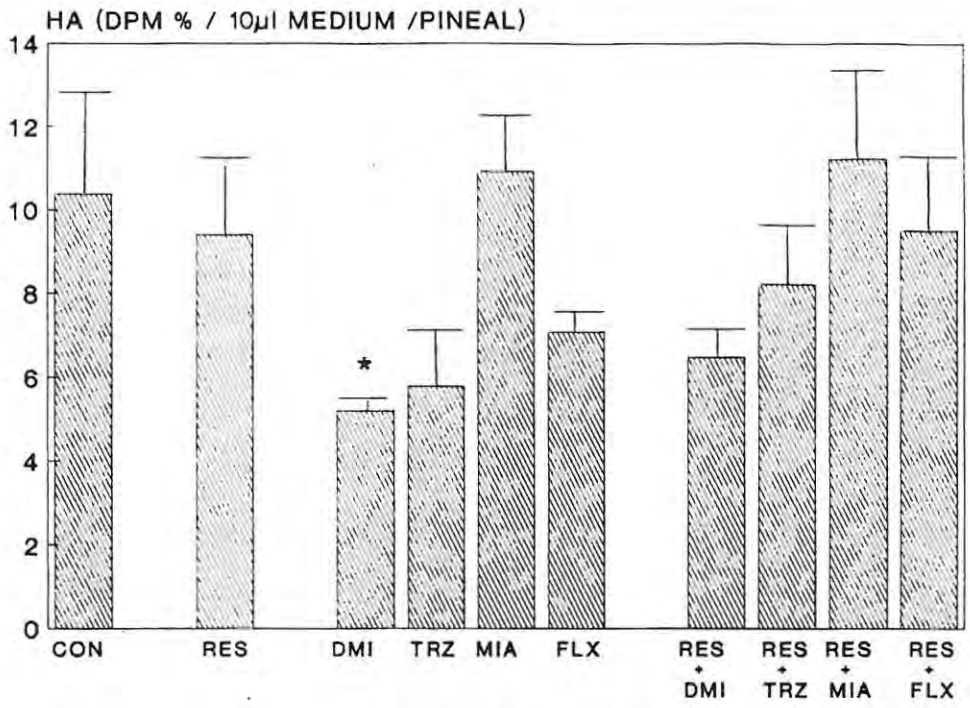


Figure 7.10 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]5-hydroxyindole acetic acid (HA) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.01$ when compared with controls.

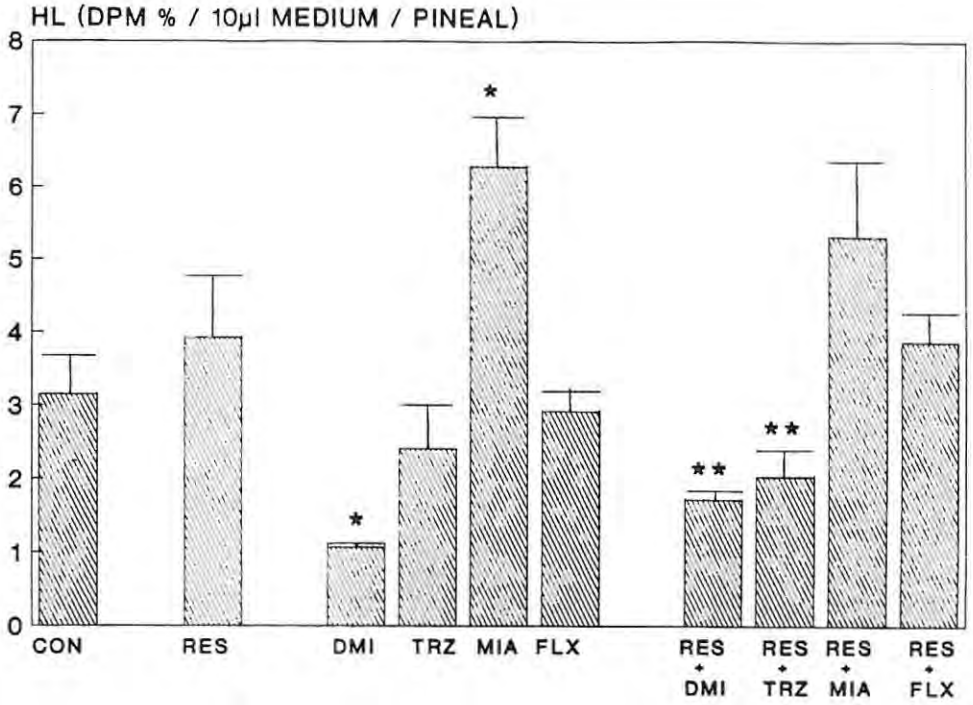


Figure 7.11 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]5-hydroxytryptophol (HL) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.01$ when compared with controls.

** $p < 0.01$ when compared with reserpine treatment.

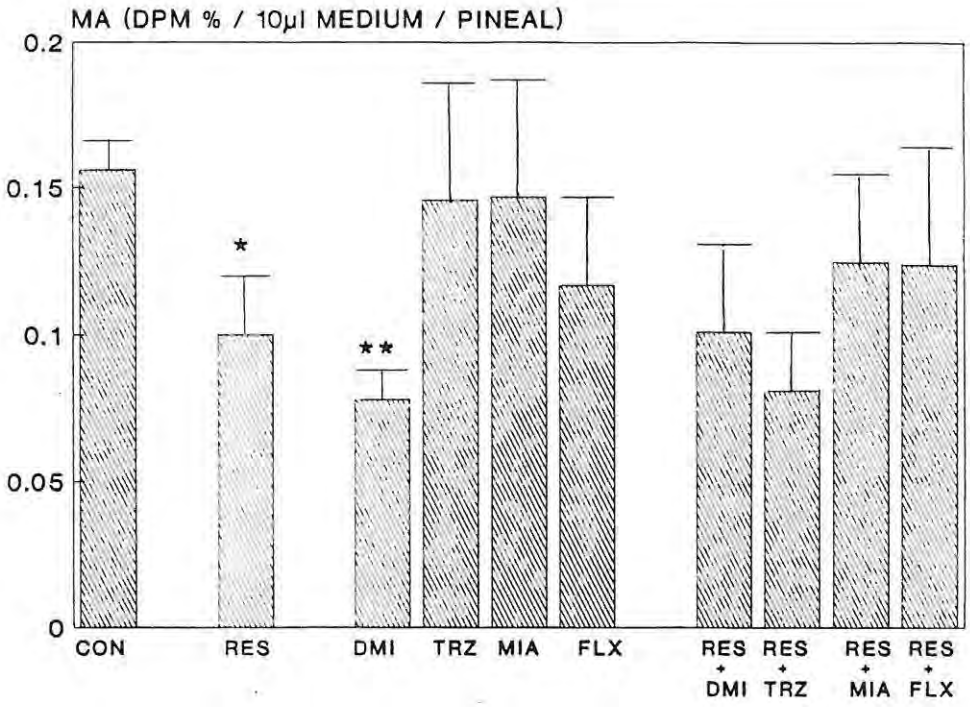


Figure 7.12 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]5-methoxyindole acetic acid (MA) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.05$ when compared with controls.

** $p < 0.01$ when compared with controls.

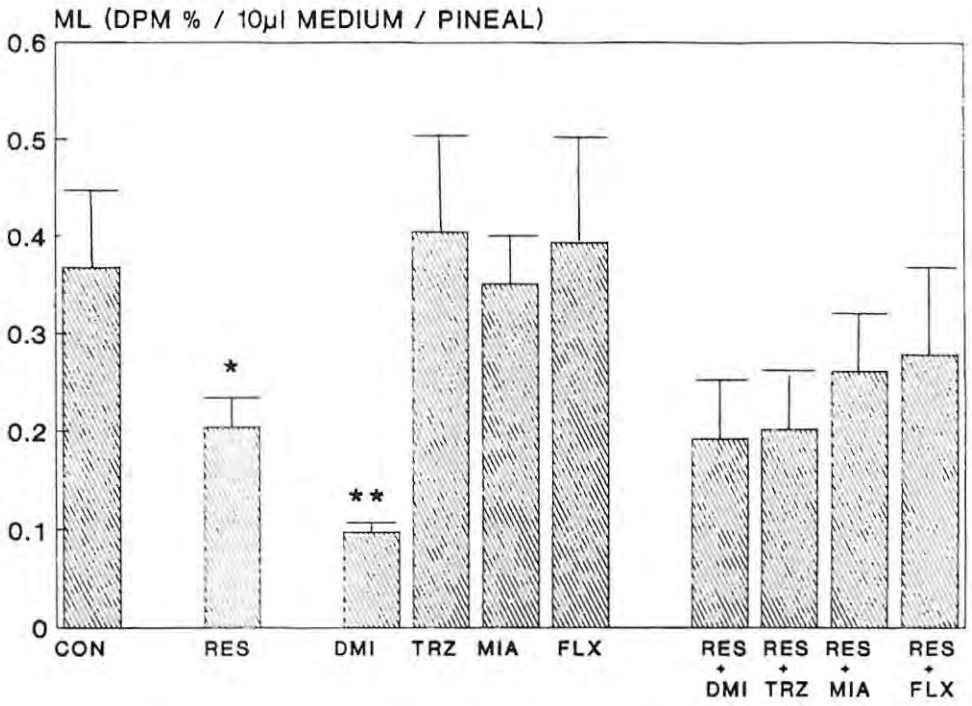


Figure 7.13 Effect of chronic antidepressant treatment on the synthesis of [^{14}C]5-methoxytryptophol (ML) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone; MIA = mianserin; FLX = fluoxetine.

* $p < 0.05$ when compared with controls.

** $p < 0.01$ when compared with controls.

the apparent drug-induced subsensitivity of the β-adrenoceptor/adenylate cyclase system, thereby increasing the synthesis of pineal N-acetylated products (Section 6.5.4). This suggestion is supported by the observation that desipramine treatment did not elicit the usual marked rise in N-acetylated product synthesis in reserpinized rats. However, the chronic administration of trazodone, mianserin or fluoxetine did not increase N-acetylated product synthesis, consistent with their inability to affect NA reuptake. In fact, fluoxetine and to a lesser extent, mianserin, were found to reduce the level of N-acetylated products. These observations suggest that fluoxetine, and possibly mianserin, may interfere with the uptake of exogenous 5-HT into the pinealocyte for conversion to melatonin. In support of this, panuramine, another selective 5-HT reuptake inhibitor, was also found to reduce the synthesis of N-acetylated products in a previous study (Section 6.5.3). Interestingly, this effect was not observed following repeated fluoxetine or mianserin treatment to chronically reserpinized rats, suggesting that these antidepressants have a variable effect on N-acetylated product synthesis in normal rats and in rats chronically depleted of NA and 5-HT.

Furthermore, the present results demonstrate that repeated treatment with desipramine, trazodone, mianserin or fluoxetine reversed the reduction in N-acetylserotonin and melatonin synthesis caused by chronic low-dose reserpine treatment. These findings are consistent with previous observations that the reserpine-induced reduction in pineal NAT activity was reversed by chronic treatment with these antidepressants (Section 7.6.3). Moreover, chronic antidepressant treatment was previously shown to antagonize the reserpine-induced supersensitivity of the β-adrenoceptor/adenylate cyclase system (see Sections 7.3.3 and 7.4.3). Taken together, these results provide further support for the hypothesis that, in reserpinized rats, synaptic levels of NA are increased by chronic treatment with antidepressants, irrespective of their acute pharmacological actions (see Section 7.3.4). Interestingly, although the antidepressants appeared to be equipotent in antagonizing the reserpine-induced reduction in N-acetyl-serotonin levels, they appeared to have differing potencies in their ability to reverse the reduction in the level of the total N-acetylated product. Thus, the order of potency was found to be desipramine ≥ trazodone > mianserin ≥ fluoxetine. The fact that fluoxetine and mianserin were found to reduce N-acetylated product levels when administered alone, may explain their weaker potencies in reversing the effects of reserpine on N-acetylated product synthesis. Furthermore, the inability of fluoxetine to counteract the effects of reserpine on β-adrenoceptor density and isoprenaline-stimulated cAMP production, may account for its weaker potency.

Chronic low-dose reserpine treatment did not affect the levels of 5-hydroxyindole acetic acid (HA) and 5-hydroxytryptophol (HL), consistent with previous results suggesting that their synthesis by the MAO enzyme is unlikely to be altered by changes in adrenergic input to the gland (Section 4.5.3.2). However, chronic reserpine treatment reduced the levels of 5-methoxyindole acetic acid (MA) and 5-methoxy-tryptophol (ML), thus supporting previous observations that the activity of the enzyme HIOMT, responsible for their synthesis, is regulated via an adrenergic mechanism (Section 4.5.3.2).

In support of previous findings, chronic desipramine treatment was found to reduce the levels of HA and HL, suggesting that more 5-HT is utilized in the melatonin biosynthetic pathway than is deaminated by the MAO enzyme (Section 6.5.3). As suggested previously, the reduced levels of HA and HL may also reflect inhibition of pineal MAO activity by desipramine (Section 6.5.4). Interestingly, chronic mianserin treatment produced an increase in the level of HL, suggesting that it may increase the oxidative deamination of 5-HT by MAO. This finding may account for the reduced levels of N-acetylated products observed with mianserin treatment. Consistent with previous studies (Section 6.5.3, *Skene* 1985), chronic treatment with trazodone and fluoxetine did not alter the level of MAO products. The finding that chronic desipramine treatment also reduced the levels of MA and ML, is in agreement with a previous study (Section 6.5.3) and suggests that the enzyme HIOMT preferentially utilizes the increased N-acetylserotonin as substrate rather than the 5-hydroxyindoles.

The simultaneous administration of antidepressants to chronically reserpinized rats appeared to have little effect on the levels of deaminated indoles, except that desipramine and trazodone were found to reduce the level of HA in reserpinized rats, thus possibly reflecting the increased utilization of 5-HT in the melatonin biosynthetic pathway.

7.8 EXPERIMENT 6 : EFFECT OF ACUTE ANTIDEPRESSANT TREATMENT ON THE RESERPINE-INDUCED ALTERATION OF PINEAL SEROTONIN N-ACETYLTRANSFERASE ACTIVITY

7.8.1 Introduction

In the present experiment, nocturnal serotonin N-acetyltransferase (NAT) activity was assessed following acute antidepressant treatment to chronically reserpinized rats.

7.8.2 Experimental Procedure

Drug Administration : Male rats were treated with either reserpine (0.2 mg/kg) or saline (1 ml/kg) i.p. once daily at 10:00 h for 2 weeks. On the day of final drug administration, the animals were additionally treated with a single i.p. dose of either antidepressant (10 mg/kg) or saline (1 ml/kg). For use in the experiment, pineal glands were collected 14 h later (midnight), frozen on solid CO_2, and stored at -70^{o}C prior to analysis.

Assay of Serotonin N-Acetyltransferase : Frozen pineal glands were thawed on ice prior to the determination of enzyme activity. The assay was performed as outlined in Section 2.5.2.

Analysis of Data : Enzyme activity is expressed as picomoles of [^{3}H]N-acetyltransferase formed per pineal per hour. Values represent means ± S.E.M. Four to eight rats were used in each experimental group.

7.8.3 Results

The results of the experiment are shown in Fig. 7.14. Repeated treatment of rats with reserpine prevented the nocturnal elevation of pineal NAT activity seen in saline-treated controls ($p < 0.01$).

Acute treatment of rats with desipramine markedly increased the nocturnal rise in NAT activity when compared with saline treatment ($p < 0.01$). However, acute treatment with trazodone produced no significant alteration of NAT activity.

The acute administration of desipramine to chronically reserpinized rats produced a slight increase in NAT activity when compared with reserpine treatment alone ($p < 0.05$). This increase, however, was not sufficient to counteract the depressant effect of reserpine on NAT activity. On the other hand, acute treatment with trazodone to chronically reserpinized rats did not alter NAT activity relative to reserpine treatment alone.

Furthermore, NAT activity following acute desipramine or trazodone treatment was markedly depressed by reserpine pretreatment ($p < 0.01$).

7.8.4 Discussion

Repeated treatment with reserpine produced the usual depression of nocturnal NAT activity that was observed previously (see Section 7.6.3). Likewise, the increase in nocturnal NAT activity following acute desipramine treatment is consistent with previous results (see Section 5.4.3). Furthermore, the observation that reserpine pretreatment prevented the increase in NAT activity caused by desipramine supports the hypothesis that reserpine induces a chronic reduction in synaptic NA levels, thus interfering with the ability of desipramine to inhibit the reuptake of this amine.

Interestingly, however, acute trazodone treatment did not increase dark-phase NAT activity in the present experiment, in contrast to previous findings (Section 5.4.3). A possible explanation for the lack of effect of trazodone may be that in the present study, NAT activity was assayed 14 h following drug administration whereas in the previous study, enzyme activity was assayed 3 h later. Thus, these results suggest that while sufficient levels of desipramine may have been present to elicit an increase in NAT activity in the present study, this may not have been the case for trazodone.

Furthermore, the present results indicate that acute treatment with desipramine or trazodone failed to reverse the reduction in NAT activity in chronically reserpinized rats. These findings suggest that, unlike chronic treatment (Section 7.6.3), acute treatment with desipramine or trazodone was unable to counteract the reserpine-induced depletion of NA levels. Consistent with this suggestion, *Jancsar* and

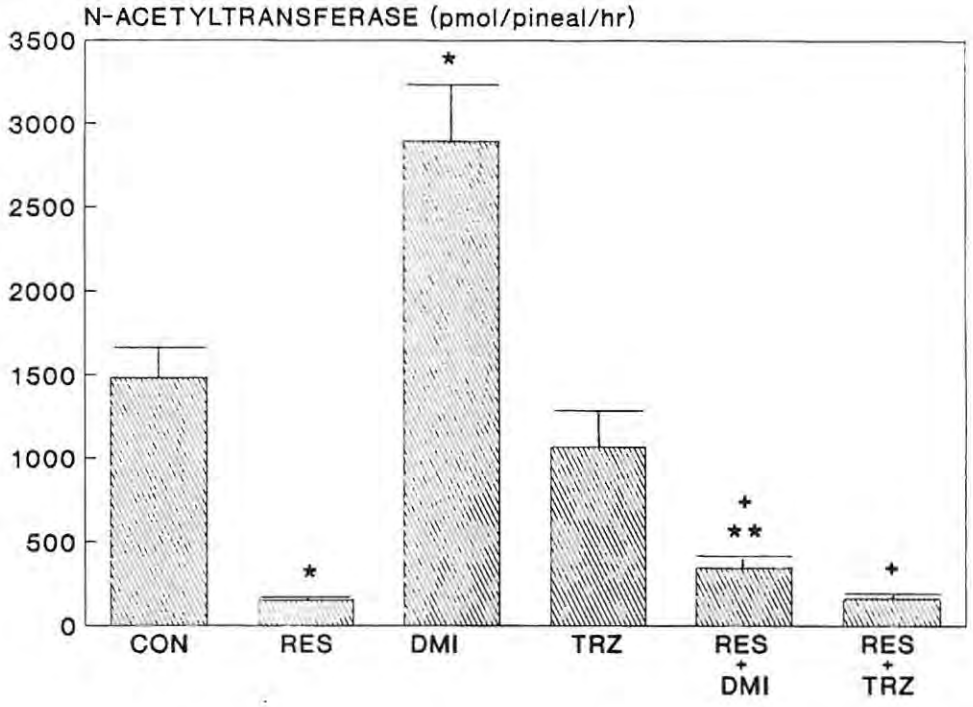


Figure 7.14 Effect of acute antidepressant treatment on nocturnal pineal N-acetyltransferase (NAT) activity in chronically reserpinized rats.
Values represent means ± S.E.M. of four to eight rats. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone.

* $p < 0.01$ when compared with controls.

** $p < 0.05$ when compared with reserpine treatment.

\+ $p < 0.01$ when compared with the respective antidepressant treatment alone.

Leonard (1983), showed that chronic, but not acute, treatment with the selective 5-HT reuptake inhibitor antidepressant, ORG-6582, reversed the reduction in NA levels in the amygdaloid cortex of chronically reserpinized rats.

7.9 EXPERIMENT 7 : EFFECT OF ACUTE ANTIDEPRESSANT TREATMENT ON THE RESERPINE-INDUCED ALTERATION IN PINEAL [^{14}C]INDOLE METABOLISM

7.9.1 Introduction

The present experiment describes the effect of acute antidepressant treatment to chronically reserpinized rats on *in vitro* pineal indole biosynthesis and metabolism.

7.9.2 Experimental Procedure

Drug Administration : Groups of male rats received either reserpine (0.2 mg/kg) or saline (1 ml/kg) i.p. once daily at 10:00 h for 2 weeks. On the day of final drug administration, the animals received, additionally, a single dose of either antidepressant (10 mg/kg) or saline (1 ml/kg). All animals were sacrificed 24 h later for use in the experiment.

Pineal Organ Culture and Analysis of [^{14}C]Indoles : The technique of pineal culture and analysis of indoles is outlined in Section 2.6.2. Briefly, pineals were cultured individually in the presence of [^{14}C]-serotonin and incubated at 37^{o}C under 95% O_2 : 5% CO_2 for 8 h. Following culture, a 10 µl aliquot of culture medium was removed and analysed for its content of radiolabeled indoles.

Analysis of Data : Results are expressed as the percentage of total radioactivity (DPM%/10 µl medium/pineal). Values represent means ± S.E.M. Four rats were used in each experimental group.

7.9.3 Results

7.9.3.1 Effect on the Synthesis of [^{14}C]N-Acetylated Indoles

The effect of acute antidepressant treatment on pineal N-acetylated indole synthesis in reserpinized rats is shown in Figs. 7.15 - 7.17. Repeated treatment with reserpine produced a significant decrease in the synthesis of N-acetylserotonin (aHT) (Fig. 7.15), melatonin (aMT) (Fig. 7.16) and N-acetylated products (Fig. 7.17) when compared with saline treatment ($p < 0.01$).

Acute treatment with desipramine increased the synthesis of aHT ($p < 0.05$), aMT ($p < 0.01$) and N-acetylated products ($p < 0.01$) when compared with saline treatment. However, acute trazodone treatment did not alter the levels of these indoles when compared with saline treatment.

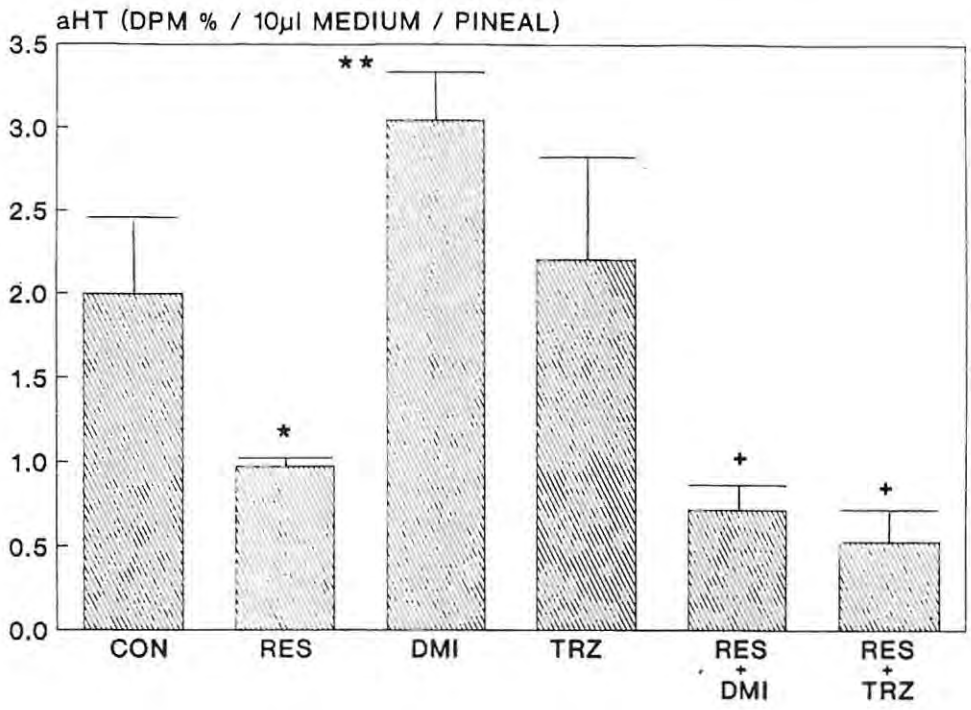


Figure 7.15 Effect of acute antidepressant treatment on the synthesis of [^{14}C]N-acetylserotonin (aHT) in pineal cultures of chronically reserpinized rats. Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone.

* $p < 0.01$ when compared with controls.

** $p < 0.05$ when compared with controls.

\+ $p < 0.01$ when compared with the respective antidepressant treatment alone.

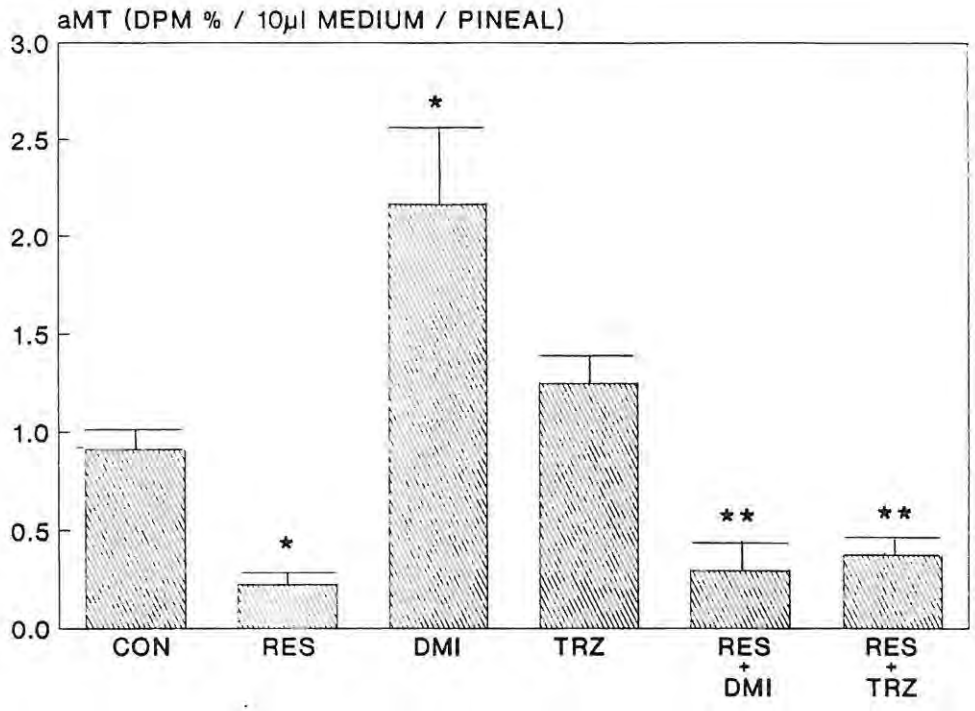


Figure 7.16 Effect of acute antidepressant treatment on the synthesis of [^{14}C]melatonin (aMT) in pineal cultures of chronically reserpinized rats. Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone.

* $p < 0.01$ when compared with controls.

** $p < 0.01$ when compared with the respective antidepressant treatment alone.

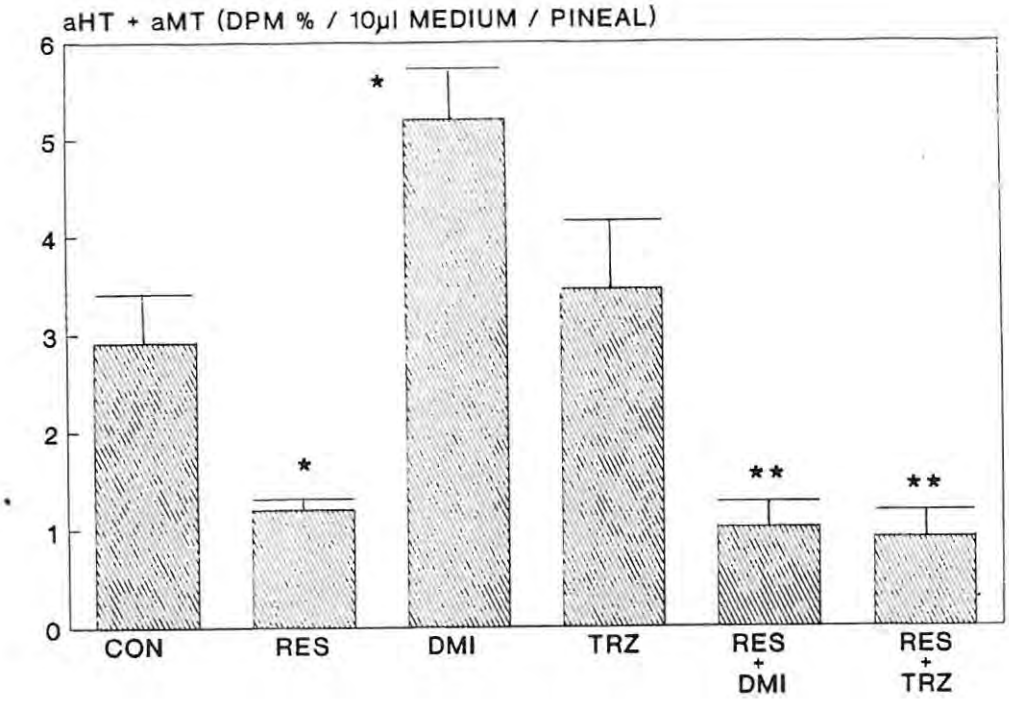


Figure 7.17 Effect of acute antidepressant treatment on the synthesis of [^{14}C]N-acetylated products (aHT + aMT) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone.

* $p < 0.01$ when compared with controls.

** $p < 0.05$ when compared with the respective antidepressant treatment alone.

The acute administration of desipramine or trazodone to reserpinized rats did not alter the synthesis of aHT, aMT or N-acetylated products when compared with reserpine treatment alone.

Furthermore, the synthesis of these indoles, following acute desipramine or trazodone administration, was markedly depressed by pretreatment with reserpine ($p < 0.01$).

7.9.3.2 Effect on the Synthesis of [^{14}C]Deaminated Indoles

The effect of acute antidepressant treatment on the synthesis of pineal deaminated indoles is presented in Figs. 7.18 - 7.21. Repeated treatment with reserpine did not alter the synthesis of 5-hydroxyindole acetic acid (HA) (Fig. 7.18) or 5-hydroxytryptophol (HL) (Fig. 7.19) when compared with chronic saline treatment. However, reserpine produced a significant decrease in 5-methoxyindole acetic acid (MA) (Fig. 7.20) and 5-methoxytryptophol (ML) (Fig. 7.21) synthesis relative to saline treatment ($p < 0.01$). Similarly, acute treatment with desipramine produced a significant decrease in MA and ML synthesis ($p < 0.01$) without altering that of HA and HL. Furthermore, the acute administration of desipramine or trazodone to reserpinized rats did not alter the synthesis of any of these indoles when compared with reserpine treatment alone.

7.9.4 Discussion

Chronic low-dose reserpine treatment produced the usual reduction in N-acetylserotonin and melatonin synthesis that was observed previously (see Section 7.7.3). Additionally, acute treatment with desipramine was found to increase the levels of N-acetylserotonin and melatonin, consistent with previous findings (see Section 5.5.3). The observation that chronic pretreatment with reserpine prevented this increase caused by desipramine supports the hypothesis that reserpine, by inducing a reduction in synaptic NA levels, interferes with the ability of desipramine to inhibit NA reuptake.

However, in contrast to previous findings (Section 5.5.3), acute treatment with trazodone did not increase N-acetylserotonin or melatonin levels in the present experiment. As suggested previously (Section 7.8.4), the longer time interval between drug administration and assay may account for this lack of effect of trazodone.

The present results also indicate that acute treatment with desipramine or trazodone failed to alter the reserpine-induced reduction in N-acetylated product synthesis. These findings are consistent with the previous observation that such treatment failed to alter the reduction in NAT activity caused by reserpine (Section 7.8.3) and suggests that, unlike chronic treatment (Section 7.7.3), acute treatment with desipramine or trazodone was unable to counteract the reserpine-induced reduction in NA levels.

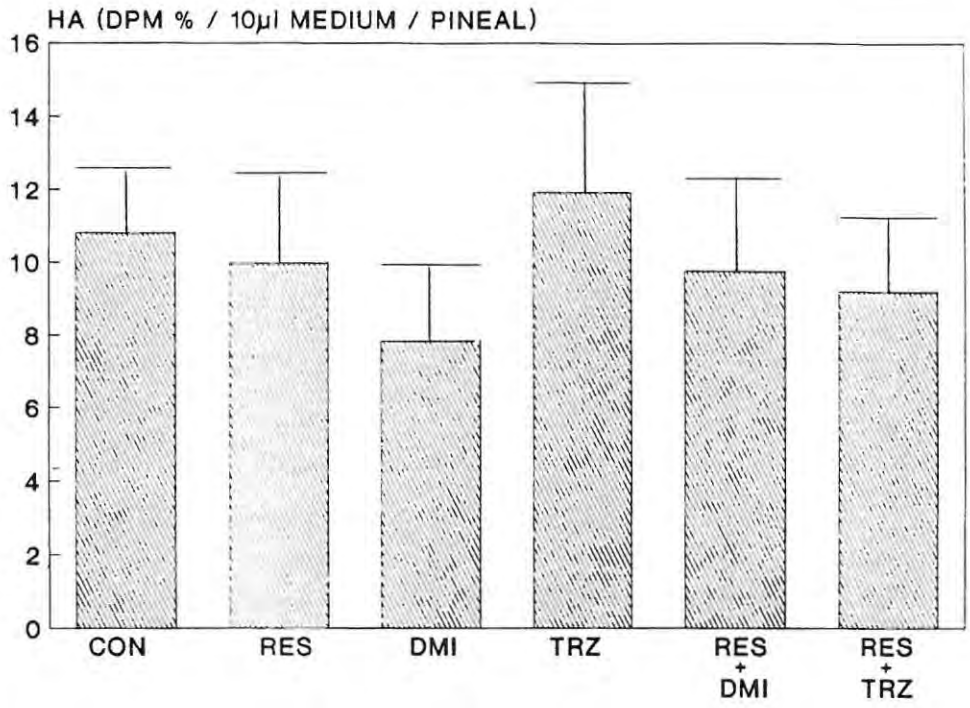


Figure 7.18 Effect of acute antidepressant treatment on the synthesis of [^{14}C]5-hydroxyindole acetic acid (HA) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone.

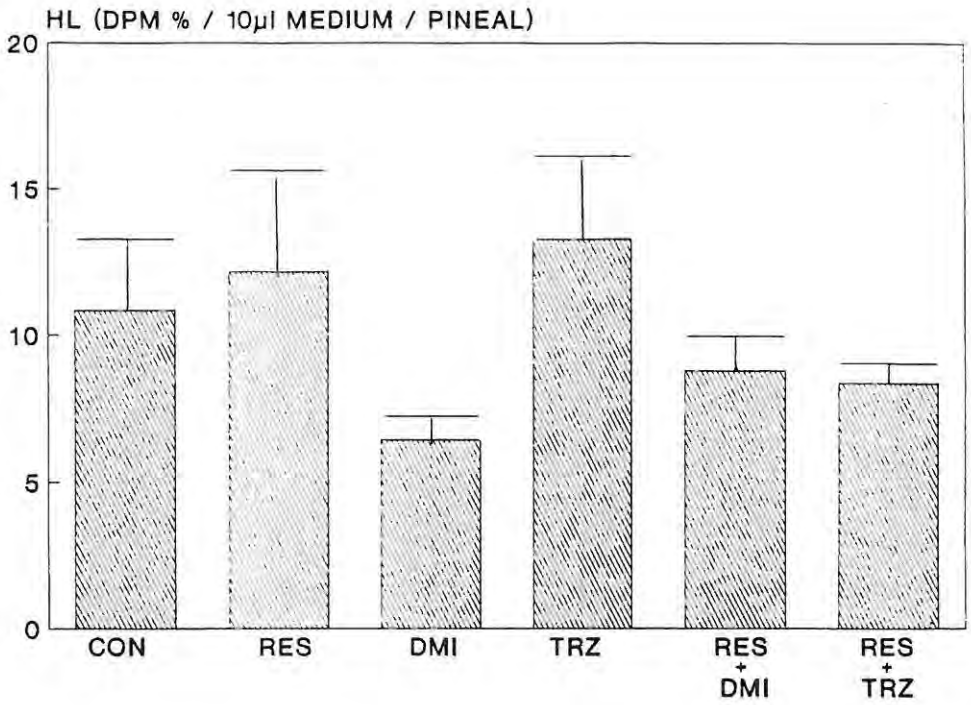


Figure 7.19 Effect of acute antidepressant treatment on the synthesis of [^{14}C]5-hydroxytryptophol (HL) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone.

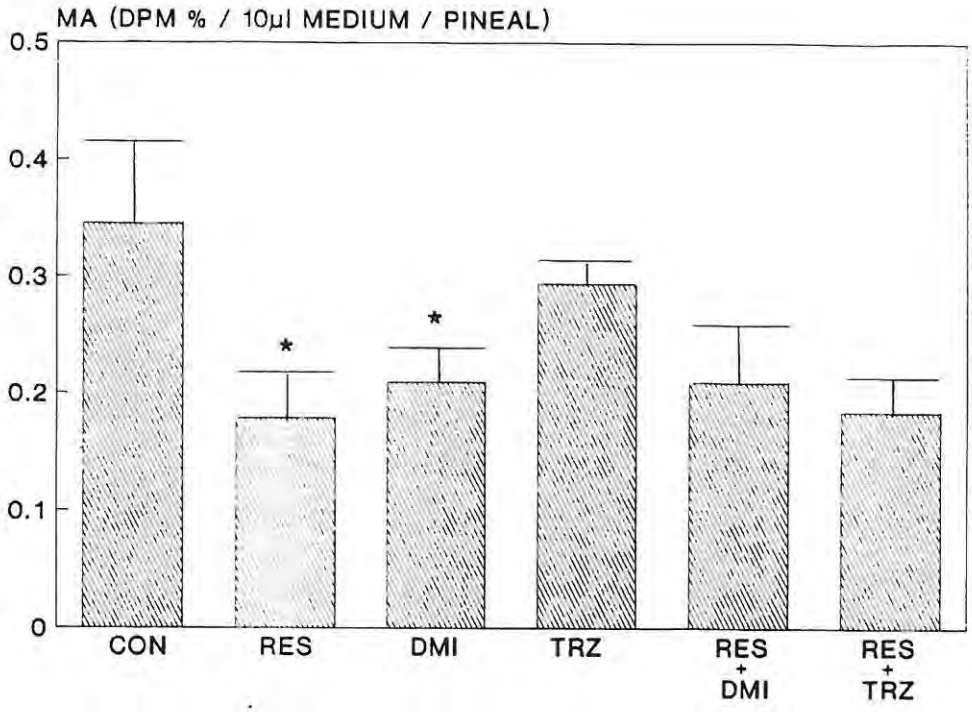


Figure 7.20 Effect of acute antidepressant treatment on the synthesis of [^{14}C]5-methoxyindole acetic acid (MA) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone.

* $p < 0.01$ when compared with controls.

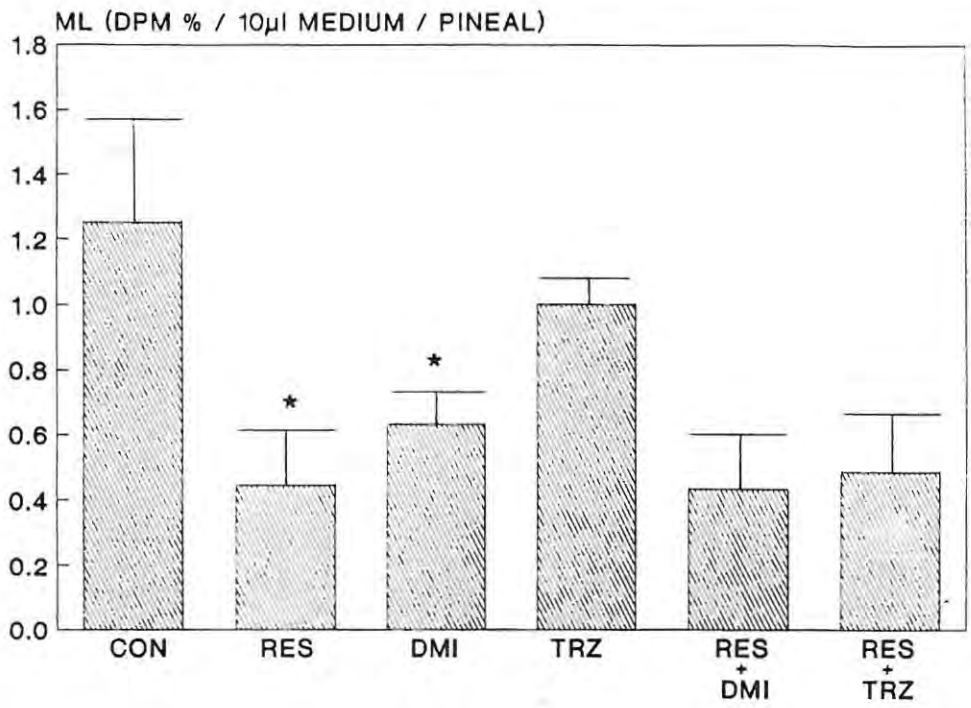


Figure 7.21 Effect of acute antidepressant treatment on the synthesis of [^{14}C]5-methoxytryptophol (ML) in pineal cultures of chronically reserpinized rats.

Values represent means ± S.E.M. Four rats were used in each experimental group. CON = control; RES = reserpine; DMI = desipramine; TRZ = trazodone.

* $p < 0.01$ when compared with controls.

7.10 CONCLUSION

Ideally, a model used to study the action of antidepressants should include measurable behavioural and biochemical alterations that are normalized by chronic, but not acute, administration of clinically efficacious antidepressant drugs from several classes. The results of the present study suggest that the pineal gland of the chronically reserpinized rat may provide a model that is characterized by several of these traits.

Chronic treatment of rats with a variety of pharmacologically different antidepressants was found to reverse the increased responsiveness of the β-adrenoceptor/adenylate cyclase system in rats treated chronically with reserpine. Moreover, chronic treatment with these antidepressants reversed the reduction in pineal NAT activity and melatonin synthesis in reserpinized rats. Acute antidepressant treatment, on the other hand, was not associated with a reversal of the reserpine-induced effects. These findings suggest that chronic treatment with antidepressants, irrespective of their acute pharmacological actions, causes an increase in the synaptic concentrations of NA and, consequently, increases melatonin synthesis in pineals of reserpinized rats. It is unlikely that the reversal of reserpine-induced effects by antidepressants is due to pharmacokinetic interactions, since compounds with different chemical structures were employed.

This effect is not unexpected in the case of the tricyclic antidepressant desipramine which inhibits the reuptake of NA from the synaptic cleft. However, the ability of the atypical and selective 5-HT reuptake inhibiting antidepressants to reverse the effects of reserpine following chronic administration is interesting. Other pharmacological challenge procedures employing reserpine (eg., reserpine-induced ptosis and hypothermia) are not sensitive to many of these antidepressants. Moreover, the present results are consistent with previous studies (see also Chapter 6) in showing that the atypical and 5-HT reuptake inhibitor group of antidepressants have either no effect or have variable effects on pineal gland function when administered chronically to normal rats. The ability of mianserin to increase synaptic NA levels may relate to its ability as a presynaptic α_2-adrenoceptor antagonist. More importantly, the present results raise the possibility that trazodone and fluoxetine may facilitate NA release from pineal noradrenergic nerve terminals as a result of increased serotonergic activity. Such an interaction between the 5-HT and NA neurotransmitter systems has been previously described in other brain areas (see Section 1.2.3.2.2). However, although 5-HT receptor sites appear to be present in the pineal gland, an interaction between these neurotransmitter systems has yet to be demonstrated. Further studies are therefore necessary to elucidate the role of 5-HT receptors in the pineal gland.

Alternatively, it is interesting to speculate that fluoxetine and trazodone may enhance the effects of the pineal noradrenergic system via a postsynaptic mechanism. While pineal β-adrenoceptors are linked in a stimulatory fashion to adenylate cyclase, pineal 5-HT receptors may be linked to phosphatidylinositol (PI) hydrolysis as has been shown in the cortex and choroid plexus (see *Sulser* 1987). The resultant formation of inositol phosphates and diacylglycerol may mediate the amplification of the cAMP and

NAT response to β-adrenoceptor activation via specific protein phosphorylation as has been described for pineal α_1-adrenoceptors (Section 1.3.3.2). Thus, the final common pathway of pineal monoaminergic receptor activation may be protein-kinase-mediated protein phosphorylation leading to changes in cellular activity. Such a mechanism, as described above, may conceivably account for the role of the extrapinealocytic pool of 5-HT. Moreover, such a phenomenon would account for the finding that fluoxetine reversed the effect of reserpine on pineal NAT activity and melatonin synthesis but not on β-adrenoceptor density. Furthermore, it is possible that such a mechanism would be observed only in the event of disruption of normal noradrenergic function since serotonergic drugs appear to be without this effect in normal rats (see also Chapter 6). Thus, 5-HT may play an important role in maintaining normal noradrenergic function.

The observation that chronic antidepressant administration increased melatonin synthesis in reserpinized rats also suggests that the overall effect of such treatment is to increase noradrenergic neurotransmission following disruption of neuronal activity. Thus, the present study provides additional evidence against the hypothesis that antidepressant drugs act by reducing noradrenergic neurotransmission and casts doubt on the importance of β-adrenoceptor down-regulation in the mechanism of antidepressant action. Furthermore, given the evidence implicating melatonin in affective disorders (Section 1.3.6), it is interesting to speculate that the increased melatonin synthesis observed in reserpinized rats and depressed patients (see Section 1.3.6.3) following chronic antidepressant administration may represent part of the therapeutic effect of these drugs. Further studies are therefore necessary to confirm these findings and to elucidate the therapeutic potential of melatonin in depressive disorders.

Thus, the pineal gland of the chronically reserpinized rat may provide an alternative approach with which to screen drugs for potential antidepressant activity and to study the neurochemical actions of antidepressants. However, the value of this model can only be fully assessed once other psychotropic drugs, with and without antidepressant activity, have been evaluated.

CHAPTER EIGHT

SUMMARY OF RESULTS

A 24-hour profile of [^{14}C]indoleamine metabolism by organ cultures of rat pineal glands was undertaken in order to determine the relative proportion of metabolites formed at any particular stage of the culture period (**Chapter 3**). Such information was necessary to provide the optimum time to terminate the culture period during subsequent studies. The β-adrenoceptor agonist isoprenaline was also evaluated for its effect on the progressive formation of pineal indoles. Pineal glands were incubated with [^{14}C]serotonin in the presence or absence of isoprenaline. The synthesis of N-acetylserotonin (aHT) and melatonin (aMT) progressively increased in isoprenaline-stimulated and control pineals up to 16 h in culture. Isoprenaline stimulation produced a sharp increase in aHT and aMT levels during the first few hours of the culture period, and aMT levels were always higher than that of aHT. In control pineals, aHT levels closely followed that of aMT up to 16 h of culture, after which aMT levels declined to a lower level than that at 16 h of culture. The levels of the other indoles, 5-hydroxyindole acetic acid (HA), 5-hydroxytryptophol (HL), 5-methoxyindole acetic acid (MA), and 5-methoxytryptophol (ML), all progressively increased up to 14-16 h in culture with HA > HL > ML > MA. During the first few hours of the culture period, however, isoprenaline produced an increase in HA and HL production with a corresponding decrease in MA and ML production. These results support the hypothesis that pineal aMT synthesis is under β-adrenergic control and suggest that the synthesis of the other pineal indoles may, likewise, be controlled by a β-adrenergic mechanism. From these results it appears that a shorter culture period may be more useful for determining the overall effect on indole metabolism following pharmacological manipulation.

The effect of reduced sympathetic input, caused by reserpine administration, on rat pineal gland function is described in **Chapter 4**. This study was undertaken, since later work involved evaluating the effect of antidepressant administration following alterations in sympathetic input to the gland. Reserpine treatment (5 mg/kg i.p. for 2 days) produced an increase in the binding of [^{3}H]DHA to pineal β-adrenoceptors. This effect was found to persist for at least 4 days following drug administration. Reserpine, by depleting endogenous catecholamines, also prevented the nocturnal rise in pineal NAT activity and reduced the conversion of radiolabeled serotonin to aHT and aMT in pineal organ cultures. The increased sensitivity of the β-adrenoceptors was associated functionally with a markedly greater induction of N-acetyltransferase (NAT) activity when stimulated with a catecholamine such as isoprenaline. Superinduction of NAT activity was also correlated with increased aHT and aMT synthesis by pineal cultures. Reserpine treatment did not alter the synthesis of the other pineal indoles with the exception of 5-methoxytryptophol (ML), the levels of which were reduced. These findings support the hypothesis that altered sympathetic input can modify the sensitivity of the β-adrenoceptor/adenylate cyclase system to catecholamines in target tissues.

The effect of acute antidepressant treatment on rat pineal gland function is described in **Chapter 5**. Acute treatment of rats (10 mg/kg i.p.) with desipramine, maprotiline or trazodone did not alter the sensitivity of the β-adrenoceptor/adenylate cyclase system as evidenced by a lack of effect on the binding of [^{3}H]DHA to pineal β-adrenoceptors and on the responsiveness of pineal NAT activity to exogenous catecholamine stimulation. However, acute treatment with desipramine, but not with maprotiline or trazodone, was found to increase daytime basal NAT activity. Moreover, treatment with desipramine and trazodone, but not with maprotiline, enhanced the nocturnal induction of NAT activity. Treatment with desipramine, maprotiline, trazodone as well as with the experimental antidepressant sertraline, increased the conversion of radiolabeled serotonin to aHT and/or aMT by cultures of rat pineal glands. Treatment with RU-25591, another experimental antidepressant, however, was not associated with increased N-acetylated indole synthesis. In addition, only desipramine and maprotiline altered the metabolism of radiolabeled serotonin by the enzyme MAO to deaminated indole products. These findings suggest that antidepressants increase pineal aMT synthesis by increasing the amount of synaptic NA available for activation of postsynaptic β-adrenoceptors. This suggestion is supported by the observation that pretreatment with the β-adrenoceptor antagonist, atenolol, prevented this effect. The ability of antidepressants such as desipramine and maprotiline to increase aMT synthesis is presumably related to their ability to inhibit the reuptake of NA. However, this effect by trazodone and sertraline, drugs which primarily affect serotonergic function, suggests the possibility that they may facilitate the release of NA from noradrenergic nerve terminals via an interaction with the serotonergic neurotransmitter system.

The effect of chronic antidepressant administration on rat pineal function was also investigated and is described in **Chapter 6**. Chronic treatment of rats (10 mg/kg i.p. for 2 weeks) with desipramine was found to reduce the binding of [^{3}H]DHA to pineal β-adrenoceptors. This effect was not observed following chronic treatment with maprotiline or trazodone. In addition, treatment with desipramine, but not with maprotiline or trazodone, reduced the responsiveness of NAT activity to exogenous catecholamine stimulation while not altering basal enzyme levels. However, treatment with desipramine was found to increase the conversion of radiolabeled serotonin to aHT and aMT by pineal cultures. This effect on N-acetylated indole synthesis was not observed following the chronic administration of maprotiline, trazodone, or the experimental antidepressants sertraline, panuramine, and CGP-6085A. However, treatment with another experimental compound, RU-25591, raised the level of N-acetylated products. Of the antidepressants tested, only desipramine was found to alter the metabolism of radiolabeled serotonin by MAO to deaminated indole products. These results demonstrate that antidepressants with different pharmacological profiles have variable effects on rat pineal function following chronic administration and suggest that the pineal gland of normal rats may not represent a suitable model for evaluating the chronic effects of antidepressant therapies.

The effect of antidepressant administration on pineal gland function of chronically reserpinized rats is described in **Chapter 7**. Repeated low-dose reserpine administration produced an increase in the sensitivity of the pineal β-adrenoceptor/adenylate cyclase system as evidenced by an increased binding

of [^{3}H]CGP-12177 to β-adrenoceptors and an enhanced accumulation of cAMP following exogenous catecholamine stimulation. This effect of reserpine is presumably due to the chronic reduction of synaptic NA levels. In addition, in the absence of exogenous catecholamine stimulation, chronic reserpine treatment prevented the nocturnal induction of NAT activity and reduced the synthesis of aHT and aMT by pineal cultures. The chronic administration of desipramine, trazodone, mianserin or fluoxetine, all of which have variable effects on the pineal noradrenergic system in normal rats, was able to reverse the neurochemical effects of reserpine. Moreover, preliminary evidence presented suggests that acute treatment with desipramine or trazodone was not associated with a reversal of the reserpine-induced reduction in NAT activity and N-acetylated indole synthesis. These findings suggest that chronic treatment with antidepressants, irrespective of their acute pharmacological actions, increase the synaptic concentrations of NA and consequently increase aMT synthesis in reserpinized rats. Thus, the pineal gland of reserpinized rats may represent an alternative model for evaluating the effects of antidepressant therapies on noradrenergic receptor function and neurotransmission in dysregulated systems.

REFERENCES

ABERG A.: (1981) *Acta Psychiatr. Scand.*, **63**(Suppl 290):244-255.

AGHAJANIAN G.K., GALLAGHER D.W.: (1975) *Brain Res*, **88**:221.

ALEXANDER R.W., DAVIS J.N., LEFKOWITZ R.J.: (1975) *Nature*, **258**:437-440.

ALOYO V.J., WALKER R.F.: (1988) *Neuroendocrinology*, **48**:61-66.

ALPHS L., LOVENBERG W.: (1984) *J. Pharmacol. Exp. Ther.*, **230**:431-437.

ANTELMAN S.M., CHIODO L.A., DE GIOVANNI L.A.: (1982) In *Typical and Atypical Antidepressants: Molecular Mechanisms* (E. Costa, G. Racagni eds.) Raven Press, New York (Advances in biochemical psychopharmacology, Vol 31, pp 121-132).

ARENDT J.: (1989) *Br. J. Psychiatry*, **155**:585-590.

ARENDT J., ALDHOUS M.: (1988) *Annu. Rev. Chronopharmacol.*, **5**:53-55.

ARENDT J., HO A.K., LAUD C., *et al.*: (1981) *Br. J. Pharmacol.*, **72**:257-262.

ARENDT J., BOJKOWSKI C., FRANEY C., *et al.*: (1985) *J. Clin. Endocrinol. Metab.*, **60**:1166-1173.

ARENDT J., ALDHOUS M., MARKS V.: (1986) *Br. Med. J.*, **292**:1170.

ARENDT J., ALDHOUS M., ENGLISH J., *et al.*: (1987) *Ergonomics*, **30**:1379-1393.

ARENDT J., ALDHOUS M., WRIGHT J.: (1988) *Lancet*, **2**:772-773.

ARMSTRONG S.M.: (1988) *Pineal Res. Rev.*, **7**:157-202.

ARMSTRONG S.M.: (1989) *Experientia*, **45**:932-938.

ARMSTRONG S.M., CHESWORTH M.J.: (1987) In *Fundamentals and Clinics in Pineal Research* (G.P. Trentini, C. DeGaetani, P. Pévet eds.) Raven Press, New York, pp 195-198.

ARMSTRONG S.M., THOMAS E.M.V., CHESWORTH M.J.: (1989) In *Advances in Pineal Research Vol 3* (R.J. Reiter, S.F. Pang eds.) John Libbey, London, pp 265-270.

ASAKURA M., TSUKAMOTO T., HASEGAWA K.: (1982) *Brain Res.*, **235**:192-197.

ASK A.-L., FOWLER C.J., HALL H., *et al.*: (1986) *Acta. Pharmacol. et Toxicol.*, **58**:16-24.

ATAMURA A.C., MAURI M.C., RUDAS N., *et al.*: (1989) *Clin. Neuropharmacol.*, **12**(Suppl):25-33.

AUERBACH D.A., KLEIN D.C., WOODARD C., *et al.*: (1981) *Endocrinology* **108**:559-567.

AURBACH G.D., FEDAK F.A., WOODARD C.J., *et al.*: (1974) *Science*, **186**:1223-1224.

AXELROD J.: (1983) In *The Pineal Gland and its Endocrine Role* (J. Axelrod, F. Fraschini, G.P. Velo eds.) Plenum Press, New York, pp 1-13.

AXELROD J., WEISSBACH H.: (1961) *J. Biol. Chem.*, **236**:211.

AXELROD J., WURTMAN R.J., SNYDER S.H.: (1965) *J. Biol. Chem.*, **240**:949-954.

AXELROD J., SHEIN H.M., WURTMAN R.J.: (1969) *Proc. Natl. Acad. Sci. USA*, **62**:544.

BALEMANS M.G.M., NOORDEGRAAF E.M., BARRY F.A.M., *et al.*: (1978) *Experientia*, **34**:887-888.

BALEMANS M.G.M., PEVET P., VAN BENTHEM J., *et al.*: (1983) *J. Neural Transm.*, **56**:53-72.

BANERJEE S.P., KUNG L.S., RIGGI S.J.: (1977) *Nature*, **278**:455-456.

BANOO S., BROWN C., DAYA S., *et al.*: (1987) *Med. Sci. Res.*, **15**:1477-1478.

BARTNESS T.J., GOLDMAN B.D.: (1989) *Experientia*, **45**:939-945.

BEARN J., FRANEY C., ARENDT J., *et al.*: (1989) *Br. J. Psychiatry*, **155**:341-347.

BECK A.T.: (1973) In *The Diagnosis and Management of Depression, 2nd Edition*, Univ. Pennsylvania Press, Philadelphia.

BECK O., JONSSON G.: (1981) *J. Neurochem.*, **36**:2013-2018.

BECK-FRIIS J.: (1983) PhD Thesis, Karolinska Institute, Stockholm, Sweden.

BECK-FRIIS J., VON ROSEN D., KJELLMAN B.F., *et al.*: (1984) *Psychoneuroendocrinol.* **9**:261-277.

BECK-FRIIS J., LJUNGGREN J.-G., THOREN M., *et al.*: (1985) *Psychoneuroendocrinol.*, **10**:173-186.

BERGSTROM D.A., KELLAR K.J.: (1979) *Nature*, **278**:363.

BERNARD C.: (1856) *Lecons de Physiologie Experimentale*, Balliér. Quoted from: K. Brendel *et al.* (1987) *TIPS*, **8**:11-15.

BHANOT R., WILKINSON M.: (1983) *Int. J. Develop. Neurosci.*, **1**:165-170.

BIECK P.R., ANTONIN K.H.: (1988) *Psychopharmacology (Berlin)*, (Suppl) 31-TU 18.03.

BIEL J.H., NUHFER P.A., HOYA W.K., *et al.*: (1962) *Ann. N.Y. Acad. Sci.*, **96**:251-262.

BINKLEY S.A.: (1981) In *The Pineal Gland, Vol 1* (R.J. Reiter ed.) CRC Press, Florida, pp 155-172.

BLACKSHEAR M.A., SANDERS-BUSH E.: (1982) *J. Pharmacol. Exp. Ther.*, **221**:303-308.

BLACKWELL B.: (1963) *Lancet*, **2**:849-851.

BLASK D.E.: (1984) In *The Pineal Gland* (R.J. Reiter ed.) Raven Press, New York, pp 253-284.

BLIER P., DE MONTIGNY C., TARDIF D.: (1984) *Psychopharmacology*, **84**:242-249.

BLIER P., DE MONTIGNY C., CHAPUT Y.: (1987) *J. Clin. Psychopharmacol.*, **7**(Suppl 6):24-35.

BLIER P., CHAPUT Y., DE MONTIGNY C.: (1988) *Naunyn Schmiedebergs Arch. Pharmacol.*, **337**:246-254.

BORUP C., MEIDAHL B., PETERSON I.M., *et al.*: (1982) *Pharmacopsychiatry*, **15**:183.

BOYCE P.M.: (1985) *Am. J. Psychiatry*, **142**:125-127.

BRANCHEY L., WEINBERG U., BRANCHEY M., *et al.*: (1982) *Neuropsychobiology*, **8**:225-232.

BREMMER J.D.: (1984) *J. Clin. Psychiatry.*, **45**:414-419.

BROGDEN R.N., HEEL R.C., SPEIGHT J.M., *et al.*: (1978) *Drugs*, **16**:273-301.

BROGDEN R., HEEL R., SPEIGHT J., *et al.*: (1979) *Drugs*, **18**:1-24.

BROGDEN R.N., HEEL R.C., SPEIGHT T.M., *et al.*: (1981) *Drugs*, **21**:401-429.

BRONSTEIN M., AXELROD J.: (1974) *Science*, **184**:163.

BROWN B.L., ALBANO J.D.M., EKINS R.P., *et al.*: (1971) *Biochem. J.*, **121**:561-562.

BROWN R., KOCSIS J.H., CAROFF S., *et al.*: (1985a) *Am. J. Psychiatry*, **142**:811-816.

BROWN R.P., CAROFF S., KOCSIS J.H., *et al.*: (1985b) *Psychopharmacol. Bull.*, **21**:579-581.

BRUNELLO N., BARBACCIA M.L., CHUANG D.M., *et al.*: (1982) *Neuropharmacology*, **21**:1145-1149.

BUBENICK G.A., BROWN G.M., GROTA L.J.: (1976) *J. Histochem. Cytochem.*, **24**:1173-1177.

BUNNEY W.E., DAVIS J.M.: (1965) *Arch. Gen. Psychiatry*, **13**:484-494.

BURROWS G.D., McINTYRE I.M., JUDD F.K., *et al.*: (1988) *J. Clin. Psychiatry*, **49**(Suppl 8):18-22.

BYLUND D.B., SNYDER S.H.: (1976) *Mol. Pharmacol.*, **12**:568-580.

CAMPBELL I.C., McKERNAN R.M.: (1982) *Br. J. Pharmacol.*, **75**:(Suppl):100.

CANTOR E.H., GREENBERG L.H., WEISS B.: (1981) *Mol. Pharmacol.*, **19**:21-26.

CARDINALI D.P., VACAS M.I.: (1978) *J. Neural Transm.*, **42**:193.

CARDINALI D.P., RITTA M.N.: (1983) *Neuroendocrinology*, **36**:152-160.

CARDINALI D.P., VACAS M.I.: (1987) *Cell Mol. Neurobiol.*, **7**:323-337.

CARDINALI D.P., LARIN F., WURTMAN R.J.: (1972) *Endocrinology*, **91**:877-886.

CARTER S.J., LAUD C.A., SMITH I., *et al.*: (1979) In *The Pineal Gland of Vertebrates including Man* (J. Ariens Kappers, P. Pévet eds.) Progress in Brain Research, 52, Elsevier, Amsterdam.

CASSONE V.M., ROBERTS M.H., MOORE R.Y.: (1988) *Am. J. Physiol.*, **255**:R332-R337.

CHARNEY D.S., MENKES D.B., HENINGER G.R.: (1981) *Arch. Gen. Psychiatry*, **38**:1160-1180.

CHECKLEY S.A., SLADE A.P., SHUR E.: (1981a) *Br. J. Psychiatry*, **138**:51.

CHECKLEY S.A., SLADE A.P., SHUR E., *et al.*: (1981b) *Br. J. Psychiatry*, **138**:248.

CHECKLEY S.A., CORN T.H., GLASS I.B., *et al.*: (1986) In *Antidepressants and Receptor Function* (Ciba Foundation 123) Wiley, Chichester, pp 126-147.

CHIK C.L., HO A.K., KLEIN D.C.: (1988) *Endocrinology*, **122**:702-708.

CHIODO L.A., ANTELMAN S.M.: (1980) *Nature*, **287**:451-454.

CHOUINARD G.: (1985) *J. Clin. Psychiatry*, **46**:32-37.

CHOUINARD G., JONES B.: (1984) *Can. Med. Assoc. J.*, **131**:1190.

CLAUSTRAT B., CHAZOT G., BRUN J., *et al.*: (1984) *Biol. Psychiatry*, **19**:1215-1228.

COHEN R.M., CAMPBELL I.C., DAUPHIN M., *et al.*: (1982) *Neuropharmacology*, **21**:293-298.

COHN J.B., WILCOX C.: (1985) *J. Clin. Psychiatry*, **43**:26-31.

COOPER B.R., HESTER T.J., MAXWELL R.A.: (1980) *J. Pharmacol. Exp. Ther.*, **215**:127-134.

COOPER D.S., GELENBERG A.J., WOJCIK J.C., *et al.*: (1981) *Arch. Int. Med.*, **141**:1023-1025.

COPPEN A.: (1967) *Br. J. Psychiatry*, **113**:1237-1264.

COPPEN A., GUPTA R., MONTGOMERY S., *et al.*: (1976) *Br. J. Psychiatry*, **129**:342-345.

COWEN P.J., FRAZER S., GRAHAME-SMITH D.G., *et al.*: (1983) *Br. J. Pharmac.*, **78**:89-96.

CRAFT C.M., MORGAN W.W., JONES D.J., *et al.*: (1985) *J. Pineal Res.*, **2**:51-66.

CROME P., NEWMAN B.: (1979) *J. Royal Soc. Med.*, **72**:649-653.

CROME P., ALI C.: (1986) *Med. Toxicol.*, **1**:411-420.

DAIGUJI M., MELTZER H.Y., TONG C., *et al.*: (1981) *Life Sci.*, **29**:2059.

DAMLOUJI N.F., FEIGHNER J.P., ROSENTHAL M.H.: (1985) In *Pharmacotherapy of Affective Disorders: Theory and Practice* (W.G. Dewhurst, G.D. Baker eds.) Croon Helm, London, pp 286-311.

DAY M.D., HEMSWORTH B.A., STREET J.A.: (1977) *J. Pharm. Pharmacol.*, **29**:52P.

DAYA S., POTGIETER B.: (1982) *S. Afr. J. Sci.*, **78**:174.

DAYA S., FATA M.: (1986) *IRCS Med. Sci.*, **14**:1153-1154.

DE MONTIGNY C., AGHAJANIAN G.K.: (1978) *Science*, **202**:1303-1306.

DE MONTIGNY C., BLIER P., CAILLE G., *et al.*: (1981) *Acta. Psychiatr. Scand*, **63**(Suppl 290):79-90.

DEAKIN J.F.W., OWEN F., CROSS A.J., *et al.*: (1981) *Psychopharmacology (Berlin)*, **73**:345-349.

DEGUCHI T.: (1973) *Mol. Pharmacol.*, **9**:184-190.

DEGUCHI T., AXELROD J.: (1972a) *Anal. Biochem.*, **50**:174-179.

DEGUCHI T., AXELROD J.: (1972b) *Proc Natl. Acad. Sci. USA*, **69**:2547-2550.

DEGUCHI T., BARCHAS J.: (1972) *Nature*, **235**:92-93.

DELINI-STULA A., VASSOUT A.: (1979) *Eur. J. Pharmacol.*, **58**:443-451.

DELINI-STULA A., HAUSER K., BAUMANN P., *et al.*: (1982) In *Typical and Atypical Antidepressants: Molecular Mechanisms* (E. Costa, G. Racagni eds.) Raven Press, New York. (Advances in Biochemical Psychopharmacology, Vol 31, pp 265-275).

DESSAIN E.C., SCHATZBERG A.F., WOOD B.T., *et al.*: (1986) *Arch. Gen. Psychiatry*, **43**:86-90.

DEVI S.P., RAO A.V., HARIHARASUBRAMINIAN N., *et al.*: (1984) *J. Steroid.Biochem.*, **20**:1477.

DICK P., FERRERO E.: (1983) *Br. J. Clin. Pharmacol.*, **15**(Suppl 3):419-425.

DICKENSON K.E.J., LEEB-LUNDBERG L.M.F., STRASSER R.H., *et al.*: (1986) *J.Neurochem.*, **46**:1153-1160.

DIFFLEY D., TRAN V.T., SNYDER S.H.: (1980) *Eur. J. Pharmacol.*, **64**:177-181.

DOOGAN D.P., CAILLARD V.: (1988) *J. Clin. Psychiatry*, **49**(Suppl 8):46-51.

DOXEY J.C., HEWLETT D.R., ROACH A.G.: (1981) In *Proceedings of the British Pharmacological Society*, pp 262P-263P.

DUMONT C., LAURENT J., GRANDADAM A., *et al.*: (1981) *Life Sci.*, **28**:1939-1945.

EASTWOOD M.R., PEACOCKE J.: (1976) *Br. J. Psychiatry*, **129**:472-475.

EBADI M.: (1984) In *The Pineal Gland* (R.J. Reiter ed.) Raven Press, New York, pp 1-37.

EBADI M., CHAN A.: (1980) *Brain Res. Bull.*, **5**(Suppl. 2):179-187.

EBADI M., GOVITRAPONG P.: (1986) *Pineal Res. Rev.*, **4**:1-54.

ELLIS G.B., McKLVEEN R.E., TUREK F.W.: (1982) *Am. J. Physiol*, **242**:R44-R50.

FANN W., DAVIS J., JANOWSKY D., KAUFMANN J., *et al.*: (1972) *Arch. Gen. Psychiatry*, **26**:158-162.

FEIGHNER J.P.: (1985) *J. Clin. Psychiatry*, **46**:369-372.

FEIGHNER J.P., COHN J.B.: (1985) *J. Clin. Psychiatry*, **46**:20-25.

FERRIS R.M., MAXWELL R.A., COOPER B.R., *et al.*: (1982) In *Typical and Atypical Antidepressants: Molecular Mechanisms* (E. Costa, G. Racagni eds.) Raven Press, New York. (Advances in Biochemical Psychopharmacology, Vol 31, pp 277-286).

FIELDS E.D.: (1982) *Drug Intell. Clin. Pharm.*, **16**:547-551.

FIGGE J., LEONARD P., RICHELSON E.: (1979) *Eur. J. Pharmacol.*, **58**:479-483.

FISCH C.: (1985) *J. Clin. Psychiatry*, **46**:42-44.

FOLDES A., HOSKINSON R.M., SCARAMUZZI R.J., *et al.*:(1983) *Neuroendocrinology*, **37**:378-385.

FRAZER A., HESS M.E., MENDELS J., *et al.*: (1978) *J. Pharmacol. Exp. Ther.*, **206**:311-319.

FRAZER A., ORDWAY G., O'DONNELL J., *et al.*: (1986a) In *Antidepressants and Receptor Function* (Ciba Foundation Symposium 123) Wiley, Chichester, pp 170-190.

FRAZER A., BROWN R., KOCSIS J.H., *et al.*: (1986b) *J. Neural Transm.*, **21**(Suppl):269-290.

FRIEDMAN E., YOCCA F.D.: (1981) *J. Pharmacol. Exp. Ther.*, **219**:121-124.

FRIEDMAN E., YOCCA F.D., COOPER T.B.: (1984) *J. Pharmacol. Exp. Ther.*, **228**:545-549.

FROST S.J., ECCLESTON D., MARSHALL E.F., *et al.*: (1983) *Curr. Ther. Res.*, **34**:239-252.

FULLER R.W.: (1987) *J. Clin. Psychiatry*, **48**(Suppl 3):5-11.

GALLAGER D.W., BUNNEY W.E.: (1979) *Naunyn Schmiedebergs Arch. Pharmacol.*, **307**:129-133.

GARCIA-SEVILLA J.A., ZIS A.P., HOLLINGSWORTH P.J., *et al.*: (1981) *Arch. Gen. Psychiatry*, **38**:1327.

GEORGOTAS A., MANN J., BUSH D., *et al.*: (1981) *Acta Psychiatr. Scand.*, **63**(Suppl 290):257-261.

GERSHON E.S., HAMOVIT J., GUROFF J.J., *et al.*: (1982) *Arch. Gen. Psychiatry*, **39**:1157-1167.

GILMAN A.G.: (1970) *Proc. Natl. Acad. Sci. USA*, **67**:305-312.

GLADSTONE R.J., WAKELY C.P.G.: (1940) In *The Pineal Gland*, Balliere, Tindall and Cox, London.

GLASS I.B., CHECKLEY S.A., SHUR E., *et al.*: (1982) *Br. J. Psychiatry*, **141**:372-376.

GOLDEN R.N., DE VANE L., LAIZURE S.C., *et al.*: (1988a) *Arch. Gen. Psychiatry*, **45**:145-149.

GOLDEN R.N., MARKEY S.P., RISBY E.D., *et al.*: (1988b) *Arch Gen Psychiatry*, **45**:150-154.

GOTTLIEB P., WANDALL T., OVERO K.F.: (1980) *Acta Psychiatr. Scand.*, **62**:236-244.

GOVITRAPONG P., EBADI M.: (1986) *Endocrinol. Res.*, **12**:293-304.

GOVITRAPONG P., MURRIM L.C., EBADI M.: (1984) *J. Pineal Res.*, **1**:215-216.

GOVITRAPONG P., EBADI M., MURRIM L.C.: (1986) *J. Pineal Res.*, **3**:223-235.

GOVITRAPONG P., PHANSUWAN-PUJITO P., EBADI M.: (1989) In *Advances in Pineal Research, Vol 3* (R.J. Reiter, S.F. Pang eds.) John Libbey, London, pp 123-126.

GREEN A.R., NUTT D.J.: (1985) In *Psychopharmacology, Vol 2* (D.G. Grahame-Smith, P.J. Cowen eds.) Elsevier, Amsterdam, pp 1-34.

GREEN A.R., HEAL D.J., GRAHAME-SMITH D.G.: (1977) *Psychopharmacology (Berlin)*, **52**:195-200.

GREEN J.P., MAAYANI S.: (1977) *Nature*, **269**:163-165.

GREENBERG L., WEISS B.: (1978) *Science*, **201**:61-63.

GUELFI J.D., DREYFUS J.F., PICHOT P.: (1983) *Br. J. Clin. Pharmacol.*, **15**:4115.

GULATI A., NATH C., DHAWAN K.N., *et al.*: (1982) *Brain Res.*, **240**:357-358.

HAAS H.L.,: (1979) *Agents Actions*, **9**:83-84.

HALBERG F.: (1967) In *Symposium Bel-Air III, Cycles Biologiques et Psychiatrie* (J. DeAjuriaguerra ed.) Masson and Co., Paris, pp 73-126.

HALL H., ÖGREN S.O.: (1981) *Eur. J. Pharmacol.*, **70**:393-407.

HALL H., SÄLLEMARK M., ROSS S.B.: (1980) *Acta. Pharmacol. Toxicol. (Copenh)*, **47**:159-160.

HARIHARASUBRAMANIAN N., NAIR N.P.V., PILAPIL C.: (1985) Abstract, Proceedings of the British Society for Chronobiology.

HERMANN H.E., KAPLAN L.M., BIERES B.E.: (1983) *Am. J. Cardiol.*, **51**:904-906.

HERTEL C., STAEHELIN M.: (1983) *J. Cell Biol.*, **97**:1538-1543.

HEURING R.E., PEROUTKA S.J.: (1987) *Neuroscience*, **7**:894-903.

HEYDORN W.E., MENDELS J., FRAZER A.: (1980) *Biochem. Pharmacol.*, **29**:1341-1346.

HEYDORN W.E., BRUNSWICK D.J., FRAZER A.: (1982) *J. Pharmacol. Exp. Ther.*, **222**:534-543.

HEYM J., KOE B.K.: (1988) *J. Clin. Psychiatry*, **49**(Suppl 8):40-45.

HIL T.M., SHRIVASTAVA R.K., MUKHERJEE S., *et al.*: (1983) *Br. J. Clin. Pharmacol.*, **15**:4335.

HIRAMATSU K.I., TAKAHASHI R., MOR A., *et al.*: (1983) *Acta Psychiatr. Scand.*, **68**(Suppl 308):41-54.

HIRATA F., HAYAISHI O., TOKUYAMA T., *et al.*: (1974) *J. Biol. Chem.*, **249**:1611.

HO A.K., KLEIN D.C.: (1987) *J. Biol. Chem.*, **262**:11764-11770.

HODDE K.C.: (1979) *Prog. Brain Res.*, **52**:39-44.

HOLLISTER L.E., CSERNANSKY J.G.: (1990) In *Clinical Pharmacology of Psychotherapeutic Drugs* (L.E. Hollister, J.G. Csernansky eds.) Churchill Livingstone, New York, pp 59-96.

HYTTEL J., LARSEN J.J.: (1985) *Acta Pharmacol. et Toxicol.*, **56**:146-153.

JANCSAR S.M., LEONARD B.E.: (1983) *Biochem. Pharmacol.*, **32**:1569-1571.

JANOWSKY D.S., EL-YOUSEF M.K., DAVIS J.M., *et al.*: (1972) *Lancet*, **2**:632-635.

JANOWSKY D.S., EL-YOUSEF M.K., DAVIS J.M., *et al.*: (1973) *Arch. Gen. Psychiatry*, **28**:542-547.

JANOWSKY A., OKADA F., MANIER D., *et al.*: (1982) *Science*, **218**:900-901.

JIMERSON D.C., LYNCH H.J., POST R.M., *et al.*: (1977) *Life Sci.*, **20**:1501-1508.

JOHNSON D.A.W.: (1979) *Acta Psychiatr. Scand*, **59**:1-8.

JOHNSON D.A.W.: (1981) *Acta Psychiatr. Scand.*, **63**:(Suppl 290):447-453.

JOHNSON R.W., REISINE T., SPOTNITZ S., *et al.*: (1980) *Eur. J. Pharmacol.*, **67**:123-127.

JONES R.L., Mc GEER P.L., GREINER A.C.: (1969) *Clin. Chim. Acta.*, **26**:281-285.

JUDD F.K., NORMAN T.R., BURROWS G.D.: (1983) *Br. Med. J.*, **287**:1592-1593.

JUE S.G., DAWSON G.W., BROGDEN R.N.: (1982) *Drugs*, **21**:1-23.

KABUTO M., NAMURA I., SAITOH.: (1986) *Endocrinology Japan*, **33**:405-414.

KAFKA M.S., VAN KAMMEN D.P., KLEINMAN J.E., *et al.*: (1980) *Psychopharmacol. Commun.*, **4**:477.

KAKU K., TSUCHIYA M., MATSUDA M., *et al.*: (1985) *Endocrinology*, **117**:2371-2375.

KAKU K., TSUCHIYA M., TAHIZAWA Y., *et al.*: (1986) *Peptides*, **7**:193-196.

KANOF P.D., GREENGARD P.: (1978) *Nature*, **272**:329-333.

KAPPERS J.A.: (1965) *Prog. Brain Res.*, **10**:87-153.

KATZ R.J., SCHMALTZ K.: (1980) *Neurosci. Lett.*, **19**:85-88.

KEBABIAN J.W., ZATZ M., ROMERO J.A., *et al.*: (1975) *Proc. Natl. Acad. Sci. USA*, **72**:3735-3739.

KELLAR K.J., CASCIO C.S., BUTLER J.A., *et al.*: (1981) *Eur. J. Pharmacol.*, **69**:515-518.

KENDALL D.A., NAHORSKI S.R.: (1985) *J. Pharmacol. Exp. Ther.*, **233**:473-479.

KILOH L.G., BALL J.R.B., GARSIDE R.F.: (1962) *Br. Med. J.*, **1**:1225-1227.

KLEIN D.C.: (1969) *Fed. Proc.*, **28**:734.

KLEIN D.C., NOTIDES A.: (1969) *Anal. Biochem.*, **31**:480-483.

KLEIN D.C., BERG G.R.: (1970) *Adv. Biochem. Psychopharmacol.*, **3**:241.

KLEIN D.C., ROWE J.: (1970) *Mol. Pharmacol.*, **6**:164-171.

KLEIN D.C., WELLER J.L.: (1970) *Science*, **169**:1093-1095.

KLEIN D.C., WELLER J.L.: (1973) *J. Pharmacol. Exp. Ther.*, **186**:516.

KLEIN D.C., WELLER J.L., MOORE R.Y.: (1971) *Proc. Natl. Acad. Sci. USA*, **68**:3107-3110.

KLEIN D.C., SMOOT R., WELLER J.L. *et al.*: (1983a) *Brain Res. Bull.*, **10**:647-652.

KLEIN D.C., SUGDEN D., WELLER J.L.: (1983b) *Proc. Natl. Acad. Sci. USA*, **80**:599-603.

KOE B.K., WEISSMAN A., WELCH W.M., *et al.*: (1983) *J. Pharmacol. Exp. Ther.*, **226**:686-700.

KOE B.K., KOCH S.W., LEBEL L.A., *et al.*: (1987) *Eur. J. Pharmacol.*, **141**:187-194.

KOIDE T., MATSUSHITA H.: (1981) *Life Sci.*, **28**:1139-1145.

KORF H.-W., MOLLER M.: (1984) *Pineal Res. Rev.*, **2**:41-87.

KRAMER B.A., KATZ J.L.: (1978) *J. Clin. Psychol.*, **39**:343-344.

KRIPKE D.F., MULLANEY D.J., ATKINSON M., *et al.*: (1978) *Biol. Psychol.*, **13**:335-351.

KRISHNA G., WEISS B., BRODIE B.B.: (1968) *J. Pharmacol. Exp. Ther.*, **163**:379-385.

KUHN R.: (1958) *Am. J. Psychiatry*, **115**:459-464.

KUPFER D.J.: (1976) *Biol. Psychiatry*, **2**:159-174.

KUPFER D.J., WEISS B.L., FOSTER G.: (1974) *Am. J. Psychiatry*, **30**:765-768.

KVEDER S., McISAAC W.M.: (1961) *J. Biol. Chem.*, **236**:3214-3220.

LAITINEN J.T., TORDA T., SAAVEDRA J.M.: (1989) *Biochem. Biophys. Res. Commun.*, **164**:645-652.

LANG V.: (1986) *Pineal Res. Rev.*, **4**:199-244.

LANGER S.Z., RAISMAN R., BRILEY M.S.: (1981) *Eur. J. Pharmacol.*, **72**:423-424.

LANGER S.Z., SETTE M., RAISMAN R.: (1983) In *Clinical Pharmacology in Psychiatry: Bridging the Experimental-Therapeutic Gap* (L.F. Gram *et al.* eds.) Macmillan, London, pp 339-348.

LANGER S.Z., RAISMAN R., TAHRAOUI L., *et al.*: (1984a) *Eur. J. Pharmacol.*, **98**:153-154.

LANGER S.Z., RAISMAN R., SECHTER D., *et al.*: (1984b) In *Frontiers in Biochemical and Pharmacological Research in Depression* (E. Usdin *et al.* eds.) Raven Press, New York, pp 113-125.

LANGER S.Z., LEE C.R., SCHOEMAKER H., *et al.*: (1985) In *Endocoids* (H. Lal *et al.* eds.) Alan R. Liss, New York, pp 441-455.

LANGER S.Z., GALZIN A.M., LEE C.R., *et al.*: (1986) In *Antidepressants and Receptor Function* (Ciba Foundation 123) Wiley, Chichester, pp 3-17.

LAPIERRE Y.D., ANDERSON K.: (1983) *Am. J. Psychiatry*, **140**:493-494.

LAPIN I.P., OXENKRUG G.F.: (1969) *Lancet*, **1**:132-136.

LARSEN J.K., HOLM P., MIKKELSEN P.L.: (1984) *Acta Psychiatr. Scand.*, **70**:254-260.

LE DONNE N.C., COFFEE C.J.: (1979) *J. Biol. Chem.*, **254**:4317-4320.

LECRUBIER Y., PUECH A.J., FRANCIS H., *et al.*: (1981) *Acta Psychiatr. Scand*, **63**(Suppl 290):173-178.

LEE T., TANG S.W.: (1982) *Psychiatry Res.*, 7:111-119.

LEFKOWITZ R.J., MUKHERJEE C., COVERSTONE M., *et al.*: (1974) *Biophys. Res. Commun.*, **60**:703-709.

LEMBERGER L., FULLER R.W., ZERBE R.L.: (1985) *Clin Neuropharmacol.*, **8**:299-317.

LEONARD B.E.: (1978) *Acta Psychiatr. Belg.*, **78**:770-780.

LEONARD B.E.: (1980) *Neuropharmacology*, **19**:1175-1183.

LEONARD B.E.: (1984) *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **8**:97-108.

LEONARD B.E.: (1988) *J. Clin. Psychiatry*, **49**(Suppl 8):12-17.

LERCHL A., KÜDERLING I.: (1989) In *Advances in Pineal Research* (R.J. Reiter, S.F. Pang eds.) John Libbey, London, pp 271-276.

LEVINE S., DEO R., MAHADEVAN K.: (1987) *Br. J. Clin. Psychiatry*, **150**:653-655.

LEVITZKI A., ATLAS D., STEER M.L.: (1974) *Proc. Natl. Acad. Sci. USA*, **71**:2773-2776.

LEWIS D.A., Mc CHESNEY C.: (1985) *Arch. Gen. Psychiatry*, **42**:485-488.

LEWY A.J., MARKEY S.P.: (1978) *Science*, **210**:741-743.

LEWY A.J., WEHR T.A., GOLD P.W., *et al.*: (1979) In *Catecholamines: Basic and Clinical Frontiers* (E. Usdin, I.J. Kopin, J. Barchas eds.) Pergamon Press, Oxford, pp 1173-1175.

LIEBOWITZ M.R., QUITKIN F.M., STEWART J.W., *et al.*: (1985) *Psychopharmacol. Bull.* **21**:558-561.

LIEBOWITZ M.R., QUITKIN F.M., STEWART J.W., *et al.*: (1988) *Arch. Gen. Psychiatry*, **45**:129-137.

LOUDON J.B., TIPLADY B., ASHCROFT G.W., *et al.*: (1981) *Acta Psychiatr. Scand.*, **63**(Suppl 290):454-463.

LOWENSTEIN P.R., CARDINALI D.P.: (1983) *Neuroendocrinology*, **37**:150-154.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L., *et al.*: (1951) *J. Biol. Chem.*, **193**:265-275.

LUCKI I., FRAZER A.: (1982) *Psychopharmacology*, **77**:205-211.

LYNCH H.J.: (1971) *Life Sci*, **10**:791-795.

LYNCH H.J., DENG M.H.: (1986) *J. Neural Transm.*, **21**(Suppl):461-474.

LYNCH T.S., CHEUNG W.Y.: (1975) *Anal. Biochem.*, **67**:130-138.

MAAYANI S., HOUGH L.B., WEINSTEIN H., *et al.*: (1982) In *Typical and Atypical Antidepressants: Molecular Mechanisms* (E. Costa, G. Racagni eds.) Raven Press, New York. (Advances in Biochemical Psychopharmacology, Vol 31, pp 133-147).

MAGGI A., U'PRICHARD D.C., ENNA S.J.: (1980) *Eur. J. Pharmacol.*, **61**:91-98.

MAITRE L., STAELHELIN M., BEIN H.J.: (1971) *Biochem Pharmacol.*, **20**:2169-2186.

MAJ J., PALIDER W., RAWLOW A.: (1979a) *J. Neural Transm.*, **44**:237-248.

MAJ J., MOGILNICKA E., KLIMEK V.: (1979b) *J. Neural Transm.*, **44**:221-235.

MAJ J., MOGILNICKA E., KLIMEK V., *et al.*: (1981) *J. Neural Transm.*, **52**:189-197.

MAJ J., MELZACKA M., MOGILNICKA E., *et al.*: (1982a) *J. Neural Transm.*, **54**:219-228.

MAJ J., PRZEGALINSKI E., MOGILNICKA E.: (1984) *Rev. Physiol. Biochem. Pharmacol.*, **100**:1-74.

MANIER D.H., GILLESPIE D.D., STERANKA L.R., *et al.*: (1984) *Experientia*, **40**:1223-1226.

MANN J.J., AARONS S.F., WILNER P.J., *et al.*: (1989) *Arch. Gen. Psychiatry*, **46**:45-50.

MASSINGHAM R., DUBOCOVICH M.L., SHEPPERSON N.B., *et al.*: (1981) *J. Pharmacol. Exp. Ther.*, **217**:467-474.

MATTHEW E., PARFITT A.G., SUGDEN D., *et al.*: (1984) *J. Pharmacol. Exp. Ther.*, **228**:434-438.

McGRATH P.J., QUITKIN F.M., HARRISON W.M., *et al.*: (1984) *Am. J. Psychiatry*, **141**:288-289.

McINTYRE I.M., McCAULEY R., MURPHY S., *et al.*: (1985) *Biochem. Pharmacol.*, **34**:3393-3394.

McMILLEN B.A., WARNACK W., GERMAN D.C., *et al.*: (1980) *Eur. J. Pharmacol.*, **61**:239-246.

MEFFORD I.N., CHANG P., KLEIN D.C., *et al.*: (1983) *Endocrinology*, **113**:1582-1586.

MENDELS J.: (1987) *J. Clin. Psychiatry*, **48**(Suppl 3):26-30.

MENDELS J., STERN S., FRAZER A.: (1976) In *Depression: Behavioral, Biochemical, Diagnostic and Treatment Concepts* (D.M. Gallant, G.M. Simpson eds.) Spectrum Publications, New York, pp 19-74.

MENDLEWICZ J., LINKOWSKI P., BRANCHEY L., *et al.*: (1979) *Lancet*, **2**:1362.

MENKES D.B., AGHAJANIAN G.K., McCALL R.B.: (1980) *Life Sci.*, **27**:45-55.

MENKES D.B., AGHAJANIAN G.K.: (1981) *Eur. J. Pharmacol.*, **74**:27-35.

MERIDETH C.H., FEIGHNER J.P.: (1983) *Acta Psychiatr. Scand.*, **68**(Suppl 308):70-79.

MHATRE M.C., VAN JAARSVELD A., REITER R.J.: (1988) *Biochem. Biophys. Res. Commun.*, **153**:1186-1192.

MICKEY J.V., TATE R., LEFKOWITZ R.J.: (1975) *J. Biol. Chem.*, **250**:5727-29.

MINNEMAN K.P.: (1977) *Mol. Pharmacol.*, **13**:735-745.

MINNEMAN K.P., IVERSEN L.L.: (1976) *Nature*, **260**:59-61.

MINNEMAN K.P., DIBNER M.D., WOLFE B.B., *et al.*: (1979) *Science*, **204**:866-868.

MISHRA R., JANOWSKY A., SULSER F.: (1980) *Neuropharmacology*, **19**:983-987.

MISHRA R., LEITH N.J., STERANKA L., *et al.*: (1981) *Naunyn Schmiedebergs Arch. Pharmacol.*, **316**:218-224.

MOBLEY P.L., SULSER F.: (1979) *Eur. J. Pharmacol.*, **60**:221-227.

MOLLER M., KORF H.-W.: (1987) *Cell Tiss. Res.*, **247**:145-153.

MOLLER M., MIKKELSEN J.D.: (1989) In *Advances in Pineal Research, Vol 3* (R.J. Reiter, S.F. Pang eds.) John Libbey, London, pp 1-10.

MOLLER M., REUSS S., OLCESE J., *et al.*: (1987) *Experientia*, **43**:186-187.

MONTGOMERY S.A., McAULEY R., RANI S.J., *et al.*: (1981a) *Acta Psychiatr. Scand.*, **63**(Suppl 290):314-327.

MONTGOMERY S.A., RANI S.J., McAULEY R., *et al.*: (1981b) *Acta Psychiatr. Scand.*, **63**(Suppl 290):219-224.

MOORE R.Y.: (1982) *Trends Neurosci.*, **5**:404-406.

MORGAN P.J., WILLIAMS L.M.: (1989) *Experientia*, **45**:946.

MORRIS J.B., BECK A.T.: (1974) *Arch. Gen. Psychiatry*, **30**:667-674.

MOYER J.A., GREENBERG L.H., FRAZER A., *et al.*: (1979) *Life Sci*, **24**:2237-2244.

MOYER J.A., GREENBERG L.H., FRAZER A., *et al.*: (1981) *Mol. Pharmacol.*,**19**:187-193.

MUKHERJEE C., CARON M.G., COVERSTONE M., *et al.*: (1975) *J. Biol. Chem.*, **250**:4869-4876.

MURAKAMI N., HAYAFUJI C., SASAKI Y., *et al.*: (1983) *Neuroendocrinology*, **36**:385-391.

MURPHY D.L., TAMARKIN L., SUNDERLAND T., *et al.*: (1986a) *Psychiatry Res.*, **17**:119-127.

MURPHY D.L., GARRICK N.A., TAMARKIN L., *et al.*: (1986b) *J. Neural Transm.*, **21**(Suppl): 291-309.

MURPHY D.L., AULAKHS C.S., GARRICK N.A., *et al.*: (1987a) In *Psychopharmacology: The Third Generation of Progress* (H.Y. Meltzer ed.) Raven Press, New York, pp 545-552.

MURPHY D.L., GARRICK N.A., HILL J.L., *et al.*: (1987b) *Psychopharmacology (Berlin)*, **92**:382-387.

NAGY A.: (1977) *J. Pharm. Pharmacol.*, **29**:104-107.

NAIR N.V.P., SCHWARTZ G.: (1982) *Curr. Ther. Res.*, **31**:969.

NAIR N.V.P., HARIHARASUBRAMANIAN N., PILAPIL C.: (1984) *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **8**:715-718.

NAKAI C., BROOKER G.: (1975) *Biochem. Biophys. Acta.*, **391**:222-239.

NIES A., ROBINSON D.S.: (1982) In *Handbook of Affective Disorders* (E.S Paykel ed.) Churchill Livingstone, Edinburgh, pp 246-261.

NILSSON B.S.: (1983) *Acta. Psychiatr. Scand.*, **68**(Suppl 308):115-119.

NIMGAONKAR V.L., GOODWIN G.M., DAVIES C.L., *et al.*: (1985) *Neuropharmacology*, **24**:279-283.

NIR I., HIRSCHMANN N.: (1983) *Biochem. Pharmacol.*, **32**:2139-2141.

NOLEN W.A.: (1989) *Prog. Neuropsychopharmacol. Biol. Psychiatr.*, **13**:155-158.

OFSTI E.: (1982) *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **6**:327-335.

OLESHANSKY M.A., NEFF N.H.: (1975) *Mol. Pharmacol.*, **11**:552-557.

OLIANAS M., OLIVER A.P., NEFF N.H.: (1982) In *Typical and Atypical Antidepressants: Molecular Mechanisms* (E. Costa, G. Racagni eds.) Raven Press, New York (Advances in biochemical psychopharmacology, Vol 31, pp 149-156).

OLIVIERI G., WELMAN A., DAYA S.: (1990) *Med. Sci. Res.*, **18**:99-100.

OXENKRUG G.F, McCAULEY R., McINTYRE I.M., *et al.*: (1985) *J. Neural Transm.*, **61**:265-270.

PANG S.F., ALLEN A.E.: (1986) *Pineal Res. Rev.*, **4**:55-95.

PARE C.M.B.: (1985) *Br. J. Psychiatry*, **146**:576-584.

PARFITT A., KLEIN D.C.: (1976) *Endocrinology*, **99**:840-851.

PARFITT A., KLEIN D.C.: (1977) *Biochem. Pharmacol.*, **26**:904-905.

PARFITT A., WELLER J.L., KLEIN D.C.: (1976) *Neuropharmacology*, **15**:353-358.

PAUL S.M., CREWS F.T.: (1980) *Eur. J. pharmacol.*, **62**:349-350.

PEET M., BEHAGEL H.: (1977) *Br. J. Clin. Pharmacol.*, **5**(Suppl):5-9.

PELAYO F., DUBOCOVICH M.L., LANGER S.Z.: (1977) *Eur. J. Pharmacol.*, **45**:317-318.

PEROUTKA S.J., SNYDER S.H.: (1980) *Science*, **210**:88-90.

PERRY E.K., MARSHALL E.F., BLESSED G., *et al.*: (1983) *Br. J. Psychiatry*, **142**:188-192.

PETROVIC S., McDONALD J.K., SNYDER G.D., *et al.*: (1983) *Brain Res.*, **261**:249-259.

PÉVET P., BALEMANS M.G.M., LEGERSTEE W.C., *et al.*: (1980) *J. Neural Transm.*, **19**:229.

PFEFFER R.I., MOSIMANN W.F., WEINER N.: (1975) *J. Pharmacol. Exp. Ther.*, **193**:533-548.

PIERPAOLI W., MAESTRONI G.J.M.: (1987) *Immunol. Lett.*, **16**:355-362.

PILLAY M., POTGIETER B.: (1984) In *Neuronal Communications* (B. Meyer, S. Kramer eds.) A.A. Balkema, Cape Town.

PINDER R.M., BROGDEN R.N., SPEIGHT T.M., *et al.*: (1977a) *Drugs*, **13**:401-421.

PINDER R.M., BROGDEN R.N., SPEIGHT T.M., *et al.*: (1977b) *Drugs*, **13**:321-352.

PLAZNIK A., DANYSZ W., KOSTOWSKI W.: (1984) *Eur. J. Pharmacol.*, **101**:305-306.

POTTAGE A., GROSCHINSKI-GRIND M.: (1983) *Acta. Psychiatr. Scand.*, **68**(Suppl 308):125-130.

PUCHALSKI W., LYNCH G.R.: (1988) *Neuroendocrinology*, **48**:280-286.

QUITKIN F.M., RIFKIN A., KLEIN D.F.: (1979) *Arch. Gen. Psychiatry*, **36**:749-760.

QUITKIN F.M., RABKIN J.G., ROSS D., *et al.*: (1984a) *Arch. Gen. Psychiatry*, **41**:782-786.

QUITKIN F.M., LIEBOWITZ M.R., STEWART J.W., *et al.*: (1984b) *Arch. Gen. Psychiatry*, **36**:777-781.

RAISMAN R., BRILEY M., LANGER S.Z.: (1979) *Nature*, **281**:148-150.

RAISMAN R., SETTE M., PIMOULE C., *et al.*: (1982) *Eur. J. Pharmacol.*, **78**:345-351.

RANDRUP A., BRAESTRUP C.: (1977) *Psychopharmacology*, **53**:309-314.

RASKIN A.: (1972) *J. Clin. Pharmacol.*, **12**:22-25.

REHAVI M., RAMOT O., YAVETZ B., *et al.*: (1980) *Brain Res.*, **194**:443-453.

REISINE T., SOUBRIE P.: (1982) *Eur. J. Pharmacol.*, **77**:39-44.

REISINE T.D., U'PRICHARD D.C., WIECH N.L., *et al.*: (1980) *Brain Res.*, **188**:587-592.

REISINE T.D., JOHNSON R., WIECH N.L, *et al.*: (1982) In *Typical and Atypical Antidepressants: Molecular Mechanisms* (E. Costa, G. Racagni eds.) Raven Press, New York (Advances in biochemical psychopharmacology, Vol 31, pp 63-67).

REITER R.J.: (1980) *Endocrin. Rev.*, **1**:109-131.

REITER R.J.: (1987) *Life Sci.*, **46**:2119-2131.

REPPERT S.M., WEAVER D.R.: (1988) *Science*, **242**:78-81.

REUSS S., MOLLER M.: (1986) *Cell Tiss. Res.*, **244**:691-694.

REUSS S., SCHRÖDER H.: (1987) *Neurosci. Lett.*, **74**:158-162.

RICHELSON E., NELSON A.: (1984) *J. Pharmacol. Exp. Ther.*, **230**:94-102.

RICKELS K., WEISE C.C., CSANALOSI I., *et al.*: (1974) *Psychopharmacologia*, **34**:361-376.

ROBINSON D.S, KAYSER A., CORCELLA J., *et al.*: (1985) *Psychopharmacol. Bull.*, **21**:562-567.

ROLLAG M.D., NISWENDER G.D.: (1976) *Endocrinology*, **93**:482-489.

ROMERO J.A., ZATZ M., KEBABIAN J.W.,*et al.*: (1975) *Nature*, **258**:435-436.

ROOS J.C.: (1983) *Br. J. Clin. Pharmacol.*, **15**:439S-445S.

ROSENBLATT J.E., PERT C.B., TALLMAN J.F, *et al.*: (1979) *Brain Res.*, **160**:186-191.

ROSENTHAL N.E., SACK D.A., GILLIN J.C., *et al.*: (1984) *Arch. Gen. Psychiatry*, **41**:72-79.

ROSENTHAL N.E., SACK D.A., JACOBSEN F.M., *et al.*: (1986) *J. Neural Transm.*, **21**(Suppl):257-267.

ROSLOFF B.N., DAVIS J.M.: (1974) *Psychopharmacology (Berlin)*, **40**:53-64.

ROSS S.B., RENYI A.L.: (1977) *Neuropharmacology*, **16**:57-63.

ROSS S.B., HALL H., RENYI A.L., *et al.*: (1981) *Psychopharmacology*, **72**:219-225.

SAAVEDRA J.M., BROWNSTEIN M., AXELROD J.: (1973) *J. Pharmacol. Exp. Ther.*, **186**:508-515.

SACH R.L., LEWY A.J., HOBAN T.M.: (1987) In *Temporal Disorder in Human Oscillatory Systems* (L. Rensing, U. van der Heiden, M.C. Mackey eds.) Springer-Verlag, Heidelberg, pp 219-224.

SACHAR E.J.: (1976) *Annu. Rev. Med.*, **27**:389-396.

SACK R.L., LEWY A.J.: (1986) *Biol. Psychiatry*, **21**:406-410.

SCHILDKRAUT J.J.: (1965) *Am. J. Psychiatry*, **122**:509-522.

SCHIWY W., DELINI-STULA A., HEATH W.R.: (1988) *Psychopharmacology (Berlin)*, (Suppl.), 31-TU 18.04.

SCHMIDT M.J., FULLER R.W., WONG D.T.: (1988) *Br. J. Psychiatry*, **153**(Suppl 3):40-46.

SCHOERLIN M.-P., MAYERSOHN M., KORN A., *et al.*: (1987) *Clin. Pharmacol. Ther.*, **42**:395-404.

SCHULTZ J.: (1976) *Nature*, **261**:417-418.

SCHWARTZ J.C., GARBERG M., QUACH T.T.: (1981) *TIPS*, **2**:122-125.

SCHWEITZER J.W., SCHWARTZ R., FRIEDHOFF A.J.: (1979) *J. Neurochem.*, **33**:377-379.

SCUVÉE-MOREAU J.J., SVENSSON T.H.: (1982) *J. Neural Transm.*, **54**:51-63.

SEGGIE J., WERSTIUK E., GROTA L., *et al.*: (1983) *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **7**:827-830.

SEGGIE J., WERSTIUK E., GROTA L., *et al.*: (1984) *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **8**:733-736.

SEGONZAC A., RAISMAN R., TATEISHI T., *et al.*: (1985) *J. Neurochem.*, **44**:349-356.

SELLINGER-BARNETTE M.M., MENDELS J., FRAZER A.: (1980) *Neuropharmacology*, **19**:447-454.

SEMM P., SCHNEIDER T., VOLLRATH L.: (1981) *J. Neural Transm.*, **50**:247-266.

SERRA G., ARGIOLAS A., KLIMEK V., *et al.*: (1979) *Life Sci.*, **25**:415-424.

SERRA G., ARGIOLAS A., FADDA F., *et al.*: (1981) *Psychopharmacology (Berlin)*, **73**:194-196.

SETTE M., BRILEY M.S., LANGER S.Z.: (1983) *J. Neurochem.*, **40**:622-628.

SHEIN H.M., WURTMAN R.J.: (1969) *Science*, **166**:519-520.

SHEPARD M., LADER M., RODNIGHT R.: (1968) In *Clinical Psychopharmacology* English Universities Press, London.

SHIOTANI Y., YAMANO M., SHIOSAKA S., *et al.*: (1986) *Neurosci. Lett.*, **70**:187-192.

SIEVER L.J., DAVIS K.L.: (1985) *Am. J. Psychiatry*, **142**:1017-1031.

SIEVER L.J., UHDE T.W., MURPHY D.L.: (1982) *Psychiat. Res.*, **6**:293.

SIMON P., LECRUBIER Y., JOUVENT R., *et al.*: (1984) *Adv. Biochem. Psychopharmacol.*, **39**:293-299.

SINGER T.P., SALACH J.: (1981) In *Essays in Neurochemistry and Neuropharmacology, Vol 5* (M.B.H. Youdim, W. Lovenberg, D.F. Sharman, J. Lagnado eds.) Wiley, Chichester, pp 131-153.

SKENE D.J.: (1985) Ph.D. Thesis, MEDUNSA, South Africa.

SMITH A.H.W., NAYLOR G.J., MOODY J.P.: (1978) *Br. J. Clin. Pharmacol.*, **5**:675-705.

SNYDER S.H., YAMAMURA H.: (1977) *Arch. Gen. Psychiatry*, **34**:236-239.

SNYDER S.H., PEROUTKA S.J.: (1982) *Pharmacopsychiatria*, **15**:131-134.

SNYDER S.H., FISCHER J., AXELROD J.: (1965) *Biochem. Pharmacol.*, **14**:363.

SPECTOR S., HIRSH C.W., BRODIE B.B.: (1963) *Int. J. Neuropharmacol.*, **2**:81-93.

SPYRAKI C., FIBIGER H.C.: (1981) *Eur. J. Pharmacol.*, **74**:195-206.

SRINIVASAN V.: (1987) *Neuroendocrinol. Lett.*, **9**:223.

SRINIVASAN V.: (1989) *Prog. Neuro-Psycho Pharmacol. Biol. Psychiatry*, **13**:653-664.

STAEHELIN M., HERTEL C.: (1983) *J. Recept. Res.*, **3**:35-43.

STARK P., HARDISON C.D.: (1985) *J. Clin. Psychiatry*, **46**:53-58.

STETSON M.H., WATSON-WHITMYRE M.: (1986) *Biol. Reprod.*, **34**:664-669.

STOCKMEIER C.A., MARTINO A.M., KELLAR K.J.: (1985) *Science*, **230**:323-325.

STRANGEWAYS T.S.P., FELL H.B.: (1926) *Proc. Royal Soc.*, **99**:340.

SUGDEN D.: (1989) *Experientia*, **45**:922-932.

SUGDEN D., KLEIN D.C.: (1983) *Endocrinology*, **113**:348-353.

SUGDEN D., KLEIN D.C.: (1984) *Endocrinology*, **114**:435-440.

SUGDEN D., KLEIN D.C.: (1988) *J. Neurochem.*, **50**:149-155.

SUGDEN A.L., SUGDEN D., KLEIN D.C.: (1987) *J. Biol. Chem.*, **262**:741-745.

SUGDEN D., HO A.K., SUGDEN A.L., *et al.*: (1988) *Endocrinology*, **123**:1425-1432.

SUGRUE M.F.: (1981a) In *Antidepressants: Neurochemical, Behavioral and Clinical Perspectives* (S.J. Enna, J.B. Malick, E. Richelson eds.) Raven Press, New York, pp 13-30.

SUGRUE M.F.: (1981b) *Pharmacol. Ther.*, **13**:219-247.

SUGRUE M.F.: (1981c) *Life Sci.*, **28**:377-384.

SUGRUE M.F.: (1982a) *Naunyn Schmiedebergs Arch. Pharmacol.*, **320**:90-96.

SUGRUE M.F.: (1982b) In *Typical and Atypical Antidepressants: Molecular Mechanisms* (E. Costa, G. Racagni eds.) Raven Press, New York (Advances in biochemical psychopharmacology, Vol 31, pp 52-62).

SUGRUE M.F.: (1982c) *J. Pharm. Pharmacol.*, **34**:446-449.

SUGRUE M.F.: (1983) *Biochem. Pharmacol*, **32**:1811-1817.

SULSER F.: (1984) *Neuropharmacology*, **23**:255-261.

SULSER F.: (1987) *J. Clin. Psychiatry*, **48**(Suppl 3):12-18.

SULSER F., VETULANI J., MOBLEY P.L.: (1978) *Biochem. Pharmacol.*, **27**:257-261.

SUNDERLAND T., MUELLER E.A., COHEN R.M., *et al.*: (1985) *Psychopharmacology (Berlin)*, **86**:432-437.

SURANYI-CARDOTTE B.E., DAM T.V., QUIRION R.: (1984) *Eur. J. Pharmacol.*, **106**:673-675.

SVENSSON T.H., USDIN T.: (1978) *Science*, **202**:1089-1091.

SVENSSON T.H., USDIN T.: (1979) In *Catecholamines: Basic and Clinical Frontiers* (E. Usdin, J. Kopin, J. Barchas eds.) Pergamon Press, New York, pp 672-674.

TAMARKIN L., BAIRD C.J., ALMEIDA O.F.X.: (1985) *Science*, **227**:714-720.

TAMMINEN T., LEHTINEN V.: (1986) Abstract, CINP Congress, Puerto Rico.

TANG S.W., HELMESTE D.M., STANCER H.C.: (1978) *Naunyn Schmiedebergs Arch. Pharmacol.*, **305**:207-211.

TANG S.W., SEEMAN P., KWAN S.: (1981) *Psychiat. Res.*, **4**:129-138.

THOMPSON W.J., APPLEMAN M.M.: (1971) *Biochemistry*, **10**:311-316.

THOMPSON C., MEZEY G., CORN T.H., *et al.*: (1985) *Br. J. Psychiatry*, **147**:389-393.

THOMPSON C., FRANEY C., ARENDT J., *et al.*: (1988) *Br. J. Psychiatry*, **152**:260-265.

TOEWS M.L., PERKINS J.P.: (1984) *J. Biol. Chem.*, **259**:2227-2235.

TOLLEFSON G.D.: (1983) *J. Clin. Psychiatry*, **44**:280-288.

TRAN V.T., CHANG R.S.L., SNYDER S.H.: (1978) *Proc. Natl. Acad. Sci. USA*, **75**:6290-6294.

TRAN V.T., LEBOVITZ R., TOLL L., *et al.*: (1981) *Eur. J. Pharmacol.*, **70**:501-509.

TROWELL O.A.: (1959) *Exp. Cell Res.*, **16**:118.

TSUCHIYA M., KABU K., MATSUDA M., *et al.*: (1987) *Biomed. Res.*, **8**:45-53.

U'PRICHARD D.C., BECHTEL W.D., ROUST B.M., *et al.*: (1979) *Mol. Pharmacol.*, **15**:47-60.

VACAS M.I., SARMIENTO I.K., CARDINALI D.P.: (1985) *J. Neural Transm.*, **26**:295-304.

VACAS M.I., SARMIENTO I.K., PEREYRA E.N., *et al.*: (1987) *Cell. Mol. Neurobiol.*, **7**:309-315.

VALLEJO J., CASTRO C., CATALAN R., *et al.*: (1987) *Br. J. Psychiatry*, **151**:639-642.

VANECEK J., SUGDEN D.: (1985) *Endocrinology*, **116**:2167-2173.

VANECEK J., PAVLIK A., ILLNEROVA H.: (1987) *Brain Res.*, **435**359-362.

VAN SCHEYEN J.P., VAN PRAAG H.M., KORF J.: (1977) *Br. Clin. Pharmacol.*, **4**(Suppl 2):179-184.

VAUGHAN G.M.: (1984) *Pineal Res. Rev.*, **2**:141-201.

VETULANI J., SULSER F.: (1975) *Nature*, **257**:495-496.

VETULANI J., PILC A.: (1982) *Eur J. Pharmacol.*, **85**:269-275.

VETULANI J., STAWARZ R.J., DINGELL J.V., *et al.*: (1976a) *Naunyn Schmiedebergs Arch. Pharmacol.*, **293**:109-114.

VETULANI J., STAWARZ R.J., SULSER F.: (1976b) *J. Neurochem.*, **27**:661-666.

VETULANI J., LEBRECHT U., PILC A.: (1981) *Eur. J. Pharmacol.*, **76**:81-85.

VETULANI J., ANTKIEWICZ-MICKALUK L., ROKOSZPELC A., *et al.*: (1986) In *Antidepressants and Receptor Function* (Ciba Foundation Symposium 123) Wiley, Chichester, pp 234-245.

VOISIN P., JUILLARD M.T., COLLIN J.P.: (1983) *Cell Tiss. Res.*, **230**:155-169.

WAINWRIGHT S.D.: (1977) *Can. J. Biochem.*, **55**:415-423.

WALDMEIER P.C.: (1982) *J. Pharm. Pharmacol.*, **34**:391-394.

WAMSLEY J.K., BYERLEY W.F., TYLER McCABE R., *et al.*: (1987) *J. Clin. Psychiatry*, **48**(Suppl 3):19-25.

WANG R.Y., AGHAJANIAN G.K.: (1980) *Commun. Psychopharmacol.*, **4**:83-90.

WARTENBERG H., GUSEK W.: (1965) *Prog. Brain Res.*, **10**:296-315.

WEHR T.A., GOODWIN F.K.: (1983) In *Circadian Rhythms in Psychiatry* (T.A. Wehr and F.K. Goodwin eds.) Boxwood Press, California, pp 1-15.

WEINER N.: (1985) In *The Pharmacological Basis of Therapeutics* (A.G. Gilman, L.S. Goodman, T.W. Rall, F. Murad eds.) Seventh Edition, Macmillan, New York, pp 208.

WEISS B.: (1969) *J. Pharmacol. Exp. Ther.*, **168**:146-152.

WEISSMAN B.A., SKOLNICK P., KLEIN D.C.: (1984) *Pharmac. Biochem. Behav.*, **21**:821-824.

WEISSMAN M.M., GERSHON E.S., KIDD K.K., *et al.*: (1984a) *Arch. Gen. Psychiatry*, **41**:13-21.

WENDT G.: (1988) *Psychopharmacology*, **96**(Suppl):13.

WETTERBERG L.: (1983) *Psychoneuroendocrinology*, **8**:75-80.

WETTERBERG L., APERIA B., BECK-FRIIS J., *et al.*: (1982) *Lancet*, **2**:100.

WHITAKER K.J., MANCHESTER E.L., JACOBSON W., *et al.*: (1984) *Brain Res. Bull.*, **12**:215-219.

WILKINSON M., WILKINSON D.A.: (1985) *Neurochem. Res.*, **10**:829-839.

WILKINSON M., JOSHI M., WERSTIUK E.S., *et al.*: (1987) *Biol. Psychiatry*, **22**:1191-1200.

WILLIAMS L.M., MORGAN P.J.: (1988) *J. Endocrinol.*, **119**:R1-R3.

WILNER P.: (1985) *Psychopharmacology*, **85**:387-404.

WIRZ-JUSTICE A., ARENDT J.: (1979) In *Biological Psychiatry Today* (J. Obiols, C. Ballus, E. Gonzalez, J. Pujol eds.) Elsevier, Amsterdam, pp 294-302.

WIRZ-JUSTICE A., ARENDT J.: (1980) *Lancet*, **1**:425.

WIRZ-JUSTICE A., ARENDT J., MARSTON A.: (1980a) *Experientia*, **36**:442-444.

WIRZ-JUSTICE A., KAFKA M.S., NABER D., *et al.*: (1980b) *Life Sci.*, **27**:341-347.

WOLFE B.B., HARDEN T.K., SPORN J.R., *et al.*: (1978) *J. Pharmacol. Exp. Ther.*, **207**:446-457.

WONG D.T., BYMASTER F.P, HORNG J.S., *et al.*: (1975a) *J. Pharmacol. Exp. Ther.*, **193**:804-811.

WONG D.T., HORNG J.S., BYMASTER F.P.: (1975b) *Life Sci.*, **17**:755-760.

WONG D.T., THRELKELD P.G., BEST K.L, *et al.*: (1982) *J. Pharmacol. Exp. Ther.*, **222**:61-65.

WOOD K., COPPEN A.: (1981) *Adv. Biol. Psychiatry*, **7**:85.

WURTMAN R.J., AXELROD J.: (1965) *Sci. Am.*, **213**:50-60.

WURTMAN R.J., AXELROD J., SEVALL G., *et al.*: (1967) *J. Pharmacol. Exp. Ther.*, **157**:487-492.

YOCCA F.D., LYNCH V.P., FRIEDMAN E.: (1983) *J. Pharmacol. Exp. Ther.*, **226**:733-737.

YOUDIM M.B.H., FINBERG J.P.M.: (1985) In *Psychopharmacology 2, Part 1: Preclinical Psychopharmacology* (D.G. Grahame-Smith ed.) Elsevier, Amsterdam, pp 35-70.

YOUNG I.M., LEONE R.M., FRANCIS P., *et al.*: (1985) *J. Clin. Endocrinol. Metab.*, **60**:114-119.

YOUNG J.P.R., COLEMAN A., LADER M.H.: (1987) *Br. J. Psychiatry*, **150**:337-340.

YUWILER A.: (1983) *J. Neurochem.*, **41**:146-153.

ZATZ M., BROWNSTEIN M.J.: (1979) *Brain Res.*, **160**:381-385.

ZATZ M., KEBABIAN J.W., ROMERO J., *et al.*: (1976) *J. Pharmacol. Exp. Ther.*, **196**:714-722.

ZEMLAN F.P., GARVER D.L.: (1980) *Prog. Neuro-Psychopharmacol. Biol Psychiat.*, **14**:503-523.

ZEMLAN F.P., BEHBEHANI M.M., MURPHY R.M.: (1988) In *Progress in Brain Research, Vol 77* (H.L. Fields ed.) Elsevier, New York, pp 349-355.

ZISAPEL N., NIR I., LAUDON M.: (1988) *FEBS*, **232**:172-176.

ZISAPEL N., LAUDON M., NIR I.: (1989) In *Advances in Pineal Research, Vol 3* (R.J. Reiter, S.F. Pang eds.) John Libbey, London, pp 189-194.

PUBLICATIONS ARISING FROM THIS WORK

Publications in Journals

BANOO S., BROWN C., DAYA S., POTGIETER B.: "A 24-hour profile of [^{14}C]serotonin metabolism by organ cultures of rat pineal glands". *Med. Sci. Res.*, 1987, 15:1477.

DAYA S., BANOO S., BROWN C., POTGIETER B.: "A profile of indoleamine metabolism by organ cultures of rat pineal glands over a 24 hour period". In : *Advances in Pineal Research, Vol 3*, 1989 (R.J. Reiter, S.F. Pang, Eds.) John Libbey, London.

Presentations at International Conferences

BANOO S., DAYA S., BROWN C., POTGIETER B.: "A 24-hour time profile of ß-adrenoceprtor-stimulated metabolism of indoleamines *in vitro* in the rat pineal gland". Symposium on Melatonin and the Pineal Gland, Hong Kong, July 1988.

Presentations at Local Conferences

BANOO S., DAYA S., POTGIETER B.: "
The effect of reduced sympathetic input caused by reserpine on rat pineal function". Eastern Cape Biochemical Symposium, Grahamstown, 1987.

BANOO S., BROWN C., DAYA S., POTGIETER B.: "The metabolism of [^{14}C]serotonin by isoprenaline-stimulated pineal glands over a 24-hour period". S.A. Pharmacological Society Annual Congress, Cape Town, 1987.

BANOO S., DAYA S., POTGIETER B.: "Reserpine alters rat pineal ß-adrenoceptor-mediated function". S.A. Academy of Pharmaceutical Sciences Annual Congress, Grahamstown, 1987.

BANOO S., POTGIETER B., DAYA S.: "A possible alternative model for the detection of antidepressive substances in rats". S.A. Pharmacological Society Annual Congress, Port Elizabeth, 1988.