

CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE



National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

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

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

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

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

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

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

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

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

LITHIUM AND CENTRAL MONOAMINERGIC NEUROTRANSMITTER SYSTEMS

by

PARDEEP AHLUWALIA, M.Sc.

A Thesis Presented to
The School of Graduate Studies
of

The University of Ottawa

In Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy
in
Pharmacology

ACKNOWLEDGEMENTS

I first wish to thank Dr. Radhey Singhal, my thesis director, for having given me the opportunity to work in his laboratory, and for his support, encouragement and guidance throughout the course of this investigation.

I also wish to thank all the members of the Department of Pharmacology for the help that they have given to me during my stay in the Department. Appreciation is extended to Shiraz Meilleur for her uncomplaining assistance, and to Diane McNeil for her expert typing of this manuscript.

The financial support received from the Ontario Mental Health Foundation during the course of this work is gratefully acknowledged.

And last, but certainly not least, my eternal thanks to my parents, who have supported and encouraged me throughout the years of my seemingly never-ending education.

CONTENTS

Acknowledgements.....	i
Contents.....	ii
List of Figures.....	v
List of Tables.....	vi
List of Abbreviations.....	viii
Abstract.....	ix

I. LITERATURE REVIEW

A. Introduction.....	1
B. The Biochemical Basis of Affective Disorders.....	2
1. Humoural Theory.....	2
2. Monoaminergic Hypothesis.....	3
C. Lithium.....	5
1. Lithium in Affective Disorders.....	5
2. Clinical Consequences of Lithium Withdrawal.....	12
3. General Methodology in Animal Studies With Lithium.....	15
a) Problems encountered in lithium research.....	15
b) Animal models of mania.....	18
4. Lithium and Biogenic Amine Neurotransmitters.....	20
a) Synthesis and metabolism of the monoaminergic neurotransmitters.....	21
i) Catecholamines.....	21
ii) 5-Hydroxytryptamine.....	28
b) Neurochemical changes due to lithium treatment.....	31
i) Noradrenaline.....	31
ii) Dopamine.....	37
iii) 5-Hydroxytryptamine.....	39
D. Tardive Dyskinesia.....	45
1. Introduction.....	45
2. Pathophysiology and Aetiology of Tardive Dyskinesia.....	46
a) Neuropathological studies.....	46
b) Behavioural studies.....	48

i) Clinical investigations.....	49
ii) Animal studies.....	51
c) Biochemical studies.....	52
3. Lithium and Tardive Dyskinesia.....	60
E. Aims of the Present Study.....	63
II. METHODS	
A. Behavioural Techniques.....	64
1. Gross Locomotor Activity.....	64
2. Monitoring of Stereotypical Behaviour.....	64
B. Biochemical Techniques.....	65
1. Brain Dissection.....	65
2. Determination of Catecholamine Synthetic Systems.....	67
a) Tyrosine hydroxylase activity.....	67
b) Tyrosine content.....	68
3. Measurement of Indoleamine Synthetic Systems.....	68
a) Tryptophan hydroxylase activity.....	69
b) Tryptophan content.....	71
4. Tissue Preparation for Monoamine Estimation.....	72
5. Determination of Tissue Catecholamine Levels.....	73
6. Determination of Tissue Indoleamine Content.....	75
a) 5-Hydroxytryptamine.....	77
b) 5-Hydroxyindoleacetic acid.....	77
7. Measurement of Catecholamine Metabolite Content.....	77
a) 3-Methoxy-4-hydroxyphenylglycol sulphate.....	77
b) Homovanillic acid.....	78
c) 3,4-Dihydroxyphenylacetic acid.....	79
8. Monoamine Uptake into a Synaptosomal Fraction.....	79
9. Measurement of Synaptic Membrane Binding Sites.....	80
a) Preparation of synaptic membranes.....	80
b) Determination of membrane binding sites.....	81
10. Determination of Protein Content.....	82
11. Determination of Lithium Levels.....	82
a) Measurement in serum.....	82
b) Measurement in brain tissue.....	83
C. Animals.....	83

D. Materials.....	83
E. Statistical Analysis.....	84

III. RESULTS

1. Determination of Gross Toxicity Following Chronic Lithium Carbonate Administration.....	85
2. Changes in Spontaneous Locomotor Activity Due to Lithium Administration and Subsequent Withdrawal.....	87
3. The Effect of Lithium Administration and Withdrawal in Various Animal Models of Mania.....	88
4. Consequences of Short-Term Lithium Administration and Subsequent Withdrawal on Central Monoamine Systems.....	92
5. Changes in Parameters of Central Monoamine Function After Treatment With and Withdrawal From Long-Term Lithium.....	103
6. Changes in Monoamine Uptake into Synaptosomes from Various Regions of Rat Brain.....	113
a) Determination of the uptake of monoamines as a function of time..	114
b) Synaptosomal uptake of a single concentration of radiolabelled ligand.....	114
c) Consequences of lithium treatment and withdrawal on synaptosomal monoamine uptake: A kinetic study.....	120
7. The Effect of Lithium Administration and Withdrawal on Binding of Spiroperidol and Dihydroalprenolol to Synaptic Membranes From Various Regions of Rat Brain.....	134
8. Effects of Haloperidol and Lithium on Biochemical Parameters Associated With Central Monoamine Function.....	138

IV. DISCUSSION

A. Lithium Treatment and Withdrawal and Central Monoaminergic Processes.....	150
1. Behavioural and Toxicological Considerations.....	150
2. Metabolic Aspects of Monoamine Function.....	153
3. Synaptosomal Uptake Systems.....	160
4. Central Catecholamine Receptor Populations.....	168
B. Lithium in an Animal Model of Tardive Dyskinesia.....	170
C. Summary.....	175
References.....	182

LIST OF FIGURES

Fig. 1. General Structure of Catecholamines.....	22
Fig. 2. General Structure of Indoleamines.....	22
Fig. 3. Main Synthetic Pathway for Catecholamines.....	23
Fig. 4. Catabolic Pathways for Noradrenaline.....	26
Fig. 5. Catabolic Pathways for Dopamine.....	27
Fig. 6. Simplified Schema for the Synthesis and Inactivation of Dopamine.....	29
Fig. 7. Biosynthetic and Catabolic Pathways for 5-Hydroxytryptamine.....	30
Fig. 8. Diagram of Rat Brain to Show Dissection Procedure.....	66
Fig. 9. Chromatograph Showing Separation of the O-Methylated Metabolites of the Catecholamines.....	76
Fig. 10. Effect of Lithium Treatment and Withdrawal on Spontaneous Locomotor Activity.....	89
Fig. 11. Effect of Behavioural Stimulants on Spontaneous Locomotor Activity....	91
Fig. 12. Effect of Lithium Treatment and Withdrawal on Spontaneous Activity in Various Animal Models of Mania.....	93
Fig. 13. Changes in Brain Dopamine Levels Following Treatment with and With- drawal from Short-Term Lithium.....	98
Fig. 14. Effect of Short-Term Lithium Administration and Withdrawal on Central Noradrenaline Content.....	99
Fig. 15. Effect of Treatment With and Withdrawal from Long-Term Lithium on Central Dopamine Levels.....	108
Fig. 16. Brain Noradrenaline Content Following Long-Term Lithium Treatment and Subsequent Withdrawal.....	109
Fig. 17. Uptake into Hypothalamic Synaptosomes as a Function of Time.....	115
Fig. 18. Noradrenaline Uptake into Hippocampal Synaptosomes.....	121
Fig. 19. Noradrenaline Uptake into Hippocampal Synaptosomes After Lithium Administration and Withdrawal.....	124
Fig. 20. 5-Hydroxytryptamine Uptake into Hippocampal Synaptosomes.....	127
Fig. 21. Effect of Lithium Treatment and Withdrawal on 5-HT Uptake into Hypothalamic Synaptosomes.....	128
Fig. 22. Dopamine Uptake into Striatal Synaptosomes.....	131
Fig. 23. Alterations in Dopamine Uptake into Striatal Synaptosomes Following Lithium Treatment and Withdrawal.....	133

LIST OF TABLES

Table 1.	Toxic Symptoms Displayed Following Chronic Lithium Treatment.....	86
Table 2.	Brain Lithium Concentrations Following Short-Term Treatment and Subsequent Withdrawal.....	95
Table 3.	Effect of Lithium Administration and Subsequent Withdrawal on Parameters of Monoamine Synthesis.....	96
Table 4.	Changes in Catecholamine Metabolites Following Short-Term Lithium Administration and Subsequent Withdrawal.....	100
Table 5.	5-Hydroxytryptamine Concentrations in Rat Brain Regions Following Administration and Withdrawal of Short-Term Lithium.....	101
Table 6.	Regional 5-Hydroxyindoleacetic acid Levels Following Short-Term Lithium Administration and Subsequent Withdrawal.....	102
Table 7.	Brain Lithium Concentrations Following Exposure to and Withdrawal from Long-Term Lithium.....	105
Table 8.	Effect of Long-Term Lithium Administration and Subsequent Withdrawal on Parameters of Monoamine Synthesis.....	106
Table 9.	Catecholamine Metabolite Levels in Rat Brain Following Long-Term Lithium Treatment and Subsequent Withdrawal.....	110
Table 10.	Changes in Central 5-Hydroxytryptamine Concentration After Long-Term Lithium Treatment and Withdrawal.....	111
Table 11.	Effect of Long-Term Lithium Treatment and Withdrawal on Regional 5-Hydroxyindoleacetic Acid Levels.....	112
Table 12.	Synaptosomal Dopamine Uptake Following Lithium Treatment and Withdrawal.....	116
Table 13.	Changes in Synaptosomal Uptake of 5-Hydroxytryptamine After Lithium Treatment and Withdrawal.....	118
Table 14.	Effect of Lithium Treatment and Subsequent Withdrawal on Synaptosomal Uptake of Noradrenaline.....	119
Table 15.	Uptake Constants for Synaptosomal Accumulation of Noradrenaline and 5-Hydroxytryptamine.....	123
Table 16.	Effect of Lithium Treatment and Withdrawal on Constants Associated with Noradrenaline Uptake.....	125
Table 17.	Constants Relating to Synaptosomal Uptake of 5-Hydroxytryptamine Following Lithium Administration and Withdrawal.....	129
Table 18.	Kinetic Constants for Dopamine Uptake by Rat Brain Synaptosomes.....	132
Table 19.	Effect of Lithium Treatment and Withdrawal on Constants of β -adrenoceptor Binding.....	135

Table 20. Constants of (³ H)Spiroperidol Binding Following Lithium Administration and Subsequent Withdrawal.....	137
Table 21. Effect of Haloperidol and Lithium on Stereotypical Responses in Rats Induced by Apomorphine.....	140
Table 22. Effect of Haloperidol and Lithium on Parameters of Monoamine Synthesis.....	142
Table 23. Central 5-Hydroxytryptamine Levels After Treatment with Haloperidol and Lithium.....	143
Table 24. Alterations in Regional Concentration of 5-Hydroxyindoleacetic Acid Following Administration of Haloperidol and Lithium.....	144
Table 25. Catecholamine Metabolites in Brain Following Administration of Haloperidol and Lithium.....	146
Table 26. Noradrenaline Content of Various Brain Regions Following Administration of Haloperidol and Lithium.....	147
Table 27. Brain Dopamine Levels Following Haloperidol and Lithium Treatment....	149

ABBREVIATIONS

DA	Dopamine
DOPA	3,4-Dihydroxyphenylalanine
DOPAC	3,4-Dihydroxyphenylacetic Acid
HEPES	N-2-Hydroxyethylpiperazine-N'-2-ethanesulphonic Acid
5-HIAA	5-Hydroxyindoleacetic Acid
5-HT	5-Hydroxytryptamine
HVA	Homovanillic Acid
MPH ₄	DL-6-Methyl-5,6,7,8-tetrahydropterine
NA	Noradrenaline
SBP	Serotonin Binding Protein

ABSTRACT

For over three decades, lithium has been employed in the treatment of bipolar affective disorders, with its use usually necessitating long-term treatment. Recent studies have indicated that while patients continue to take lithium their condition is stable, but on withdrawal of the drug, a «rebound» mania may present itself. In addition to its efficacy in the treatment and prophylaxis of manic depression, lithium is considered to be of value in other conditions. One of these is tardive dyskinesia, a slow developing buccal-lingual-masticatory movement disorder which results from long-term neuroleptic use, and it is suggested that if lithium is co-administered with the neuroleptic agent the onset of the dyskinetic state may be prevented.

Despite the period of time for which lithium has been in use in psychiatric medicine, its mechanism of action is still not known, although it is thought that the monoaminergic neurotransmitters may play a role. This idea is derived from the biogenic amine hypothesis of affective disorders. If this is indeed so, then it is reasonable to assume that these same neurotransmitters would be involved in the genesis of any withdrawal phenomenon. This study therefore set out to examine various parameters of monoaminergic function to determine whether they may be involved in the mechanism of action of lithium, and to see if any form of neurochemical «rebound» occurs following withdrawal of the drug. In addition, it was decided to examine the effect of lithium in an animal model of tardive dyskinesia to investigate whether the utility of this agent in preventing the dyskinetic state is related to alterations in metabolic functions of the monoaminergic neurotransmitters.

Preliminary toxicological testing showed that a dose of 2 mEq lithium/kg was the maximal dose of the drug tolerated by rats without the appearance of overt toxic effects, and so this amount was utilized in all studies carried out in this investigation. Examination of spontaneous locomotor activity showed that

this behaviour was attenuated following 9 days of treatment with lithium, with the reduction being maintained with drug administration. When lithium was withdrawn, locomotor activity had returned to essentially control values after 2 days, and so 48 hours was utilized as the withdrawal period in all subsequent studies. No apparent «rebound» effect was observed in this behaviour following lithium withdrawal. Examination of the effects of lithium treatment and withdrawal in various animal models of mania showed that these analogues of the clinical condition were not affected by either treatment regime, casting doubts on their utility in the investigation of anti-manic agents. For this reason, «normal» animals were employed in all subsequent studies.

The effects of treatment with and withdrawal from short- and long-term lithium on central monoamine systems in rat brain were investigated. Treatment with the drug for either 12 or 26 days resulted in the accumulation of similar amounts of the cation in various brain regions; serum levels of lithium were also similar in both groups. Following withdrawal, tissue and serum lithium levels declined to approximately the same degree in both treatment groups. Synthetic parameters for the catecholamine and the indoleamine neurotransmitters were found to be unchanged after both treatment regimes, but abrupt withdrawal resulted in enhanced activity of tyrosine hydroxylase. NA in most brain regions examined was depressed following both short- and long-term lithium administration, with a further decrease in pons and an enhancement of cortical levels on withdrawal. DA displayed regionally variable changes after both lithium treatment and withdrawal, while 5-HT content was found to be reduced after lithium treatment with a return towards control values on withdrawal. Lithium administration resulted in elevated DOPAC levels, with long-term treatment producing an enhancement of 5-HIAA content in certain brain regions. Withdrawal of lithium caused an enhancement of HVA and depression of MOPEG levels, with a return towards control values for DOPAC and 5-HIAA. It appears that the changes in central monoamine systems are qualitatively similar following both short- and long-term lithium treatment, as are the consequences

of abrupt withdrawal. It is suggested that lithium administration results in a disruption of monoamine storage processes, while withdrawal from lithium does not result in a simple return towards normal states of catecholamine function.

The uptake systems for NA and 5-HT into synaptosomal preparations were found to consist of two components, a low-capacity and a high-capacity process. While administration of lithium for 12 days increased the activity of the low-capacity uptake process for NA, it markedly inhibited the high-capacity system for this neurotransmitter. Following withdrawal of lithium, the activity of the low-capacity system returned towards control values, but there was a profound enhancement of the high-capacity uptake process for NA. Both the low- and high-capacity uptake systems for 5-HT were found to be inhibited by lithium administration. The reduced activity of the high-capacity system due to lithium administration was still present 2 days following cessation of drug treatment, whereas the low-capacity process was found to be further depressed. The uptake of DA into synaptosomal preparations appears to be more complex than that for NA and 5-HT, since the shape of the substrate-velocity curve implies the presence of some form of multiple and co-operative process. Lithium administration resulted in an enhancement of DA uptake in all brain regions examined, while 2 days of withdrawal from the drug caused a reduction in the amount of DA taken up to below control values. It therefore seems that alterations in monoaminergic uptake processes may play a part in lithium's mode of action and may be involved in the genesis of the lithium withdrawal phenomenon.

Lithium administration for 12 days caused a regionally variable effect on the binding of dihydroalprenalol to β -adrenergic binding sites on synaptic plasma membranes, while a return towards control values was noted following lithium withdrawal. Examination of the binding of spiroperidol to dopaminergic binding sites following lithium treatment and subsequent withdrawal appeared to result in highly equivocal results.

When lithium was tested in an animal model of tardive dyskinesia, it was found that when the anti-manic agent was administered in conjunction with haloperidol, the apomorphine-induced stereotypical response was attenuated when compared to that resulting from treatment with the neuroleptic alone. This was not the case when lithium was administered following haloperidol administration. Examination of various parameters of dopaminergic function indicates that co-administration of lithium and haloperidol cause an enhanced turnover of DA, whereas the changes in noradrenergic and serotonergic parameters following this treatment are similar to those observed when lithium is administered following haloperidol. These data suggest that alterations in metabolic aspects of dopaminergic neurotransmission may be involved in lithium's amelioration of tardive dyskinesia, while the other two monoaminergic neurotransmitters do not play a part in this effect.

I

LITERATURE REVIEW

A. INTRODUCTION

The field of Neuropsychopharmacology has become one of increasing importance in the past two decades, as it applies the basic sciences of pharmacology and neurochemistry to the clinical problems of psychiatry and psychology. The combination of these disciplines has allowed for much investigation into the pathophysiology and treatment of disease states such as the affective disorders, schizophrenia, Huntington's chorea and Parkinson's disease.

The affective disorders are a group of psychotic disorders characterized by exaggerated mood states, which have been sub-divided into two major categories. The unipolar affective disorder is defined as recurrent depression, while the bipolar illness is a situation in which there are cycles of mania and depression. These can be further sub-divided into primary and secondary affective disorders, with the major difference being that in the secondary illnesses, the disorder occurs with either a pre-existing non-affective psychiatric illness or a life-threatening or incapacitating medical illness.

A problem which has existed with regard to the primary affective disorders was a lack of specificity and confusing terminology which had been used to describe them. In order to improve the diagnostic categories used in psychiatry, Feighner et al. (1972) suggested the following criteria for the clinical assessment of affective disorders:

Diagnosis of depression. The following criteria should be fulfilled: (A) dysphoric mood characterized by symptoms such as the following; depressed, sad, blue, despondent, hopeless, «down in the dumps», irritable, fearful, worried or discouraged. (B) at least five of the following criteria are required for «definite» depression; four are required for «probable depression»: 1) poor appetite or weight loss; 2) sleep difficulty (including insomnia or hypersomnia; 3) loss of energy, e.g. fatigability, tiredness; 4) agitation or retardation; 5) loss of interest in usual activities or decrease in sexual drive; 6) feelings of self reproach or guilt (either may be delusional); 7) complaints of or actually diminished ability to

think or concentrate, such as slow thinking or mixed up thoughts; 8) recurrent thoughts of death or suicide, including thoughts of wishing to be dead. (C) A psychiatric illness lasting at least one month with no pre-existing psychiatric conditions.

Diagnosis of mania. The following conditions should be met: (A) euphoria or irritability; (B) at least three of the following: 1) hyperactivity (includes motor, social and sexual activity); 2) push of speech (pressure to keep talking); 3) flight of ideas (racing thoughts); 4) grandiosity (may be delusional); 5) decreased sleep; 6) distractibility. (C) A psychiatric illness lasting at least two weeks with no pre-existing psychiatric conditions.

Hypomania is also possible. This condition is milder and more common than mania. The mood elevation is moderate and accompanied by overactivity, distractibility, heightened energy and irritability, but the flights of ideas or delusional convictions are absent.

B. THE BIOCHEMICAL BASIS OF AFFECTIVE DISORDERS

1. Humoural Theory

The earliest theory regarding the biochemical basis of depression comes from Hippocrates and other ancient Greek physicians, who believed that the liver removed toxic humours, including black bile, from food. If the liver malfunctioned, then there was an accumulation of black bile leading to melancholia, or severe depression (Berger and Barchas, 1977). Aretaeus of Cappadocia however, in the second century A.D., proposed that depression was caused by purely psychological factors and had little to do with the bodily humours. He was also the first to associate mania with depression in certain cases, and to consider both conditions as part of a single disease entity (Arieti and Bemporad, 1978). This theory was not accepted by Aretaeus' successors, including Galen, who subscribed to the humoural hypothesis of mental illness, which remained in vogue up to the scientific revolution in the 19th century. During this latter period, some investigators began to look upon mental illness as a physiological phenomenon, although the major focus at this time

and for a considerable period thereafter was on defining and categorizing the symptoms rather than determining a cause for the disorders. Also, due to the predilection for psychoanalysis which flourished at the turn of the century, most psychiatric practitioners still considered the affective disorders to be a purely psychological problem.

However, there were many factors which appeared to provide evidence for a biological basis for the disorders, particularly the somatic symptoms of severe depression, i.e. disturbances of sleep, appetite, gastrointestinal function and sex drive, together with the diurnal pattern of depression and in many cases, the association of depression with other medical illnesses (Baldeasarini, 1975). Also, since there appeared to be a lack of association with obvious stress factors in the initiation of many severe depressions and manias, further evidence was provided for the existence of some form of endogenous cause of the illness.

2. Monoaminergic Hypothesis

There were two major clinical findings in the early 1950's that laid the groundwork for a neurochemical basis of affective disorders. One of these was the discovery that some patients receiving reserpine for the treatment of hypertension became depressed (Bunney and Davis, 1965; Schildkraut, 1965; Himwich and Alpers, 1970; Moskovitz and Klawans, 1977; Maas, 1979). Despite the fact that many reviewers give the impression that this was a widespread side-effect of this anti-hypertensive regime, examination of the literature tends to suggest that reserpine-induced depression was not particularly common, ranging from 5% to 20% of all patients treated (Achor et al., 1955; Muller et al., 1955; Lemieux et al., 1956). Also, from the same reports, it seems that many of the patients in whom a depressive reaction to reserpine was noted had previous histories of depression, although it appears that the administration of reserpine may well have been the final precipitating factor in the genesis of these depressive episodes. A number of studies also showed that reserpine caused a depletion of both noradrenaline (NA) and 5-hydroxytryptamine (5-HT) stores in both peripheral and central tissues

(Holzbauer and Vogt, 1956; Pletscher et al., 1956; Quinn et al., 1959). However, the importance of each neurotransmitter, or its lack, in producing the reserpine syndrome was still uncertain, with some authors favouring the depletion of 5-HT as the causative effect producing depression (Pletscher et al., 1956; Quinn et al., 1959; Spector et al., 1960), while others regarded NA as being the prime agent (Holzbauer and Vogt, 1956; Bertler, 1961). Carlsson and Lindqvist (1967) took the catecholamine predominance one step further and suggested that the major neurotransmitter in the behavioural syndrome was, in fact, dopamine (DA). It later became accepted that the behavioural syndrome noted following reserpine administration was related to the general depletion of brain monoamines (Schildkraut, 1965; Berger, 1977).

- The second important clinical discovery was the recognition of one of the major side-effects of iproniazid, an agent introduced for the treatment of tuberculosis. The principal side-effect, when considering the genesis of affective disorders, was described by Selikoff et al. (1952) as «return of strength, energy and sense of well-being.» This expression of a euphoric feeling was also described by other investigators examining this anti-bacterial drug, and led to the suggestion that it may be of utility in the treatment of depression. Ayd (1957), Dally (1958), DeVerteuil and Lehmann (1958), Ferreira and Freeman (1958) and Pare and Sandler (1959) all tested this theory using patients classified as clinically depressed, and found an improvement in the mental state of certain of their subjects. Iproniazid was known to cause an inhibition of monoamine oxidase activity (Zeller and Barsky, 1952; Brodie et al., 1956; Spector et al., 1958), causing a concomitant elevation in the brain levels of NA and 5-HT (Spector et al., 1960), which provided further evidence for the involvement of central monoamines in the genesis of mood and affective disorders.

These findings led to the development of the biogenic amine hypothesis of affective disorders, which in its simplest form states that depression is associated with a functional deficit of biogenic neurotransmitter at critical

synapses, while the opposite is true in mania (Baldessarini, 1975; Berger, 1977). This view is now considered to be too simplistic as the affective disorders are not a clinically or neurochemically homogeneous group of illnesses, and it also appears that alterations in two or more amine systems may be present in different types of affective disease (Maas, 1979); however, despite these criticisms, the hypothesis still provides the major impetus for research into the affective disorders.

C. LITHIUM

1. Lithium in Affective Disorders

Lithium salts have had a varied past in medicine, having been recommended for use, at various times, in the treatment of gout, rheumatism, epilepsy, syphilis, diabetes, hypertension and as a diuretic (Cade, 1978; Prien, 1978), although their application was not particularly widespread. Lithium's greatest initial public use was in the 1940's as a substitute for salt in patients requiring low-sodium diets. The toxicity which resulted from this application of lithium chloride (Corcoran et al., 1949; Hanlon et al., 1949) appeared to signal the end of the therapeutic usefulness of lithium salts, and resulted in the subsequent slow acceptance of the cation into psychiatric medicine in later years, particularly in North America.

The finding that lithium is of value in the treatment of bipolar affective disorders was purely a chance discovery, and provides convincing proof that serendipity does play an important role in the discovery of new therapeutic agents.

The discovery was made by John Cade, a psychiatrist working alone in a small chronic care centre in Australia. He thought that the manic-depressive syndrome may be the result of some form of abnormal metabolism in his patients, and that the product of this metabolism may be excreted in urine. His problem was to demonstrate the presence of this anomalous metabolite, and he devised a crude toxicity test to determine whether there was a difference in the urine of various patient groups. He therefore administered varying amounts of concentrated urine obtained from manics, melancholics, schizophrenics and normal subjects by intra-

peritoneal injection to guinea-pigs. Cade found that the urine from certain of his manic patients was more toxic than that from any other group, and further determined that urea was the toxic agent, producing powerful convulsions prior to death.

His next question was - what makes the urine from manic patients more toxic than that from other groups? Cade considered the answer to lie with uric acid or creatinine, which possibly modified the toxicity of urea, and determined that uric acid enhanced the toxic effects of urea, while creatinine showed potent protective properties.

The next step was to determine the amount of uric acid required to increase the toxicity of urea. Cade ran into a technical problem here, in that uric acid is relatively insoluble in water, and so he chose the most soluble salt with which to work - lithium urate. Rather than enhancing the toxicity of urea, this salt seemed to exert a protective effect.

The lithium salts themselves were then examined, and Cade found that large doses of lithium carbonate given intraperitoneally to guinea pigs produced lethargic and unresponsive animals. This being the case, he next tried lithium carbonate on one of his manic patients, and found that after 5 days of lithium therapy, the patient was returning towards normal and was eventually released from hospital care (Cade, 1978).

The case was reported in the Medical Journal of Australia in September 1949 (Cade, 1949) and was noted by a number of other investigators, primarily in Australia and Europe (Schou, 1957) who tested lithium in their own clinics. These groups noted an improvement in the majority of their manic subjects (Schlagenhauf et al., 1966), but tended to report their findings in a rather subjective fashion. Schou et al. (1954) were the first to attempt to demonstrate the efficacy of lithium using «open» and «double-blind» treatment regimes, with controls for placebo effect. They found that approximately 40% of their subjects

became less manic within two weeks following commencement of lithium therapy, thus confirming the earlier findings of the Australian researchers.

Subsequent to this, many other investigators reported the efficacy of lithium in the treatment of manic episodes, although the use of empirical methods without the types of control used by Schou et al. (1954) were still prevalent. Rice (1956) described a study involving 58 patients, of whom 41 were diagnosed as suffering from affective or predominantly affective disorders, while the remaining 17 displayed schizoform illnesses. Of those with the affective illnesses, 38 either recovered or displayed marked improvement following initiation of lithium therapy. No depression was noted in any of the subjects. With regard to the schizoform patients, 5 showed signs of improvement. Gershon and Yuwiler (1960) reported the first study carried out in North America with lithium, and examined a number of patient types, including subjects suffering from recurrent mania, various forms of schizophrenia, epilepsy, and behavioural disorders. They found that lithium therapy was effective in the treatment of mania, even in cases that were unresponsive to electroconvulsive shock, which had been the mainstay of therapy for the affective disorders until the introduction of reserpine, phenothiazines and lithium. However, it was only useful in the treatment of non-manic states in which hyperactivity was a feature of the symptomology, and in these cases, lithium's efficacy was based purely on its ability to decrease psychomotor activity and not due to an improvement of the basic condition. At the same time that Gershon and Yuwiler (1960) were investigating lithium, Kingstone (1960) was examining the effect of lithium administration in both manic and non-manic patients. Of his 17 manic subjects, all except one responded to lithium, while there was little change in the patients in whom excitement was not manic in nature. He did note however, that «although these patients' moods and activity were returned to a normal state, the urge to remain elated and facilitated was still present. The feeling of internal curbing resulted from the inability of the patient to respond to this desire in thought, speech or act.» Schou (1963) mentions this feeling of being «kept down» and

suggests that it is due to the treatment not having had its full effect since the feeling later dissipates. Maggs (1963) carried out the second reported «blind» study involving lithium, and found no significant differences between his results and those of the previous blind (Schou et al., 1954) or open studies (Rice, 1956; Yuwiler and Gershon, 1960; Kingstone, 1960). In all of these investigations, a positive response to lithium was noted within two weeks of the commencement of therapy.

Bunney et al. (1968) carried out one of the first longitudinal studies of lithium administration during acute mania and although only two subjects were involved, the authors provide elegant proof that the inhibition of manic symptoms is directly related to serum lithium levels. Goodwin et al. (1969) extended this study to include 12 manic subjects, and again showed that the degree of improvement in lithium responders corresponds to time from commencement of lithium therapy.

Stokes et al. (1971) examined the efficacy of lithium using an «on-off» treatment regime, with «blind» behavioural raters and demonstrated a specific effect of lithium on decreasing the severity of mania during acute attacks. This amelioration of manic symptoms due to lithium (75%) was almost twice that noted with placebo (40%). It was also found that the decrease in mania rating during lithium administration was greater when the pre-treatment manic score was high. These effects were all seen following 7-10 days of treatment. In fact, Post and Cutler (1979) have stated that in a number of uncontrolled and single-blind studies utilizing 413 patients, an overall improvement was noted in 81% of the subjects.

These reports were all important since they demonstrated that lithium was a valuable psychopharmacological tool in the treatment of mania. A number of investigators however, were showing that lithium's abilities were more profound than simply the control of acute mania.

Schou et al. (1954) had noted that «many patients can be kept in a normal state by administration of a maintenance dose» of lithium, while both Hartigan (1963) and Baastrup (1964) found that long-term treatment with lithium salts

prevented the occurrence of new manic attacks. These observations led to the hypothesis that lithium could be used not only for the treatment of manic attacks, but also as a prophylactic agent.

Baastrup and Schou (1967) tested this hypothesis using 88 subjects admitted to hospital for manic-depressive psychosis, which included patients also suffering from recurrent primary depression and a number of atypical cases, and followed them for a period of 6 1/2 years. Lithium treatment was started during a psychotic episode in attempts to establish a constant starting point for therapy. They suggested that if lithium did interfere with the course of the manic-depressive syndrome, this may be displayed in four possible ways: 1) the treatment might produce a change in the frequency with which psychotic episodes occurred without interfering with the duration of the episodes; 2) lithium treatment might lead to a change in the duration of the episodes without interfering with the relapse frequency; 3) it might alter the intensity of the symptoms; 4) a combination of all or any of the above. The parameters which were chosen for the study were the relapse frequency (i.e. the average number of episodes per year) and the psychosis rate (i.e. the average number of months per year spent in a psychotic state). It was found that there was a significant decrease in both of the parameters measured, since prior to lithium administration, relapses occurred on average every 8 months but during lithium treatment, they occurred only every 60 months, and the average time spent in a psychotic state before lithium was 13 weeks per year as compared to only 1 1/2 weeks during lithium therapy. Relapses, when they did occur, also tended to be shorter during lithium treatment than they were prior to drug administration. This prophylactic effect was found to be strongest in purely affective disorders, i.e. manic-depressive psychosis and recurrent depression, and less so when paranoid tendencies were present.

Blackwell and Shepherd (1968) however, took issue with the report of Baastrup and Schou (1967) and re-examined the data presented in the original

paper. They concluded that a prophylactic effect had not been demonstrated and that the initial report was flawed due to inappropriate selection of subjects, criteria of prophylaxis, and evaluation of results, coupled with an observer bias. In addition, since the study of Baastrup and Schou (1967) was carried out using an «open» treatment regime, there were, in the opinion of Blackwell and Shepherd (1968), no adequate controls used.

Baastrup and Schou (1968) responded to the criticisms of Blackwell and Shepherd (1968) and supplied a critique of the criticisms, which were shown to be «either irrelevant or invalid» and again stated their feeling that «the study succeeds in proving its point beyond a reasonable doubt.» However, in order to provide more conclusive evidence for a prophylactic effect, Baastrup et al. (1970) carried out a double-blind comparison of matched patient groups treated with either placebo or lithium. The procedure which was used was to switch subjects on maintenance lithium therapy in a double-blind fashion to either lithium or placebo. The trial was terminated when the patient relapsed into either a manic or depressive episode, at which point antimanic or antidepressant therapy was administered and open lithium reinstituted. The patients chosen were diagnosed as suffering from either bipolar manic-depression or recurrent endogenous depression, and had received lithium for at least one year prior to the study. It was found that no relapses occurred in the groups maintained on lithium, while there were 12 relapses from 22 manic-depressives and 9 relapses from recurrent depressives. These data strengthened the view that lithium did exert a prophylactic effect, not only in manic-depressive subjects, but also in recurrent depressives.

These reports were supplemented by independent reports from other investigators. Coppen et al. (1971) used a total of 120 patients, and found that the time spent in hospital and the time undergoing an outpatient episode (expressed as a percentage of the total time in the study) were significantly less in patients on lithium therapy than on placebo. This was true both of subjects

diagnosed as suffering from unipolar as well as bipolar affective disorders.

Hullin et al. (1972) and Stallone et al. (1973) further confirmed these findings.

Hullin et al. (1972) also noted that unipolar manic patients and unipolar depressives responded better to lithium than did the bipolar manic-depressives.

This latter study also brought up another interesting facet of the clinical utility of lithium, which is the question of whether lithium is effective in the treatment of recurrent depression. If this is indeed the case, then lithium could well warrant the label applied to it by Schou (1963), i.e. that of normothymotic or mood-normalizer, rather than simply an anti-manic agent. The normothymotics act on the mood and turns a pathologically altered mood-state into a normal one, and the classification is based on certain findings that therapeutically, lithium and the imipramines act in a similar fashion (Schou, 1963).

However, the evidence for the efficacy of lithium in recurrent endogenous depression is not unequivocal. In his original report, Cade (1949) found that lithium neither helped nor worsened depression, a conclusion also reached by Noack and Trautner (1951), Gershon and Yuwiler (1960), Fieve et al. (1968) and Zall et al. (1968). In contrast to these, Hartigan (1963) found a definite therapeutic effect in his small number of the unipolar depressed patients. Dyson and Mendelson (1968) reported that subjects in the depressive phase of the manic-depressive cycle responded well to lithium therapy, and that lithium maintenance had been found to have a prophylactic effect. Similar responses to lithium therapy were also noted by Baastrup et al. (1970), Coppen et al. (1971), Goodwin et al. (1972), Hullin et al. (1972), Prien et al. (1973) and Stallone et al. (1973).

Goodwin et al. (1969), in their longitudinal study of lithium therapy, included not only manic patients, but also bipolar depressed subjects and non-cyclic depressives. They determined that in addition to a 75% response in their manic patients (9 of 12), 77% of the bipolar depressed subjects also showed a degree of improvement (10 of 13). However, only 2 of the 5 non-cyclic depressed

patients displayed a response, with neither of these being classified as a complete and unequivocal recovery. In the bipolar depressed group, it was found that one of the lithium non-responders was successfully treated with imipramine.

In the majority of the studies mentioned above, it should be mentioned that when the patient groups evaluated in determining the efficacy of lithium in depression were subdivided into bipolar depressed and unipolar depressives, the predominant effect of lithium in ameliorating the symptoms was found in the bipolar depressed groups. In fact, it has been suggested (Ramsey and Mendels, 1978) that the unipolar patients who responded to lithium may have been suffering from bipolar affective disorder, which follows from the work of Perris (1966) who found that 14% of patients diagnosed as unipolar depressives based on three episodes of depression will have a manic episode in the future, and therefore require reclassification.

Although the use of lithium as an antidepressant drug is as yet unconfirmed in the treatment of all subtypes of depression, its usefulness as both a therapeutic and prophylactic agent in bipolar affective disorder is well established, with the evidence being more convincing for its efficacy in the manic rather than the depressive phase.

2. Clinical Consequences of Lithium Withdrawal

The prophylactic aspect of lithium therapy has, of necessity, resulted in a situation in which treatment is long-term, and thus has raised the ethical question of whether a drug which may no longer be required should be continued. This is particularly true in light of recent clinical observations suggesting renal impairment following chronic lithium administration (Bakris et al., 1981; Moskovitz et al., 1981). The consequences of cessation of lithium in patients stabilized on this anti-manic agent are, however, controversial.

In his account of one of his initial patients, Cade (1978) described a subject who was stabilized on lithium and subsequently released from hospital,

but who required re-admission 6 months later with severe manic symptoms because he had ceased lithium intake. Gershon and Yuwiler (1960) noted that «protracted cases of mania respond to treatment by elimination or significant decrease in symptomology so long as lithium is administered but discontinuation of treatment leads to a recurrence of symptomology.» Hartigan (1963) found that withdrawal of lithium or a reduction in its dosage to below a minimum effective level usually led to a rapid relapse, while Baastrup (1964) states that «discontinuation of lithium always led to relapse.» Bunney et al. (1968) also found that when placebo was substituted for lithium, a recurrence of manic symptoms occurred within 24 hours, data which was substantiated by Goodwin et al. (1969). Stokes et al. (1971) disagreed with the previous groups of investigators (Bunney et al., 1968; Goodwin et al., 1969) with regard to the time-course of the manic relapse, but did note an increase in mania rating whenever placebo was substituted for lithium. It should be noted that in some of these studies, the patients under examination were suffering from acute mania, and lithium was discontinued during the course of treatment for this acute state (Bunney et al., 1968; Goodwin et al., 1969). In the other reports however, lithium was being administered on a long-term basis, and while recurrence of manic symptoms would be expected if drug therapy was withdrawn during the course of a manic attack, this would not necessarily be the case if lithium was discontinued some time after stabilization.

These findings led to the suggestion that there may indeed be a lithium withdrawal phenomenon. This theory was first tested by Small et al. (1971) who withdrew lithium from five patients who had been on lithium therapy for at least 18 months prior to placebo substitution. Of these five subjects, three had both manic and depressive attacks, one suffered only recurrent depression, while the fifth had a circular illness with some atypical features which were not specified. They found that when lithium was replaced with a placebo, the atypical patient relapsed within 2 days, and the other patients all displayed symptoms 2-4 weeks after substitution. In all cases, the clinical deterioration

was reversed when lithium was re-introduced. Lapierre et al. (1980) described a study in which well-documented bipolar manic depressives who had been stabilized on lithium for at least one year were subjected to lithium withdrawal for 5 days. It was found that 20% of their subjects suffered relapses within this time period, necessitating hospitalization for some of them. In some cases, the deterioration continued despite resumption of lithium intake. Wilkinson (1979) also reported a case of rapid mental deterioration 2 days following lithium withdrawal. In this case, an acute confusional state was diagnosed rather than a manic relapse. Klein et al. (1981) examined 21 patients with previous histories of affective disorders who had been maintained on lithium for at least 7 months. Within 14 days of lithium withdrawal, 11 of their subjects relapsed into severe psychotic states with paranoid, manic and depressive syndromes, the relapse state corresponding to their previous illness. A rapid remission occurred in all cases when lithium was re-administered. Klein et al. (1981) therefore stated that «the immediate onset of psychotic symptoms suggests rebound effects due to lithium withdrawal rather than a spontaneous recurrence of the underlying disease during lack of lithium protection.»

This, however, is at variance with the views of other investigative groups. Maggs (1963) used a treatment regime which involved 14 days of lithium treatment, followed by 28 days with no lithium, and found no evidence of increased manic symptoms after lithium withdrawal. Baastrup and Schou (1967) discontinued lithium in some of their subjects, and found that 88% of these had relapses within periods ranging from a few days to 12 months. They stated that «the episodes reappear with the same frequency and duration as before lithium treatment was started.» Baastrup et al. (1970) later repeated that «there was no indication of rebound or abstinence effects» following lithium withdrawal, a view also held by Rifkin et al. (1975) who studied the effects of abrupt withdrawal of lithium in twelve patients following 6 weeks of lithium treatment. In fact, Schou (1980) stated that «abstinence phenomena have never been seen after discontinuation of lithium.»

As can be seen, there is as yet no compelling evidence for either the presence or absence of a lithium withdrawal syndrome, leaving the issue, in terms of its clinical importance, unresolved.

3. General Methodology in Animal Studies With Lithium

(a) Problems encountered in lithium research

There are many problems encountered in investigating the mechanism of action of lithium, some of which are common to all pharmacological investigations, e.g. selection of dosage, route and frequency of administration and species of test subject. Various routes of administration have been used to treat test animals, which are usually rats although other species have also been used, e.g. mice (Kuriyama and Speken, 1970; Shaw and Ratcliffe, 1976) and cats (Sangdee and Franz, 1980; Swann et al., 1981). Among the routes of administration employed are the inclusion of lithium salts in diet (Hesketh et al., 1978; Gallagher and Bunney, 1979; Ebstein et al., 1980; Lerer et al., 1980; Rosenblatt et al., 1980; Staunton et al., 1982), in drinking water (Eroglu et al., 1981; Guerri, 1982), by subcutaneous injection (Grahame-Smith and Green, 1974; Atterwill and Tordoff, 1982), by slow-release tablets (Swann et al., 1981), but most commonly by intraperitoneal injection (Friedman and Gershon, 1973; Poitou and Bohuon, 1975; Shaw and Ratcliffe, 1976; Collard, 1978a; Allikmets et al., 1979; Cameron and Smith, 1980; Sangdee and Franz, 1980; Teixeira and Karniol, 1982). In the cases of administration by injection, the frequency of treatment is variable, lithium having been given either once daily (Friedman and Gershon, 1973; Collard, 1978a; Allikmets et al., 1979; Swann et al., 1981) or twice daily (Grahame-Smith and Green, 1974; Poitou and Bohuon, 1975; Shaw and Ratcliffe, 1976; Sangdee and Franz, 1980; Atterwill and Tordoff, 1982).

The oral route of administration tends to produce animals with a more constant blood lithium level than do the parenteral routes, but the disadvantage of this route of administration is that it is not possible to know beforehand the amount of lithium which the animal will ingest. This disadvantage was, however,

considered to be minimal, since it was deemed to be more important to have stable blood lithium levels, particularly for chronic use. This theory was recently challenged by Plenge et al. (1981) who found that a relatively constant serum lithium concentration is more harmful to the kidney than a peak value followed by a period with a declining lithium concentration, and they suggest that this may be due to an inhibition of regenerative processes in damaged kidney cells by high levels of lithium. The popularity of the twice-daily administration is probably related to the changes in blood lithium levels, in that the variations between the high and low lithium concentrations would be reduced. Also, using the twice-daily treatment regime would enable a high, relatively constant, lithium concentration to be reached faster than by using once-daily administration. However, Plenge et al. (1982) have determined that in a clinical setting, functional and structural changes in kidney were more pronounced when patients were given lithium in divided doses throughout the day rather than as a single dose. Perry et al. (1981) compared twice-daily administration of lithium to single daily dosage and found that there was no significant difference in the average steady-state lithium serum concentration, although the high and low limits were more exaggerated with the once-daily treatment regime. These data therefore suggest that a single daily treatment with lithium is preferable to administration of divided doses of the drug in order to reduce the possibility of toxic side-effects.

With regard to the selection of dose of lithium to be administered, many investigators have attempted to achieve clinically relevant blood levels of the cation (0.6 - 1.2 mEq/l) in test species. However, it is possible that this may not be a valid target at which to aim in non-human subjects. Gallager and Bunney (1979) achieved serum lithium concentrations of 0.8 mEq/l, but noted a marked polyuria in these rats, while Rosenblatt et al. (1979) induced blood levels of 0.7 - 0.9 mEq/l, and found a 15% decrease in body weights relative to control after 3-4 weeks. Ebstein et al. (1980) used two dosage regimes with 22-25 days

of oral administration of lithium. At their lower dose, rats achieved serum levels of 0.56 ± 0.03 mEq/l, while at the high dose serum lithium levels were 1.56 ± 0.10 mEq/l. At the higher dose, it was found that the animals did not gain weight and also displayed polyuria and polydipsia, while at the lower lithium dose, growth was «almost normal.» Lerer *et al.* (1980) used an oral administration schedule which resulted in mean lithium blood levels of 0.87 mEq/l, but noted that while control animals gained 114 ± 2 g in the 19 day treatment period, the lithium-fed rats gained only 12 ± 3 g. Swann *et al.* (1981) administered lithium to cats and after three days of treatment found plasma lithium concentrations of 0.70 ± 0.31 mEq/l with a weight loss of 0.34 kg, while fourteen days of lithium administration produced plasma levels of 1.01 ± 0.27 mEq/l with a weight loss of 0.37 kg. Since polyuria and lack of weight gain, or a frank weight loss, are considered to be toxic effects of lithium, it seems that the levels of lithium required to produce therapeutic effects in the clinic are in excess of those tolerated by many laboratory animals. An alternative approach in choosing a dose of lithium to be administered may be to use the maximum amount of lithium tolerated by the species before the onset of toxic signs. This follows from the fact that the clinical plasma concentration of lithium used to achieve therapeutic effects (0.6 - 1.2 mEq/l) is close to that at which toxic symptoms become manifest (<2.0 mEq/l) (Schou, 1980).

A factor which is of major importance in the evaluation and comparison of many animal studies using lithium is the blood lithium level, since this is often the only effective way of assessing the suitability of the lithium dose administered. Unfortunately, comparisons are often made difficult by the variations in the time from the last lithium administration to the time of blood sampling, particularly in cases where treatment is parenteral. While some authors have not reported time from last lithium administration to blood sampling (Ho *et al.*, 1970), others have used a variety of times, including 60 minutes (Friedman and Gershon, 1973), 5 hours (Poitou and Bohoun, 1975), 8-10 hours (Sangdee and Franz,

1980), 12 hours (Kuriyama and Speken, 1970), 16 hours (Cameron and Smith, 1980; Shaw and Ratcliffe, 1976), 17 hours (Grahame-Smith and Green, 1974), 19 hours (Atterwill and Tordoff, 1982), 21 hours (Slotkin et al., 1980) and 24 hours (Collard, 1978a; Swann et al., 1981). Many of these reports state that the blood lithium concentration at the time of sampling compares well to the clinical level aimed for (Friedman and Gershon, 1973; Grahame-Smith and Green, 1974; Atterwill and Tordoff, 1982), but this type of comparison must be viewed with caution. Studies have shown a rapid decline in blood lithium concentrations following parenteral lithium administration (Cameron and Smith, 1980; Plenge et al., 1981). Since blood lithium measurements are made in the clinic approximately 12 hours after the last lithium treatment (Schou, 1980), studies which demonstrate «clinically relevant» blood levels at sampling times significantly greater or less than this are probably doing so erroneously.

(b) Animal models of mania

Another problem prevalent in the investigation of a psychotherapeutic agent is that of choice of an appropriate model of the disorder against which the test agent is effective. Murphy (1977) suggested that an animal model of a human behavioural disorder would utilize some component of the disorder. Most models can be classified according to their ability to resemble the human condition in terms of symptoms, aetiology, mediating mechanisms, or treatment responses. Since one of the major clinical symptoms of mania appears to be a hyperactive state, most animal models which have been used for investigating this disorder are based primarily on hyperactivity. However, behavioural hyperactivity is very non-specific, and so models based on this behavioural element would, at best, provide a poor fit with the clinical syndrome. Some of these hyperactivity analogues of mania are described together with their possible shortcomings.

i) Hyperactivity due to neonatal hyperthyroidism. The rationale for the use of this model derives from the fact that a number of clinical studies have

demonstrated that excessive thyroid secretion produces psychological symptoms including emotional lability, restlessness, irritability, over-reactiveness with predominant anxiety; and tension (Eayrs, 1960; Whybrow and Ferrel, 1974).

Evidence also exists suggesting some neurochemical and behavioural similarities between hyperthyroidism and mania (Maletzky and Blachley, 1971). It has been shown that neonatal hyperthyroidism is accompanied by hypermobility, in addition to increased synthesis and turnover of NA, DA and 5-HT in specific areas of the brain (Rastogi and Singhal, 1976; Singhal and Rastogi, 1978). However, there is no evidence indicating an increase in thyroid function during mania, and it is known clinically that lithium will in itself affect thyroid activity, resulting in hypothyroidism with or without goitre (Wolff, 1979; Mann and Gershon, 1980).

Since lithium appears to alter thyroid function, the question to be asked with respect to the neonatal hyperthyroidism model of mania is whether the neurochemical effects seen following lithium administration are due to lithium per se, or are sequelae to alterations in thyroid function confounded by direct lithium action on central neurotransmitter systems.

ii) Amphetamine-induced hyperactivity. In this model, low doses of amphetamine are administered in combination with barbiturates or chlordiazepoxide. The resulting hyperactivity was found to consist of «fast and co-ordinated, but characteristically repetitive and apparently compulsive walking, as well as other activities, depending on the species of animal and on the kind of test environment used» (Davies et al., 1974). This type of activity was said to be different to the stereotyped behaviour seen after large doses of amphetamine alone, and has been used by a number of investigators (Davies et al., 1974; Poitou et al., 1975; Engel and Berggren, 1980; Berggren et al., 1981). Unfortunately, this model does not correlate well with the clinical picture seen in mania, since the time-course of amphetamine-induced hyperactivity would be in the order of hours and not days, as in mania. Also, Davies et al. (1974) state that «in clinical practice lithium usually only becomes effective after several days, in our

experiments with rats the most striking results were obtained after only a single pretreatment.» This would tend to suggest that the mechanism of lithium action in this model is not the same as that required for its therapeutic effectiveness.

Another problem encountered in this model with regard to biochemical determinations is due to the inherent neuronal effects of amphetamine, which would result in a confounding of the neurochemical changes due to lithium alone.

iii) Reserpine-induced hyperactivity. This particular analogue of mania has been described by Delini-Stula and Meier (1976). Hyperactivity is induced by administration of reserpine following treatment with pargyline, a monoamine oxidase inhibitor. This combination results in elevated levels of monoamine neurotransmitters in the synaptic cleft, a situation which is thought to be present in mania. Unfortunately, this model has certain of the drawbacks present in the amphetamine-induced hyperactivity model, particularly those relating to time-course and to attempting to carry out neurochemical studies in these animals.

iv) 6-Hydroxydopamine-induced hyperactivity. This animal model of mania was recently proposed by Petty and Sherman (1981), and involves the intraventricular administration of 6-hydroxydopamine. This treatment is said to increase irritability, aggression, hyperemotionality, hyperreactivity and vocalization. The data presented by these authors indicate that chronic lithium, electroconvulsive shock and acute chlorpromazine all reduce the hyperreactivity due to shock following 6-hydroxydopamine administration. Petty and Sherman (1981) propose that since the behavioural response is abolished by three treatments efficacious in the clinical treatment of mania, this is a pharmacologically valid model of the disorder. However, it again appears that its usefulness is limited by the inability to carry out neurochemical studies.

4. Lithium and Biogenic Amine Neurotransmitters

Much of the research regarding the neurochemical mode of action of lithium has centred on examination of the monoaminergic neurotransmitters. This has

primarily been due to the fact that the biogenic amine hypothesis of affective disorders, despite its deficiencies, has tended to dominate thinking regarding the genesis of mood dysfunctions. If this theory holds true, even partially, then lithium must exert its effects, to some extent, through alterations in some aspect of monoamine neurotransmitter function.

(a) Synthesis and metabolism of the monoaminergic neurotransmitters

Strictly speaking, catecholamines are all organic compounds containing a catechol nucleus and an alkylamine group (Fig. 1), although the term tends to be reserved for the neurotransmitters which are composed of these, i.e. DA, NA and adrenaline. The indolealkyl amines, commonly referred to as indoleamines, also have an amine substituent, but are based on an indole nucleus (Fig. 2), and include tryptamine, 5-HT and melatonin.

i) Catecholamines. The synthesis and metabolism of the catecholamines has been the subject of many excellent reviews over the years, including detailed examination of the subject by Euler (1972) and Bowman and Rand (1980) and will therefore only be briefly summarized here.

The starting point for the synthesis of catecholamines is with the amino acids, phenylalanine and tyrosine, which are derived from proteins in the diet. Although the dietary intake of tyrosine is usually more than adequate for catecholamine synthesis, phenylalanine can enter the synthetic pathway via conversion to tyrosine by phenylalanine hydroxylase (Fig. 3). Since phenylalanine hydroxylase is primarily a liver enzyme, the primary substrate for the catecholamine synthetic pathway can be considered to be tyrosine.

Tyrosine hydroxylase catalyses the addition of a 3-hydroxy group to tyrosine, forming 3,4-dihydroxyphenylalanine (DOPA). The enzyme requires tetrahydropteridine as a co-factor, and is activated by Fe^{2+} . This conversion of tyrosine to DOPA is a relatively slow process when compared to the subsequent enzymatic transformations, and is therefore the rate-limiting step in this pathway.

Fig. 1: General Structure of Catecholamines

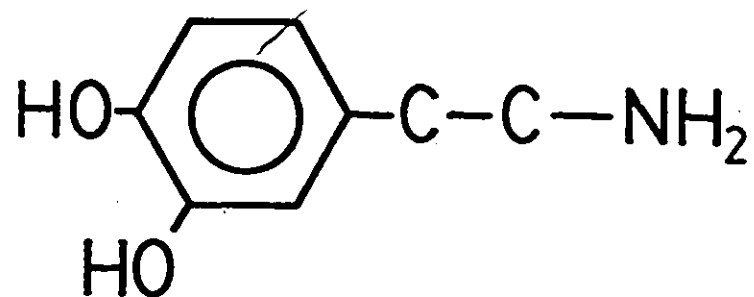


Fig. 2: General Structure of Indoleamines

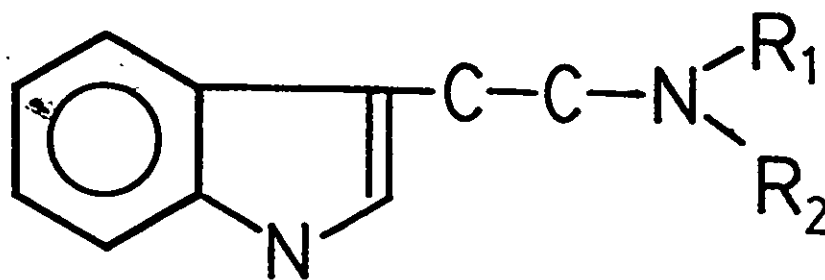
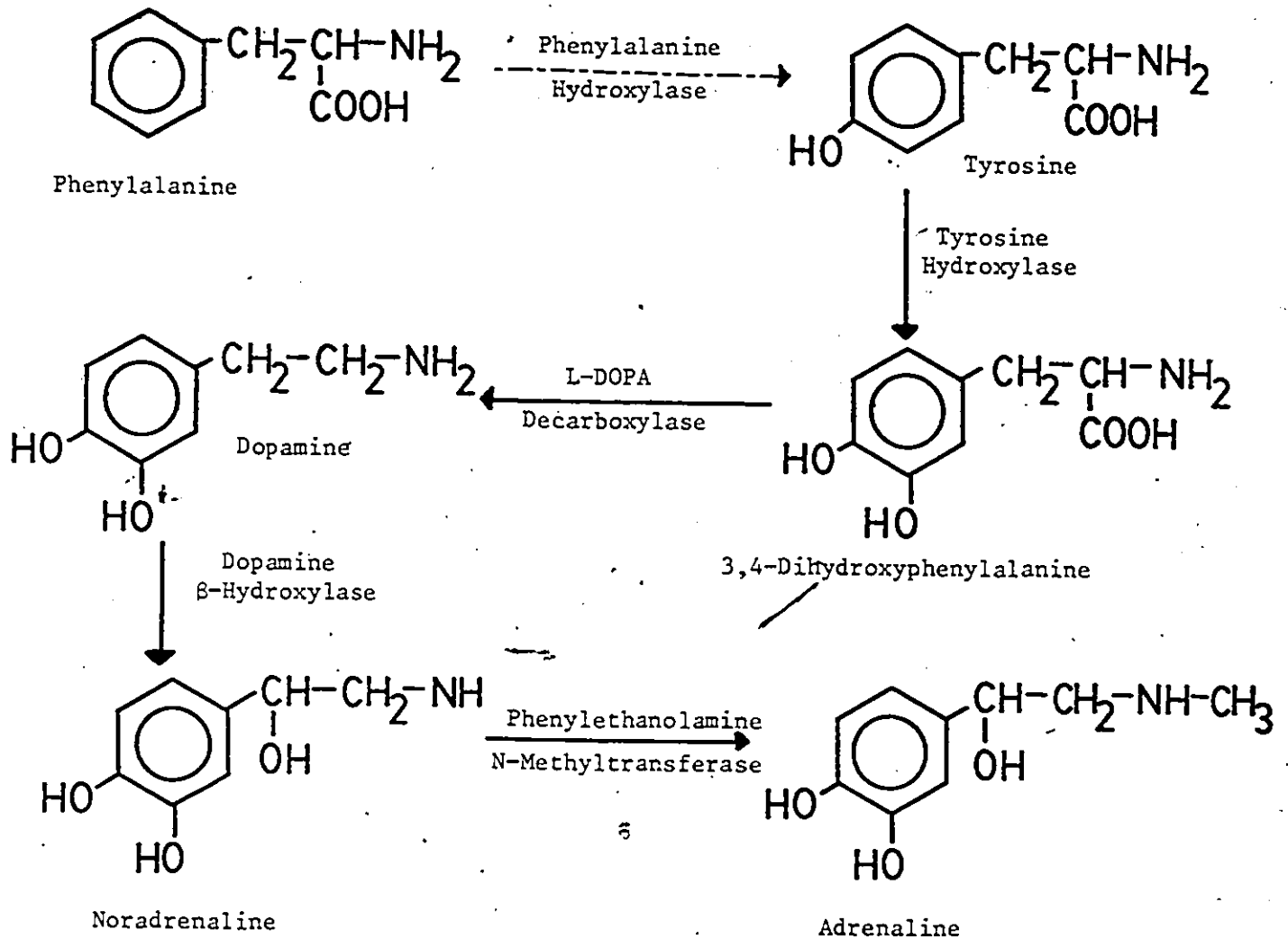


Fig. 3: Main Synthetic Pathway for Catecholamines



Tyrosine hydroxylase is synthesized in the cell body and is passed along the axons in association with the endoplasmic reticular apparatus, to which it remains loosely bound. The rate of enzyme synthesis is proportional to the neuronal activity, being increased when activity is high and decreased during periods of low neuronal traffic. Tyrosine hydroxylase is subject to an end-product inhibition by NA in peripheral nerves, and is likely to show similar characteristics in brain tissue. It is also probable that in nerves lacking dopamine β -hydroxylase, i.e. dopaminergic structures, DA exerts a similar effect.

The next step in the pathway is the decarboxylation of the side-chain of DOPA to form DA with DOPA decarboxylase as the catalytic enzyme. This is a rapid transformation and for this reason, DOPA does not accumulate in nervous tissue. The enzyme appears free in the cytoplasm and not bound to any subcellular structures. The co-factor required for DOPA decarboxylase activity is pyridoxal phosphate. The name DOPA decarboxylase is derived from the early discovery that the enzyme removes carboxyl groups from L-DOPA, but it was subsequently found that the enzyme will act on all naturally occurring aromatic L-amino acids, e.g. histidine, tyrosine, tryptophan and phenylalanine, and is therefore more appropriately referred to as L-aromatic amino acid decarboxylase.

The next enzyme in the pathway, dopamine β -hydroxylase, is absent in nerves which utilize DA as a neurotransmitter, but is present in adrenergic structures. This enzyme catalyzes the conversion of DA to NA by the addition of a hydroxy group to the β -position on the side-chain. Dopamine β -hydroxylase is a Cu^{2+} -containing protein, and requires ascorbic acid as a co-factor. It appears to be incorporated in the catecholamine storage granules, with part being bound to the organelle membrane and part being releasable. In neurones in which NA is the transmitter substance, catecholamine synthesis is completed at this point, since the enzyme responsible for the formation of adrenaline is absent.

7. This enzyme is phenylethanolamine-N-methyl transferase (PNMT) and it is responsible for the conversion of NA to adrenaline by the transfer of a methyl

group from S-adenosylmethionine to the nitrogen atom. Although PNMT is primarily found in the chromaffin cells of the adrenal medulla, the presence of adrenaline and its synthetic enzyme have been reported in brain (Ciaranello et al., 1969; Koslow and Stumpf, 1974). Also, using immunohistochemical techniques, Hokfelt et al. (1974) have provided evidence for the existence of adrenaline neurones in the brain, although their contribution to central function is as yet unknown.

There are two major enzymes involved in the termination of action of released catecholamines, catechol O-methyl transferase and monoamine oxidase (Fig. 4, Fig. 5). Catechol O-methyl transferase catalyzes the transfer of a methyl group from S-adenosylmethionine to the 3-hydroxy group of catechol compounds, and requires magnesium or other divalent cations for this activity. It is found in the cytoplasm of most animal tissue, being particularly abundant in liver and kidney. The precise cellular localization of the enzyme is not known, although it has been suggested to function extraneuronally. Monoamine oxidase brings about the oxidative deamination of a variety of amines, including catecholamines, to form an aldehyde. The aldehyde intermediate is rapidly metabolized by oxidation or reduction to the corresponding acid or alcohol. In brain, if the initial substrate contains a β -hydroxyl group, e.g. NA, then the favoured route of metabolism of the aldehyde is reduction to an alcohol, with the end-product being a glycol. Monoamine oxidase is a mitochondrial enzyme and is thought to be located between the outer and inner membranes of mitochondria. Because of this localization, the enzyme is only able to act on intracellular monoamines if they are free in the cytoplasm, and not on amines which are bound in the amine storage vesicles. The two enzymes usually act in concert, with the 3-methoxy compounds formed from catecholamines by catechol O-methyl transferase being substrates for monoamine oxidase, while the catechols with alcoholic or acid side-chains produced by monoamine oxidase are acted on by catechol O-methyl transferase.

Fig. 4. Catabolic Pathways for Noradrenaline

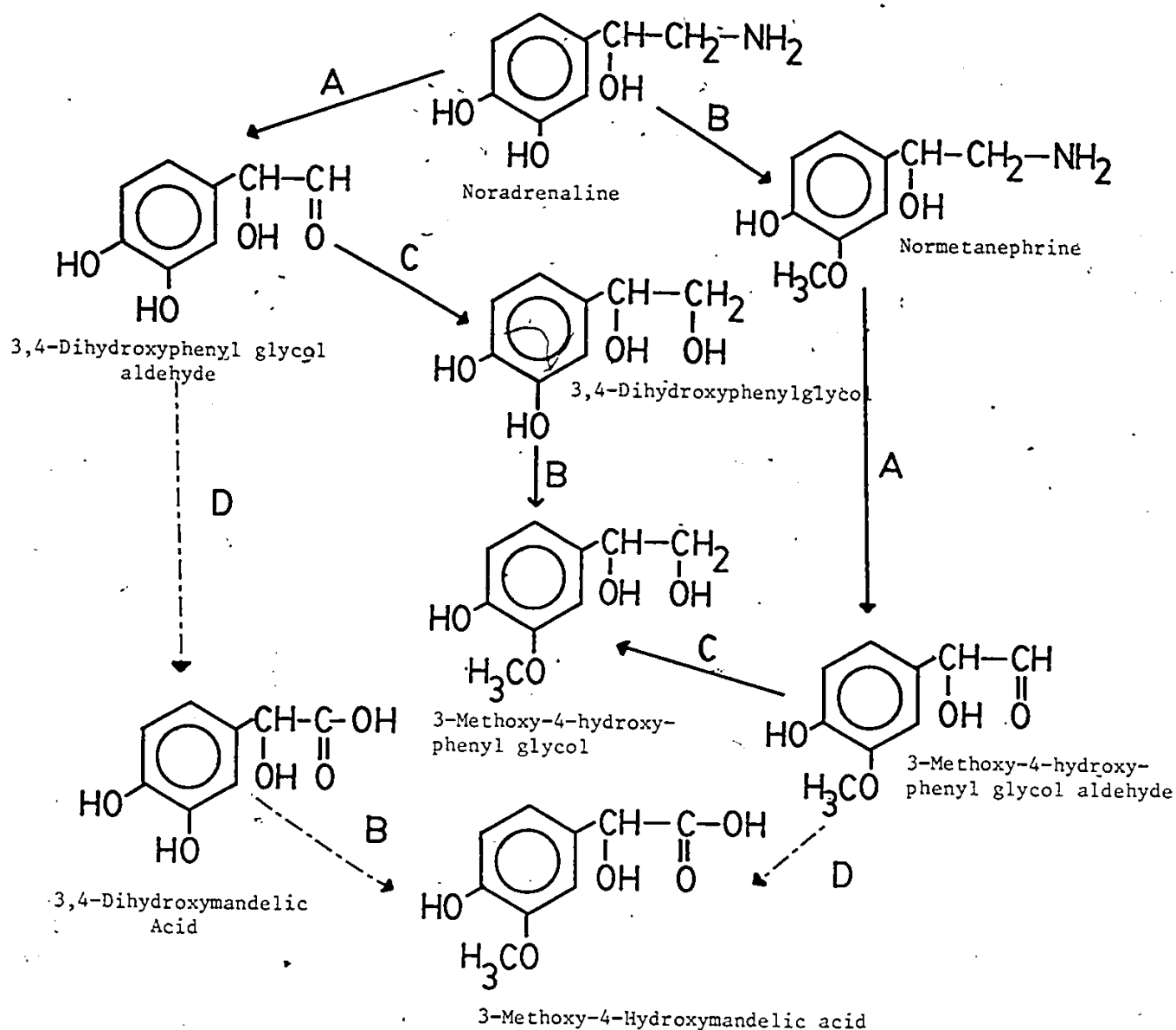
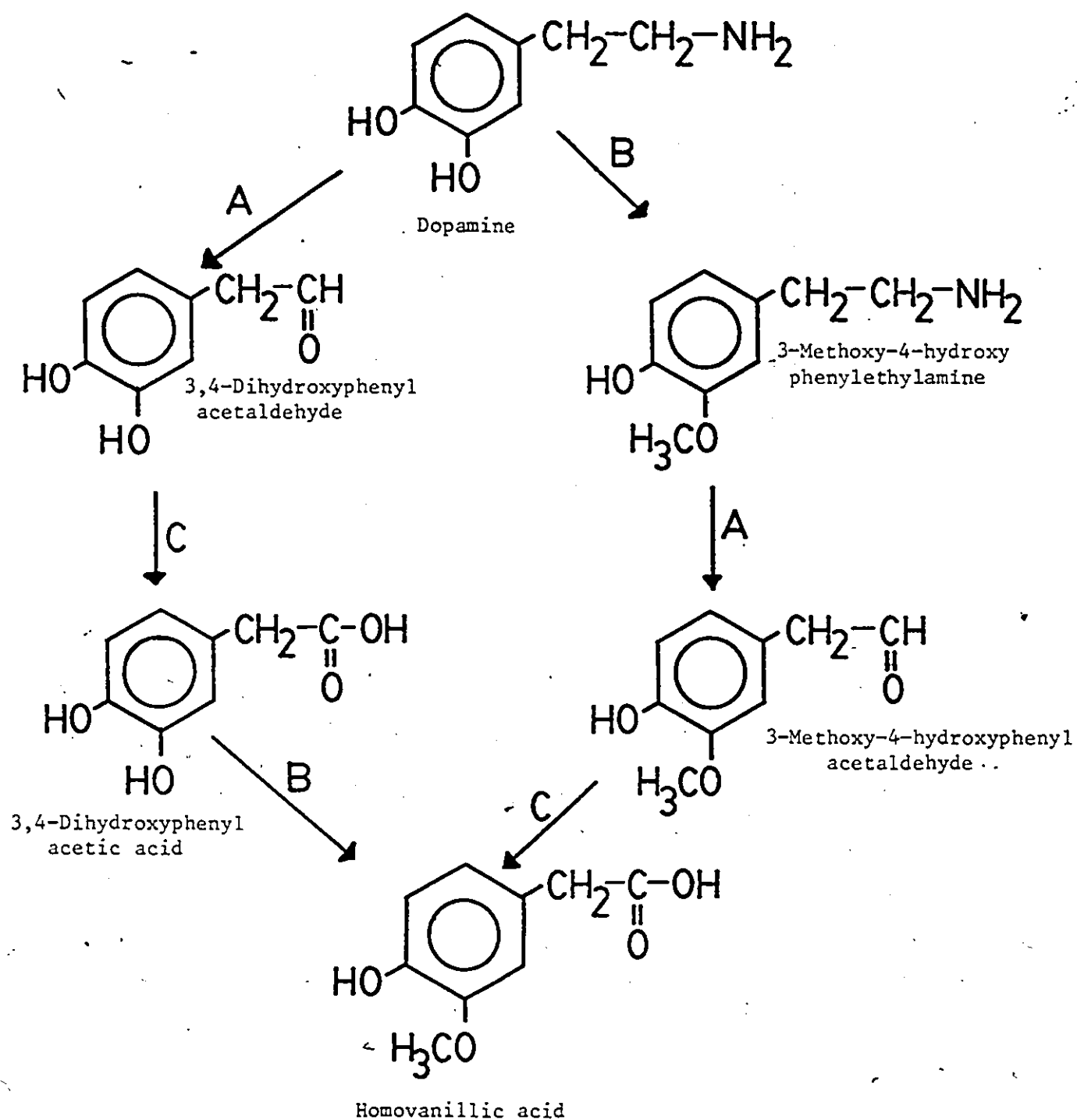


Fig. 5. Catabolic Pathways for Dopamine



In addition to enzymatic degradation, physical processes take place which terminate the action of released neurotransmitter. The neurotransmitter is removed from the synaptic cleft by diffusion away from the receptor sites and subsequent absorption into the circulation, or by an active re-uptake of the catecholamine through the presynaptic membrane into neurones, where it can either re-enter the storage granules or undergo enzymatic metabolism (Fig. 6). The re-uptake process is considered to be more important for the termination of monoamine transmitter function than is enzymatic degradation. While all parts of the neurone have the ability to take up catecholamines across the neuronal membrane, the process is functionally more important at axonal terminals because the terminals are the site of release and hence reuptake, and are in close proximity to the sites of action of the neurotransmitter.

ii) 5-Hydroxytryptamine. The precursor for 5-HT synthesis is the amino acid tryptophan, which is obtained through the diet. Tryptophan is hydroxylated at the 5-position to form 5-hydroxytryptophan (Fig. 7), the enzyme responsible being tryptophan hydroxylase. Like tyrosine hydroxylase, tryptophan hydroxylase utilizes tetrahydropteridine as a co-factor and seems to provide the rate-limiting step in the biosynthesis of 5-HT. In contrast to tyrosine hydroxylase, tryptophan hydroxylase does not appear to be associated with any subcellular organelle, but appears to exist as a soluble cytoplasmic enzyme. Following its synthesis, 5-hydroxytryptophan is decarboxylated to 5-HT by L-aromatic amino acid decarboxylase, the same enzyme responsible for the formation of DA from DOPA.

The inactivation of 5-HT in many ways resembles the termination of action of the catecholamine neurotransmitters. The principal enzyme concerned with its degradation is monoamine oxidase, which causes the oxidative deamination of 5-HT to 5-hydroxyindole-3-acetaldehyde. This compound is then rapidly converted to either 5-hydroxyindole-3-acetic acid (5-HIAA) or to 5-hydroxytryptophol, the product formed being dependent upon the relative abundance of NAD and NADH. However, under normal conditions, the major metabolite is 5-HIAA (Elliot et al.,

Fig. 6. Simplified Schema for the Synthesis and Inactivation of Dopamine

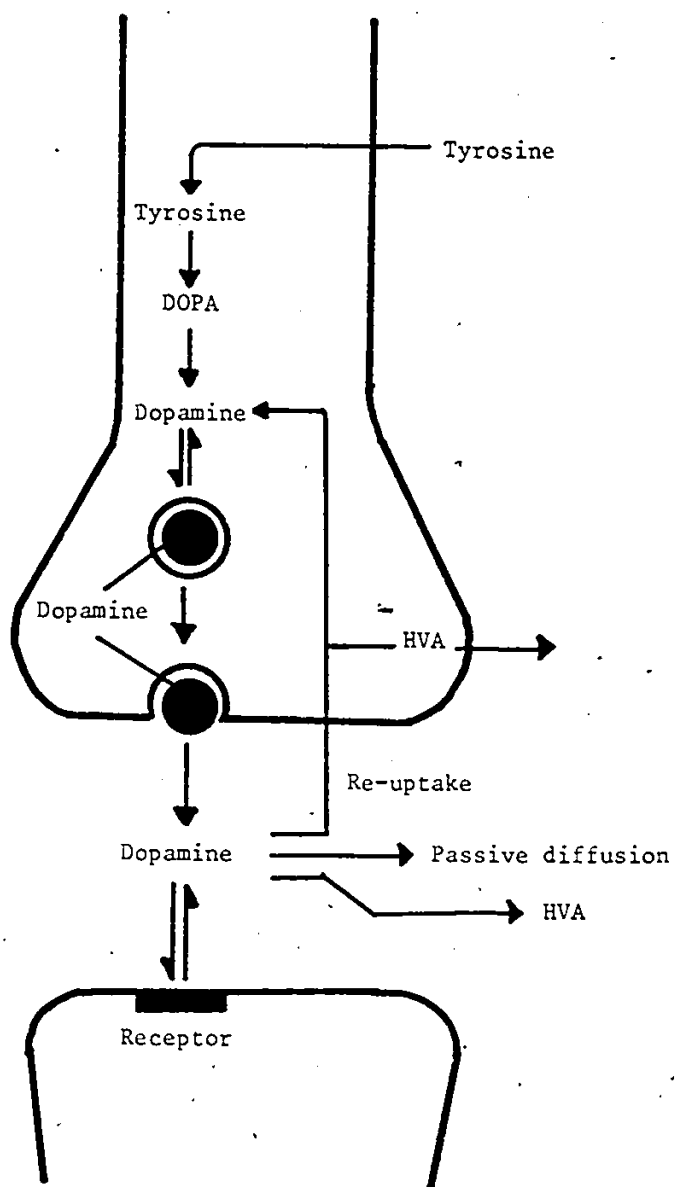
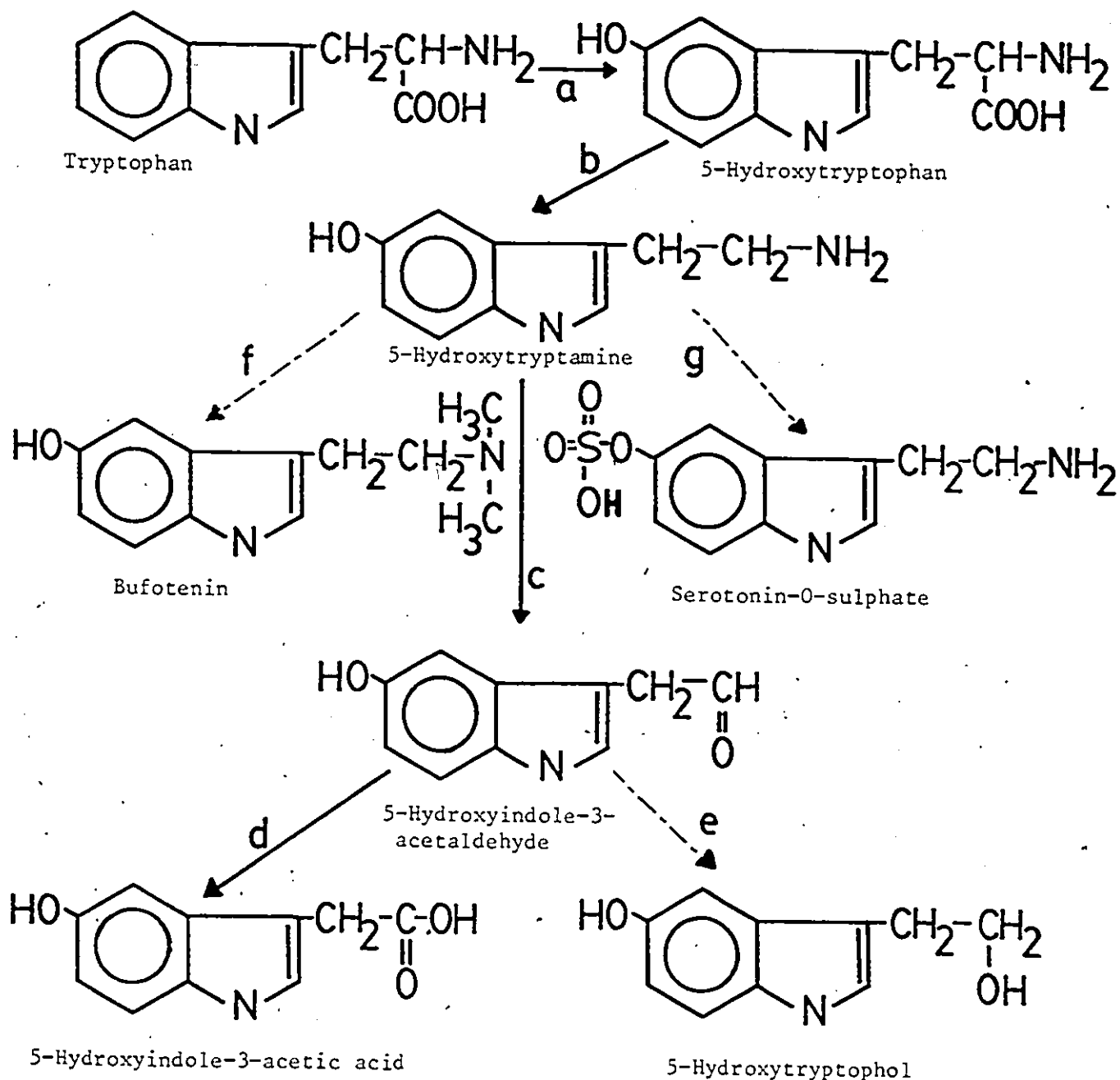


Fig. 7. Biosynthetic and Catabolic Pathways for 5-Hydroxytryptamine



→ Major pathways

- a. Tryptophan hydroxylase
- b. 5-Hydroxytryptophan decarboxylase
- c. Monoamine oxidase
- d. Aldehyde dehydrogenase

- e. Aldehyde reductase
- f. Serotonin N-methyl transferase
- g. Serotonin sulphotransferase

1977). In addition to this major catabolic pathway, it has been suggested that formation of a 5-sulphate ester of 5-hydroxytryptamine may be an important metabolic pathway, as may the N-methylation of the neurotransmitter.

In keeping with the similarities to the inactivation of catecholamines, physical processes are probably also important in terminating the action of 5-HT, and so the schema for DA depicted in Fig. 6 (without the involvement of catechol-O-methyl transferase) may well be applicable to 5-HT also.

(b) Neurochemical changes due to lithium treatment

(i) Noradrenaline. The influence of lithium treatment on various processes associated with central noradrenergic function has been examined over the years, often providing conflicting data. Corrodi *et al.* (1969) examined the disappearance of NA from the brain following the inhibition of tyrosine hydroxylase with H44/68 (α -methyl-tyrosine methyl ester). Three weeks of dietary lithium did not influence the depletion of NA from rat brain following inhibition of the synthetic enzyme, nor did it cause any change in the steady-state brain concentration of the monoamine. Schubert (1973) administered lithium in the diet for 7 days and examined the accumulation and disappearance of labelled catecholamines in rat brain after intravenous infusion of (14 C)tyrosine. He demonstrated that accumulation of labelled NA was not different in lithium-treated animals when compared to controls, while the levels of endogenous tyrosine and NA were in the same range in different treatment groups. The rate of decline of labelled neurotransmitter was also unchanged by lithium administration. Segal *et al.* (1975) examined the activity of tyrosine hydroxylase in the cell-body and nerve-ending regions of the dorsal noradrenergic pathway (i.e. the locus coeruleus and hippocampus-cortex) and found that eight daily injections of lithium chloride (1.5 mEq/kg) did not affect the activity of this enzyme in these regions. These data would tend to suggest that chronic lithium treatment does not affect the biosynthesis and depletion of NA.

In contrast to these reports however, Corrodi et al. (1967) found that administration of acute doses of lithium (up to 15 mEq/kg) resulted in a greater depletion of NA in the presence of H44/68 than that produced by the inhibitor alone. They therefore suggested that since the effect appeared to be correlated with the amount of lithium in the brain, it may simply be a local effect of lithium on the nerve terminals. Alternatively, the presence of lithium may result in higher activity of noradrenergic neurones, a hypothesis which was not supported by the data from their chronic study (Corrodi et al., 1969). Schildkraut et al. (1969a) examined the effects of both acute and chronic lithium administration in rats. Tritiated NA was injected intracisternally and followed (60, 120 and 180 min later) by intraperitoneal lithium chloride (2.4 + 1.2 + 1.2 mEq/kg), with the animals being sacrificed 270 min after the intracisternal injection. It was found that the levels of labelled NA and normetanephrine were lower in the brains of lithium-treated rats than in control animals, while the concentrations of tritiated deaminated catechol metabolites were higher, suggesting an increased turnover of NA and an enhanced rate of deamination. In the chronic study, lithium chloride was administered intraperitoneally (2.4 mEq/Kg twice daily) for 7 days. Labelled NA was injected 3.5 hr after the last drug treatment. These authors found that the levels of tritiated NA were significantly less in lithium-treated rats than control animals 150 min after the intracisternal injection, although 6 min after administration of the labelled catecholamine brain levels were not significantly different in the two treatment groups. Increased amounts of tritiated deaminated catechol metabolites and free deaminated O-methylated metabolites were noted in the lithium-treated rats relative to controls. These data suggest that acute and chronic lithium administration increases the turnover of NA in brain, and produces alterations in the metabolism of the amine such that deamination is increased. The effect of lithium on the turnover rate of NA was also investigated by Stern et al. (1969), who gave four injections of 3.75 mEq/kg lithium chloride (48, 38, 24 and 14 hr prior to studying turnover).

They determined that while the whole brain steady-state levels of the neurotransmitter were unaffected by lithium treatment, the turnover rate of NA was increased by 95%, while the turnover rate in heart was not altered significantly. Greenspan et al. (1970) administered lithium carbonate (1, 2 and 3 mEq/kg daily) for 10 days, and then injected (^3H)NA intraventricularly. At various times following the administration of the labelled neurotransmitter, rats were sacrificed and the levels of tritiated NA and its metabolites measured. At the highest lithium dose used, the rate of NA turnover was more than twice that of control, while a smaller increase (approximately 40%) was produced by 2 mEq lithium/kg. No change was found in the animals treated with 1 mEq lithium/kg. None of these treatment regimes caused a change in the amount of radioactivity present as deaminated O-methylated metabolites of NA in lithium-treated rats as compared to controls (cf. Schildkraut et al., 1969a). However, in contrast to Schildkraut et al. (1969a), Greenspan et al. (1970) found no change in the amount of labelled normetanephrine present in the brains of control and lithium-treated rats. Poitou and Bohuon (1975) used both short- and long-term lithium administration, and found no alteration in NA levels following either treatment regime. It is noteworthy that these authors did find that after 5 days of lithium treatment (2 mEq/kg, twice daily) there was a two-fold increase in the rate of synthesis of NA indicating an enhanced turnover rate, while after 15 days of treatment (2 mEq/kg daily), there was no modification of NA metabolism. It was therefore suggested that the effects of lithium on catecholamine metabolism are time-dependent.

There is, however, evidence that lithium does not induce an increase in NA turnover. Ho et al. (1970) treated rats for 28 days with lithium chloride (2 mEq/kg) and in accordance with other investigators (Corrodi et al., 1967; Stern et al., 1969; Greenspan et al., 1970; Poitou and Bohuon, 1975) found that chronic lithium treatment had no effect on NA levels in any of the brain regions examined. However, when rates of NA turnover were measured, a decrease was found

in the hypothalamus. Matussek and Muller (1975) also showed that following inhibition of tyrosine hydroxylase, there was a decrease in the rate of disappearance of NA from the brains of lithium-treated rats. Berggren et al. (1980) were also able to provide an alternate interpretation for the data obtained by Poitou and Bohuon (1975), when they determined that the effects of acute lithium on catecholamine synthesis, as measured by the accumulation of DOPA following inhibition of L-aromatic amino acid decarboxylase, were dose-dependent, resulting in a decrease in DOPA accumulation which suggests a decrease in catecholamine turnover. These authors (Berggren et al., 1980) also found that acute lithium treatment results in reduced tyrosine levels in both striatum and limbic fore-brain, which confirmed the data of Leonard (1975) who also noted decreased tyrosine content in brain after acute lithium administration. This decrease in central tyrosine levels has been suggested to be due to a decreased uptake of tyrosine by brain as a result of lithium treatment (Berggren et al., 1980). If this is so, then the change in tyrosine uptake must occur at the level of the individual neurones, since Ehrlich et al. (1980) have shown that neither acute nor chronic lithium administration affects the transport of tyrosine across the blood brain barrier.

Another aspect of noradrenergic function which has been investigated is that of neuronal re-uptake. Colburn et al. (1967) examined the uptake of NA into synaptosomes prepared from the brains of rats which had been treated with dietary lithium for 5 to 7 days. Synaptosomal accumulation of (³H)NA was measured using a single concentration of ligand (7 nM), and it was found that lithium pretreatment increased the net uptake of the catecholamine by approximately 30%. Administration of lithium by gastric intubation was used by Baldessarini and Yorke (1970), who treated rats with a daily dose of 6-8 mEq lithium/kg for 3 to 5 days, and then examined (³H)NA uptake into synaptosomes. Using substrate concentrations of 20-30 nM, they found a small increase in uptake (8-10%).

They further determined that when lithium (4 and 10 mM) was added to the incubation medium of synaptosomes prepared from control animals, there was a significant decrease in the amount of NA taken up by the synaptosomes (11 and 17%, respectively). Kuriyama and Speken (1970) prepared synaptosomes from the brains of mice treated for 5 days with lithium chloride (3.75 mEq/kg twice daily) and incubated them with 3 μ M (3 H)NA. In agreement with the previous authors, Kuriyama and Speken (1970) noted a small increase in synaptosomal uptake of NA, but in contrast to Baldessarini and Yorke (1970) found that addition of 2-10 mM lithium in vitro did not alter NA uptake. Cameron and Smith (1980) examined (3 H)NA uptake into brain slices obtained from rats treated with lithium, again using a single concentration of tritiated ligand (0.1 μ M). They found a time-dependent effect, with short-term lithium administration causing an increase in the amount of NA taken up, with a return to control values after long-term treatment. Katz et al. (1968) however, had previously shown that acute lithium administration (2.5 and 7.5 mEq/kg) did not alter the uptake of radiolabelled NA into tissue slices obtained from striatum, while Schildkraut et al. (1969a) had found no significant change in (3 H)NA uptake into brain in vivo after chronic administration of lithium chloride (2.4 mEq/kg twice daily for 7 days).

Slotkin and his associates examined the effects of lithium on vesicular uptake of NA. They determined that while in vitro lithium concentrations of up to 5 mM did not alter significantly the uptake of NA, concentrations of lithium ranging from 10 to 100 mM markedly inhibited NA uptake into synaptic vesicles (Slotkin et al., 1978). However, the relevance of this finding to the in vivo situation is not known, particularly in view of the high levels of the cation required to achieve the inhibition. The action of acute and chronic lithium administration on the storage and release of NA from synaptic vesicles was also investigated (Slotkin et al., 1980). It was found that both of these treatment regimes caused an impairment of vesicular retention of the catecholamine, resulting

in a more rapid spontaneous release than was seen in the vesicles prepared from control animals. This effect was not seen when lithium was added to the incubation medium in vitro (concentrations up to 100 mM). The possibility that lithium treatment could affect the storage capabilities of the central noradrenergic neurons, and hence reduce the effectiveness of central noradrenergic neurotransmission had previously been mentioned by Greenspan et al. (1970) and Kuriyama and Speken (1970). Since the storage of the neurotransmitter appears to be essential prior to release into the synaptic cleft, then a disruption in the storage capacity of the vesicles could explain the reduction in release of NA from brain slices of rats treated with lithium chloride following electrical stimulation (Katz et al., 1968). This early report seems to be in agreement with that of Slotkin et al. (1980), since Katz et al. (1968) had found that addition of lithium (up to 2 mM) to the perfusion medium did not alter the rate of spontaneous release of the catecholamine from control brain slices, although the stimulation-induced release of NA was reduced.

Alterations in adrenergic receptor populations as a result of lithium treatment have also been investigated. Rosenblatt et al. (1979) administered dietary lithium for 3 weeks, and then examined the β -adrenoceptor population in whole brain. They found a reduction in (^3H)dihydroalprenolol binding of 10-13%, which was attributed to a decrease in the number of specific binding sites. Similar changes were noted in cerebral cortex by Treiser and Kellar (1979), while Schultz et al. (1981) reported that chronic lithium treatment (75-85 mg lithium chloride/kg/day for 9 to 12 days) had no significant effect on (^3H)dihydroalprenolol binding in cerebellum. Rosenblatt et al. (1979) also investigated the binding of (^3H)WB-4101 to α -adrenoceptors in cerebral cortex. The observed alterations in adrenergic receptor populations appear to be adaptive changes to chronic in vivo administration of lithium, since Rosenblatt et al. (1979) had found that the addition of lithium to rat brain membranes in vitro (0.03-3.0 mM) did not affect binding to either α - or β -adrenoceptors.

(ii) Dopamine. The case for dopaminergic involvement in the central action of lithium is as contradictory as is that for NA. Schubert (1973) examined the accumulation and disappearance of DA formed from (^{14}C)tyrosine after 7 days of dietary lithium, and showed that neither the formation nor the rate of decline of the labelled catecholamine was altered by administration of this cation. In addition, lithium treatment did not affect the endogenous levels of the neurotransmitter or its metabolite, homovanillic acid (HVA). This observation that endogenous DA content is not altered by lithium treatment was in agreement with the findings of investigators using both acute (Friedman and Gershon, 1973) and chronic administration (Corrodi et al., 1969; Ho et al., 1970; Friedman and Gershon, 1973; Hesketh et al., 1978). Other groups however, did find changes in DA content. Corrodi et al. (1967) noted a slight decrease in DA levels following acute lithium administration, as did Leonard (1975). Poitou and Bohoun (1975) found a decrease in brain DA after 15 days of lithium treatment (2 mEq/kg daily), while Rastogi and Singhal (1977) demonstrated that lithium carbonate administration (60 mg/kg daily for 10 days) resulted in decreased DA levels in striatum only. Eroglu et al. (1981) reported increased levels of DA in striatum after 2 weeks administration of lithium chloride in drinking water, with no alterations in the tissue content of the neurotransmitter in frontal cortex.

Corrodi et al. (1969) also found that the decline in brain DA levels after tyrosine hydroxylase inhibition was less pronounced in animals chronically treated with lithium than in controls, suggesting a decrease in DA turnover. Data from Friedman and Gershon (1973) seem to support this observation since they found a significant decrease in striatal DA synthesis following 14 days of lithium administration (1 and 2 mEq/kg), as did Poitou and Bohoun (1975). In contrast, Segal et al. (1975) noted a significant increase in tyrosine hydroxylase activity in the dopaminergic nigro-striatal pathway after 8 days of lithium treatment (1.5 mEq/kg daily), while Hesketh et al. (1978) demonstrated increased concent-

rations of striatal HVA and 3,4-dihydroxyphenylacetic acid (DOPAC), but no alteration in tyrosine hydroxylase activity. Ho et al. (1970) however, found no significant changes in DA turnover in four brain regions following 28 days of lithium administration (2 mEq/kg).

Little is known regarding the effects of lithium on processes regulating the neuronal uptake of DA. One of the few reports regarding this aspect of lithium's action comes from Stefanini et al. (1976) who investigated both the in vitro and in vivo effects of lithium on synaptosomal uptake. When rat caudate nucleus synaptosomes were incubated with (^3H)DA (0.09 μM) in the presence of varying amounts of lithium (1-10 mM), it was found that the amount of radiolabelled catecholamine accumulated by the synaptosomes was significantly reduced. However, when rats were chronically treated with lithium chloride (2 mEq/kg twice daily for 20 days) and the uptake of (^3H)DA into caudate nucleus synaptosomes prepared from these animals examined, Stefanini et al. (1976) found that the amount of catecholamine taken up into the synaptosomes was 23% greater than that in the corresponding controls. They postulate that the enhancement of DA uptake following chronic treatment with lithium is an adaptive response to an initial inhibition of this process. Alternatively, it may be a response to alterations in other parameters of dopaminergic function.

The effects of lithium on dopaminergic receptors have been a subject of considerable interest in recent years, particularly in view of its potential utility in preventing neuroleptic-induced dopaminergic supersensitivity. Since this particular aspect of lithium's activity is more involved with the treatment and/or prevention of tardive dyskinesia, it will be examined later.

Pert et al. (1978) studied the binding of (^3H)spiroperidol to synaptic membranes prepared from the striata of chronically lithium-treated rats, and found no difference when compared to the control preparations. Similar observations were made by Rosenblatt et al. (1979) and Staunton et al. (1982). In contrast, Rosenblatt et al. (1980) later found that (^3H)spiroperidol binding

to striatal membranes was reduced following 14 and 21 days of dietary lithium (by 26.3 and 26.9%, respectively). These authors also showed that after cessation of lithium administration (^3H)spiroperidol binding returned to control values within 3 days, and that there was no evidence of a «rebound» effect with regard to dopaminergic receptors following lithium withdrawal.

(iii) 5-Hydroxytryptamine. The involvement of this neurotransmitter in the mechanism of action of lithium has been the subject of much investigation in recent years. In their early study of the effects of chronic lithium administration, Corrodi *et al.* (1969) found no change in the brain concentration of 5-HT, an observation confirmed by other authors (Schubert, 1973; Shaw and Ratcliffe, 1976; Collard, 1978a; Furukawa *et al.*, 1979). This is not a consistent finding however, as Ho *et al.* (1970) noted significant reductions in 5-HT concentrations of hypothalamus and brain stem following 28 days of lithium injections (2 mEq/kg daily), while Leonard (1975) found that acute lithium treatment (50 mg lithium chloride/kg, equivalent to 1.2 mEq lithium/kg) resulted in decreased transmitter content. In contrast, Perez-Cruet *et al.* (1971) noted a dose-dependent increase in brain 5-HT after 5 days of lithium administration, while Rastogi and Singhal (1977) found that 10 days treatment with lithium (1.6 mEq/kg) caused regionally variable changes in the central levels of the amine, with increased amounts relative to controls seen in pons-medulla, midbrain and striatum, but a decrease in cerebellum. Atterwill and Tordoff (1982) examined the sub-cellular distribution of 5-HT and found that lithium administration (3 mEq/kg twice daily for 3 days) did not alter the transmitter content of nuclear, crude synaptosomal or soluble fractions, nor did this treatment change the levels of 5-HT in either the cytoplasmic or vesicular fractions obtained from hypo-osmotically disrupted synaptosomes. Unfortunately, this last observation did not correspond well with that of Kuriyama and Speken (1970) who had previously reported a decrease in the synaptosomal content of 5-HT in preparations obtained from the brains of mice which had been treated with lithium for 5 days (3.75 mEq/kg twice daily).

In addition to detecting no change in central 5-HT, Schubert (1973) found no alterations in the endogenous level of its metabolite, 5-HIAA. This finding was supported by data from Grahame-Smith and Green (1974) who treated rats with lithium (3 mEq/kg twice daily for 3 days), and Shaw and Ratcliffe (1976), who administered lithium to mice (2 mEq lithium carbonate/kg twice daily for 3 days). At variance with these data are those of Leonard (1975) who noted a decrease in the brain concentrations of 5-HIAA. Perez-Cruet et al. (1971) however, noted elevated levels of the metabolite, as did Collard (1978a) who measured forebrain levels of 5-HIAA in rats after 10 days of lithium (0.75 mEq/kg daily), and Furukawa et al. (1979) who measured brain levels after acute lithium treatment. Rastogi and Singhal (1977) found that the changes in 5-HIAA concentration paralleled those of 5-HT.

Since alterations in the level of the transmitter substance and its metabolite may be indicative of an alteration in turnover, this has also been a subject of investigation. Corrodi et al. (1969) examined the depletion of 5-HT from rat brain after administration of a tryptophan hydroxylase inhibitor, H22/54 (α -propyl-dopacetamide) and found that the rate of disappearance of the amine was reduced in lithium-treated animals. Schildkraut et al. (1969b) injected radio-labelled 5-HT intracisternally into rats treated acutely with lithium, and in accord with the data of Corrodi et al. (1969), noted a slowing in the disappearance of the radio-active amine. These data suggest a reduction in the turnover of the neurotransmitter. Ho et al. (1970) measured 5-HT turnover following pargyline administration and found decreased turnover of the monoamine in several brain regions of lithium-treated rats, the exception being cerebellum, in which an increase was noted. However, Perez-Cruet et al. (1971) examined the disappearance of 5-HIAA after monoamine oxidase inhibition with pargyline and found that while the decline of brain levels of the metabolite was not influenced by prior lithium treatment, the rate of synthesis of 5-HT was increased by 61-82%. Similar data were obtained by Grahame-Smith and Green (1974) who inhibited monoamine oxidase with

both pargyline and tranylecypromine and noted increased turnover rates of approximately 70% after lithium treatment. Shaw and Ratcliffe (1976) found a significant increase in 5-HT accumulation in lithium-treated mice after pargyline administration, together with an elevation in the activity of 5-hydroxytryptophan decarboxylase, while Rastogi and Singhal (1977) have reported an increase in tryptophan hydroxylase activity as a result of chronic lithium treatment. Schubert (1973) has also shown that following the infusion of tritiated tryptophan, the accumulation of labelled 5-HT and its major metabolite was enhanced in lithium-treated rats. In addition, Schubert (1973) demonstrated an increase in the brain levels of tryptophan, a change which was also reported by Perez-Cruet et al. (1971) and by Singhal and Rastogi (1977). However, Grahame-Smith and Green (1974) found no alteration in central tryptophan concentration. The increase in the concentration of brain tryptophan could be due to either a decrease in 5-HT synthesis or an increase in tryptophan uptake. Knapp and Mandell (1975) showed that administration of lithium stimulated the uptake of tryptophan by striatal synaptosomes, and demonstrated that this increase was due to the high-affinity uptake system which is present in these organelles. In their examination of the conversion of tryptophan to 5-HT, Knapp and Mandell (1975) also noted a biphasic effect of lithium on serotonergic systems. There was an initial increase in 5-HT synthesis due to the stimulation of tryptophan uptake into striatal synaptosomes and conversion of the precursor to the neurotransmitter, followed some time later by a compensatory decrease in the activity of brain tryptophan hydroxylase. It was stated that these changes in the biosynthetic parameters of 5-HT served to stabilize serotonergic function. Swann et al. (1980; 1981) have also found an increase in synaptosomal uptake of tryptophan and, like Knapp and Mandell (1975), attributed this to the high-affinity uptake system. However, unlike Knapp and Mandell (1975), Swann et al. (1981) suggest that lithium does not cause a stabilization of the serotonergic system, but makes it more flexible by changing the responsiveness of tryptophan

uptake to changes in 5-HT utilization. This theory was proposed after experiments in which lithium-exposed rats were subjected to other drug treatments. Neither lysergic acid diethylamide (LSD) nor chlorimipramine altered striatal tryptophan uptake in control animals. However, after 5 weeks of dietary lithium, Swann et al. (1981) found that LSD (which decreases the firing rate of, and transmitter, efflux from, serotonergic neurones) decreased tryptophan synaptosomal uptake, while chlorimipramine (which increases 5-HT efflux but not serotonergic firing rate) caused an enhancement of tryptophan uptake. It therefore appears that lithium treatment results in changes in the tryptophan uptake process which renders it more sensitive to the synthetic requirements of neurones for the replacement of neurotransmitter stores.

It is possible that this change in precursor uptake is related to alterations in the re-uptake process for the neurotransmitter itself, such that if the 5-HT uptake process is impaired as a result of lithium treatment, then the neurone is obliged to synthesize more transmitter than would normally be generated in order to replace transmitter lost due to loss into the synaptic cleft. Kuriyama and Speken (1970) examined the uptake of radiolabelled 5-HT into synaptosomes prepared from mouse brain after 5 days of lithium treatment (3.75 mEq lithium/kg twice daily) and found a small, although non-significant, reduction in the amount of tritiated amine taken up by the synaptosomes after incubation with 2 μ M substrate. Collard (1978a) however, reported that after incubating synaptosomes prepared from rat forebrain after 10 days of lithium administration (0.75 mEq/kg) with 0.1 μ M (3 H)5-HT, there was no alteration in uptake of the neurotransmitter.

An alternative theory regarding lithium's effect on the serotonergic system is that it alters the ability of the neurones to store 5-HT. This follows from the findings of Grahame-Smith and Green (1974) and Collard (1978a) that chronic lithium treatment increases the intraneuronal deamination of 5-HT, and of Kuriyama and Speken (1970) who noted a decrease in the synaptosomal content of the monoamine. Collard (1978b) has also reported a decrease in the amount of 5-HT in

the cytoplasmic fraction of synaptosomes, together with an increase in the concentration of the metabolites of the neurotransmitter in this fraction after 10 days of lithium administration (0.75 mEq/kg daily), although vesicular concentrations of 5-HT were unaffected. Since monoamine oxidase activity is also not affected by lithium treatment (Shaw and Ratcliffe, 1976; Collard, 1978b; Guerri, 1982), the increase in deaminated metabolites of the monoamine must be due to the presence of elevated levels of «free» 5-HT in the cytoplasm. However, as neither the uptake of the neurotransmitter (Kuriyama and Speken, 1970; Collard, 1978a) nor the vesicular concentration of the amine are affected by lithium treatment (Collard, 1978b), the «free» 5-HT must come from some other source. One possibility is that the «free» amine which is acting as extra substrate for the monoamine oxidase is excess 5-HT synthesized by the neurone as a result of enhancement of the activities of the serotonergic synthetic enzymes (Shaw and Ratcliffe, 1976; Rastogi and Singhal, 1977). Alternatively, the «free» 5-HT may well be the amine which is no longer bound to a cytoplasmic binding site. Tamir and Huang (1974) have demonstrated the presence of a soluble cytoplasmic protein present in synaptosomes which has a high affinity for 5-HT, and whose regional distribution appears to parallel that of areas rich in the neurotransmitter. This protein, termed serotonin-binding-protein (SBP), displays a relatively high specificity, in addition to high affinity, for 5-HT and it has been suggested that it may function as a storage site for 5-HT in the cytoplasm (Tamir and Rapport, 1976). If SBP does function as a neurotransmitter storage site, it is possible that chronic treatment with lithium affects the protein, either by altering its chemical properties or by inhibiting its synthesis, and so causes an increase in the amount of 5-HT «free» in the cytoplasm available for deamination by monoamine oxidase. A decrease in the storage capabilities of the serotonergic nerve terminal could explain the finding of Katz et al. (1968) that acute lithium treatment 24 or 48 hr prior to sacrifice decreased the evoked release of 5-HT from rat striatal slices. Further evidence for a

disruption in 5-HT storage comes from Weilosz and Kleinrok (1976) who noted head-twitch responses in rats within 15-20 min of lithium chloride administration (150 mg/kg, equivalent to 3.5 mEq lithium/kg). The effect was transient, and may reflect an initial disruption of transmitter storage resulting in an overflow of 5-HT onto receptors from the presynaptic neurones.

If a disruption of the storage mechanism of 5-HT does occur due to lithium administration, it is possible that in order to compensate for reduced amounts of transmitter in the synaptic cleft the serotonergic receptors could become supersensitive. This, when coupled with the lithium-induced reduction in 5-HT release, may result in a situation where normal-function is little affected, but large transient increases or decreases in activity in serotonergic pathways are buffered. This theory was proposed by Harrison-Read (1978), based on data from a number of behavioural studies and appears to be supported, at least in part, by the findings of Carruba et al. (1979) who demonstrated that prolonged blockade of serotonergic neuronal transmission in the central nervous system may well result in receptor supersensitivity. However, a degree of doubt is cast on this hypothesis by Maggi and Enna (1980) who reported that receptor binding saturation studies following chronic lithium administration (21 days of dietary lithium) resulted in a significant decrease in 5-HT binding to hippocampal and striatal membranes, which was due to a loss of binding sites with no change in the affinity of the receptor. This decrease in binding was also observed by Treiser and Kellar (1980) following 4 or 6 weeks of dietary lithium. These data would tend to suggest that rather than causing a supersensitivity of the serotonergic receptors, lithium treatment in fact results in a receptor subsensitivity.

As is obvious, despite extensive research regarding the effects of lithium on central monoaminergic neurotransmitter systems, there is little uniformity in the data obtained, leaving the role of these substances in the mechanism of action of lithium unresolved.

D. TARDIVE DYSKINESIA

1. Introduction

In the late 1950's, two groups of European physicians reported the occurrence of late and persistent dyskinesias following prolonged treatment with phenothiazines (Delay et al., 1959; Sigwald et al., 1959). These were followed in the early part of the next decade by a number of reports describing the occurrence of similar persistent dyskinesias, nearly always among chronic psychiatric patients, in the course of treatment with phenothiazine antipsychotic agents.

Uhrbrand and Faurbye (1960) examined a group of patients being treated with chlorpromazine for a variety of disorders, and documented the course of the dyskinesias which developed. The dyskinesias were found to persist during the treatment periods and also following termination of therapy. Uhrbrand and Faurbye (1960) called the syndrome a bucco-linguo-masticatory dyskinesia, and described the symptoms as «consisting of incessant involuntary munching and masticulatory movements of the jaw during which the tongue is protruded at short intervals with vigorous grimaces of the lips. In the most serious cases, there are also rocking and torsionary body movements and incessant tripping and shuffling movements so that the patient can not stand still.»

This bucco-linguo-masticatory syndrome was to be later dubbed tardive dyskinesia by Faurbye et al. (1964) and was said to generally occur only after treatment with anti-psychotic agents for a long period of time, was seldom seen during the first 6 months of treatment and may occur even after several years of symptom-free treatment. In a review of the syndrome, Baldessarini and Tarsy (1979) stated that various studies strongly support an association between the use of neuroleptic drugs and the development of persistent or relatively transient forms of tardive dyskinesia. While there also appears to be evidence that prolonged exposure, or exposure to large total amounts of drug may be a contributing factor, there is no definitive correlation with the size of average doses. Tepper and

Haas (1979) carried out an examination of epidemiological studies, as did Kane (1982) and consistently noted a higher incidence of tardive dyskinesia in the elderly and in females, with no other conclusive predisposing factors for the syndrome. Prevalence of the dyskinesia was estimated at between 24-56% of chronic neuroleptic users.

2. Pathophysiology and Aetiology of Tardive Dyskinesia

(a) Neuropathological studies

Since tardive dyskinesia is prolonged and frequently irreversible, it is possible that permanent structural alterations of the brain may be responsible for the disorder. Various studies have been carried out to determine whether these alterations are morphological in nature. Mackiewicz and Gershon (1964) treated guinea-pigs with chlorpromazine (10 mg orally/day) for periods ranging from 4-13 weeks. At the end of each treatment period, the animals were sacrificed and the brains examined histologically. Changes in morphology were noted, predominantly after the longer treatment regimes (i.e. 11 and 13 weeks). These were found to consist of vacuolization of nerve cells in the anterior part of the brain, hyperplasia of the cellular elements of the capillaries, mobilization of microglia and the presence of large numbers of these elements about the reticular formation, while in the cerebellum, there was a fall-out of Purkinje cells and a marked glial reaction along the lines of the Purkinje cells. From their data, Mackiewicz and Gershon (1964) concluded that the area of brain most affected by the chronic chlorpromazine treatment was the reticular formation of the medulla oblongata. They further suggested that these changes may not be totally irreversible, while the focal destruction in the granular layer of the cerebellum was irreversible.

Other studies have disagreed with these authors as to the site of possible damage. Christensen et al. (1970) carried out a neuropathological investigation of 28 brains from patients who had tardive dyskinesia at the time of death. The most conspicuous differences between the brains from dyskinetic subjects and those from controls were cell degeneration in the substantia nigra, and gliosis

in the midbrain and brain stem. Pakkenberg et al. (1973) examined the effect of perphenazine in rats. The animals used were 3 months old at the start of the study and were administered perphenazin enanthate every second week. The treatment period was 1 year and each animal received a total of 30.9 mg of perphenazine enanthate. At the end of this time, the brains of these rats were examined and Pakkenberg et al. (1973) found that the number of nerve cells in the basal ganglia were significantly reduced in neuroleptic-treated rats, while there was little difference in the cortices of treated and control animals. On histological examination of sections from these brains, no difference was seen between the two groups, either with respect to the morphology of the nerve cells or to the placing of the glial cells. Peripheral tissues were found to be normal in all cases. Fog et al. (1976) also investigated the effects of perphenazine, but used a different treatment strategy. In this later study, Fog et al. (1976) administered 40 mg perphenazine enanthate/kg every second week to rats for a period of 6 months. When cell counts were carried out, there was no difference between treatment groups in the number of cells in either basal ganglia or cortex, and electron microscopy of the corpora striatum showed no morphological differences between control and treated animals. These data were different to those obtained by Pakkenberg et al. (1973). Since the treatment period in the second study was only 6 months, as compared to one year in the first, Fog et al. (1976) proposed that the time factor may be more important than the dose. Also, the animals were said to have been younger at the time of sacrifice in the latter study than in the former. However, it appears that the findings from the two studies are in accordance with the clinical reports that the neurotoxic effects of neuroleptics are seen primarily in elderly patients after many years of constant neuroleptic treatment.

Since the introduction of computerized axial tomography scanning, it has been possible to carry out non-invasive radiological examination of the ventricles and surrounding tissues, so that it is possible to examine patients suffering

from the dyskinetic syndrome. Such a study was carried out by Gelenberg (1976), who examined 8 patients aged 17 to 56, all of whom were diagnosed as suffering from tardive dyskinesia following exposure to a variety of neuroleptics. Normal scans were observed in 7 of the subjects, while in the eighth, a 40 year old woman who had been taking phenothiazines for 16 years, a mild generalized cortical atrophy was seen.

None of these investigations provided convincing proof of gross morphological changes resulting from prolonged neuroleptic use, although Christensen et al. (1970) and Pakkenberg et al. (1973) have indicated alterations in cell numbers in the basal ganglia, while Mackiewicz and Gershon (1964) and Christensen et al. (1970) have suggested that the morphology of brain stem may be affected. Gelenberg (1976) therefore proposed that if a brain lesion is indeed responsible for the development of tardive dyskinesia, it may be in the form of a very fine lesion. Alternatively, there may be no morphological lesion underlying the syndrome, but rather a biochemical change, such as neuronal supersensitivity to DA.

(b) Behavioural studies

The possibility that the dyskinetic syndrome may be related to dopaminergic supersensitivity was suggested by Klawans (1973), and is based on the fact that chlorpromazine and other neuroleptics exhibit DA receptor blockade as one of their major properties. Therefore, by causing a blockade of these receptors, neuroleptics may produce a «chemical denervation» of the DA receptors, particularly in the striatum where there is a profuse dopaminergic innervation. If the blockade is complete, the receptor is unable to receive its normal neurotransmitter input, and so denervation supersensitivity may result after prolonged blockade. Many investigations, both clinical and animal, have been carried out to determine whether such a hypersensitivity does exist following neuroleptic treatment, and whether the tardive dyskinesia syndrome is a manifestation of dopaminergic over-reactiveness.

(i) Clinical investigations. Kazatmatsuri et al. (1974) examined the effects of haloperidol, a DA receptor blocking agent, and tetrabenazine, a central amine depleting agent, in chronic psychotic in-patients suffering from tardive dyskinesia. After discontinuation of their primary medication, the subjects were treated with one of the test drugs for 18 weeks. It was found that the 7 patients receiving haloperidol displayed a reduction in the frequency of oral dyskinesia which was most evident in the first 2 weeks. Those subjects receiving tetrabenazine also had a reduction in dyskinetic symptoms, although it did not appear to be as effective as haloperidol in suppressing abnormal movements. Bateman et al. (1979) also investigated the effects of haloperidol in tardive dyskinesia, and in addition, examined metaclopramide, a DA receptor blocker considered to be ineffective as an anti-psychotic. Intravenous administration of both of these agents was found to improve the dyskinetic symptoms displayed, although the authors considered the dose of metaclopramide required (40-mg) to have been high. These findings, together with reports indicating that other agents which interfere with dopaminergic function, e.g. clozapine, phenothiazines, pimozide, reserpine, α -methyldopa, α -methyltyrosine, have an ameliorating effect on tardive dyskinesia (Gerlach et al., 1974; Tarsy and Baldessarini, 1976; Carroll et al., 1977) helped to further advance the dopaminergic hypersensitivity theory.

If a post-synaptic supersensitivity situation does exist, then DA agonists should exacerbate the dyskinetic symptoms. Smith et al. (1977) tested this hypothesis by administering a directly acting agonist, apomorphine, and an indirectly-acting agonist, d-amphetamine, to patients with tardive dyskinesia. Apomorphine did not increase the dyskinetic symptoms in these subjects, and in fact caused a reduction in dyskinesia in some patients. In contrast, d-amphetamine produced an increase in tardive dyskinesia in most subjects. Similar results were reported by Carroll et al. (1977). The fact that d-amphetamine administration caused a worsening of the dyskinetic symptoms seems to support

the hypersensitivity theory, although the paradoxical responses to apomorphine reported by these two groups (Carroll et al., 1977; Smith et al., 1977) would tend to be in conflict with the hypothesis. In order to explain these unexpected effects with apomorphine, it was suggested that apomorphine at the doses used (2-6 mg s.c. - Carroll et al., 1977; 0.75-6 mg s.c. - Smith et al., 1977) may have had a preferential effect at the presynaptic DA autoreceptors, thus inhibiting the release of the neurotransmitter. It was not possible for either of these groups of investigators to examine higher doses of apomorphine to produce a post-synaptic response due to the emetic effect of the drug.

Smith et al. (1977) also used a neuroendocrine approach to the investigation of the dopaminergic hypersensitivity theory. They proposed that since DA plays a role in the modulation of prolactin and growth hormone release (Frohman and Stachura, 1975), the stimulation of supersensitive DA receptors in patients with tardive dyskinesia should result in a greater decrease in prolactin and a greater increase in growth hormone levels than would be seen in control subjects. It was found that apomorphine did not produce greater neuroendocrine responses than were noted for controls. Smith et al. (1977) suggested that this does not provide evidence for the lack of dopaminergic hypersensitivity, but simply reflects the choice of an inappropriate indicator, since the receptors influencing the release of prolactin and growth hormone are part of the tubuloinfundibular DA system, and so are distinct from the nigrostriatal DA pathway which modulates motor control. It is therefore reasonable to accept these data as showing that repeated neuroleptic administration may have regionally variable effects in the brain.

In addition to the evidence indicating a dopaminergic hypersensitivity in tardive dyskinesia, there are reports which suggest that a cholinergic hypersensitivity may exist in this syndrome (Gerlach et al., 1974; Tarsy and Baldessarini, 1976; Jeste and Wyatt, 1979; de Montigny et al., 1979; Ansell, 1981), although this possibility has been less well explored due to the predominance of the DA supersensitivity theory.

(ii) Animal studies. If tardive dyskinesia is indeed related to enhanced activity of DA at striatal dopaminergic receptors, then an animal model of the disorder should also be based on striatal dopaminergic function (Klawans et al., 1977). For this reason, many investigators have carried out studies in animals using amphetamine- and apomorphine-induced stereotypical behaviour. Rubovits et al. (1973) examined stereotyped behaviour in guinea-pigs after amphetamine administration and found that chronic pretreatment with chlorpromazine resulted in a reduction in the threshold for amphetamine-induced stereotypy. A similar reduction in the threshold to apomorphine-induced stereotypy was noted by Tarsy and Baldessarini (1974). This enhancement of stereotypical response after apomorphine administration to chronically neuroleptic-treated animals has been observed not only in rats (Tarsy and Baldessarini, 1974; Smith and Davis, 1976; Clow et al., 1979; Tye et al., 1979), but also in monkeys (Barany and Gunne, 1979), and has also been noted after challenge with DA applied to the nucleus accumbens or corpus striatum (Jackson et al., 1975).

It has also been found that this change in receptor sensitivity is a relatively long-lasting phenomenon. Rubovits et al. (1973) determined that the reduction in the amphetamine-induced stereotypy threshold was maintained for up to 4 weeks after discontinuation of neuroleptic administration in guinea-pigs, while the magnitude of the stereotypical response to apomorphine challenge was also found to be maintained for some weeks after cessation of neuroleptic treatment (Tarsy and Baldessarini, 1974; Smith and Davis, 1976; Tye et al., 1979). In addition, Smith and Davis (1976) noted that rats withdrawn from chronic thioridazine or clozapine displayed an increase in spontaneous locomotor activity for up to 10 days after withdrawal from neuroleptic treatment.

These data all provided evidence for an enhanced DA receptor sensitivity after long-term treatment with and withdrawal from neuroleptics, and therefore appeared to provide a useful animal model for the investigation of the biochemical

disruption which occurs following prolonged administration of antipsychotic agents. However, these investigators appeared to assume that dopaminergic hypersensitivity was the only cause of the dyskinetic syndrome. Barany and Gunne (1979) examined agents which are active on other neurotransmitter systems in Cebus monkeys which had received chronic haloperidol treatment. In addition to determining that neuroleptics alleviated the tardive dyskinesia symptoms, they found that both apomorphine and L-DOPA produced a degree of depression of the dyskinetic signs, and this was attributed in both cases to stimulation of dopaminergic presynaptic inhibitory receptors. The central cholinergic agonists examined, deanol and RS86, both reduced tardive dyskinesia, lending support to the argument for cholinergic hypofunctioning. Drugs active within the serotonergic system were tested in this model of tardive dyskinesia and it was found that 5-hydroxytryptophan caused a profound reduction in dyskinesia, whereas cyproheptadine, which has 5-HT antagonist properties, caused an increase in symptoms, suggesting that the serotonergic system may also play a part in this disorder.

(c) Biochemical studies

Since the dopaminergic receptor hypersensitivity hypothesis of tardive dyskinesia has tended to dominate thought regarding this essentially iatrogenic disorder, the majority of studies carried out to determine the underlying biochemical dysfunction have focused on alterations in DA receptor populations. The first group to demonstrate a change in these receptors were Burt et al. (1977), who examined the binding of (³H)haloperidol to membranes obtained from the corpus striatum of rats chronically treated with neuroleptics. They observed that treatment with haloperidol, reserpine and fluphenazine (all of which have been used clinically as anti-schizophrenics) for 3 weeks, followed by a withdrawal period of 5-7 days, resulted in an enhancement of the specific binding of the radiolabelled ligand, whereas similar treatment with promethazine, a phenothiazine which lacks anti-schizophrenic activity, did not alter the amount of haloperidol binding to the membranes. Burt et al. (1977) further determined that the increased binding observed after

termination of haloperidol administration was due to an increase in the number of binding sites present, rather than to a change in the affinity of the receptors. Pert et al. (1978) also found increased binding to dopaminergic binding sites after chronic haloperidol using (^3H)spiroperidol as the ligand, and also attributed this to an increase in the number of sites with no change in affinity. Lai et al. (1981) examined tritiated spiroperidol binding to rat striatal membranes after chronic treatment with zotepine, thioridazine or haloperidol, and found that all three neuroleptics enhanced the concentration of binding sites, again with no alteration in the dissociation constant of the binding. After prolonged administration of haloperidol or sulpiride to rats, Jenner et al. (1982) noted increases in the specific striatal binding sites only, when binding measurements were carried out using (^3H)spiroperidol, (^3H)sulpiride or (^3H)N,n-propylnorapomorphine as the ligand.

Hitri et al. (1978) used an experimental design similar to that of Burt et al. (1977) in that rats were administered haloperidol for 14 days followed by 7 days of saline treatment. Dopaminergic binding sites in the corpus striatum and nucleus accumbens were then determined by measuring the binding of tritiated DA to membranes obtained from these brain regions. In accord with the data of Burt et al. (1977), Pert et al. (1978), Lai et al. (1981) and Jenner et al. (1982), Hitri et al. (1978) found an increase in the number of binding sites in striatum, but in contrast to these authors, Hitri et al. (1978) also noted a large increase in the binding affinity. No alterations in either the number of binding sites or the binding affinity was seen in nucleus accumbens. Hitri et al. (1978) attributed the difference in their data to that of Burt et al. (1977) to the different radiolabelled ligands used in the two studies. They suggested that their data provide a more accurate measure of the alterations which occur in the supersensitivity phenomenon following neuroleptic withdrawal since the natural agonist may well be more specific for DA receptors. This is particularly true

in light of the fact that haloperidol is thought to bind not only to DA receptors, but also to other sites, e.g. serotonergic, α -adrenergic, muscarinic, haloperidol (Hitri et al., 1978; Peroutka and Snyder, 1980), which could influence the data obtained using the antagonist. This argument could well apply to other studies which have utilized a DA antagonist as the binding ligand, as Peroutka and Snyder (1980) have demonstrated that many neuroleptics compete for binding sites associated with neurotransmitter substances other than DA. *

The majority of the studies described so far utilized neuroleptic treatment periods of 2-3 weeks, and it has been suggested that treatments of this duration may not accurately reflect the clinical dyskinetic syndrome since tardive dyskinesia tends to be manifested after several months, or even years, of neuroleptic administration (Owen et al., 1980), and often appears during the period in which the patient is receiving the medication (Clow et al., 1979). Some investigative groups have therefore examined changes in receptor populations resulting from treatment periods of some months. Clow et al. (1979) administered trifluoperazine to rats for a total of 6 months, and measured striatal DA receptors using (^3H)spiroperidol binding, at various times during this treatment period. An initial decrease in striatal ligand binding was noted (after 1 month's neuroleptic administration) due to decreased receptor affinity, while after 6 months of trifluoperazine treatment, total specific radioligand binding was greater than in age-matched controls with the change being due to an increased receptor affinity with no alteration in the number of binding sites. Similar results were said to have been obtained with thioridazine (Clow et al., 1979). Owen et al. (1980) treated rats with haloperidol for a period of 9 months following which (^3H)spiroperidol binding to striatal membranes was determined. It was found that there was an increase in the number of specific binding sites present, and this was also noted in animals allowed a 7-10 day drug withdrawal period at the end of the 9 month treatment schedule. When the dissociation constant for the (^3H)spiroperidol binding was calculated however, an increase in the dissociation

constant was seen only in the animals which had not received a drug-washout period. Owen et al. (1980) suggested that this alteration in affinity of the in vitro binding may be an artifact, and caused by the presence of the drug in the tissue from which the membrane fractions were prepared.

However, the effects of anti-psychotic neuroleptics are not confined to changes in receptor populations. In his review on the effects of neuroleptic drugs on central DA metabolism, Westerink (1979) has shown that there is considerable evidence for a pre-synaptic component to the action of these agents, although many of the changes in DA biosynthesis and catabolism observed following acute administration of these drugs may be regulated through DA receptors located both pre- and post-synaptically. Carlsson and Lindqvist (1963) had initially proposed that there was a strionigral loop which acted as an inhibitory feedback mechanism activated following occupation of post-synaptic DA receptors by an agonist, and that the enhancement of DA turnover following neuroleptic administration was due to blockade of the receptor sites by the antagonist which prevented activation of the inhibitory feedback mechanism. However, DiChiara et al. (1978) have shown that following intrastriatal administration of kainic acid, a glutamic acid analogue which damages receptors, haloperidol treatment stimulated the synthesis of DOPA, which would tend to imply that post-synaptic receptors are not an essential requirement for the neuroleptic-induced enhancement of DA turnover. The second possibility therefore, is that DA metabolism is regulated by the actions of the neurotransmitter at pre-synaptic autoreceptors, which have an inhibitory action on dopaminergic neurotransmission (Strombom, 1977; Westerink, 1979), and that by blocking the autoreceptors, neuroleptics stimulate DA turnover.

Unfortunately, the situation is further complicated by the fact that the effects of acute neuroleptic administration are different to the changes observed following chronic treatment, and it has been proposed that a tolerance develops

following long-term administration of these agents (Westerink, 1979; Casu et al., 1980; Meller, 1980; Nicolaou, 1980). Casu et al. (1980) administered haloperidol both acutely and chronically, and found that acute administration of the neuroleptic resulted in enhanced levels of DOPA and DOPAC in the caudate nucleus, together with an increase in the activity of tyrosine hydroxylase. Following chronic administration, the enhancement of DOPA and DOPAC content was not observed, although tyrosine hydroxylase still displayed an increased activity. Treatment with kainic acid did not alter tolerance development, suggesting that post-synaptic sites are not an important component to the development of the tolerance (Casu et al., 1980). Engel et al. (1980) also noted an enhanced activity of tyrosine hydroxylase following chronic treatment with penfluridol, but in contrast to Casu et al. (1980), found that there was still an increase in the accumulation of DOPA following inhibition of L-aromatic amino acid decarboxylase. The data of Engel et al. (1980) therefore suggest that the synthesis of DA is enhanced after chronic neuroleptic treatment, whereas those of Casu et al. (1980) appear to demonstrate that while the activity of the synthetic mechanism is increased, the actual rate of synthesis is not affected. A number of other studies have also noted that the increase in the striatal content of DA metabolites following chronic neuroleptic treatment is attenuated relative to the increase found after acute administration (Meller et al. 1980; Nicolaou, 1980; Stanley and Wilk, 1980), while Meller et al. (1980) and Nicolaou (1980) also determined that the enhancement of DA turnover seen in the substantia nigra following acute neuroleptic treatment was not attenuated following chronic administration, indicating a regional variation in the response to chronic neuroleptic administration.

It should be noted however, that although the turnover of DA in these studies was attenuated following chronic neuroleptic administration relative to acute treatment, it was still generally greater than was noted in controls. It is therefore possible that the dyskinetic symptoms seen following long-term neuroleptic use may be due to a combination of the pre-synaptic component of

neuroleptic action, i.e. increased synthesis and hence spillover of DA, coupled to the post-synaptic component, i.e. supersensitive DA receptors, rather than simply to hypersensitive post-synaptic receptors. This is particularly true in the case of dyskinesia noted following neuroleptic withdrawal, as it has been shown that not only is there a dopaminergic receptor hypersensitivity which exists for some time following neuroleptic withdrawal (Rubovits et al., 1973; Tarsy and Baldessarini, 1974; Smith and Davis, 1976; Tye et al., 1979), but there is still an enhanced neurotransmitter synthesis (Engel et al., 1980), which is suggestive of an elevated rate of DA turnover.

A limited number of investigations have been carried out examining the effects of the neuroleptic anti-psychotic agents on other monoamine neurotransmitter systems. Carlsson et al. (1977) administered haloperidol acutely to rats, and found that the drug caused no change in the brain content of tryptophan or 5-HT and did not alter the rate of tryptophan hydroxylation. Barkai (1979) examined 5-HT turnover in rabbits following acute administration of haloperidol. Turnover of the neurotransmitter was estimated from the rate of appearance of 5-HIAA in the cerebrospinal fluid compartment during ventriculocisternal perfusion. Barkai (1979) determined that haloperidol administration caused an almost two-fold increase in 5-HT turnover. Rastogi et al. (1981) noted that acute administration of either haloperidol or chlorpromazine to rats elicited an increase in the levels of 5-HT and its primary metabolite in the mid-brain together with enhanced activity of tryptophan hydroxylase, indicating an increase in transmitter turnover. Bacopoulos et al. (1982) however, found that acute haloperidol or fluphenazine treatment of the Vervet monkey did not alter the concentration of 5-HIAA in any brain region examined, while Boadle-Biber (1982) determined that haloperidol (0.5 mM) added to the perfusion medium of brain-stem slices in vitro did not affect the activity of tryptophan hydroxylase. Rastogi et al. (1981) further noted that chronic administration of either haloperidol or chlorpromazine to rats

caused pronounced increases in tryptophan hydroxylase, in conjunction with elevated levels of both 5-HT and 5-HIAA in various brain regions. In contrast, Bacopoulos et al. (1982) saw no significant alteration in the 5-HIAA content of any brain region of Vervet monkey after chronic treatment with either haloperidol or fluphenazine. However, there is a growing body of evidence indicating that the serotonergic neurotransmitter system has a modulatory effect on dopaminergic function (Balsara et al., 1979; Carter and Pycock, 1979; Waldmeier and Delini-Stula, 1979; Waldmeier, 1980; Fang and Shian, 1981), with the modulation taking the form of an inhibitory action. If there is indeed an increase in 5-HT turnover, as postulated by Rastogi et al. (1981), it is possible that this could be the cause of the dopaminergic tolerance to chronic neuroleptic administration. It is also possible that with a treatment period of sufficient duration, the serotonergic system could itself become tolerant to the effects of chronic neuroleptic administration. If this happens, then the inhibitory action of the serotonergic system on dopaminergic function could be reduced, with the disinhibition allowing the dopaminergic system to become more active causing a greater release of DA onto supersensitive dopaminergic receptors resulting in the appearance of dyskinetic symptoms.

With regard to the effects of neuroleptics on central noradrenergic function, Kizer et al. (1978) examined the turnover of catecholamines in hypothalamus following short-term haloperidol administration. It was found that while 4 days of haloperidol treatment increased the rate of decline in DA levels of the median eminence in treated rats following administration of α -methyl-p-tyrosine, there was no alteration in the disappearance of NA. These data however, do not correlate well with previous studies demonstrating increased turnover in brains of mice (Nyback and Sedvall, 1970) and rats (Anden et al., 1970; Bartholini et al., 1973), and in rat brain stem (Burki et al., 1975) following acute neuroleptic administration. The most probable explanation for this discrepancy is that Anden et al.

(1970), Nyback and Sedvall (1970), Bartholini et al. (1973) and Burki et al. (1975) studied the turnover of this catecholamine in gross brain regions, incorporating many noradrenergic pathways, whereas Kizer et al. (1978) used a discrete hypothalamic nucleus which contains a more limited number of noradrenergic terminals, which may be unaffected by neuroleptic treatment. It has also been found that the evoked release of NA from rat cerebral cortex, using as a stimulus either potassium (Arbilla et al., 1978) or electrical stimulation (Gross and Schumann, 1980), was enhanced in the presence of neuroleptics in vitro, which would appear to correlate well with the data showing enhanced NA turnover after acute neuroleptic administration.

In addition to examining the effects of neuroleptics on catecholamine turnover, Anden et al. (1970) determined the ratio of dopaminergic receptor blockade to noradrenergic receptor blockade. These authors determined that while some neuroleptics blocked only DA receptors, e.g. pimozide, others mainly blocked DA receptors, e.g. spiroperidol, haloperidol, fluphenazine, while a third group, e.g. chlorpromazine, thioridazine, blocked DA and NA receptors to the same extent. Anden et al. (1970) therefore suggested that noradrenergic receptor blockade by the neuroleptic drugs may cause a compensatory feedback which results in an activation of the presynaptic noradrenergic neurone, a theory which also appeared to explain the enhanced NA turnover noted by Bartholini et al. (1973) and Burki et al. (1975). With the advent of the hypothesis which linked stimulation of presynaptic α -adrenoceptors with inhibition of NA release (Langer, 1974), an alternative explanation for the neuroleptic-induced enhancement of NA turnover became possible, i.e. that by blocking presynaptic α -adrenergic receptors, the anti-psychotic neuroleptics enhanced the stimulated release of neurotransmitter, hence increasing NA turnover.

Marwaha et al. (1981) investigated the electrophysiological interactions of certain anti-psychotic drugs with cerebellar noradrenergic fibres and found that

acute administration of neuroleptics such as fluphenazine, haloperidol and α -flupenthixol were able to produce a complete blockade of noradrenergic neurotransmission, and they suggested that the neuroleptics therefore have a post-synaptic component to their mechanism of action. These authors (Marwaha et al., 1981) also determined that this effect was present after chronic administration of fluphenazine, implying that if a tolerance to the noradrenergic blockade does develop, the duration of the neuroleptic treatment period required is greater than is that necessary to observe dopaminergic function tolerance. Marwaha et al. (1981) also suggest that if their data were generalized to the central nervous system as a whole, then the mechanism of action of neuroleptic drugs involves the noradrenergic system in addition to the dopaminergic. They further propose that the functional consequence of a blockade of noradrenergic neurotransmission may be to diminish the sensitivity of post-synaptic neurones to other neurotransmitters, since NA increases the sensitivity of neurones to other afferent synaptic inputs. If this is so, then the increase in the number of DA receptors found after chronic neuroleptic administration may be an adaptive response to a decreased sensitivity of the post-synaptic neurone to neurochemical transmission, while the tardive dyskinesic symptoms seen may be due to a late developing tolerance of the noradrenergic system to the presence of the neuroleptic agent, resulting in a decrease in the blockade of noradrenergic neurotransmission.

It therefore appears that while the primary cause of tardive dyskinesia may be alterations in dopaminergic function, particularly in the basal ganglia, these changes may be modulated by neuroleptic-induced dysfunctions in other monoaminergic neurotransmitter systems.

3. Lithium and Tardive Dyskinesia

In addition, to its efficacy in the treatment and prophylaxis of bipolar affective disorders, a few brief reports in the early part of the last decade indicated that lithium may be useful in the treatment of tardive dyskinesia

(Dalen, 1973; Simpson, 1973; Reda et al., 1974). Klawans et al. (1977) therefore tested lithium in an animal model of tardive dyskinesia. Guinea-pigs were treated with haloperidol for 3 weeks, or with haloperidol in conjunction with a lithium-containing diet for the same period of time. Treatment in both these groups was terminated 1 week prior to behavioural testing. A third experimental group received haloperidol for 3 weeks, followed by a lithium diet for 1 week. It was found that haloperidol treatment caused a decrease in the threshold for amphetamine-induced stereotypy, which was prevented by the simultaneous administration of lithium. Lithium given after haloperidol did not alter the haloperidol-enhanced response to amphetamine. Similar changes were noted in animals challenged with apomorphine. Klawans et al. (1977) therefore suggested that while lithium treatment may not ameliorate the dyskinetic symptoms once they have developed, concurrent administration of the drug may prevent the onset of the syndrome. Allikmets et al. (1979) examined the effect of lithium treatment on apomorphine-induced aggression following chronic haloperidol administration. In this study, rats were treated with haloperidol for 2 weeks, or haloperidol plus lithium, or lithium alone for 2 weeks, and behavioural assessments carried out 5 days following cessation of drug therapy. Following apomorphine challenge, these authors found that chronic haloperidol prolonged the stereotyped behaviour elicited as a result of DA agonist administration, which was prevented by lithium given simultaneously with the neuroleptic agent. Interestingly, Allikmets et al. (1979) also noted a decreased stereotypical response to apomorphine challenge in the lithium-treated rats indicating that lithium alone may decrease the sensitivity of DA receptors (cf. Rosenblatt et al., 1980). When high doses of apomorphine were administered to paired male rats, chronic haloperidol caused an enhancement of the aggressive behaviour produced, which concurrent lithium administration prevented. Allikmets et al. (1979) therefore proposed that simultaneous chronic treatment of lithium with haloperidol prevents the development of dopaminergic

supersensitivity due to neuroleptic administration, a view which is in concurrence with Klawans et al. (1977).

The biochemical basis behind this «protective» effect of lithium has been the subject of limited investigation. Pert et al. (1978) used treatment groups similar to those employed by Klawans et al. (1977) and also noted that the enhanced apomorphine-induced stereotypy in rats following chronic haloperidol was abolished in those animals receiving lithium in conjunction with haloperidol. Pert et al. (1978) took this investigation one step further and examined (³H)spiroperidol binding to striatal membranes of rats 7 days after the termination of haloperidol or haloperidol plus lithium. It was found that while chronic haloperidol produced an elevation in the specific binding of the ligand, due to an increase in the number of binding sites, cotreatment with lithium reduced the ability of the neuroleptic to induce new binding sites. However, while Staunton et al. (1982a) agree that chronic lithium attenuated the behavioural response due to dopaminergic supersensitivity, and that haloperidol treatment increases the number of binding sites for (³H)spiroperidol (Staunton et al., 1982b), they found that lithium administration in conjunction with neuroleptic treatment had no effect on the density of binding sites with respect to the effect of haloperidol alone (Staunton et al., 1982b).

Eroglu et al. (1981) examined DA metabolism in two brain regions of rat following concurrent administration of chlorpromazine and lithium. These authors noted that while chronic treatment with either the neuroleptic or the anti-manic agent caused no alteration in DA metabolism in the frontal cortex, simultaneous administration of both drugs appeared to reduce dopaminergic activity in this brain region. In the striatum, Eroglu et al. (1981) found that chlorpromazine reduced DA turnover while lithium alone appeared to have no effect. The result of the combined treatment seemed to be a return towards normal metabolism of the neurotransmitter. These data indicate that there may well be a regionally

variable effect associated with the combined administration of lithium and neuroleptic drugs.

However, the usefulness of the administration of lithium in preventing or treating the tardive dyskinesia syndrome remains equivocal, while there are still unanswered questions regarding the neurochemical consequences of this combination treatment.

E. AIMS OF THE PRESENT STUDY

As can be seen from the preceding sections, there is as yet little consensus in the literature regarding the neurochemical consequences of lithium treatment, while the subject of lithium withdrawal has received almost no attention. It was therefore felt that a further investigation of the effects of lithium administration on certain parameters of monoaminergic neurotransmitter function was warranted. This would not only provide new information on the functioning of the monoamine systems following chronic treatment with this anti-manic agent, but would also serve as a basis for comparison of the effects of abrupt lithium withdrawal on these parameters of monoamine neurotransmission.

In addition, since the limited information available indicates that lithium is able to modify the dopaminergic effects of neuroleptic administration, it was decided to investigate in more detail the interaction of these two psychoactive drugs with respect to DA metabolism. Since NA and 5-HT have also been implicated in the central action of both of these agents, it seemed appropriate to extend the study to include an examination of these two monoamine neurotransmitters in addition to DA.

II METHODS

A. BEHAVIOURAL TECHNIQUES

1. Gross Locomotor Activity

Activity was measured using a two-channel Columbus Selective Activity Meter (Model SE, Columbus, Ohio). The observation chambers were clear polycarbonate cages with dimensions of 43x23x15 cm (lxwxh). Each channel was used to record one animal, with each subject being sampled for 30 sec in every minute. The meters were set at 57 μ A for sensitivity and 16 μ A for tuning, and at these settings limb and body movements and gross, but not minor, tremor were recorded. The observation chambers were located within a wooden enclosure, subdivided such that the two chambers were isolated from each other. Each section of the enclosure received equal artificial white light, and was equipped with a white-noise generator set to mask external noise.

Typically, animals were placed in the observation chambers and allowed a 5 min adaptation period. Activity was then measured in a 30 min observation period.

2. Monitoring of Stereotypical Behaviour

Behavioural observations were conducted using the method of Merali (unpublished). The observation chambers (clear polycarbonate cages, 43x23x15 cm) were equipped with an array of 10 infrared light beams. One beam was located immediately below an array of holes on the floor of the cage to monitor the frequency and duration of poking behaviour. A grid of five beams was located 1 cm above floor level, and these beams were used to detect horizontal and lateral displacement of the animal, and could differentiate between locomotion and localized floor activity by dividing the cage floor into 6 sections. A curtain of four beams located approximately 14 cm above the floor detected rearing, and measured both frequency and duration of this behaviour. An inverted cage of the same dimensions as the observation chamber, but with a metal grid floor, served as a lid allowing adequate ventilation and room for rearing. A custom designed

Z-80 microprocessor-based controller performed the timing and scoring functions. This system consisted of 12 chambers, with all beams being sampled once every second.

Typically, animals were placed in the observation chambers and allowed a 5 min adaptation period. Activity was then measured in a 20 min observation session.

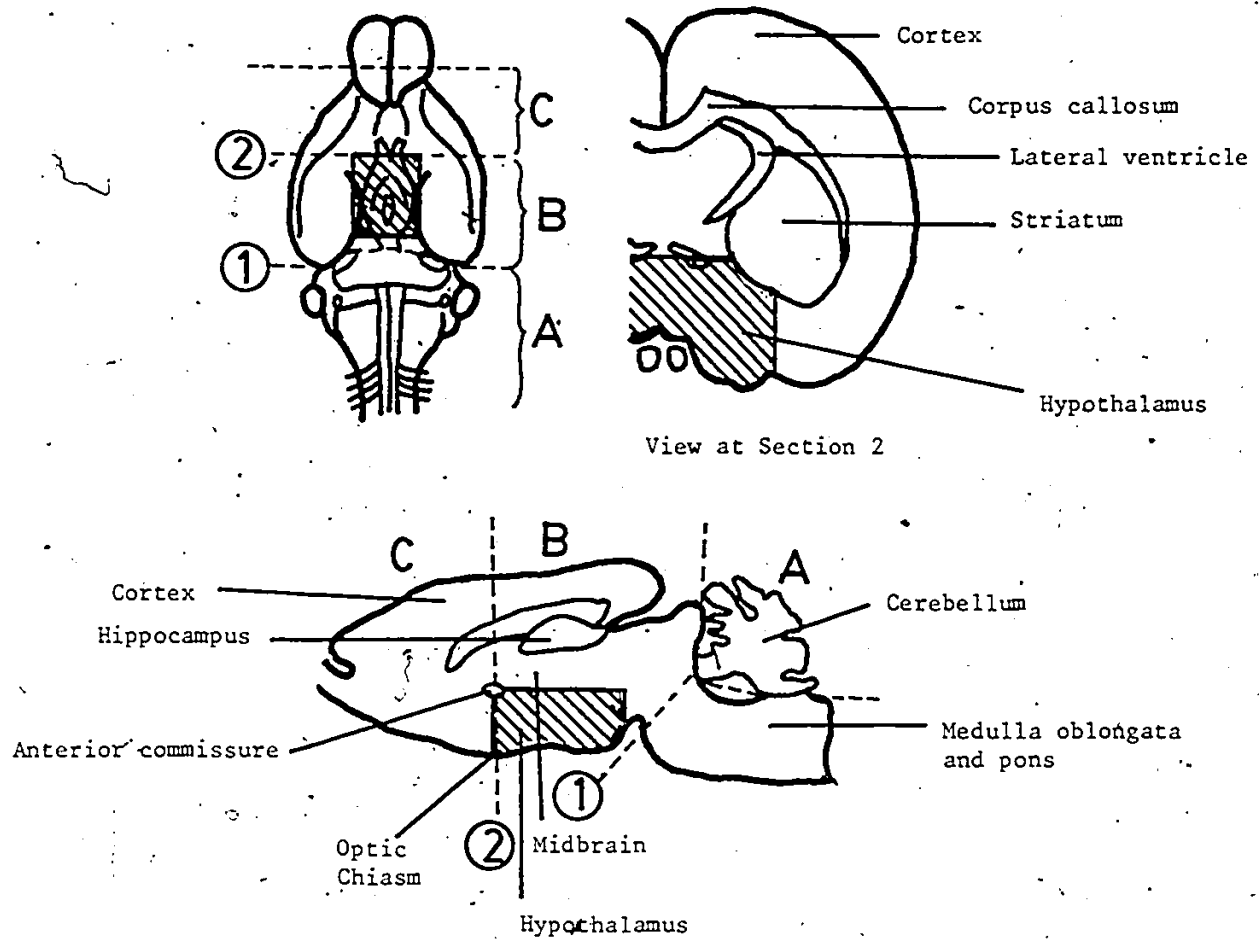
B. BIOCHEMICAL TECHNIQUES

1. Brain Dissection

Animals were sacrificed by decapitation using a rodent guillotine. The skull was removed as rapidly as possible and the brain excised. After being placed on a glass plate resting on crushed ice, grossly visible blood vessels and meningeal tissue were removed from the surface of the brain, which was then sectioned into the following regions: i) cerebellum; ii) pons-medulla, which comprised the medulla oblongata and the pons; iii) hypothalamus; iv) striatum, composed of the putamen nucleus, caudate nucleus and the globus pallidus, i.e. the basal ganglia without the amygdala; v) hippocampus; vi) midbrain, corresponding to the midbrain, thalamus and subthalamus; vii) cortex, containing both white and grey matter of the cerebral cortex.

The dissection was carried out freehand, essentially using the method of Glowinski and Iversen (1966). The cerebellum was first teased away from the rest of the brain exposing the pons-medulla, which was then removed (Fig. 8, Section 1). A transverse section was made at the level of the optic chiasm (Fig. 8, Section 2), splitting the brain into two parts. The more caudal of these was sub-divided into five portions. The hypothalamus was removed, taking the anterior commissure as the horizontal reference and the line between the posterior hypothalamus and the mammillary bodies as the caudal limit. The striatum was dissected using the external walls of the lateral ventricles and the corpus callosum as the inner and outer reference points, respectively. The frontal portions of the striatum,

Fig. 8. Diagram of Rat Brain to Show Dissection Procedure



Dotted lines indicate positions of initial sections.
(Taken from Glowinski and Iversen, 1966).

located in the rostral part of the two brain sections were also removed and added to the rest of the striatum. The cortex was then carefully split along the midline and peeled away, exposing the hippocampus which was removed. The remaining tissue after removal of the remnants of the cortex, was the midbrain. Cortical tissue removed from this portion of the brain was added to that from the rostral part of the two brain sections.

2. Determination of Catecholamine Synthetic Systems

Tyrosine content and tyrosine hydroxylase activity were measured in striatum only because of their relative preponderance in this particular region of the brain.

a) Tyrosine hydroxylase activity

This assay is modified from those of McGeer *et al.* (1967) and Shiman *et al.* (1971) and is based on the formation of (14 C)DOPA from (14 C)tyrosine by tyrosine hydroxylase present in the sample.

Tissue was homogenized in 50 vol (w/v) of ice-cold distilled water using a Teflon-glass homogenizer. The homogenate was centrifuged at 6,000 g for 10 min, and the resultant pellet discarded. The supernatant fluid was used to estimate both tyrosine hydroxylase activity and tyrosine content.

50 μ l of the supernatant fluid was placed in an incubation tube kept on ice. The incubation was carried out in a total volume of 130 μ l containing, in addition to the sample, 2.5 μ M (14 C)tyrosine, 180 mM 2-mercaptoethanol (to allow reactivation of the co-factor), 1.2 mM tetrahydrobiopterin (the natural co-factor for tyrosine hydroxylase; Weiner, 1979), 0.8 mM NSD-1034 (a non-specific decarboxylase inhibitor), 4 mM ferrous sulphate and 0.4 M potassium phosphate (pH 6.0), for 10 min at 37°C. The reaction was terminated by the addition of 1.25 ml of 0.4 M perchloric acid containing cold carrier DOPA, and the perchlorate precipitated material removed by centrifugation. The supernatant fluid from this centrifugation was added to 1.5 ml of 0.2 M EDTA and 3 ml of 0.4 M potassium phosphate. The solution was

7

adjusted to between pH 8.8 and 9.2 using dilute sodium hydroxide, and approximately 400 mg of acid-washed alumina immediately added to the sample and the mixture stirred for 4 min. It was then poured into a glass column plugged with cotton wool, and the alumina rinsed from the mixing beaker into the column with 25 ml of distilled water. The passage of fluid through the column was helped by the use of slight vacuum. When the water had passed through the alumina layer, the column was removed from the aspirator and the (^{14}C)DOPA removed by elution with 4 ml of 0.5 M acetic acid, which was placed in a scintillation vial to which was added 14 ml of scintillant (100 g naphthalene + 4 g Omnifluor/l of dioxane). Radioactivity was measured using a Beckman LS-230 Liquid Scintillation Counter, and the amount of (^{14}C)DOPA formed was calculated.

b) Tyrosine content

Tyrosine levels were measured in the same sample as was tyrosine hydroxylase activity, essentially using the method of McGeer et al. (1967). To 0.5 ml of the sample supernatant was added 2 ml of 8% (w/v) trichloroacetic acid. After standing for 10 min, the sample was centrifuged and 0.5 ml of the resultant supernatant fluid added to 1 ml of a 0.1% (w/v) methanolic solution of 1-nitroso-2-naphthol and 1 ml of 2.5 M nitric acid. The solution was then incubated at 60°C for 30 min. After cooling in ice, 2.5 ml of ethylene dichloride was added and mixed to extract the unreacted nitrosonaphthol into the organic phase. After centrifugation, the aqueous layer was removed and the fluorescence measured using either a Turner spectrofluorometer (Model 430) or an Aminco-Bowman spectrofluorometer. Activation and fluorescence wavelengths were 460 nm and 570 nm, respectively. Tyrosine content was then determined by comparing the sample fluorescence to a curve prepared using known amounts of the substrate.

3. Measurement of Indoleamine Synthetic Systems

Tryptophan content and tryptophan hydroxylase activity were measured in mid-brain only due to their relatively high amounts in this region when compared to other parts of the brain.

a) Tryptophan hydroxylase activity

This assay is modified from that of Kizer et al. (1975), and is based on the conversion of L-(^{14}C)tryptophan to (^{14}C)5-hydroxytryptophan by tryptophan hydroxylase present in the sample, followed by decarboxylation of the hydroxylated product to (^{14}C)5-hydroxytryptamine by exogenous L-aromatic amino acid decarboxylase added in excess.

i) Preparation of L-aromatic amino acid decarboxylase. This enzyme was prepared using the method described by Kizer et al. (1975). Rats were treated with p-chlorophenylalanine (325 mg/kg of the methyl ester, i.p.) and sacrificed 24 hr later. The livers were removed and homogenized in 2 vol (w/v) of 0.1 M sodium phosphate buffer (pH 7.2), and centrifuged at 78,000 g for 90 min. The supernatant fluid was removed and to it was slowly added solid ammonium sulphate to produce an approximately 50% saturated solution. After stirring for 1 hr at 4°C, the solution was centrifuged at 16,000 g for 20 min. The supernatant fluid was removed and to it was slowly added further solid ammonium sulphate to give an approximately 75% saturated solution. This was stirred at 4°C for 1 hr and then centrifuged at 16,000 g for 20 min. The precipitate was resuspended in 100 ml of an approximately 75% saturated solution of ammonium sulphate in 0.1 M sodium phosphate buffer (pH 7.2). After standing for 1 hr at 4°C, the preparation was centrifuged at 12,000 g for 20 min and the precipitate resuspended in a minimal volume of 0.02 M sodium phosphate buffer (pH 7.2). This solution was submitted to successive dialyses (3 hr each) against 200 vol of 0.02 M sodium phosphate buffer (pH 7.2). After dialysis, the precipitate was removed by centrifugation. To the supernatant was added ethylene glycol (10% w/v) and sodium EDTA and dithiothreitol to give final concentrations of 0.1 mM and 1.0 mM, respectively. The enzyme preparation was then divided into 1 ml aliquots and stored at -70°C.

ii) Assay of L-aromatic amino acid decarboxylase. The activity of the enzyme was determined as described by Kizer et al. (1975). In incubation tubes were

placed 10 μ l of a 1:10 dilution or 10 μ l of an undiluted aliquot of the enzyme preparation. To these incubation tubes were added 200 μ l of a solution containing pyridoxal phosphate (100 mM), sodium EDTA (30 mM) and ascorbic acid (0.003% w/v) in 0.1 M sodium phosphate buffer (pH 7.2). 50 μ l of a 2 mM (14 C)DOPA solution were then added and the tubes capped with rubber stoppers holding a plastic well into which had been placed a small piece of filter paper and 200 μ l of NCS solubilizer to trap the carbon dioxide liberated by the reaction. The incubation was started by transferring the reaction tubes from an ice bath to a Dubnoff metabolic shaker at 37°C. After 20 min, 0.5 ml of 10% (w/v) trichloroacetic acid was added to each tube to terminate the reaction, and the tubes incubated for a further 1 hr to release all of the labelled carbon dioxide which had been formed. At the end of this period, the plastic wells were removed and placed in scintillation vials. 50 μ l of glacial acetic acid was added to each, followed by 10 ml of scintillant (40 ml Liquifluor/1 of toluene).

It was found that 1 ml of the enzyme preparations used during the course of these studies decarboxylated 2.15 - 2.60 μ mol of L-DOPA per hr.

iii) Assay of tryptophan hydroxylase. Tissue was homogenized in 50 vol of ice-cold 0.05 M HEPES buffer (pH 7.4) containing 2 mM dithiothreitol, and the homogenate centrifuged at 6,000 g for 10 min in a refrigerated centrifuge. The supernatant fluid was removed and used to estimate both tryptophan hydroxylase activity and tryptophan content.

50 μ l of the sample supernatant were placed in an incubation tube kept on ice. The incubation was carried out in a total volume of 75 μ l containing, in addition to the sample, 0.1 mM L-(14 C)tryptophan, 0.21 mM pargyline (to inhibit any monoamine oxidase which may be present), 0.82 mM MPH₄ (an artificial co-factor) and 50 mg/ml albumin, for 30 min at 37°C. The reaction was terminated by the addition of 25 μ l of 0.4 M hydrochloric acid and the incubation tubes placed on ice.

The 5-hydroxytryptophan formed during this incubation was then converted to 5-hydroxytryptamine as follows: to the incubation tube from the first reaction was added 100 μ l of a solution made up of 115 parts of 1 M dipotassium hydrogen phosphate and 1 part of 10 M sodium hydroxide containing 20 μ g/ml o-phenanthroline (to inhibit any phenylalanine hydroxylase which may be present in the L-aromatic amino acid decarboxylase preparation). To this was added 33 μ l of a mixture containing equal volumes of the decarboxylase enzyme preparation and 0.1 mM hydrochloric acid. The incubation was then carried out at 37°C for 30 min. This reaction was terminated by the addition to the incubation tube of 1 ml of ice-cold perchloric acid/acetic acid (1.75 ml of 70% perchloric acid and 0.6 ml of glacial acetic acid/100 ml).

The reaction tubes were centrifuged for 10 min in a bench-top centrifuge to remove the particulate matter, and the supernatant fluid removed. The residue was washed with 2 ml of the perchloric acid/acetic acid mixture, and the resulting supernatant combined with that initially obtained, to which was added 50 μ l of a 1 mg/ml solution of 5-hydroxytryptamine in 0.1 M hydrochloric acid. To the sample was added 5 ml of 0.5 M Tris buffer (pH 7.0), and the sample adjusted to pH 7.0 with sodium hydroxide. The pH adjusted sample was then poured into a column packed with pre-soaked Amberlite GC-50 resin to a height of 2.5 cm (0.5 cm diameter). After the sample had passed through, the column was washed with 5 ml of distilled water, followed by 50 ml of 10 mM Tris acetate buffer (pH 7.0). 5-Hydroxytryptamine was then eluted from the column with 5 ml of 4 M acetic acid, which was placed in a scintillation vial to which was added 14 ml of scintillant (100 g of naphthalene + 4 g Omnifluor/1 of dioxane). Radioactivity was measured using a Beckman LS-230 Liquid Scintillation Counter and the amount of (¹⁴C)5-hydroxytryptamine formed was calculated.

b) Tryptophan content

The levels of this amino acid were measured in the same sample as was tryptophan hydroxylase, using a modification of the method of Hess and Udenfriend (1959).

To 0.5 ml of the sample supernatant was added 2 ml of 8% (w/v) trichloroacetic acid. After standing for 10 min, the sample was centrifuged and 0.5 ml of the resultant supernatant fluid added to 2 ml of water and 0.1 ml of 18% (v/v) formaldehyde solution. After mixing, the sample was incubated for 20 min in a boiling water bath. After cooling, 0.1 ml of 3% (v/v) hydrogen peroxide was added to the sample, and the solution re-incubated for a further 20 min in a boiling water bath. The sample was cooled to room temperature, and the fluorescence measured with either a Turner Spectrofluorometer (Model 430) or an Aminco-Bowman spectrofluorometer, using activation and fluorescence wavelengths of 365 nm and 440 nm, respectively. The tryptophan content was determined by comparing the sample fluorescence to a curve prepared using known amounts of the amino acid.

4. Tissue Preparation for Monoamine Estimation

Noradrenaline, dopamine, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were extracted from brain tissue essentially by the procedure of Maickel et al. (1968).

Cortex was homogenized in 10 vol (w/v) of n-butanol acidified with concentrated hydrochloric acid (1000:1), while all other brain regions (weighing less than 300 mg) were homogenized in 3 ml of acidified butanol (Curzon and Green, 1970). After centrifugation at 10,000 g for 10 min at 0°C, 2.5 ml of the supernatant fluid was added to a test tube containing 5 ml of n-heptane and 1 ml of 0.1 M hydrochloric acid and mixed for 10 min using a mechanical shaker. The phases were then separated by centrifugation, the aqueous acidic phase removed and kept for determination of noradrenaline, dopamine and 5-hydroxytryptamine.

To the organic phase was added 0.6 ml of 0.5 M Tris buffer (pH 7.0), and the tube mixed for 10 min using a mechanical shaker. The phases were separated by centrifugation, the organic phase removed by aspiration and discarded, while the aqueous phase was kept for 5-hydroxyindoleacetic acid measurement.

5. Determination of Tissue Catecholamine Levels

This assay procedure is based on the O-methylation of catecholamines by catechol O-methyl transferase using (^3H)S-adenosylmethionine as the methyl donor in the presence of magnesium and dithiothreitol and is modified from the methods of DaPrada and Zurcher (1976) and Peuler and Johnson (1977).

i) Preparation of catechol O-methyl transferase. The enzyme preparation described here is a modification of the method of Axelrod and Tomchick (1958).

Adult rats were sacrificed by cervical dislocation and exsanguination, and the livers removed and chilled. Tissue was combined from up to 6 rats, following which the livers were homogenized in 4 vol (w/v) of isotonic potassium chloride (1.9% w/v). The homogenate was centrifuged at 78,000 g for 30 min at 4°C to remove the cellular debris. The supernatant fluid was removed and titrated to pH 5.0 with 1 M acetic acid, following which it was stirred for 20 min at 4°C. At the end of this time, the acidified supernatant was centrifuged at 10,000 g for 20 min in a refrigerated centrifuge. Solid ammonium sulphate was slowly added to the resulting supernatant fluid to produce a 30% saturated solution. This solution was stirred at 4°C for 1 hr and then centrifuged at 10,000 g for 20 min, following which further solid ammonium sulphate was added to the resultant supernatant to produce a 55% saturated solution. After being stirred for 1 hr at 4°C, this solution was centrifuged at 10,000 g for 20 min, and the supernatant fluid discarded. The precipitate obtained was resuspended in water, and solid ammonium sulphate added to produce a 55% saturated solution. After standing for 1 hr at 4°C, the solution was centrifuged at 10,000 g for 20 min, and the resulting supernatant fluid discarded. The precipitate obtained from this centrifugation was dissolved in 1 mM Tris buffer (pH 7.4) containing 1 mM dithiothreitol, to give a solution containing 2 g original tissue/ml. This solution was dialyzed against 1 mM Tris buffer (pH 7.4) containing 1 mM dithiothreitol for 20 hr, using a total buffer volume of 2 l. Following dialysis, the solution was centrifuged at 10,000 g for 20 min to remove the debris. Aliquots of the enzyme preparation were then frozen and stored at -70°C until used.

ii) Assay of catechol O-methyl transferase. The activity of this enzyme was determined as described by McCaman (1965). In incubation tubes were placed 20 μ l of a 1:4 dilution of the enzyme and 20 μ l of an undiluted aliquot of the enzyme preparation. The incubation was then carried out in a total volume of 125 μ l containing, in addition to the enzyme sample, 1 mM noradrenaline, 44 μ M (3 H)S-adenosylmethionine, 5 mM magnesium chloride and 80 mM potassium phosphate buffer (pH 7.8) for 60 min at 37°C. At the end of this time, the reaction was stopped by the addition of 20 μ l of 3 M hydrochloric acid and the tubes placed on ice. The normetanephrine formed was then extracted in 0.5 ml of ethyl acetate. After separating the phases by centrifugation, 250 μ l of the ethyl acetate phase was transferred to a scintillation vial and 10 ml of scintillant (20:1, toluene:Liquifluor) added. The radioactivity was measured in a Beckman LS-230 Liquid Scintillation Counter.

It was found that 1 ml of the enzyme preparations used in the course of these studies O-methylated 90.0-97.5 μ moles of noradrenaline per hour.

iii) Measurement of catecholamine content. To 30 μ l of the acid phase obtained from the extraction of monoamines from brain tissue was added 30 μ l of a mixture containing 2 M Tris buffer (pH 9.6), 7 μ M magnesium chloride, 9 mM dithiothreitol and 1.5 μ M (3 H)S-adenosylmethionine. After mixing, 20 μ l of the catechol O-methyl transferase preparation was added. An internal standard was utilized for this assay and so duplicates of the above mixtures were prepared to which were added 100 pg each of noradrenaline and dopamine. The reaction mixtures were incubated at 37°C for 60 min, at the end of which time the incubation was stopped by the addition of 100 μ l of ice-cold borate buffer (pH 11.0) containing 8 mM normetanephrine, 4 mM metanephrine and 4 mM 3-methoxytyramine to act as non-labelled carriers at a later stage. To each reaction tube was added 2 ml of a toluene/isoamyl alcohol (3:2) mixture. After vortexing for 60 sec and subsequent separation, an aliquot of the organic phase was removed and added to 200 μ l of 0.1 M acetic acid. This was vortexed for 30 sec and after separation, the organic phase was

aspirated and discarded. The aqueous layer was then washed with a further 1 ml of the toluene/isoamyl alcohol (3:2) mixture. An aliquot of the aqueous phase was added to 100 µl methanol and mixed well. This alcoholic solution was then spotted onto silica gel thin layer chromatography plate (silica gel GF plates, 250 µm thick) and the plate developed using a mixture of t-amyl alcohol/benzene/40% aqueous methylamine solution (6:2:3). After the plates had dried, they were visualized under UV light (wavelength 254 nm) and it was possible to clearly see three zones: 3-methoxytyramine (top), metanephrine (middle) and normetanephrine (bottom) (Fig. 9). The individual zones were marked on the plate.

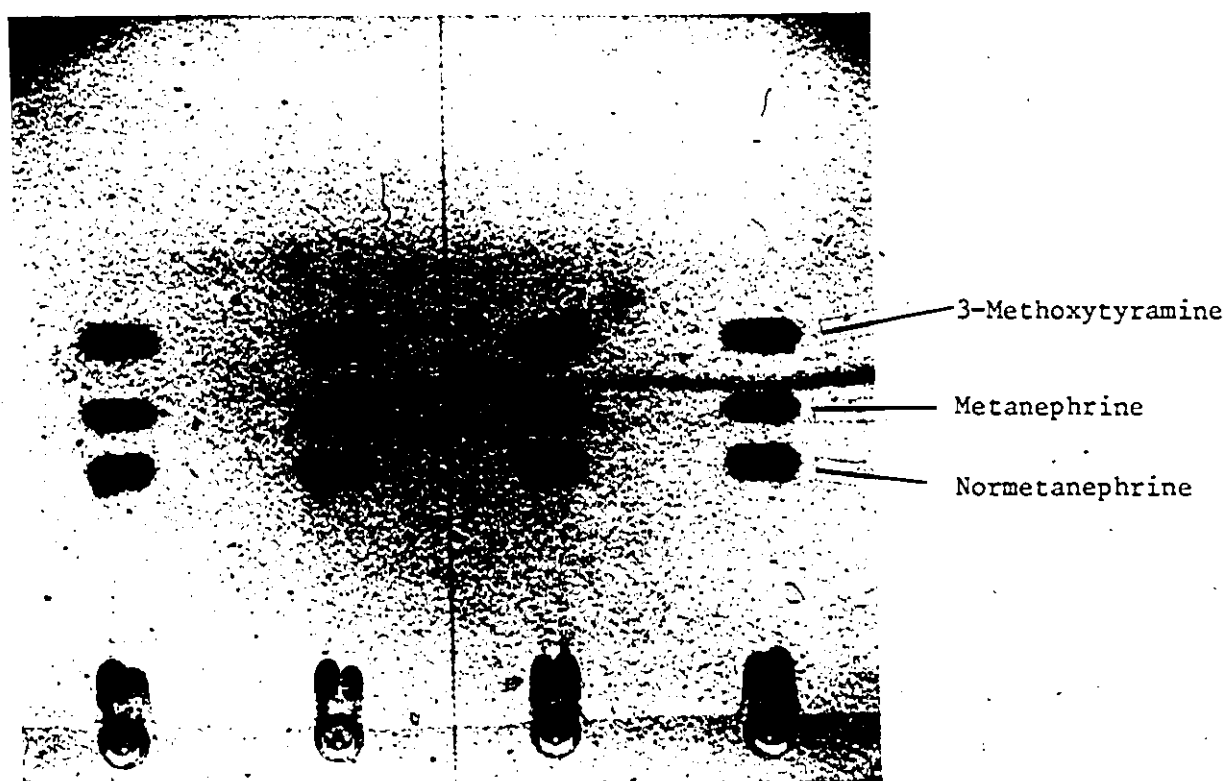
For the measurement of dopamine, the 3-methoxytyramine spot was scraped into a scintillation vial, and 1 ml of 50 mM ammonium hydroxide added to elute the metabolite from the silica gel. 3-Methoxytyramine was then extracted from the ammonium hydroxide into 10 ml of a toluene/isoamyl alcohol mixture containing Liquifluor (70:30:5) which acted as a scintillant, and the radioactivity measured in a Beckman LS-230 Liquid Scintillation Counter.

For the determination of noradrenaline, the normetanephrine spot was scraped into a scintillation vial, and the metabolite eluted from the silica gel with 1 ml of 50 mM ammonium hydroxide. To this was added 50 µl of 4% sodium periodate to oxidize the normetanephrine to vanillin, a reaction which was stopped 5 min later by the addition of 50 µl of a 10% (v/v) glycerol solution. The mixture was acidified with 1 ml of 0.1 M acetic acid and the vanillin extracted into 10 ml of toluene containing Liquifluor (20:1). Radioactivity was measured in a Beckman LS-230 Liquid Scintillation Counter. The final reaction in this assay, i.e. the conversion of metanephrine to vanillin is incorporated to help reduce the blank counts which improves the sensitivity of the assay (DaPrada and Zurcher, 1976; Peuler and Johnson, 1977).

6. Determination of Tissue Indoleamine Content

After extraction from tissue, both 5-hydroxytryptamine and 5-hydroxyindole-acetic acid were measured by fluorometric means, using modifications of the

Fig. 9. Chromatograph Showing Separation of the O-Methylated
Metabolites of the Catecholamines



methods of Curzon and Green (1970).

a) 5-Hydroxytryptamine

200 μ l of the acid phase obtained from the extraction of monoamines from brain tissue were pipetted into a test tube and to this was added 1.2 ml of 0.004% (w/v) o-phthalaldehyde in 10 M hydrochloric acid. After mixing, 20 μ l of a 1% (w/v) L-cysteine solution was added and the sample incubated at 60°C for 15 min. After cooling, the fluorescence of the sample was measured using either a Turner spectrofluorometer (Model 430) or an Aminco-Bowman spectrofluorometer. Activation and fluorescence wavelengths were 360 nm and 470 nm, respectively.

5-Hydroxytryptamine content was determined by comparing the sample fluorescence to a curve prepared using known amounts of the indoleamine.

b) 5-Hydroxyindoleacetic acid

400 μ l of the buffered aqueous phase obtained from the extraction of monoamines from brain tissue were pipetted into a test tube, and to this was added 50 μ l of a 1% (w/v) L-cysteine solution, followed in sequence (with mixing between each addition) by 1 ml of concentrated hydrochloric acid, 50 μ l of a 0.1% (w/v) solution of o-phthalaldehyde in methanol, and 50 μ l of a 0.02% (w/v) solution of potassium periodate. After incubation at 60°C for 15 min, the tubes were allowed to cool and the fluorescence read in a spectrofluorometer using activation and fluorescence wavelengths of 360 nm and 470 nm, respectively.

5-Hydroxyindoleacetic acid content was determined by comparing the sample fluorescence to a curve prepared using known amounts of the substrate.

7. Measurement of Catecholamine Metabolite Content

a) 3-Methoxy-4-hydroxyphenylglycol sulphate

A modification of the fluorometric procedure of Meek and Neff (1972) was used to measure the tissue levels of this noradrenaline metabolite.

Rat brains were homogenized in 4 vol of 0.2 M zinc sulphate. To the homogenate was added 4 vol of 0.2 M barium hydroxide and after mixing, the homogenates were centrifuged at 23,000 g for 15 min at 0°C. The supernatant fluid was passed

through a 5 x 30 mm column of DEAE Sephadex A-25, and the effluent discarded. The column was washed with 8 ml of 0.06 M hydrochloric acid, and then the MOPEG eluted from the column with 5.5 ml of 0.15 M hydrochloric acid. To the eluate was added 200 μ l of 1.5% (w/v) L-cysteine hydrochloride and 300 μ l of 60% perchloric acid. 2 ml aliquots of the acidified eluate were then used for sample, internal standard and blank analysis. The internal standard consisted of 1 μ g MOPEG, which was added to one of the aliquots. The tubes containing the sample and the standard were heated at 100°C for 12 min in a water bath while the blank was left at room temperature. After cooling, 300 μ l of ethylene diamine was added to all tubes, including blanks and heated at 100°C for 5 min. After cooling, the fluorescence was measured using either a Turner spectrofluorometer (Model 430) or an Aminco-Bowman spectrofluorometer. Activation and fluorescence wavelengths were 320 nm and 465 nm, respectively.

b) Homovanillic acid .

The striatum was homogenized in 2 ml of ice cold 0.1 M hydrochloric acid and the homogenate transferred to a polypropylene centrifuge tube. The homogenizer was washed with 2 ml of ice cold water, and the washings added to the homogenate. 120 μ l of 70% perchloric acid was then added and mixed with the homogenate. Solid potassium chloride was then added in an amount just in excess of that required to saturate the homogenate (approximately 750 mg) and mixed thoroughly. This removes the perchlorate ion as the insoluble potassium salt, yet leaves the solution acid. The homogenate was centrifuged at 15,000 g for 5 min at 0°C and a 3.5 ml aliquot of the supernatant fluid transferred to a screw-capped centrifuge tube to which was added a few crystals of potassium chloride (to ensure that the solution was saturated) and 10 ml of n-butyl acetate. The tube was then gently shaken using a mechanical shaker for 5 min. The phases were separated by centrifugation and two 4.5 ml aliquots of the organic phase taken. To one portion was added 2 ml of a 0.6% (w/v) Tris solution, and the tube shaken on a mechanical shaker for

3 min. The second aliquot was used for the measurement of DOPAC (to be described). The phases were separated by centrifugation, and the n-butyl acetate phase aspirated and discarded. A 0.6 ml aliquot of the Tris extract was transferred to a culture tube and to it was added 1 ml of 0.002% (w/v) potassium ferricyanide in 5 M ammonium hydroxide. 4 min after mixing, 200 μ l of 1% (w/v) L-cystein was added and mixed. The fluorescence of the sample was then read in either a Turner Spectrofluorometer (Model 460) or an Aminco-Bowman spectrofluorometer (activation wavelength 315 nm, fluorescence wavelength 420 nm) and compared to a curve constructed using known amounts of substrate.

c) 3,4-Dihydroxyphenylacetic acid

To the second n-butyl acetate aliquot obtained above was added 2.2 ml of a mixture containing 35 ml distilled water, 1 ml of 2 M hydrochloric acid and 1.5 ml of ethylenediamine. The tube was shaken thoroughly on a mechanical shaker for 3 min, and the phases separated by centrifugation. The organic phase was aspirated and discarded. A 1 ml aliquot of the ethylenediamine extract was transferred to a culture tube and heated at 60°C for 20 min in the dark. After cooling, 300 μ l of a 1:1 (v/v) dilution of concentrated hydrochloric acid was added and mixed. The sample was left on ice for 10 min, then neutralized by the addition of 300 μ l of a 1:9 (v/v) dilution of ethylenediamine. The fluorescence was measured in a spectrofluorometer using an activation wavelength of 385 nm and a fluorescence wavelength of 450 nm. The DOPAC content was then determined by comparing the sample's fluorescence to a curve constructed using known amounts of the substrate. The methods of Murphy et al. (1969) were used for the measurement of HVA and DOPAC.

8. Monoamine Uptake Into a Synaptosomal Fraction

The method of Slotkin et al. (1978) was used to prepare synaptosomes and to determine monoamine uptake.

Tissue was homogenized in 4 vol of 300 mM sucrose containing 25 mM Tris (pH 7.4) and 10 μ M iproniazid, using a Teflon-glass homogenizer. The homogenate

was centrifuged at 1,000 g for 10 min. The supernatant was then sedimented by centrifugation at 10,000 g for 20 min and the crude synaptosomal pellet washed by gently resuspending the precipitate in 4 vol of Krebs-Henseleit medium (120 mM sodium chloride, 5 mM potassium chloride, 1.2 mM magnesium sulphate, 1.9 mM calcium chloride, 1.2 mM potassium dihydrogen phosphate, 25 mM sodium bicarbonate, 10 mM dextrose) modified by the addition of 2 μ M ascorbic acid and 1.25 μ M iproniazid. After recentrifugation at 10,000 g for 20 min, the supernatant fluid was discarded and the washed pellet resuspended in 10 vol modified Krebs-Henseleit medium. This final resuspension therefore contained 100 mg original tissue per ml.

100 μ l aliquots of the synaptosomal preparation (equivalent to 10 mg of original brain tissue) were mixed with 100 μ l of the modified Krebs-Henseleit medium. The sample was pre-incubated at 37°C for 5 min, at the end of which time tritiated monoamine was added in a volume of either 10 μ l or 20 μ l. After a 10 min incubation period, uptake was stopped by the addition of 1 ml of ice-cold modified Krebs-Henseleit medium and placing the tubes on ice. Duplicate tubes kept on ice throughout the incubation period served as blanks. Labelled synaptosomes were trapped by filtration under vacuum through cellulose-acetate filters (0.2 μ m pore size). After three washes with ice-cold modified Krebs-Henseleit medium (2 ml per wash), the filters were oven-dried. Scintillation fluid (10 ml of PCS) was added to each filter and the radioactivity counted using a Beckman LS-230 Liquid Scintillation Counter.

Uptake was determined by subtracting the 0°C blank from the 37°C sample, and expressed in terms of pmol of monoamine taken up either per gram of original tissue or per mg protein.

9. Measurement of Synaptic Membrane Binding Sites

a) Preparation of synaptic membranes

Synaptic membranes were prepared from synaptosomes according to modifications of the procedures of Slotkin et al. (1978) and Maggi and Enna (1980).

Tissue was homogenized in 4 vol of ice cold 0.32 M sucrose containing 50 mM Tris (pH 7.4) using a Teflon-glass homogenizer and the homogenate centrifuged at 1,000 g for 10 min. The supernatant was sedimented by centrifugation at 10,000 g for 20 min. The resultant pellet was homogenized in 5 vol of ice-cold water using a Polytron PT-10. This homogenate was centrifuged at 45,000 g for 20 min, and the precipitate resuspended in 5 vol of the appropriate buffer (for dihydro-alprenolol binding, 50 mM Tris, pH 8.0 at 37°C; for spiroperidol binding, 50 mM Tris-HCl, 120 mM sodium chloride, 5 mM potassium chloride, 2 mM calcium chloride, 1 mM magnesium chloride, 0.1% (w/v) ascorbic acid, pH 7.1 at 37°C). After recentrifugation at 45,000 g for 20 min, the supernatant fluid was discarded and the pellet resuspended in 10 vol of the appropriate buffer.

b) Determination of membrane binding sites

i) β -Adrenergic binding sites. 100 μ l aliquots of the final membrane preparation were incubated at 37°C for 10 min with varying concentrations of (3 H) dihydro-alprenolol in a final incubation volume of 1 ml. The incubation was terminated by the addition of 4 ml of ice-cold buffer to the sample, and rapid filtration of the membrane preparation under vacuum through glass-fibre filters (Whatman GF/B). The filters were washed three times with 4 ml of ice-cold buffer, placed in scintillation vials and oven dried. Scintillation fluid (10 ml of Econofluor) was added to each vial and the radioactivity measured in a Beckman LS-7800 Liquid Scintillation Counter. Specific receptor binding was defined as the difference between radioactivity bound in the absence and presence of 10 μ M alprenolol.

ii) D₂-Dopaminergic binding sites. 100 μ l aliquots of the final membrane preparation were incubated at 37°C for 10 min with varying concentrations of (3 H) spiroperidol in a final incubation volume of 1 ml. The incubation was terminated by the addition of 4 ml of ice-cold buffer to the sample, and rapid filtration of the membrane preparation through glass fibre filters (Whatman GF/B). The filters were washed three times with 4 ml of ice-cold buffer, placed in scintillation vials and oven dried. 10 ml of scintillant (NEN Econofluor) was

added to each vial and the radioactivity measured in a Beckman LS-7800 Liquid Scintillation Counter. Specific receptor binding was defined as the difference between total binding in the presence of 10 μ M (-)-butaclamol and non-specific binding in the presence of 10 μ M (+)-butaclamol.

10. Determination of Protein Content

This assay is modified from that of Lowry et al. (1951).

To an aliquot of the sample was added 5 ml of a mixture of 1% copper sulphate, 2% potassium-sodium tartrate, and 2% alkaline sodium carbonate (1:1:100), which was incubated at 45°C for 10 min. After cooling on ice, 0.5 ml of a 1:1 dilution of Folin-Ciocalteu reagent was added to the sample, mixed immediately and left at room temperature for 20 min, after which the intensity of the developed colour was measured using a Klett colourimeter with a No. 64 filter (620-680 nm). The amount of protein present was determined by comparing the colour developed to a curve prepared using known amounts of protein (bovine serum albumin).

11. Determination of Lithium Levels

a) Measurement in serum

When the animals were ~~were~~ decapitated, trunk blood was collected in polypropylene centrifuge tubes, and left at room temperature for approximately 1 hr to clot. The clotted blood was centrifuged at 10,000 g for 10 min, and the serum removed. This was stored frozen at -20°C until lithium determinations could be carried out.

250 μ l of thawed serum was added to 5 ml of a 5.25% (w/v) solution of trichloroacetic acid, mixed and allowed to stand at room temperature for 10 min, following which it was centrifuged using a clinical centrifuge for 10 min. The deproteinized supernatant fluid was removed and used for lithium determination.

To measure lithium content of the deproteinized serum, a Jarrel-Ash Model 850 atomic absorption spectrophotometer was used in its flame emission mode. The operating parameters were as follows: detection wavelength 6708Å; spectral band pass 1Å; oxidizing flame using acetylene and air. In order to compensate for

other ions which would be present in the serum, the lithium standards were prepared in a solution which also contained 150 mEq/l sodium, 6 mEq/l potassium and 6 mEq/l calcium. Lithium content of serum samples was determined by comparison to a curve prepared using known lithium standards.

b) Measurement in brain tissue

After weighing, brain sections were oven-dried overnight at 105°C. To the dry tissue was added 1.5 ml of concentrated nitric acid, which was gently heated until the tissue was completely digested. The digest was then concentrated to a volume of 1 ml over low heat, following which 1.5 ml of a 5% (w/v) solution of trichloroacetic acid was added, mixed and allowed to stand at room temperature for 10 min. The precipitate formed was removed by filtration and the filtrate diluted with an equal volume of distilled water. The level of lithium in this solution was then measured by atomic absorption spectrophotometry, using the operating parameters described above. The lithium content of the tissue samples was determined by comparison to a curve prepared using known lithium standards.

C. ANIMALS

All animals used in these studies were male Sprague-Dawley rats obtained from Canadian Breeding Farms Ltd., Montreal. At the start of each study, rats weighed 150-175 g and were maintained in groups of 3 per cage under constant environmental conditions (24°C, 60% relative humidity and regular alternate cycles of 12 hr light and darkness) with food and water ad libitum.

D. MATERIALS

The following radiolabelled compounds were obtained from either Amersham Corp., Oakville, Ont. or New England Nuclear (Canada) Ltd., Lachine, Que.:
L-(U-¹⁴C)tyrosine (>450 mCi/mmol), L-(side chain-3-¹⁴C)tryptophan (40-60 mCi/mmol), L-3,4-dihydroxyphenyl(1-¹⁴C)alanine (5-20 mCi/mmol), S-adenosyl-L-(methyl-³H)methionine (5-15 Ci/mmol), 5-hydroxy(G-³H)tryptamine creatinine sulphate (10-20 Ci/mmol), DL-(7-³H)noradrenaline hydrochloride (8-20 Ci/mmol), 3,4-(³H(G)dihydroxy-

phenylethylamine (2-10 Ci/mmol), L-(ring, propyl-³H(N) dihydroalprenalol hydrochloride (90-120 Ci/mmol), (benzene ring-³H) spiroperidol (20-40 Ci/mmol).

Also obtained from Amersham Corp. were PCS scintillation cocktail and NCS solubilizer, while New England Nuclear (Canada) Ltd. supplied Econofluor scintillation cocktail and Omnifluor and Liquifluor pre-mixed liquid scintillation fluors.

The 5,6,7,8-tetrahydro-L-biopterin was obtained from Hoffman La Roche, Montreal while the NSD-1034 came from Smith and Nephew, Lachine, Que. (+) and (-)-Butaclamol hydrochloride were purchased from Research Biochemicals Inc., Wayland, Mass. McNeil Labs (Canada) Ltd., Stouffville, Ont. provided the haloperidol. The silica gel GF thin-layer chromatography plates were supplied by Mandel Scientific Co. Ltd., Rockwood, Ont.

All other chemicals were the purest grade available from commercial suppliers.

E. STATISTICAL ANALYSES

Mean values and their standard errors were calculated and were compared by a two-tailed analysis using Student's t-test. Differences were considered significant if $p = 0.05$ or less. In cases where the use of the t-test was inappropriate, i.e. multiple comparisons, data were compared using a one-way analysis of variance and if a significant difference was found ($p < 0.05$), they were further subjected to the Duncan multiple-range test to isolate the differences.

III

RESULTS

1. Determination of Gross Toxicity Following Chronic Lithium Carbonate Administration

Many investigators have examined the effects of lithium in animals after achievement of «clinically effective» blood lithium concentrations. However, certain reports have indicated that lithium levels of the magnitude used in the clinical situation may produce toxic manifestations in commonly used laboratory animals. An alternative approach to the selection of a lithium dosage to be used is to utilize the maximum sub-toxic dose, i.e. the highest amount of the drug tolerated by the animal without the appearance of any toxic effect.

Singly housed, male Sprague-Dawley rats were therefore treated with varying amounts of lithium carbonate for a period of 30 days. The doses used ranged from 0.25 to 2.5 mmol/kg (equivalent to 0.5-5.0 mEq lithium/kg), administered intra-peritoneally once daily in a volume of either 5 ml/kg (0.25, 0.5, 1.0 and 1.5 mmol/kg) or 10 ml/kg (2.0 and 2.5 mmol/kg). The lithium carbonate solutions were adjusted to pH 7.4 with citric acid immediately prior to use. Control animals received equivalent volumes of physiological saline.

Eighteen hours after each drug administration, the rats were checked for signs of toxicity. Among the symptoms for which the animals were examined were polyuria, diarrhoea, piloerection, tremor, gross motor dysfunction (i.e. disturbed gait) and mortality.

None of the saline-treated control animals displayed any toxic symptoms, and they gained weight at the same rate as untreated rats. No toxic symptoms were noted in animals administered either 0.5 mEq lithium/kg or 1 mEq lithium/kg, but in the group treated with 2 mEq lithium/kg, two animals displayed signs of polyuria (day 3 to day 6) and one of these also suffered a brief period of diarrhoea (day 3 to day 5) (Table 1). However, since these signs were only present in a small number of animals for a short period of time, it is likely that they may not have been related to lithium toxicity. Administration of 3 mEq lithium/kg resulted in polyuria in 3 animals, which was delayed in onset

Table 1. Toxic Symptoms Displayed Following Chronic Lithium Treatment

Symptoms	Amount of Lithium Administered (mEq/kg)					
	0.5	1.0	2.0	3.0	4.0	5.0
Polyuria	0	0	**	***	*** ***	*** ***
Diarrhoea	0	0	*	***	***	*** ***
Piloerection	0	0	0	*** **	*** *	*** **
Tremor	0	0	0	***	*** *	*** **
Motor Dysfunction	0	0	0	*	**	*** *
Mortality	0	0	0	0	***	*** **

Each dose of lithium was administered daily (i.p.) to 6 rats for 30 days. Each animal was examined daily for toxic symptoms.

0 = the symptom was not observed in the group during the treatment period.

* = the symptom was observed in one animal at some point during the treatment period.

(starting days 13-17), but persisted for the remainder of the test period. Diarrhoea was also noted in these rats, starting between days 21 and 25, and also lasting to the end of the test period. Five animals suffered from piloerection for varying periods of time, while 3 exhibited pronounced tremor. Disturbed gait was also seen in one animal. Weight gain in this group was not different to control animals. Polyuria was noted in all rats treated with 4 mEq lithium/kg, while diarrhoea was seen in 3 animals. Piloerection and tremor were displayed by 4 rats, with 2 also exhibiting disturbed gait. Three animals died during the treatment period (days 23, 26 and 27). Weight gain was also reduced in this treatment group (76% of control). With the highest treatment regime, all animals suffered polyuria and diarrhoea, 5 exhibited piloerection and severe tremor, and 4 displayed a disturbed gait. Five of the rats in this treatment group died before the end of the treatment period (days 17, 19 (2), 22 and 23).

Since 2 mEq lithium/kg appeared to be the highest dose of lithium tolerated by the animals after a 30 day treatment period, this was the dose chosen for use in subsequent studies.

2. Changes in Spontaneous Locomotor Activity Due to Lithium Administration and Subsequent Withdrawal

Clinical experience has shown that there is a time-lag of between 5 and 12 days associated with the start of lithium treatment and the onset of its anti-manic effect. It is therefore reasonable to assume that there is a delay period following the commencement of lithium treatment in experimental animals and the alteration of neurochemical parameters resulting from this treatment. In order to determine whether this was in fact so, it was decided to examine the changes in gross behavioural parameter, spontaneous locomotor activity, with the assumption being that lithium would reduce spontaneous activity. In addition, it was decided to examine the consequences of withdrawal of lithium following a suitable

treatment period, since certain clinical studies had suggested that a «rebound phenomenon» became manifest on termination of lithium therapy.

Groups of rats were therefore treated with lithium carbonate (1 mmol/kg, equivalent to 2 mEq lithium/kg) i.p. daily, with a total treatment period of 16 days. One of the groups of animals treated with lithium was to undergo lithium withdrawal, the start of the withdrawal period to be determined by the behavioural response. Control animals received an equivalent volume of physiological saline daily.

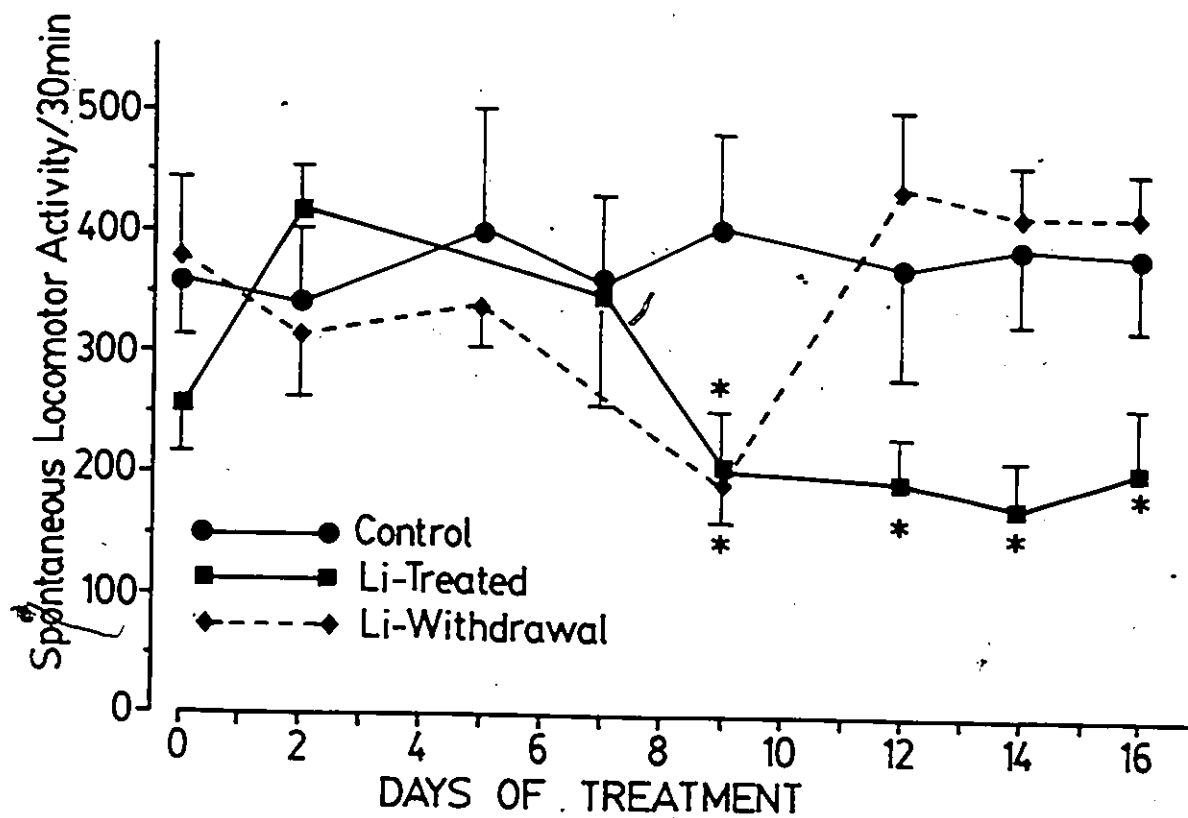
Behavioural testing was carried out 18-24 hr after the previous drug administration, using a test period of 30 min. As can be seen in Fig. 10, after 9 days of lithium administration, there was a significant decrease in spontaneous locomotor behaviour; the decrease being maintained for the remainder of the treatment. Since a decrease in behavioural activity was seen after 9 days of treatment, it was decided that the lithium-withdrawal group should receive 10 days of lithium administration, followed by 6 days of an equivalent volume of physiological saline. As Fig. 10 shows, 12 days after the start of the treatment period, i.e. 2 days into the withdrawal period, spontaneous locomotor activity was slightly, but not significantly greater than control (119%), and with time more closely approached control values.

It therefore appeared that a two-day withdrawal period was appropriate, although no conclusive evidence for a behavioural «rebound» phenomenon was found, and this was used in subsequent studies.

3. The Effect of Lithium Administration and Withdrawal in Various Animal Models of Mania

Various models of «manic» behaviour have been proposed for the behavioural testing of known and novel anti-manic agents, including reserpine-induced hyperactivity in the presence of a monoamine oxidase inhibitor, and amphetamine-induced hyperactivity in the presence of chlordiazepoxide or barbiturate. It has been said

Fig. 10. Effect of Lithium Treatment and Withdrawal on Spontaneous Locomotor Activity



Each point represents the mean $\pm$ S.E. mean for 6 animals.

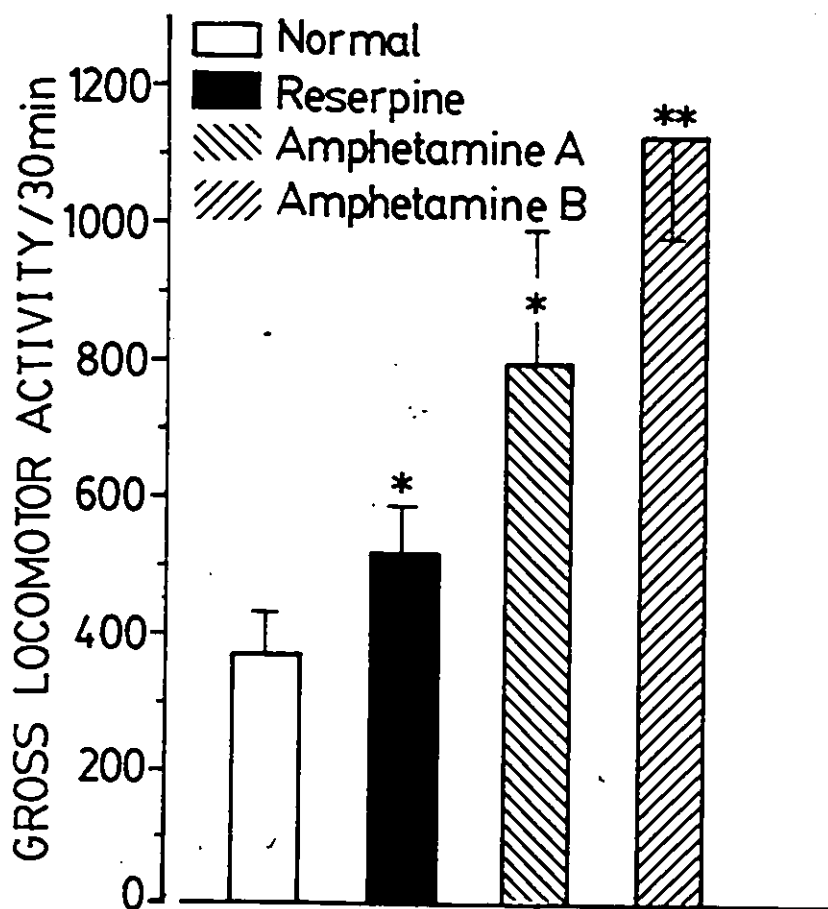
Significantly different to control (*p < 0.05).

that the behavioural hyperactivity due to neuronal release of neurotransmitters following treatment with either reserpine or amphetamine provides a more accurate method of determining the effectiveness of anti-manic agents than does the utilization of «normal» animals. It was therefore decided to evaluate the effects of lithium treatment in these analogues of mania, and to compare them to changes in locomotor activity in «normal» lithium-treated animals. In addition, the consequences of lithium withdrawal were examined in these behavioural paradigms.

Groups of animals were treated with lithium carbonate (1 mmol/kg) i.p. daily for 12 days, or with the salt (1 mmol/kg) i.p. for 10 days followed by 2 days of saline. Control rats received an equivalent volume of physiological saline for 12 days. Prior to measurement of locomotor activity, which took place 18-24 hr after the last drug administration, animals were divided into four groups: (a) normal - no drug pre-treatment immediately prior to locomotor testing; (b) reserpine-induced hyperactivity - the animals were administered pargyline (50 mg/kg i.p.) followed 30 min later by reserpine (5 mg/kg i.p.). Locomotor testing was carried out 30 min after reserpine administration (Delini-Stula and Meier, 1976); (c) amphetamine-induced hyperactivity «A» - the animals were treated with a mixture of dexamphetamine (1.18 mg/kg) and chlordiazepoxide (12.5 mg/kg), administered i.p. 30 min before measuring locomotor activity (Davies et al., 1974); (d) amphetamine-induced hyperactivity «B» - as above, but using 2.36 mg/kg dexamphetamine and 25 mg/kg chlordiazepoxide (Davies et al., 1974).

As can be seen in Fig. 11, there was a significant enhancement of recorded spontaneous activity in control animals following treatment with reserpine and both amphetamine regimes as compared to the «normal» rats, with amphetamine displaying a dose-dependent effect. This increase in activity was more pronounced in the amphetamine-treated groups than in those animals receiving reserpine, but the causes of the enhanced activity appear to be different. In the case of the reserpine-treated animals, the rats seemed to exhibit more voluntary activity in

Fig. 11. Effect of Behavioural Stimulants on Spontaneous Locomotor Activity



Each value represents the mean + S.E. mean for 6 rats per group. Control animals were administered the behavioural stimulants as described. Locomotor activity was measured for 30 min following a 10 min adaptation period.

Significantly different from control («normal»); *p < 0.05; **p < 0.01.

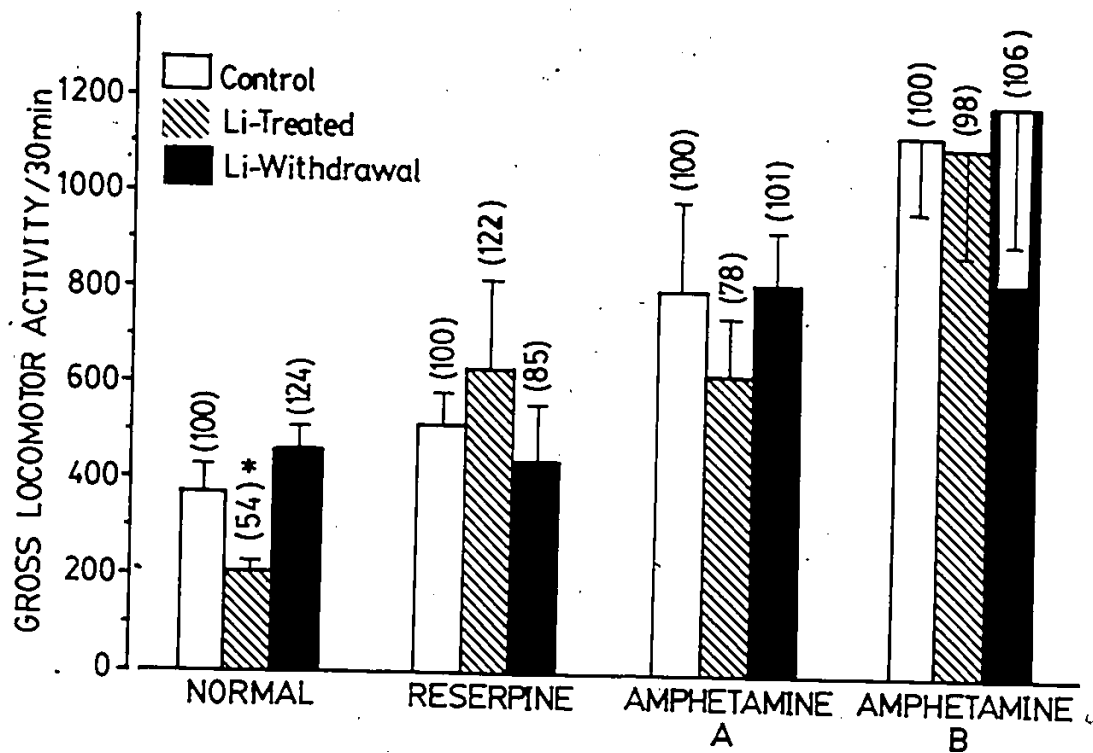
the home cages and the test cage than did the «normal» rats, and displayed no other overt behavioural symptoms. In contrast to this, the animals treated with amphetamine, particularly at the higher dose, appeared to have a marked impairment of voluntary movement, as manifested by locomotion, but displayed severe tremor. Therefore, the increased spontaneous activity recorded when these animals were placed in the test cages does not represent spontaneous locomotor activity. Attempts to alter the sensitivity and tuning of the measuring instrument to eliminate the recording of this severe tremor resulted in a situation in which the recording of actual locomotor activity, as determined by the use of «normal» animals, was impaired.

When the effect of 12 days of lithium administration was examined using the behavioural paradigms described, it can be seen (Fig. 12) that the only model in which lithium produced a significant effect was in the «normal» animals, where a reduction in spontaneous activity was noted. Following withdrawal of lithium, the spontaneous activity of the «normal» animals was slightly, but non-significantly enhanced relative to the control animals. Again, no change in spontaneous activity was seen following lithium withdrawal in the other behavioural analogues of mania tested. It should be noted that the overt behavioural symptoms displayed by the control rats receiving the amphetamine-chlordiazepoxide mixtures were also seen in the animals subjected to lithium administration and withdrawal and then treated with this drug mixture.

4. Consequences of Short-Term Lithium Administration and Subsequent Withdrawal on Central Monoamine Systems

The central biogenic amine neurotransmitters have been heavily implicated in the genesis of mood and mood disorders, which has led to the investigation of their role in the mechanism of action of lithium. This study was designed to investigate the changes which occur in the monoaminergic neurotransmitter systems of rat brain following short-term lithium administration, and to determine whether any «rebound» effects are present following abrupt cessation of lithium treatment.

Fig. 12. Effect of Lithium Treatment and Withdrawal on Spontaneous Activity in Various Animal Models of Mania



Each value represents the mean $\pm$ S.E. mean for 6 rats per group. The figures in parentheses express data as a percentage of control (100%).

Significantly different from control; *p < 0.05.

Three treatment groups were used in this study. One group received lithium carbonate (1 mmol/kg, equivalent to 2 mEq lithium/kg) i.p. for 12 days, while the second group was given the salt (1 mmol/kg) i.p. for 10 days followed by 2 days administration of physiological saline. Eighteen hours after the last injection, animals were sacrificed and the trunk blood collected for lithium analysis. The brains were rapidly removed and sectioned, and unless used for the measurement of enzyme activity, were frozen in liquid nitrogen and stored at -70°C until assayed. Series of brains were used for the measurement of tyrosine, tryptophan, NA, DA, 5-HT, MOPEG, 5-HIAA, HVA, DOPAC and tyrosine hydroxylase and tryptophan hydroxylase activities. In addition, lithium levels in the various brain regions were determined.

i) Lithium concentrations. 18 hr after the last drug administration, serum lithium levels in the lithium-treated animals were 0.320 ± 0.036 mEq/l, while in the rats withdrawn from lithium for 2 days, serum concentration was 0.018 ± 0.002 mEq lithium/l. Lithium levels in the control animals were not detectable. The tissue levels of lithium detected following this treatment regime are shown in Table 2. As can be seen, following 12 days of lithium administration, the cation appears to be distributed relatively evenly throughout the brain, with the highest concentration being in the striatum. After 2 days of withdrawal from lithium treatment, tissue levels of the ion were significantly reduced in all regions of the brain. No lithium was detectable in any region of control brains.

ii) Effect of lithium treatment and withdrawal on precursor levels and synthetic enzyme activity. Neither lithium administration nor its subsequent withdrawal had any effect on the parameters of serotonergic synthesis examined, i.e. tryptophan levels and tryptophan hydroxylase activity (Table 3). With regard to the catechol-aminergic synthetic parameters, no change was noted following lithium treatment. An increase in the activity of tyrosine hydroxylase was seen in the group withdrawn from lithium (131% of control), although this increase did not reach statistical significance.

Table 2. Brain Lithium Concentrations Following Short-Term Treatment and Subsequent Withdrawal

	Li-Treated	Li-Withdrawal
Pons	0.286±0.020	0.055±0.005
Hypothalamus	0.225±0.020	0.070±0.012
Striatum	0.445±0.028	0.039±0.003
Hippocampus	0.327±0.013	0.033±0.002
Midbrain	0.247±0.018	0.052±0.004
Cortex	0.335±0.042	0.035±0.004

Each value is the mean $\pm$ S.E. mean for 6 animals per group. Data are expressed as mEq lithium/kg wet tissue.

Table 3. Effect of Lithium Administration and Subsequent Withdrawal on Parameters of Monoamine Synthesis

Treatment	Tyrosine	Tyrosine Hydroxylase	Tryptophan	Tryptophan Hydroxylase
Control	34.22±6.2 (100)	361.97±56.4 (100)	10.79±2.63 (100)	3.73±0.5 (100)
Li-treated	39.01±5.6 (114)	357.16±56.1 (99)	9.74±0.83 (90)	3.33±1.1 (89)
Li-withdrawal	31.03±1.2 (91)	473.48±66.3 (131)	12.14±1.50 (112)	4.69±0.4 (126)

Each value is the mean ± S.E. mean for 6 animals per group.
Data in parentheses express results as percentage of control (100%).

Tyrosine and tryptophan values expressed as µg/g tissue.
Tyrosine hydroxylase and tryptophan hydroxylase activities expressed as nmol/g tissue/hr.

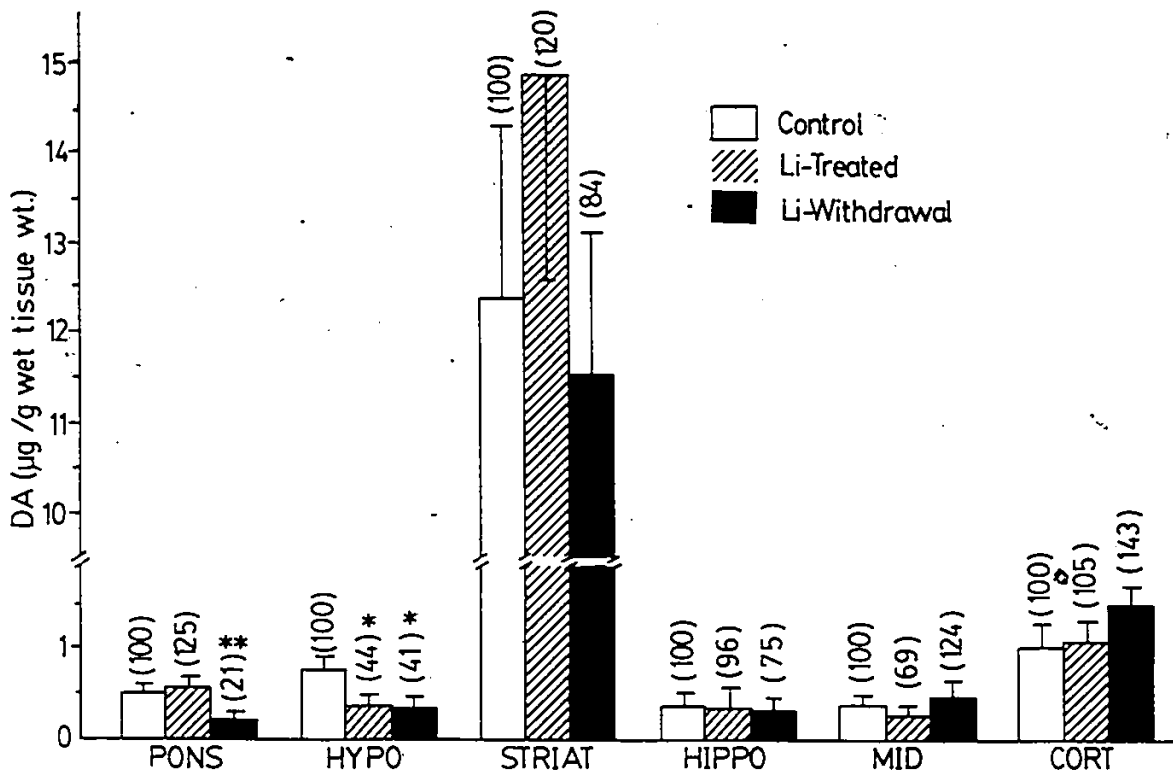
iii) Changes in regional catecholamine levels following lithium administration and withdrawal. After 12 days of lithium administration, the only change in tissue DA content was found in hypothalamus, where a significant decrease relative to control was noted (Fig. 13). Following lithium withdrawal, hypothalamic DA content was still depressed relative to control (41% of control, $p < 0.05$) while the level of neurotransmitter in pons-medulla was reduced (21% of control, $p < 0.01$). Cortical DA content was found to be elevated in the lithium withdrawal group (143% of control), although this change did not reach statistical significance.

Short-term lithium treatment resulted in decreased NA levels in hypothalamus, striatum and hippocampus (Fig. 14). After 2 days of withdrawal from lithium administration, those brain regions which displayed a reduced NA content as a result of 12 days lithium administration did not display a return towards control values. There was also a decrease, although statistically non-significant, in pons-medulla of the withdrawal group, while cortical NA levels were enhanced (187% of control, $p < 0.05$).

iv) Alterations in catecholamine metabolite levels following lithium treatment and withdrawal. Examination of MOPEG and DOPAC tissue levels showed that there was no significant alteration in these parameters as a result of either 12 days of lithium administration or 2 days of lithium withdrawal (Table 4). No alteration in striatal HVA content was noted as a result of lithium treatment, although a significant increase (161% of control, $p < 0.05$) was seen in the withdrawal group.

v) Effect of lithium treatment and withdrawal on regional 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentrations. As can be seen from Table 5, lithium administration for 12 days resulted in a significant decrease in 5-HT content of the mid-brain (65% of control, $p < 0.01$). Following 2 days of withdrawal from lithium treatment, all the brain regions examined, with the exception of hypothalamus, showed a tendency for 5-HT levels to return towards the control values. Hypothalamic content of the neurotransmitter showed a slight, but non-significant increase (114% of control) following lithium withdrawal.

Fig. 13. Changes in Brain Dopamine Levels Following Treatment With and Withdrawal from Short-Term Lithium

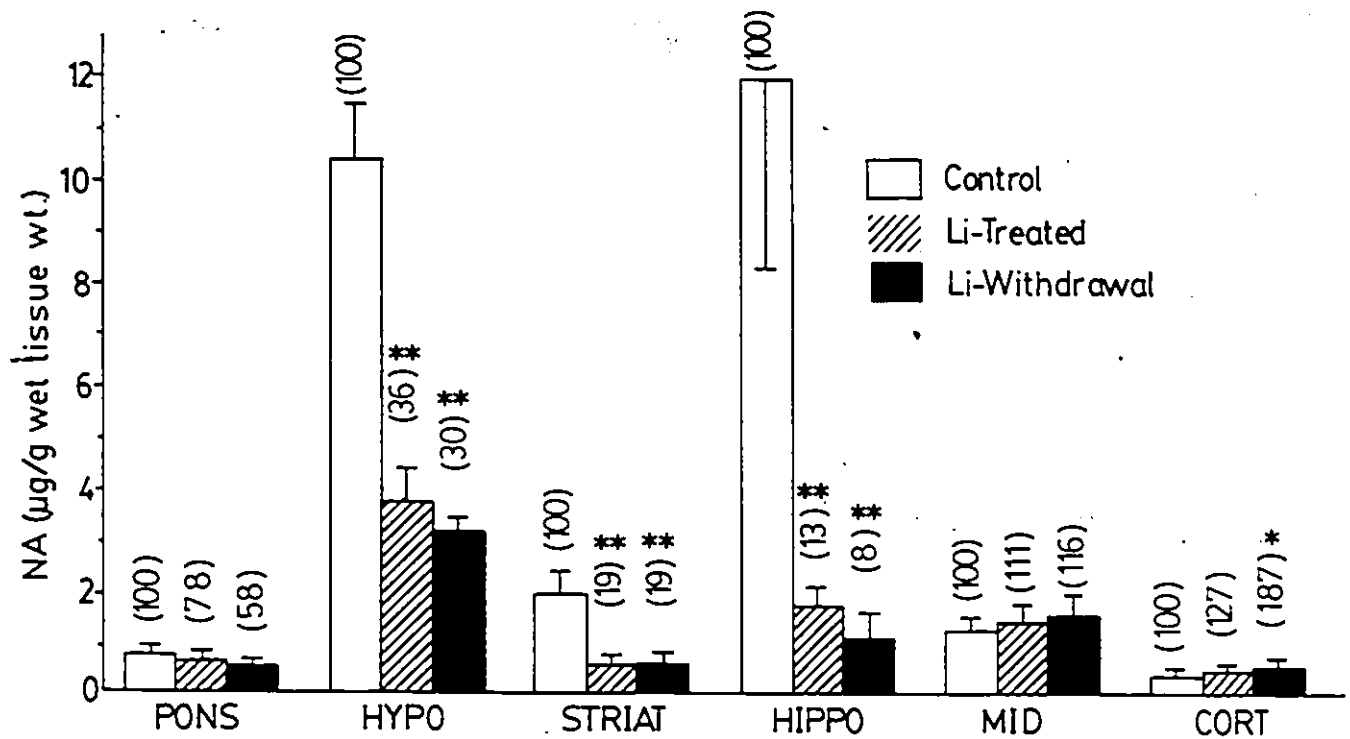


Each value represents the mean $\pm$ S.E. mean for 6 animals per group. Data in parentheses express results in percentages of control values (100%).

Significantly different to control (*p < 0.05; **p < 0.01).

Abbreviations: Pons = pons medulla; Hypo = hypothalamus; Striat = striatum; Hippo = hippocampus; Mid = midbrain; Cort = cortex.

Fig. 14. Effect of Short-Term Lithium Administration and Withdrawal on Central Noradrenaline Content



Each value represents the mean $\pm$ S.E. mean for 6 animals per group. Data in parentheses express results as a percentage of control (100%).

Significantly different to control (*p < 0.05; **p < 0.01).

For abbreviations, see Fig. 13.

Table 4. Changes in Catecholamine Metabolites Following Short-Term Lithium Administration and Subsequent Withdrawal

Metabolite	Control	Li-Treated	Li-Withdrawal
HVA	9.44±1.4 (100)	6.23±1.6 (66)	13.29±3.2 (161)*†
DOPAC	20.98±2.8 (100)	17.82±3.7 (85)	16.24±3.3 (77)
MOPEG	0.288±0.06 (100)	0.240±0.04 (86)	0.213±0.02 (76)

Each value is the mean $\pm$ S.E. mean for 6 animals per group. Values are expressed as $\mu\text{g/g}$ tissue. Data in parentheses express results as percentage of control (100%).

Statistically significant difference when compared to control (*p < 0.05)

Statistically significant difference when compared to Li-treated rats (†p < 0.05).

Table 5. 5-Hydroxytryptamine Concentrations in Rat Brain Regions Following Administration and Withdrawal of Short-Term Lithium

	Control	Li-treated	Li-withdrawal
Pons	1.55±0.08 (100)	1.65±0.08 (106)	1.58±0.12 (102)
Hypothalamus	1.01±0.07 (100)	1.00±0.07 (99)	1.15±0.11 (114)
Striatum	0.28±0.02 (100)	0.25±0.01 (89)	0.26±0.03 (90)
Hippocampus	0.10±0.01 (100)	0.09±0.01 (89)	0.10±0.01 (99)
Midbrain	1.37±0.16 (100)	0.89±0.07 (65)**	1.02±0.10 (75)*
Cortex	0.59±0.03 (100)	0.52±0.04 (90)	0.56±0.04 (95)

Each value is the mean $\pm$ S.E. mean for 6 animals. Values are expressed as $\mu\text{g/g}$ tissue. Data in parentheses express results as a percentage of control (100%).

Significantly different to control values (*p <0.05; **p <0.01).

Table 6. Regional 5-Hydroxyindoleacetic Acid Levels Following Short-Term Lithium Administration and Subsequent Withdrawal

	Control	Li-treated	Li-withdrawal
Pons	1.59±0.08 (100)	1.49±0.06 (94)	1.32±0.15 (83)
Hypothalamus	2.06±0.25 (100)	1.47±0.09 (71)*	1.95±0.15 (94)
Striatum	1.30±0.05 (100)	1.30±0.06 (100)	1.32±0.10 (102)
Hippocampus	0.93±0.06 (100)	1.03±0.13 (110)	1.02±0.06 (109)
Midbrain	1.43±0.14 (100)	1.23±0.14 (86)	1.29±0.08 (90)
Cortex	0.68±0.04 (100)	0.66±0.04 (97)	0.77±0.06 (113)

Each value is the mean ± S.E. mean for 6 animals. Values are expressed as µg/g tissue. Data in parentheses express results as a percentage of control (100%).

Significantly different to control (*p <0.05).

Significantly different to lithium-treated animals (†p <0.05).

On examination of the regional levels of the 5-HT metabolite, 5-HIAA, in the 12 day lithium treated group, a significant decrease (71% of control, $p < 0.05$) was found in the hypothalamus (Table 6). No significant differences to the control animals were found following lithium withdrawal, while hypothalamic levels returned towards control values.

5. Changes in Parameters of Central Monoamine Function After Treatment With and Withdrawal From Long-Term Lithium

Clinical evidence has shown that lithium is of value in the treatment of manic-depressive episodes, and it appears that the monoamine neurotransmitter systems may well be involved in lithium's anti-manic action. In addition to this acute action, lithium has been found to provide prophylaxis against the recurrence of this affective disorder. However, it is not known if the underlying process(es) involved in its efficacy in returning manic subjects to normal is the same as that involved in its prophylactic usefulness. This study therefore set out to determine whether the effect of long-term («prophylactic») administration of lithium is qualitatively the same as that seen following short-term («therapeutic») treatment. In addition, this study examined the consequences of withdrawal from the long-term treatment regime. The withdrawal period utilized in this study was the same as that used in the short-term administration regime, i.e. 2 days.

Three treatment groups were again used: one group of animals received lithium carbonate (1 mmol/kg, equivalent to 2 mEq lithium/kg) i.p. daily for 26 days, while the second group received the salt for 24 days followed by 2 days of saline treatment. Control animals were administered an equivalent volume of physiological saline daily for 26 days. Eighteen hours after the last injection, animals were sacrificed and the trunk blood collected for lithium measurement. The brains were rapidly removed and sectioned, and unless used for the measurement of enzyme activity, were frozen in liquid nitrogen and stored at -70°C until assayed. Series of brains were used for the measurement of various parameters of catecholaminergic and serotonergic function, and for the determination of tissue levels of lithium.

i) Lithium concentrations. Eighteen hours after the last drug administration, serum lithium levels in the lithium-treated rats were found to be 0.345 ± 0.04 mEq/l, while in the withdrawn animals lithium concentrations were 0.036 ± 0.006 mEq/l. No lithium was detectable in the serum obtained from control rats. The serum lithium levels in the lithium-treated group were found to be comparable to those found following 12 days of lithium treatment, while they were slightly higher in the rats withdrawn from long-term treatment when compared to the levels in animals withdrawn from short-term administration.

As was the case with serum levels, the concentrations of lithium in the various brain regions after long-term lithium administration were comparable to those obtained after short-term treatment (Table 7). Two days of withdrawal from the long-term treatment regime also resulted in brain lithium levels which were similar to those found after withdrawal from short-term lithium administration.

ii) Effect of lithium treatment and withdrawal on precursor levels and synthetic enzyme activities. The effects of long-term lithium administration and its subsequent withdrawal on serotonergic synthetic processes resemble those of short-term lithium treatment and withdrawal in that no significant changes in tryptophan content or tryptophan hydroxylase activity were seen (Table 8). With regard to the catecholaminergic synthetic parameters examined, long-term lithium treatment did not change either tyrosine levels or tyrosine hydroxylase activity. Following 2 days of withdrawal however, tyrosine content was significantly reduced (42% of control, $p < 0.01$), while the activity of tyrosine hydroxylase was enhanced (131% of control, $p < 0.05$). This increase in tyrosine hydroxylase activity is of the same magnitude as that noted following withdrawal from short-term lithium administration (Table 3).

iii) Changes in regional catecholamine levels following lithium administration and withdrawal. After 26 days of lithium administration, significant decreases in DA levels were seen in hypothalamus (63% of control, $p < 0.05$) and hippocampus

Table 7. Brain Lithium Concentrations Following Exposure to and Withdrawal from Long-Term Lithium

	Li-Treated	Li-Withdrawal
Pons	0.317±0.028	0.043±0.004
Hypothalamus	0.251±0.017	0.064±0.011
Striatum	0.520±0.037	0.064±0.006
Hippocampus	0.374±0.046	0.030±0.002
Midbrain	0.279±0.017	0.041±0.003
Cortex	0.387±0.018	0.040±0.011

Each value is the mean + S.E. mean for 6 animals per group.
Data are expressed as mEq lithium/kg wet tissue.

Table 8. Effect of Long-Term Lithium Administration and Subsequent Withdrawal on Parameters of Monoamine Synthesis

	Tyrosine	Tyrosine Hydroxylase	Tryptophan	Tryptophan Hydroxylase
Control	8.26±0.7 (100)	360.80±54.1 (100)	5.91±0.69 (100)	11.00±1.1 (100)
Li-treated	8.24±1.4 (99)	393.27±34.5 (109)	5.30±0.13 (90)	9.23±1.9 (84)
Li-withdrawal	3.48±1.0 (42)** ++	474.26±28.5 (131)*	4.90±0.44 (83)	10.00±0.6 (91)

Each value is the mean + S.E. mean for 6 animals per group. Data in parentheses express results in percentage of controls (100%).

Tyrosine and tryptophan levels expressed as µg/g wet tissue wt..
Enzyme activities expressed as nmol/g wet tissue wt./hr.

Statistically significant difference when compared to control
(*p <0.05; **p <0.01).

Statistically significant difference when compared to lithium-
treated rats (++p <0.01).

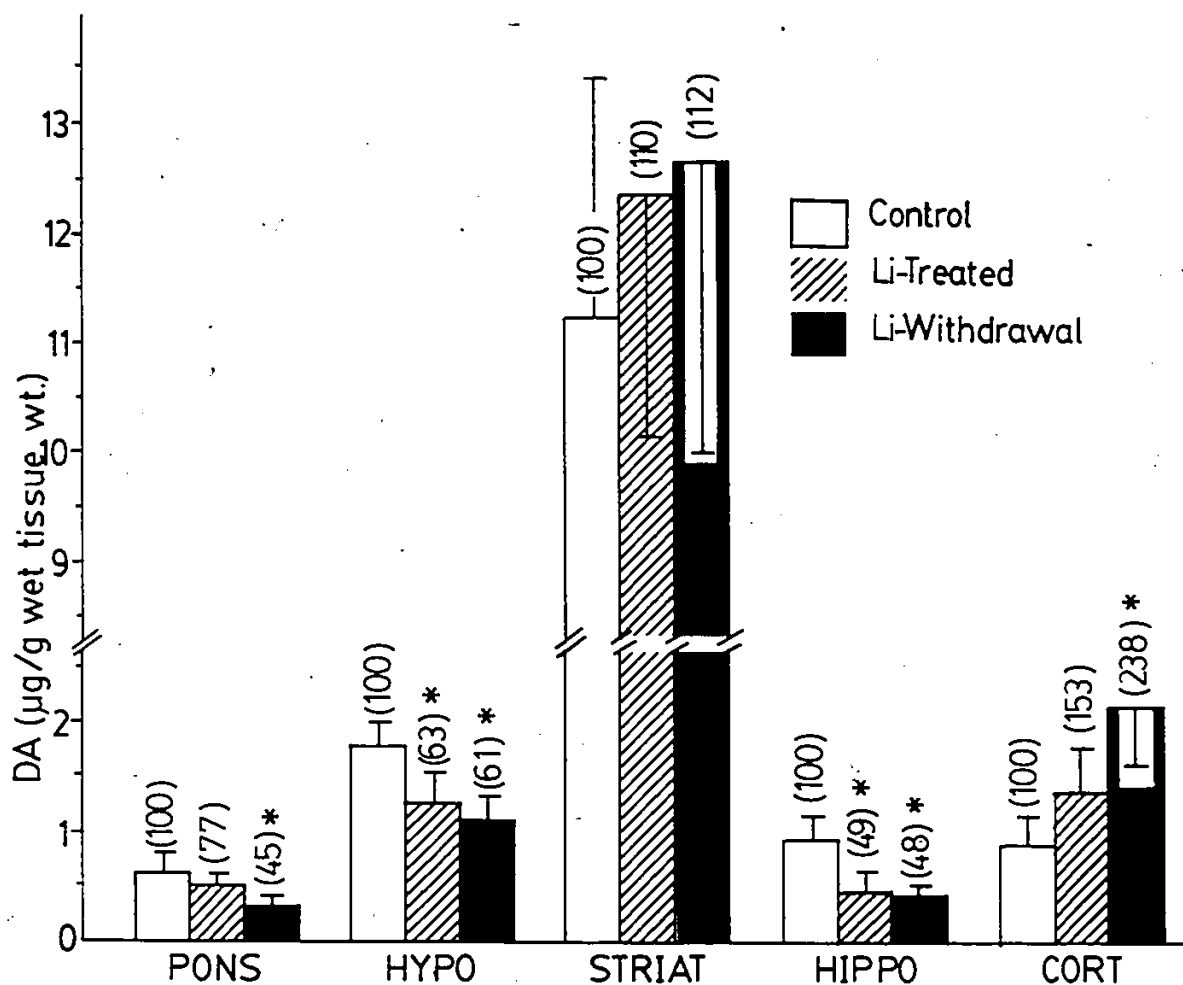
(49% of control, $p < 0.05$) (Fig. 15). DA content of pons-medulla was also depressed (77% of control), while the concentration of this neurotransmitter in cortex was elevated (153% of control), although neither of these changes achieved statistical significance. In the withdrawal group, the reduction in DA content in hypothalamus and hippocampus was maintained, while a further decrease was noted in DA concentration of pons-medulla (to 45% of control, $p < 0.05$). The increase in neurotransmitter levels in cortex seen after long-term lithium administration was enhanced as a result of 2 days withdrawal from lithium (238% of control, $p < 0.05$).

Long-term treatment with lithium resulted in decreased NA content in hypothalamus (65% of control, $p < 0.05$), striatum (26% of control, $p < 0.01$) and hippocampus (58% of control, $p < 0.05$) (Fig. 16). Cessation of lithium after long-term treatment produced a further decrease in hippocampal concentration of NA (to 17% of lithium-treated rats; $p < 0.01$), while pontine levels of the neurotransmitter were also found to be depressed (29% of control, $p < 0.05$). Cortical NA content was elevated in these animals (223% of control, $p < 0.05$).

iv) Alterations in catecholamine metabolite levels following lithium treatment and withdrawal. Lithium administration for 26 days resulted in a significant enhancement in the striatal content of DOPAC (182% of control, $p < 0.05$), while HVA and MOPEG levels were unaltered (Table 9). Following lithium withdrawal, the concentration of DOPAC returned essentially to control values, while there was a significant decrease in MOPEG content (36% of control, $p < 0.01$). There was also an increase in the concentration of striatal HVA in the withdrawal group (165% of control, $p < 0.05$), the magnitude of which was comparable to that found following withdrawal from short-term lithium administration.

v) Effect of long-term lithium treatment and subsequent withdrawal on regional 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentrations. Administration of lithium carbonate to rats for 26 days caused decreased levels of 5-HT in hypothalamus, striatum and mid-brain (75%, 73% and 59% of control, respectively) (Table 10). As was noted after short-term administration of lithium, 2 days of

Fig. 15. Effect of Treatment With and Withdrawal From Long-Term Lithium on Central Dopamine Levels

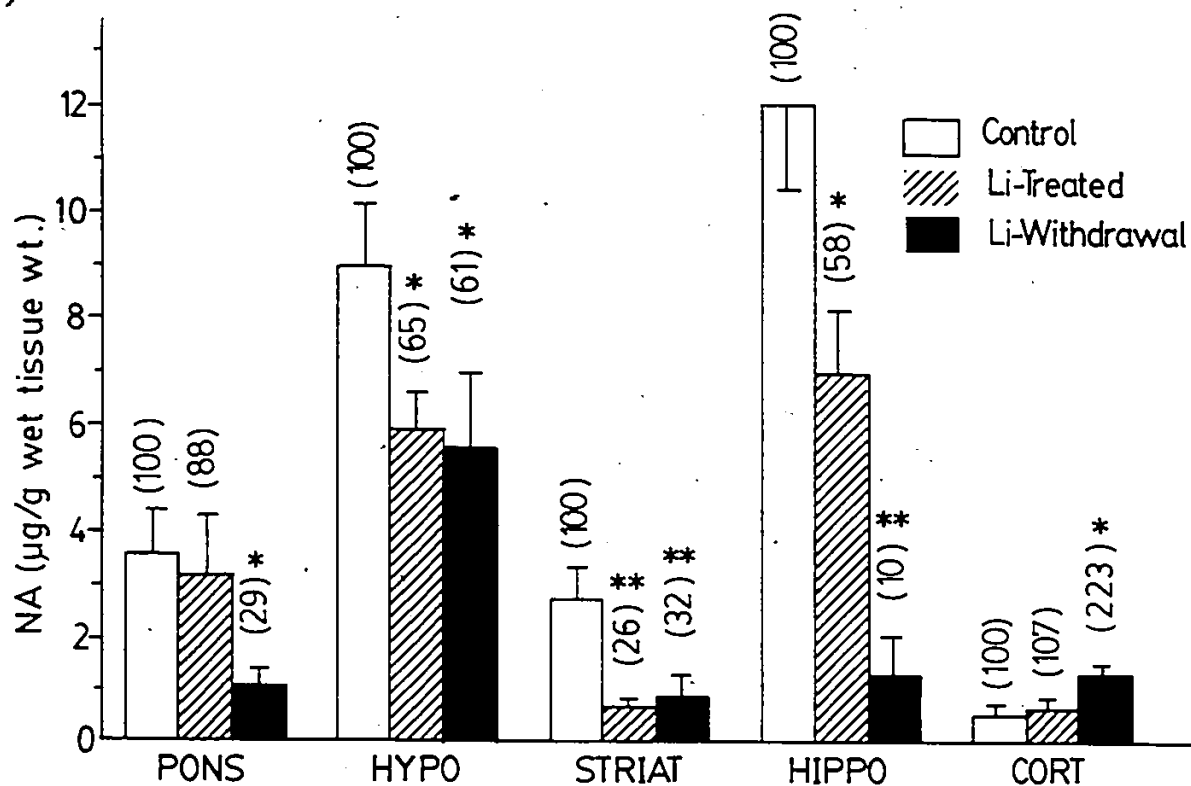


Each value represents the mean $\pm$ S.E. mean for 6 animals per group. Data in parentheses express results in percentages of control (100%).

Significantly different to control (* $p < 0.05$).

Abbreviations: Pons = pons-medulla; Hypo = hypothalamus; Striat = striatum; Hippo = hippocampus; Cort = cortex.

Fig. 16. Brain Noradrenaline Content Following Long-Term Lithium Treatment and Subsequent Withdrawal



Each value represents the mean ± S.E. mean for 6 animals per group. Data in parentheses express results in percentages of control values (100%).

Significantly different to control (*p < 0.05; **p < 0.01).

For abbreviations, see Fig. 15.

Table 9. Catecholamine Metabolite Levels in Rat Brain Following Long-Term Lithium Treatment and Subsequent Withdrawal

	Control	Li-treated	Li-withdrawal
HVA	2.01±0.2 (100)	2.08±0.3 (104)	3.31±0.6 (165)*†
DOPAC	5.12±0.5 (100)	9.32±1.7 (182)*	4.34±0.29 (85)††
MOPEG	0.246±0.02 (100)	0.226±0.03 (92)	0.088±0.02 (36)**††

Each value is the mean $\pm$ S.E. mean for 6 animals per group. Values are expressed as $\mu\text{g/g}$ tissue. Data in parentheses express data as percentage of control (100%).

Statistically significant difference when compared to control (*p < 0.05; **p < 0.01).

Statistically significant difference when compared to lithium-treated rats (†p < 0.05; ††p < 0.01).

Table 10. Changes in Central 5-Hydroxytryptamine Concentration After Long-Term Lithium Treatment and Withdrawal

	Control	Li-treated	Li-withdrawal
Pons	1.01±0.10 (100)	0.96±0.12 (95)	1.13±0.08 (112)
Hypothalamus	2.09±0.12 (100)	1.57±0.09 (75)**	2.28±0.15 (109)†
Striatum	1.34±0.18 (100)	0.98±0.05 (73)*	1.23±0.10 (92)
Hippocampus	0.85±0.09 (100)	0.85±0.12 (99)	0.80±0.07 (93)
Midbrain	1.43±0.06 (100)	0.84±0.10 (59)**	1.21±0.09 (84)*†
Cortex	0.78±0.05 (100)	0.73±0.06 (94)	0.79±0.09 (102)

Each value is the mean ± S.E. mean for 6 animals. Values are expressed as µg/g tissue. Data in parentheses express results as a percentage of control (100%).

Significantly different to control values (*p < 0.05; **p < 0.01).

Significantly different to lithium-treated animals (†p < 0.05).

Table 11. Effect of long-term lithium treatment and withdrawal on regional 5-hydroxyindoleacetic acid levels

	Control	Li-treated	Li-withdrawal
Pons	0.86±0.13 (100)	0.73±0.20 (85)	0.81±0.15 (94)
Hypothalamus	0.60±0.07 (100)	1.16±0.07 (194)**	0.57±0.11 (95)††
Striatum	1.29±0.12 (100)	1.36±0.13 (106)	1.44±0.10 (111)
Hippocampus	1.28±0.06 (100)	1.19±0.20 (93)	1.57±0.24 (123)
Midbrain	0.99±0.10 (100)	1.25±0.04 (126)**	0.74±0.10 (75)††
Cortex	0.56±0.07 (100)	0.48±0.08 (86)	0.63±0.07 (112)

Values are expressed as $\mu\text{g/g}$ tissue, and each is the mean $\pm$ S.E. mean for 6 animals. Data in parentheses express results as a percentage of control (100%).

Significantly different to control values (**p <0.01) or to lithium-treated animals (††p <0.01).

withdrawal resulted in a tendency for 5-HT levels to return towards the control values. Hypothalamic content of the neurotransmitter again showed a slight, but non-significant increase (109% of control) following lithium withdrawal.

Levels of 5-HIAA were found to be elevated in hypothalamus and midbrain (194% and 126% of control, respectively) after 26 days of lithium administration (Table 11). Following 2 days of withdrawal from the long-term treatment regime, concentrations of the metabolite in most brain regions displayed a return towards control levels. In the midbrain however, there was a decrease, although non-significant, relative to control (75%).

6. Changes in Monoamine Uptake into Synaptosomes from Various Regions of Rat Brain Following Lithium Administration and Withdrawal

It has long been known that re-uptake of NA into nervous tissue is a major route of inactivation for the neurotransmitter, and it is assumed that the same type of mechanism would apply for the other monoamine transmitter substances. Many psychotherapeutic agents (e.g. tricyclic antidepressants) have the ability to alter uptake, and it is thought that at least part of their therapeutic efficacy is due to changes in this process. If the biogenic amine hypothesis of affective disorders is indeed correct, then mania is a consequence of a functional excess of monoaminergic neurotransmitter at critical synapses. Should this be the case, it would be reasonable to assume that lithium may exert an effect on this inactivation process. A limited number of studies have examined lithium-induced changes in NA uptake, and have shown that lithium treatment may well enhance synaptosomal NA uptake. However, an early in vivo study had shown that lithium administration did not affect the uptake of NA into brain tissue. This study therefore set out to examine the consequences of short-term lithium administration and its subsequent withdrawal on the uptake of NA, DA and 5-HT into synaptosomes prepared from various regions of rat brain.

(a) Determination of the uptake of monoamines as a function of time

Synaptosomal fractions were prepared from each brain region of drug-naive rats as described previously. Aliquots of these preparations were incubated at 37°C for 5 min. At the end of this time, 20 μ l of buffer containing one of the radiolabelled monoamines was added to the synaptosomal aliquots, and the fraction incubated for a further period of time ranging from 30 sec to 20 min. The concentrations of tritiated ligands used were 0.05 μ M and 5 μ M.

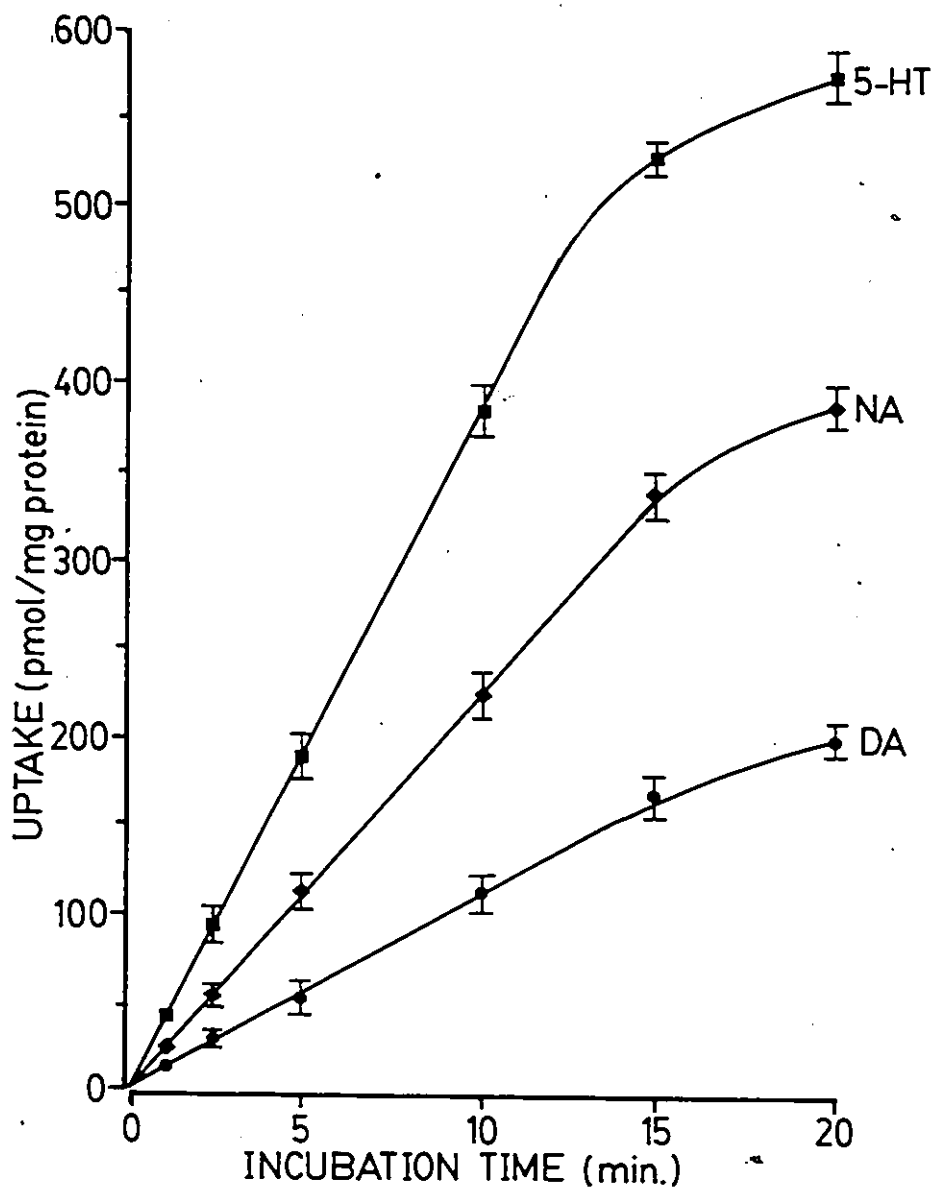
Figure 17 shows the uptake of monoamines into hypothalamic synaptosomal fractions using a substrate concentration of 5 μ M. As can be seen, synaptosomal uptake of all three monoamines was linear for at least 10 min, and this was found to be the case for all brain regions examined at both concentrations of substrate, and for this reason, 10 min was chosen as the appropriate incubation period in subsequent uptake studies.

(b) Synaptosomal uptake of a single concentration of radiolabelled ligand

Three treatment groups were used in this study: i) received 1 mmol/kg lithium carbonate i.p. for 12 days; ii) received lithium carbonate (1 mmol/kg) i.p. for 10 days followed by physiological saline for 2 days; iii) received an equivalent volume of physiological saline for 12 days. Eighteen hours after the last injection, animals were sacrificed, the brains removed, sectioned and synaptosomal fractions prepared from each brain region. Aliquots of the synaptosomal preparations were then incubated with 100 μ Ci of radiolabelled monoamine, resulting in final concentrations of 310 nM (3 H)NA, 1.03 μ M (3 H)DA and 160 nM (3 H)5-HT.

Following 12 days of treatment with 2 mEq lithium/kg, the amount of (3 H)DA taken up by the synaptosomal preparations showed a highly significant increase in all regions examined (Table 12), with the greatest relative increase being in the hippocampus (250% of control). When the animals had been withdrawn from lithium for 2 days, all brain regions examined, with the exception of the hippo-

Fig. 17. Uptake Into Hypothalamic Synaptosomes as a Function of Time



Each point is the mean $\pm$ S.E. mean for 3 determinations. Aliquots of the synaptosomal preparation were incubated at 37°C in the presence of 5 μ M monoamine.

Table 12. Synaptosomal Dopamine Uptake Following Lithium Treatment and Withdrawal

	Control	Li-treated	Li-withdrawal
Pons	256.2±6.6 (100)	426.7±30.0 (167)**	242.4±5.9 (95)++
Hypothalamus	132.0±6.8 (100)	301.4±17.0 (228)**	145.0±3.5 (110)++
Striatum	139.4±2.5 (100)	247.1±9.4 (177)**	327.6±10.3 (235)**++
Hippocampus	161.1±8.9 (100)	404.3±4.5 (250)**	385.7±5.8 (239)**
Midbrain	153.9±3.9 (100)	348.5±5.1 (227)**	192.4±2.6 (125)**++
Cortex	139.3±8.2 (100)	346.2±8.4 (249)**	159.8±5.2 (115)++

Values are the mean ± S.E. mean of 6 determinations per group and are expressed as pmol/g tissue/10 min. Data in parentheses express results as percentages of control (100%).

Significantly different to control values (**p <0.01).

Significantly different to lithium-treated rats (++p <0.01).

campus and striatum, showed a significant decrease in uptake relative to the 12-day treated rats, i.e. the uptake values tended to return towards those of the control preparations. The hippocampus showed no significant change from the 12-day treated animals while the striatal preparations displayed a highly significant increase.

In the case of (^3H)5-HT uptake (Table 13), twelve days of lithium administration resulted in significant decreases in uptake in all regions, with the greatest relative decrease being in the midbrain (to 37% of control). Following withdrawal from lithium, significant increases in uptake values, i.e. tending towards control, were noted in pons-medulla, hippocampus, hypothalamus and cortex, while no differences to the 12-day treated animals were seen in striatal and midbrain preparations.

Uptake of (^3H)NA also showed regional variation, with the maximal uptake in striatum, and the least in hypothalamus (Table 14). The influence of 12 days of lithium treatment appeared to be a combination of the effects seen with (^3H)5-HT and (^3H)DA uptake. The amount of (^3H)NA taken up by synaptosomes was significantly enhanced in the hypothalamus and striatum, but decreased in the midbrain and cortex, with no significant changes in the pons-medulla or hippocampus. On cessation of lithium, the uptake values in the regions which showed change due to lithium all displayed a tendency to return towards control.

As can be seen, lithium treatment appeared to have profound effects on the synaptosomal uptake of all three monoamines, with the changes due to lithium withdrawal being confined to dopaminergic and serotonergic systems. However, the data obtained in this study should be treated with caution since uptake is expressed in terms of pmol/g wet tissue weight/10 min. It is known that the amount of monoamine taken up by synaptosomes is proportional to the amount of protein present in the sample, and no allowances were made in this study for the possibility of varying amounts of protein in the different aliquots of synapto-

Table 13. Changes in Synaptosomal Uptake of 5-Hydroxytryptamine After Lithium Treatment and Withdrawal

	Control	Li-treated	Li-withdrawal
Pons	70.4±0.8 (100)	44.7±1.7 (64)**	61.7±2.6 (88)*††
Hypothalamus	69.0±2.2 (100)	26.6±3.0 (39)**	34.9±1.6 (51)**
Striatum	56.6±2.2 (100)	34.2±4.0 (60)**	32.5±1.8 (57)**
Hippocampus	51.4±6.3 (100)	21.1±4.0 (41)**	33.2±3.4 (64)*†
Midbrain	80.0±4.0 (100)	29.8±11.8 (37)*	28.6±0.8 (36)**
Cortex	48.1±6.7 (100)	30.5±1.9 (63)*	34.0±3.3 (71)*

Each value is the mean + S.E. mean for 6 determinations per group. Values are expressed as pmol/g tissue/10 min. Data in parentheses express results as percentages of control values (100%).

Statistically significant difference when compared to control (*p <0.05; **p <0.01).

Significantly different to lithium-treated rats (†p <0.05; ††p <0.01).

Table 14. Effect of Lithium Treatment and Subsequent Withdrawal on Synaptosomal Uptake of ³H-Noradrenaline

	Control	Li-treated	Li-withdrawal
Hypothalamus	15.5±2.0 (100)	49.6±4.0 (321)**	29.2±3.1 (189)*†
Striatum	66.7±5.4 (100)	266.2±6.8 (399)**	136.7±19.0 (205)*††
Midbrain	50.9±8.4 (100)	11.9±3.0 (23)*	30.3±7.2 (60)†
Cortex	40.9±5.8 (100)	21.8±6.4 (53)*	34.8±3.2 (85)

Values are expressed as pmol/g tissue/10 min and each is the mean ± S.E. mean for 6 determinations per group. Data in parentheses express results as percentages of control (100%).

Significantly different when compared to control (*p <0.05; **p <0.01).

Significantly different to lithium-treated rats (†p <0.05; ††p <0.01).

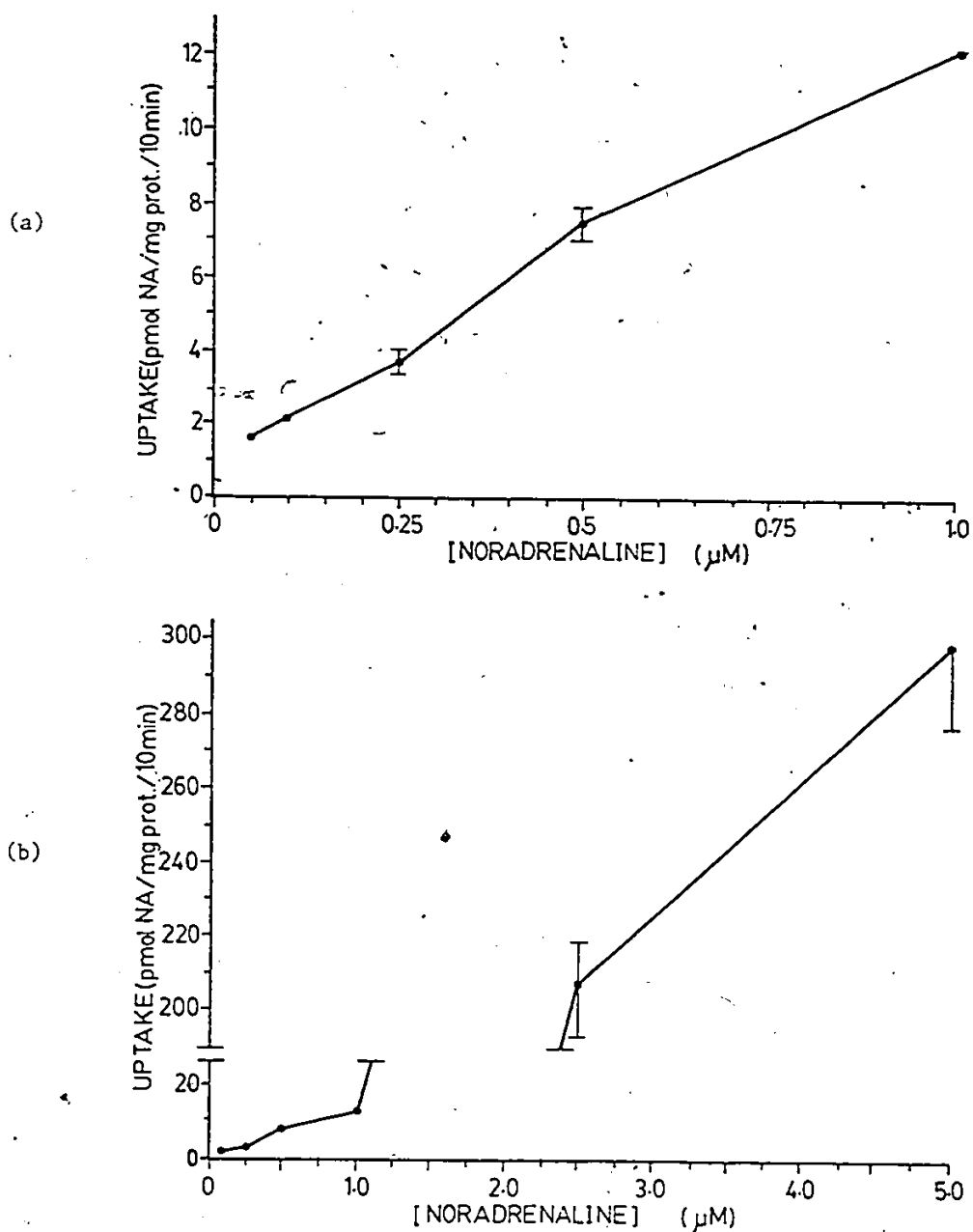
somes used. Despite this, it is probable that at least part of the changes in synaptosomal uptake noted are due to lithium per se, but the use of a single concentration of ligand results in the accumulation of limited information, and it was therefore decided to examine the uptake of the monoamines using a range of substrate concentrations.

(c) Consequences of lithium treatment and withdrawal on synaptosomal monoamine uptake: A kinetic study

Series of animals were treated as described previously. Eighteen hours after the last injection the rats were sacrificed, the brains removed and sectioned, and synaptosomal fractions prepared from each brain region. Aliquots of the synaptosomal preparations were incubated in a modified Krebs-Henseleit buffer containing radiolabelled monoamine in concentrations ranging from 0.05 μM to 5.0 μM . Portions of the synaptosomal preparations were also used for the determination of protein content. Plots of uptake velocity vs. substrate concentration were then constructed for each brain region for each of the three monoamines examined.

Figure 18 displays the uptake of (^3H)NA into hippocampal synaptosomes. As can be seen in Fig. 18a, NA uptake into synaptosomes prepared from this brain region displayed linear characteristics for substrate concentrations ranging from 0.05 μM to 1 μM . However, when higher substrate concentrations (2.5 μM and 5.0 μM) were incorporated into the graphic display (Fig. 18b), a second uptake component became apparent. This was true of all the brain regions examined. The presence of two uptake components for NA uptake was further confirmed by the finding that it was not possible to obtain a linear plot for the uptake of NA using double-reciprocal transformation methods (Lineweaver-Burk plot). When the uptake of NA into synaptosomal fractions was treated as a two-component system, it was possible to calculate K_m and V_{max} values for the low-capacity system, but not for the high-capacity system due to the lack of sufficient data.

Fig. 18. Noradrenaline Uptake into Hippocampal Synaptosomes



Each point is the mean $\pm$ S.E. mean for 3 determinations (S.E. mean not shown when smaller than symbol).

points on which to base the Lineweaver-Burk plot. As can be seen in Table 15, the low-capacity uptake system in synaptosomes prepared from the 6 brain regions examined have similar affinities for NA, and similar maximal velocities of substrate uptake.

Following lithium administration for 12 days, when synaptosomes were incubated with (^3H)NA at concentrations of up to 1 μM , there was an enhancement in the ability of synaptosomes to take up the catecholamine (Fig. 19a). When the uptake constants were calculated for the NA uptake into the synaptosomes prepared from lithium-treated rats, there was a tendency for an increase in the V_{max} for all 6 brain regions (Table 16), with the greatest relative increase being in the striatal synaptosomes. There was also a concomitant decrease in the affinities of the synaptosomes for the substrate (as demonstrated by an increase in the K_{m} value) in all regions except hypothalamus, in which there was no difference when compared to control. When uptake at higher NA concentrations was examined (Fig. 19b), an even more surprising change is seen. At substrate concentrations of 2.5 μM and 5.0 μM , (^3H)NA uptake into synaptosomes prepared from all regions of the brains of lithium-treated rats was significantly reduced with respect to control values.

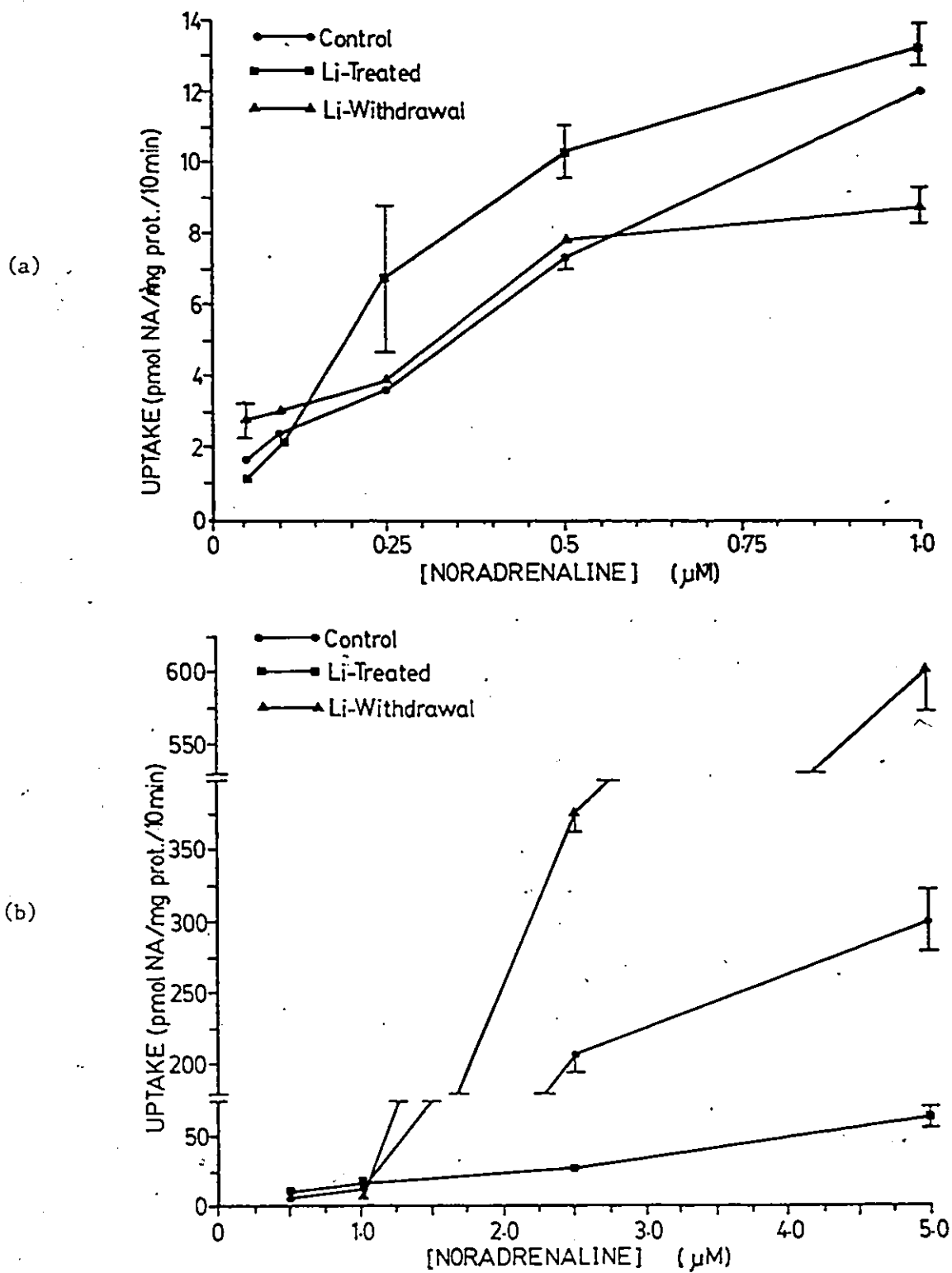
Synaptosomal preparations from rats withdrawn from lithium displayed a return towards control values of NA uptake in the substrate concentration range of 0.05 μM to 1.0 μM (Fig. 19a). As can be seen from Table 16, K_{m} and V_{max} values at this time also returned to, or towards, control values for both these constants, except in hypothalamus and cortex. In both of these regions, V_{max} values were found to be lower in the lithium-withdrawal group with respect to control, together with an increase in affinity for the substrate. At the higher concentrations of NA however, there was a marked elevation in the amount of NA taken up into synaptosomes prepared from all brain regions (Fig. 19b), with the exception of cortex. Uptake of the catecholamine via the high-capacity system

Table 15. Uptake Constants for Synaptosomal Accumulation of Noradrenaline and 5-Hydroxytryptamine

	NA		5-HT	
	Km	Vmax	Km	Vmax
Pons	1.020 ±0.01	20.46 ±1.04	0.403 ±0.06	10.64 ±0.16
Hypothalamus	1.253 ±0.06	26.73 ±0.57	0.366 ±0.01	11.14 ±0.02
Striatum \	0.657 ±0.03	24.83 ±1.63	0.534 ±0.02	13.48 ±0.05
Hippocampus	0.627 ±0.02	15.34 ±0.74	0.399 ±0.02	9.38 ±0.07
Midbrain	0.818 ±0.04	22.89 ±1.40	0.329 ±0.04	6.55 ±0.09
Cortex	1.019 ±0.55	29.84 ±15.03	0.302 ±0.02	8.88 ±0.04

Each value is the mean + S.E. mean for 3 determinations. Km expressed as 10^{-6} M, and Vmax expressed as pmol/mg Protein/10 min.

Fig. 19. Noradrenaline Uptake into Hippocampal Synaptosomes After Lithium Administration and Withdrawal



Each point is the mean $\pm$ S.E. mean for 3 determinations (S.E. mean not shown when smaller than symbol).

Table 16. Effect of Lithium Treatment and Withdrawal on Constants Associated With Noradrenaline Uptake

	Control		Li-treated		Li-withdrawal	
	Km	Vmax	Km	Vmax	Km	Vmax
Pons	1.020 ±0.01	20.46 ±1.04	3.544 ±1.00*	40.72 ±14.03	1.544 ±0.23	19.79 ±4.10
Hypothalamus	1.253 ±0.06	26.73 ±0.57	1.066 ±0.22	44.97 ±8.52	0.505 ±0.03**	8.969 ±0.16***
Striatum	0.657 ±0.03	24.83 ±1.63	3.518 ±0.72**	105.47 ±10.38**	0.738 ±0.25	17.06 ±3.85
Hippocampus	0.627 ±0.02	15.34 ±0.74	1.561 ±0.25*	40.48 ±8.01*	1.175 ±0.14*	22.18 ±2.52
Midbrain	0.818 ±0.04	22.89 ±1.40	2.862 ±0.42**	32.02 ±3.06*	1.544 ±0.35	17.09 ±3.36†
Cortex	1.019 ±0.55	29.84 ±15.03	3.616 ±0.62*	48.96 ±9.60	0.390 ±0.04††	7.71 ±0.27††

Each value is the mean ± S.E. mean for 3 determinations. Km expressed as 10^{-6} M and Vmax expressed as pmol/mg protein/10 min.

Significantly different to control (*p < 0.05; **p < 0.01).

Significantly different to lithium-treated rats (†p < 0.05; ††p < 0.01).

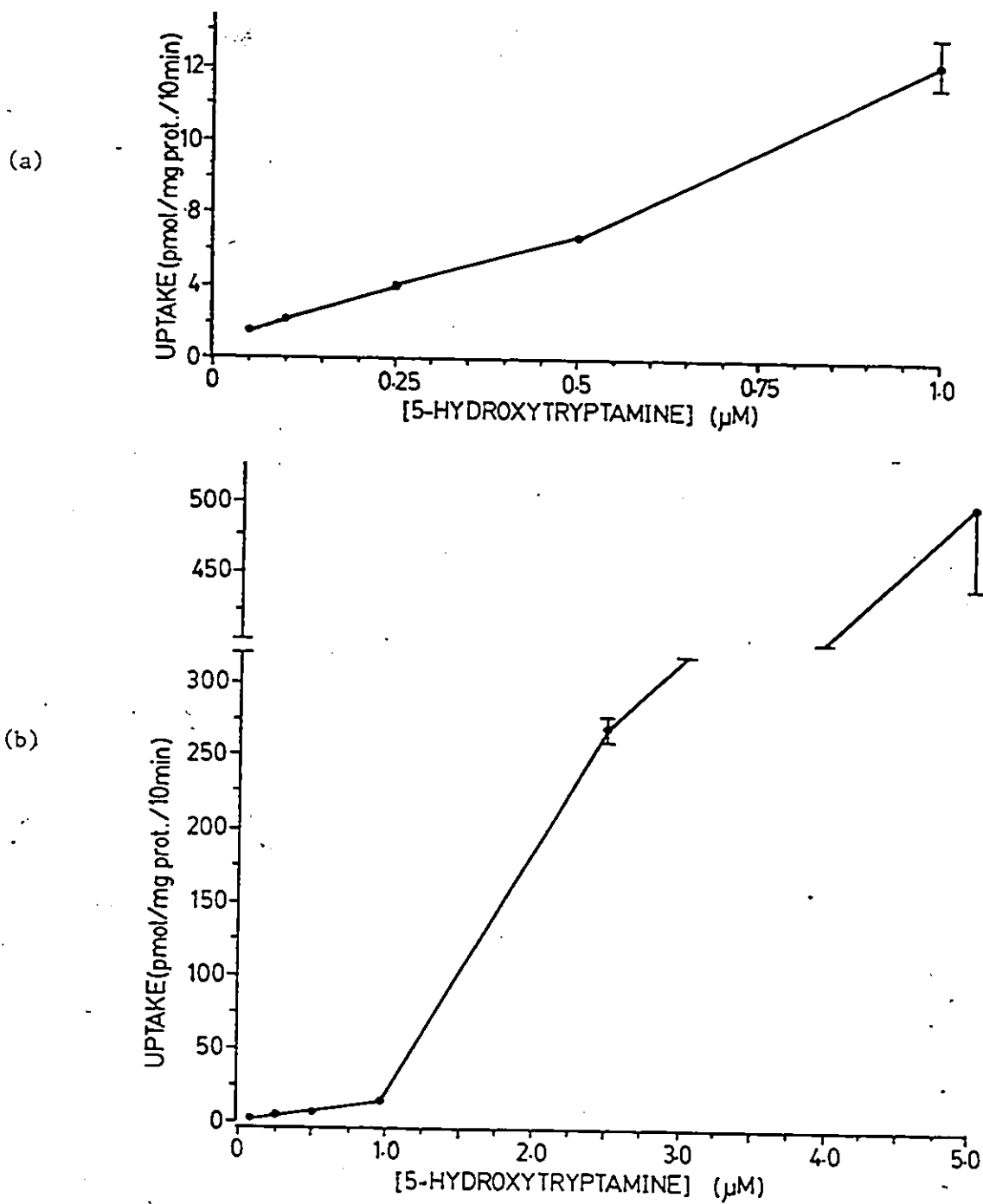
in this brain area was essentially the same as that noted in control preparations, although it was significantly greater than was seen in synaptosomal fractions obtained from lithium-treated animals.

When the synaptosomal uptake of (^3H)5-HT was examined, a similar situation to that noted for NA was observed. With exogenous 5-HT concentrations of up to 1 μM , uptake appeared to be linear, with a second high-capacity system becoming apparent at substrate concentrations of 2.5 μM and 5.0 μM (Fig. 20). Again, it was only possible to calculate K_m and V_{max} values using Lineweaver-Burk transformations for the low-capacity system due to the lack of data regarding the high-capacity uptake process. As shown in Table 15, the values of these constants for the low-capacity system are similar in all brain regions examined, but were consistently lower than those calculated for NA. These data indicate that the uptake process for 5-HT present at low substrate concentrations has a higher affinity, but a lower capacity, than the corresponding system for NA.

Following lithium administration, 5-HT uptake was found to be slightly reduced in all brain regions with substrate concentrations of up to 1 μM (Fig. 21a). This was found to be due primarily to a decrease in V_{max} (Table 17), which was accompanied in some cases by an increase in affinity. At higher substrate concentrations, it was found that there was a decrease in uptake also (Fig. 21b).

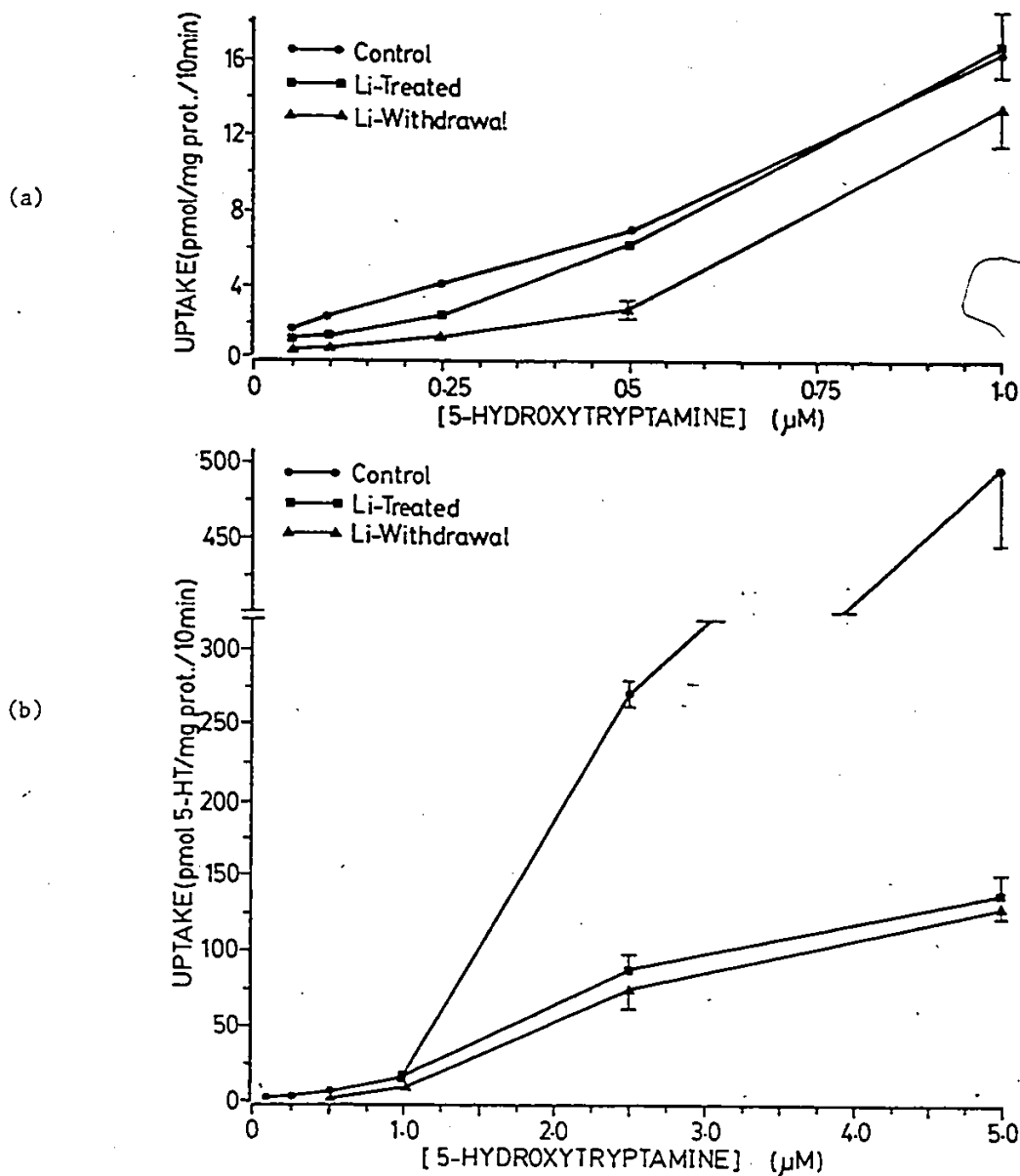
However, it was in the synaptosomal preparations obtained from rats which had been withdrawn from lithium that the greatest change in 5-HT uptake parameters was apparent. In all 6 brain regions examined, there was a reduction in the amount of monoamine taken up by the synaptosomal fraction at substrate concentrations of up to 1 μM (Fig. 21a). On transformation of the data, it was found that there was a significant reduction in V_{max} , relative to control, in all brain regions with the exception of pons-medulla (Table 17). In this brain region, there was a tendency towards reduced values of this parameter, although

Fig. 20. 5-Hydroxytryptamine Uptake into Hypothalamic Synaptosomes



Each point is the mean $\pm$ S.E. mean for 3 determinations (S.E. mean not shown when smaller than symbol).

Fig. 21. Effect of Lithium Treatment and Withdrawal on 5-HT Uptake into Hypothalamic Synaptosomes



Each point is the mean $\pm$ S.E. mean for 3 determinations (S.E. mean not shown when smaller than symbol).

Table 17. Constants Relating to Synaptosomal Uptake of 5-Hydroxytryptamine Following Lithium Administration and Withdrawal

	Control		Li-treated		Li-withdrawal	
	Km	Vmax	Km	Vmax	Km	Vmax
Pons	4.030 ±0.56	10.64 ±1.56	4.273 ±0.12	10.21 ±0.23	5.267 ±0.75	7.25 ±1.20
Hypothalamus	3.663 ±0.14	11.14 ±0.19	4.387 ±0.42	7.13 ±0.35**	4.230 ±1.03	3.85 ±0.85***†
Striatum	5.340 ±0.16	13.48 ±0.51	3.013 ±0.12**	5.42 ±0.49**	3.390 ±0.21**	4.22 ±0.01**
Hippocampus	3.993 ±0.15	9.38 ±0.72	3.057 ±0.02**	7.85 ±0.44	2.147 ±0.40*	3.06 ±0.64***††
Midbrain	3.290 ±0.41	6.55 ±0.81	2.160 ±0.05*	4.88 ±0.18	1.053 ±0.03***††	2.69 ±0.16***††
Cortex	3.023 ±0.20	8.88 ±0.35	3.367 ±0.66	7.45 ±0.76	3.357 ±0.11	2.78 ±0.18***††

Each value is the mean $\pm$ S.E. mean for 3 determinations. Km expressed as 10^{-7} M and Vmax expressed as pmol/mg protein/10 min.

Significantly different to control (*p <0.05; **p <0.01).

Significantly different to lithium treatment (†p <0.05; ††p <0.01).

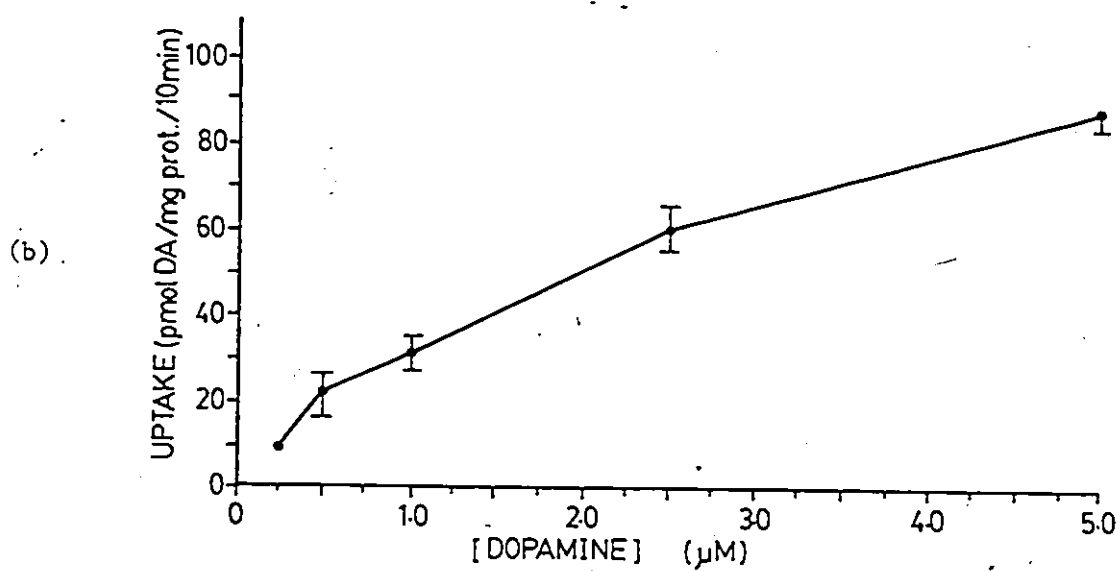
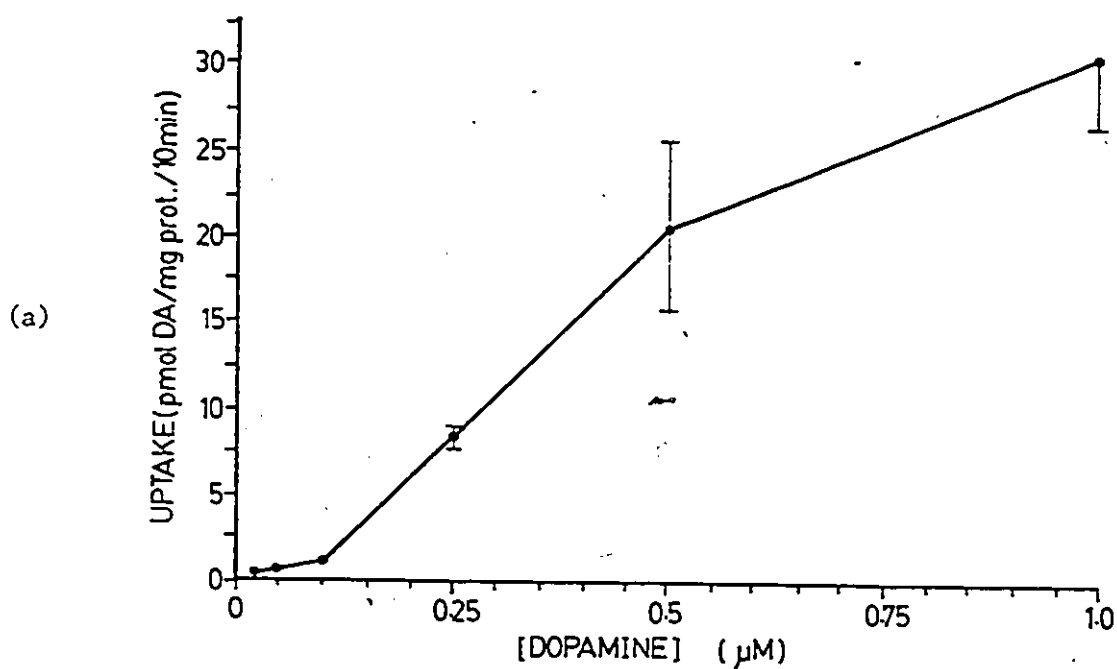
the change did not reach statistical significance. In addition, significant alterations in the K_m of the uptake process were noted in striatum, hippocampus and midbrain. When synaptosomal uptake of 5-HT was examined at the higher substrate levels (Fig. 21b), it was found that there was no difference in the amount of monoamine taken up by synaptosomes prepared from either the lithium-treated or the lithium-withdrawal rats, although both were significantly lower than control.

The uptake of DA into striatal synaptosomes is shown in Fig. 22. As can be seen, there appeared to be more than one uptake process present for the catecholamine. When Lineweaver-Burk plots were constructed for DA uptake, curves were produced which could be resolved into two linear components. The kinetic constants for these two components are shown in Table 18, and it can be seen that the dissociation constants for Uptake₂ are similar in all brain regions examined, as are the V_{max} values. The values for K_m and V_{max} for Uptake₁ are also given in Table 18, and it can be seen that the dissociation constants for this uptake process are two orders of magnitude greater than for Uptake₂, with a corresponding increase in V_{max} . It was not possible to calculate the kinetic constants for the high-capacity process for DA uptake into pons-medulla.

When DA uptake into synaptosomes prepared from the brains of lithium-treated rats was examined (Fig. 23), it was generally found that there was an increase in the amount of amine accumulated at most concentrations used. However, it was not possible to calculate the kinetic constants for DA uptake into synaptosomes prepared from lithium-treated rats, as transformation of the data into double-reciprocal plots did not allow for linearization, even when a two-component system was assumed.

Following lithium withdrawal, uptake of DA into regional synaptosomes tended to show a decrease relative to both control and lithium-treated preparations (Fig. 23). Again, it was not possible to linearize the data to allow calculation of the kinetic constants.

Fig. 22. Dopamine Uptake into Striatal Synaptosomes



Each point is the mean $\pm$ S.E. mean for 3 determinations (S.E. mean not shown when smaller than symbol).

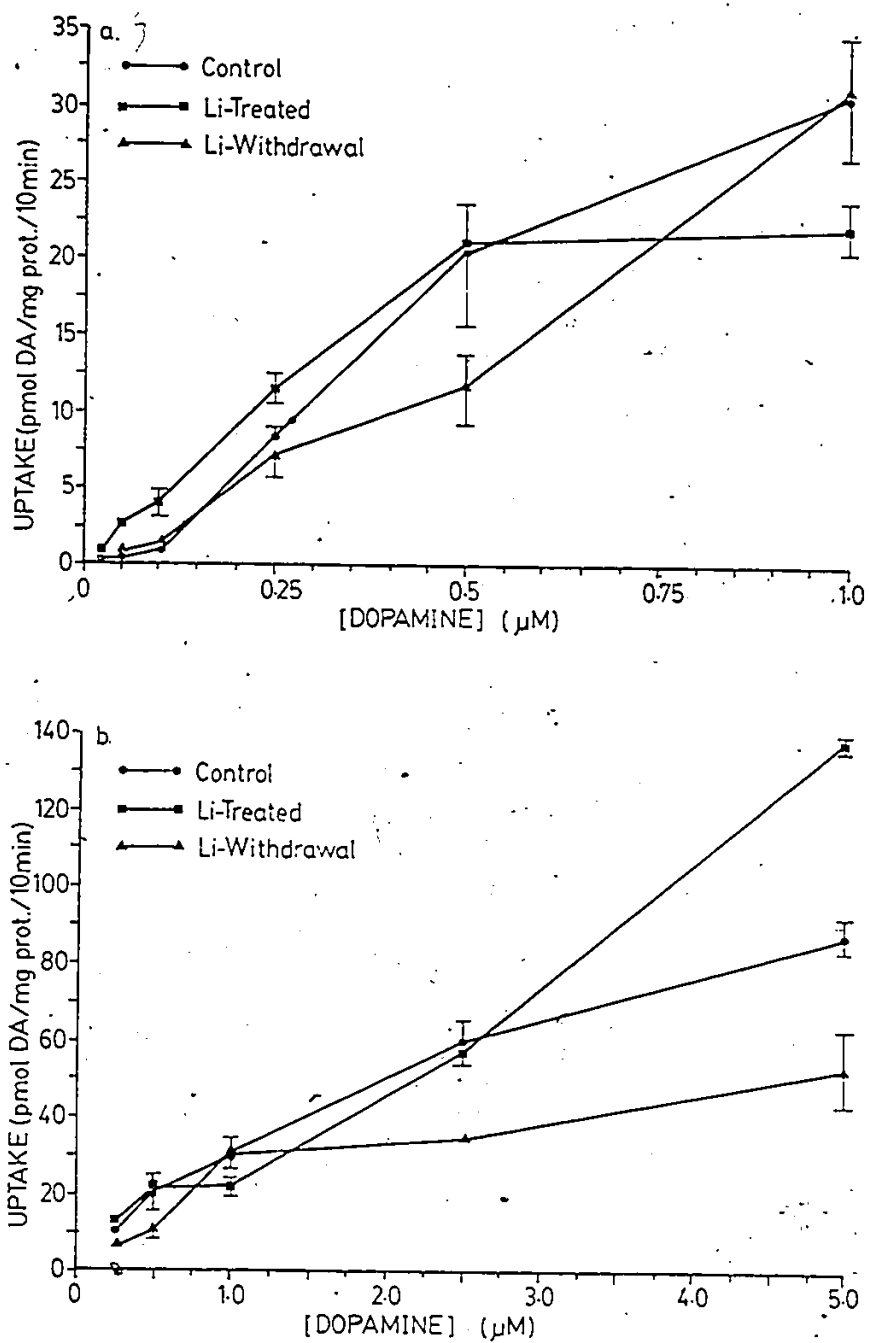
Table 18. Kinetic Constants for Dopamine Uptake by Rat Brain Synaptosomes

	Uptake _a		Uptake _b	
	Km	Vmax	Km	Vmax
Pons	0.53 ±0.007	0.442 ±0.066	NA	NA
Hypothalamus	0.049 ±0.003	0.456 ±0.018	2.776 ±0.224	67.58 ±9.41
Striatum	0.050 ±0.001	0.574 ±0.083	4.180 ±1.676	151.86 ±31.30
Hippocampus	0.036 ±0.008	0.473 ±0.091	5.050 ±0.370	172.49 ±4.99
Midbrain	0.033 ±0.013	0.413 ±0.147	9.166 ±1.931	76.22 ±17.09
Cortex	0.038 ±0.005	0.677 ±0.105	18.575 ±6.885	317.40 ±122.06

Each value is the mean ± S.E. mean for 3 determinations. Km is expressed as 10⁻⁶M and Vmax as pmol/mg protein/10 min.

NA - constants could not be calculated.

Fig. 23. Alterations in Dopamine Uptake into Striatal Synaptosomes Following Lithium Treatment and Withdrawal



Each point is the mean $\pm$ S.E. mean for 3 determinations (S.E. mean not shown when smaller than symbol).

7. The Effect of Lithium Administration and Withdrawal on Binding of Spiroperidol and Dihydroalprenolol to Synaptic Membranes from Various Regions of Rat Brain

Many recent studies have focused on the effects of various psychotropic drugs on central receptor populations, and lithium has been no exception, although the investigations regarding this anti-manic agent have been somewhat limited. This study therefore set out to examine further the changes in β -adrenergic and dopaminergic receptors following lithium treatment, and to determine whether abrupt cessation of lithium administration caused an effect on these populations.

The three treatment schedules described previously were again utilized, and after 12 days of treatment the animals were sacrificed and synaptic membrane fractions prepared. Aliquots of these preparations were then incubated with concentrations of either (^3H)dihydroalprenolol (for β -adrenoceptor determination) or (^3H)spiroperidol (for dopaminergic binding sites) ranging from 0.5 to 20.0 nM. Binding constants were determined by Scatchard analysis of the binding data obtained.

i) Changes in dihydroalprenolol binding following lithium treatment and withdrawal.

The values for the dissociation constants (K_D) and total number of binding sites (B_{max}) for each brain region examined for dihydroalprenolol binding are shown in Table 19, and as can be seen, the B_{max} values show more regional variation than is seen with the K_D values. The effect of 12 days of lithium treatment also displays regional variability, with enhanced B_{max} values in hypothalamus, striatum and cortex, and decreases in pons-medulla and midbrain. Hippocampus displayed little change in this parameter or in K_D value. A decreased K_D value was noted in pons-medulla, in which a decrease in B_{max} was observed, while increased K_D values were found in hypothalamus and striatum, both of which areas has increased numbers of binding sites.

Following lithium withdrawal, B_{max} values returned towards control in hypothalamus, striatum and cortex. In hippocampus and midbrain however, the number

Table 19. Effect of Lithium Treatment and Withdrawal on Constants of β -Adrenoceptor Binding

	Control		Li-treated		Li-withdrawal	
	K _D	B _{max}	K _D	B _{max}	K _D	B _{max}
Pons	3.89	169.3	1.80	108.8	NA	
Hypothalamus	3.88	95.2	9.47	176.8	1.94	130.4
Striatum	1.13	22.1	3.83	328.5	3.64	182.8
Hippocampus	2.13	33.4	1.95	40.6	8.83	206.9
Midbrain	3.61	157.2	3.18	128.7	4.89	239.7
Cortex	3.45	88.27	3.40	126.7	2.69	79.5

Values are the result of one assay carried out in triplicate. K_D values are expressed as nM, while B_{max} is expressed as fmol/mg protein.

NA - values not available

of binding sites was found to increase following lithium withdrawal. With regard to the dissociation constants, these remained elevated in striatum, but declined relative to control and lithium-treated preparations in hypothalamus and cortex. Binding of dihydroalprenolol to membranes obtained from lithium-withdrawn rats displayed elevated K_D values (relative to control and lithium treatment) in hippocampus and midbrain.

ii) Consequences of lithium treatment and withdrawal on spiroperidol binding to synaptic membranes. The dissociation constants for spiroperidol binding are shown in Table 20, and appear to be similar in all brain regions examined, with the exception of pons-medulla. In this area, the calculated binding affinity was greater than in the other regions. B_{max} values displayed a regional variation, as had these values for dihydroalprenolol binding. Following lithium treatment, regional alterations in the number of binding sites were found, with increased B_{max} values in pons-medulla, striatum and hippocampus, and a decrease in hypothalamus. K_D values were elevated in these brain regions also, but were essentially unaltered in hypothalamus and cortex. In preparations obtained from rats withdrawn from lithium, B_{max} values were found to be depressed relative to both control and lithium-treated animals in hypothalamus, striatum and cortex. In pons-medulla, the number of binding sites calculated was not different to those in lithium-treated preparations, while hippocampus displayed a return towards control values. Binding affinities for spiroperidol were also altered as a result of lithium withdrawal, with a return towards control K_D values in pons-medulla, striatum and hippocampus. Hypothalamic values for this parameter were essentially unaltered with respect to control and lithium-treated animals. In cortex however, lithium withdrawal appeared to cause an increase in affinity for the ligand with respect to the other two treatment groups.

It should be noted however, that the data shown in this study are the results of a single, unreplicated experiment and hence were not subjectable to statistical

Table 20. Constants of (³H)Spiroperidol Binding Following Lithium Administration and Subsequent Withdrawal

	Control		Li-Treated		Li-Withdrawal	
	K _D	B _{max}	K _D	B _{max}	K _D	B _{max}
Pons	0.79	94.44	11.42	243.66	1.77	231.15
Hypothalamus	4.14	765.12	3.33	332.30	4.20	221.71
Striatum	3.95	173.77	13.55	600.98	5.40	34.24
Hippocampus	3.12	129.48	32.86	381.30	2.06	146.26
Cortex	7.37	682.82	6.61	686.21	1.57	182.39

Values are the result of one assay carried out in triplicate.
K_D values are expressed as nM, while B_{max} is expressed as fmol/mg protein.

analysis. For this reason, any conclusions drawn from these data are open to question, but they may give an indication as to the effects of lithium treatment and withdrawal on these two receptor populations.

8. Effects of Haloperidol and Lithium on Biochemical Parameters Associated with Central Monoamine Function

One of the major iatrogenic disorders seen in psychiatry is tardive dyskinesia, a late-developing bucco-linguo-masticatory dyskinesia, which usually results from prolonged administration of neuroleptic drugs. It has been suggested that the onset of the dyskinesia is due to the development of supersensitive post-synaptic DA receptors resulting from a «chemical denervation» caused by receptor blockade by the neuroleptics. A limited number of studies have demonstrated that lithium may be efficacious in preventing the appearance of increased numbers of DA receptors caused by the prolonged presence of anti-schizophrenic drugs, if the anti-manic agent is co-administered with the neuroleptics.

However, studies with neuroleptic agents have demonstrated that these drugs also affect biochemical processes involved with dopaminergic neurotransmission, and in addition cause changes in certain of these parameters associated with the other monoaminergic transmitter substances. Since lithium has been shown to have profound effects on biochemical functions related to biogenic amine neurotransmission, it may be that the consequences of co-administration of lithium and a neuroleptic are due to a change in these aspects of monoaminergic function, rather than a simple stabilization of DA receptor numbers. This study was therefore designed to investigate the interaction of lithium and haloperidol on certain aspects of monoaminergic function.

The treatment groups used were essentially those described by Klawans et al. (1977). Rats were subjected to one of the following treatment regimes:

- a) lithium carbonate (1 mmol/kg daily, i.p.) for 26 days (Li);
- b) saline for 10 days, followed by haloperidol (0.75 mg/kg i.p.) for 10 days, then saline for 6 days (Sal + Hal + Sal);

- c) lithium carbonate (1 mmol/kg, i.p.) for 10 days, followed by haloperidol (0.75 mg/kg i.p.) plus lithium for 10 days, then lithium alone for a further 6 days (Li + Hal-Li + Li);
- d) saline for 10 days, followed by haloperidol (0.75 mg/kg i.p.) for 10 days, then lithium carbonate (1 mmol/kg i.p.) for 6 days (Sal + Hal + Li).
- e) physiological saline for 26 days (Control)
- (a) Apomorphine-induced stereotypical behaviour following lithium and haloperidol administration

Eighteen hours after the last drug administration, animals received an injection of apomorphine hydrochloride (0.5 mg/kg s.c.) and were placed in individual behavioural cages. After a 5 min adaptation period, stereotypical activity was measured in a 20 min observation session using a microprocessor-based scoring system.

Scores were generated for rearing frequency, poking frequency, locomotion and floor activity, and these data are given in Table 21. It should be noted that most of these behaviours are mutually exclusive, i.e. if an animal is displaying a rearing response, it is unlikely to display marked locomotion. Also, the administration of apomorphine to the animals of one group did not result in all the animals of that group exhibiting the same behavioural response, and this accounts for the high standard errors given in Table 21. For these reasons, the individual behavioural scores for each animal were summed, and these are given in Table 21 as Gross Activity. Although no statistically significant differences between groups were observed when this measure of behavioural response was examined, it is possible to make qualitative comparisons. The greatest degree of activity was seen in the animals which received haloperidol alone (Sal + Hal + Sal), an effect which was attenuated when there was a co-administration of lithium (Li + Hal-Li + Li). An unexpected effect was seen when lithium was given following haloperidol

Table 21. Effect of Haloperidol and Lithium on Stereotypical Responses in Rats Induced by Apomorphine

	Rearing Frequency	Poking Frequency	Locomotion	Floor Activity	Gross Activity
Control	42.0±16.1 (100)	6.8±4.3 (100)	103.0±21.6 (100)	261.3±54.2 (100)	413.2±81.0 (100)
Li	35.2±16.8 (84)	2.5±0.9 (37)	119.2±40.9 (116)	319.0±101.7 (122)	475.8±137.6 (115)
Sal + Hal + Sal	52.2±30.6 (124)	3.5±0.6 (51)	121.8±14.6 (118)	394.2±44.6 (151)	517.7±65.1 (138)
Li + Hal-Li + Li	5.8±3.7 (14)	1.8±0.9 (27)	55.8±21.0 (54)	189.2±43.5 (72)	252.7±58.7 (61)
Sal + Hal + Li	48.3 22.0 (115)	2.3 0.9 (34)	101.2 29.1 (98)	285.7 80.4 (109)	437.5 98.0 (106)

Each value is the mean ± S.E. mean for 6 animals per group. Data in parentheses express results as percentage of control (100%).

treatment (Sal + Hal + Li), in that the administration of lithium subsequent to haloperidol appears to produce an amelioration of the haloperidol response.

Although these results are not unequivocal, it does appear that haloperidol treatment followed by a withdrawal period produces an elevated response to a DA agonist, and that co-treatment with lithium antagonizes this effect.

b) Changes in central monoamine function following treatment with haloperidol and lithium

Further series of rats were subjected to the drug treatment schedules described and utilized for biochemical determinations. Eighteen hours after the last drug administration, the animals were sacrificed, the brains rapidly removed and sectioned, and used for the measurement of tyrosine, tryptophan, NA, DA, 5-HT, MOPEG, HVA, DOPAC, 5-HIAA and tyrosine hydroxylase and tryptophan hydroxylase activities.

i) Effect of neuroleptic and lithium administration on monoamine precursor levels and synthetic enzyme activity. As shown in Table 22, none of the treatment regimes altered the parameters of indoleamine synthesis. In the case of the catecholaminergic system, although no significant changes were found in either the levels of tyrosine or the activities of striatal tyrosine hydroxylase, there did appear to be a tendency towards an increase in enzyme activity in the three groups which received haloperidol as part of their treatment schedule.

ii) Alterations in indoleamine content of brain regions due to haloperidol and lithium treatment. Changes in 5-HT content were found in certain of the brain regions examined (hypothalamus, striatum, midbrain), but these decreases in tissue concentration were only significant in animal groups which received lithium as part of their drug treatment (Table 23). In the case of the indoleamine metabolite, 5-HIAA, elevated levels relative to control were noted in hypothalamus in the three treatment groups which received lithium (Table 24). In the animals which received haloperidol alone (Sal + Hal + Sal), decreases in 5-HIAA levels relative

Table 22. Effect of Haloperidol and Lithium on Parameters of Monoamine Synthesis

	Tyrosine	Tyrosine Hydroxylase	Tryptophan	Tryptophan Hydroxylase
Control	8.26±0.7 (100)	360.8±54.1 (100)	5.92±0.69 (100)	11.0±1.1 (100)
Li	8.24±0.2 (99)	399.0±18.6 (110)	5.30±0.13 (89)	9.2±1.9 (84)
Sal + Hal + Sal	9.12±1.4 (110)	475.1±30.2 (132)	4.61±0.20 (78)	11.5±1.2 (104)
Li + Hal-Li + Li	9.62±1.0 (116)	468.6±45.7 (130)	5.02±0.35 (85)	9.1±2.9 (83)
Sal + Hal + Li	9.91±1.0 (120)	454.5±24.1 (126)	4.86±0.96 (82)	10.1±1.4 (92)

Each value is the mean ± S.E. mean of 6 animals per group. Data in parentheses express results as percentage of control (100%).

Tyrosine and tryptophan levels expressed as µg/g tissue.

Tyrosine hydroxylase and tryptophan hydroxylase activities expressed as nmol/g tissue/hr.

Table 23. Central 5-Hydroxytryptamine Levels After Treatment With Haloperidol and Lithium

	Pons	Hypothalamus	Striatum	Hippocampus	Midbrain	Cortex
Control	1.10±0.04 (100)	1.32±0.15 (100)	1.52±0.17 (100)	1.00±0.03 (100)	1.68±0.05 (100)	0.82±0.09 (100)
Li	1.05±0.03 (95)	0.81±0.16 (61) ^a	0.98±0.05 (64) ^{a,c}	0.99±0.05 (99)	1.06±0.06 (63) ^a	0.77±0.05 (94)
Sal + Hal + Sal	0.99±0.03 (90)	0.92±0.15 (69)	1.39±0.13 (91)	0.89±0.05 (89)	1.49±0.06 (89)	0.76±0.06 (93)
Li + Hal-Li + Li	1.02±0.05 (93)	0.68±0.17 (51) ^a	0.93±0.07 (61) ^{a,c}	1.17±0.24 (116)	1.53±0.51 (91)	0.84±0.04 (102)
Sal + Hal + Li	1.06±0.04 (96)	0.77±0.18 (58) ^a	0.93±0.07 (61) ^{a,c}	0.96±0.04 (95)	1.21±0.13 (72)	0.78±0.03 (95)

Each value is the mean ± S.E. mean for 6 animals per group and is expressed as µg/g tissue. Data in parentheses represent results as percent of control (100%).

Significantly different to control (^aF>2.76, df 4,25; p <0.05).

Significantly different to Sal + Hal + Sal (^cF>2.76, df 4,25; p <0.05).

Table 24. Alterations in Regional Concentration of 5-Hydroxyindoleacetic Acid Following Administration of Haloperidol and Lithium

	Pons	Hypothalamus	Striatum	Hippocampus	Midbrain	Cortex
Control	0.89±0.13 (100)	0.58±0.08 (100)	1.31±0.12 (100)	1.56±0.06 (100)	0.98±0.10 (100)	0.50±0.06 (100)
Li	0.74±0.21 (83)	1.16±0.07 (198) ^a	1.65±0.13 (125)	1.07±0.23 (68) ^a	1.25±0.14 (126)	0.33±0.07 (66)
Sal + Hal + Sal	0.40±0.08 (45) ^{a,d}	0.81±0.07 (139)	1.07±0.24 (82) ^b	1.36±0.07 (87) ^b	0.44±0.06 (44) ^{a,b}	0.49±0.04 (97)
Li + Hal-Li + Li	1.02±0.08 (114)	0.99±0.06 (171) ^a	1.30±0.06 (99)	1.54±0.05 (98) ^b	1.10±0.14 (112) ^c	0.43±0.07 (86)
Sal + Hal + Li	0.79±0.15 (88)	1.10±0.21 (189) ^a	1.32±0.06 (100)	1.56±0.07 (100) ^b	1.20±0.07 (122) ^c	0.43±0.07 (85)

Values are the mean ± S.E. mean for 6 animals per group and are expressed as µg/g tissue. Data in parentheses represent data as percent of control (100%).

Significantly different to control (^aF>2.76, df 4,25; p <0.05).

Significantly different to Li (^bF>2.76, df 4,25; p <0.05).

Significantly different to Sal + Hal + Sal (^cF>2.76, df 4,25; p <0.05).

to control were found in pons-medulla and midbrain. Unexpectedly, a reduction in hippocampal content of this metabolite was also observed following lithium treatment alone.

iii) Effect of haloperidol and lithium on brain catecholamine metabolites. Although the tissue content of HVA, the deaminated O-methylated metabolite of DA, did not show significant change following any of the treatment regimes used, a tendency towards an increase was noted after three of the drug schedules, with the greatest relative elevation being in the haloperidol-treated rats (Table 25). In the case of DOPAC, a significant increase was found following lithium administration. Enhanced tissue content was also observed after lithium and haloperidol co-treatment (Li + Hal-Li + Li), although this increase did not reach statistical significance.

With regard to MOPEG, it was found that brain levels of this NA metabolite were significantly reduced in two groups which received haloperidol (Sal + Hal + Sal and Li + Hal-Li + Li), while the Li and Sal + Hal + Li groups displayed no significant difference in comparison to the control values.

iv) Catecholamine levels in various brain regions following lithium and haloperidol treatment. With regard to regional NA content, lithium administration alone resulted in decreased transmitter levels in most brain regions (Table 26), while haloperidol caused regionally variable changes. As can be seen, the Sal + Hal + Sal treatment schedule resulted in significantly decreased tissue concentration in the hippocampus, while in the cortex there was a significant enhancement. When lithium was administered in combination with haloperidol (Li + Hal-Li + Li), the alteration noted was in the same direction as the predominant effect seen with either lithium or haloperidol alone, and decreases in NA levels were therefore observed in hypothalamus, striatum and hippocampus, with a significant increase in cortical tissue. Administration of lithium following haloperidol (Sal + Hal + Li) caused changes which appeared to be similar to those observed after lithium alone (i.e. decreased NA levels).

Table 25. Catecholamine Metabolites in Brain Following Administration of Haloperidol and Lithium

	MOPEG	HVA	DOPAC
Control	0.246±0.02 (100)	2.01±0.19 (100)	5.12±0.49 (100)
Li	0.231±0.04 (82)	2.45±0.57 (122)	7.68±0.60 (150) ^a
Sal + Hal + Sal	0.125±0.03 (51) ^{a,b}	2.80±0.40 (139)	6.11±0.57 (119) ^b
Li + Hal-Li + Li	0.131±0.02 (53) ^{a,b}	2.25±0.29 (112)	7.35±0.92 (144)
Sal + Hal + Li	0.172±0.02 (70)	2.00±0.28 (100)	5.06±0.37 (99) ^b

Each value is the mean ± S.E. mean for 6 animals per group. Values are expressed as µg/g tissue. Data in parentheses express results as percent of control (100%).

Significantly different to control (^aF>2.76, df 4,25; p <0.05).

Significantly different to Li (^bF>2.76, df 4,25; p <0.02).

Table 26. Noradrenaline Content of Various Brain Regions Following Administration of Haloperidol and Lithium

	Hypothalamus	Striatum	Hippocampus	Cortex
Control	14.78±6.02 (100)	3.48±1.06 (100)	12.00±0.76 (100)	0.58±0.11 (100)
Li	5.84±0.74 (40) ^a	1.14±0.47 (33) ^a	6.91±0.84 (58) ^a	0.67±0.21 (115)
Sal + Hal + Sal	13.51±1.64 (91) ^b	3.90±1.17 (112) ^b	0.81±0.18 (7) ^{a,b}	1.37±0.52 (236) ^{a,b}
Li + Hal-Li + Li	4.18±0.33 (28) ^{a,c}	1.77±0.52 (51) ^{a,c}	4.39±0.78 (36) ^{a,b,c}	1.87±0.55 (322) ^{a,b}
Sal + Hal + Li	2.61±0.58 (18) ^{a,c}	0.39±0.12 (11) ^{a,c}	1.30±0.57 (11) ^{a,b}	0.23±0.07 (40) ^{a,b,c,d}

Each value is the mean ± S.E. mean for 5 or 6 animals per group and is expressed as µg/g tissue. Data in parentheses represent results as percent of control (100%).

Significantly different to control (^aF>2.76, df 4,25; p <0.05).

Significantly different to Li (^bF>2.76, df 4,25; p <0.05).

Significantly different to Sal + Hal + Sal (^cF>2.76, df 4,25; p <0.05).

Significantly different to Li + Hal-Li + Li (^dF>2.76, df 4,25; p <0.05).

When DA levels are examined (Table 27), it can be seen that lithium administration resulted in significantly decreased neurotransmitter content in hypothalamus and hippocampus, and that there was an increase, although non-significant, in striatal tissue concentration. Six days after haloperidol administration, hypothalamic DA content was reduced, with again a statistically non-significant increase in striatal levels. In both of these treatment groups (Li and Sal + Hal + Sal) cortical DA was found to be slightly, although non-significantly, altered also. As was the case with NA, when lithium was given concurrently with the neuroleptic (Li + Hal-Li + Sal), the changes observed followed the direction seen after the administration of each agent alone, and so decreases were found in hypothalamus and hippocampus, while striatal and cortical levels were significantly elevated with respect to controls. Following administration of lithium after the neuroleptic (Sal + Hal + Li), the alterations in DA content observed were essentially the same as those observed following lithium alone (Li).

Table 27. Brain Dopamine Levels Following Haloperidol and Lithium Treatment

	Pons	Hypothalamus	Striatum	Hippocampus	Cortex
Control	0.59±0.11 (100)	1.77±0.19 (100)	11.22±2.76 (100)	0.98±0.18 (100)	0.90±0.25 (100)
Li	0.45±0.05 (76)	1.22±0.21 (69) ^a	16.39±5.15 (146)	0.46±0.15 (47) ^a	1.06±0.40 (118)
Sal + Hal + Sal	0.44±0.07 (74)	0.97±0.14 (55) ^a	17.58±4.36 (157)	0.97±0.21 (99) ^b	1.14±0.31 (127)
Li + Hal-Li + Li	0.77±0.17 (130)	0.94±0.13 (53) ^a	23.32±3.31 (208) ^a	0.48±0.08 (49) ^{a,c}	1.74±0.29 (193) ^a
Sal + Hal + Li	0.26±0.09 (44) ^d	0.55±0.04 (31) ^{a,b}	14.26±2.47 (127) ^d	0.54±0.14 (55)	1.01±0.18 (112)

Values are the mean ± S.E. mean and are expressed as µg/g tissue. Data in parentheses represent results as a percentage of control (100%).

Significantly different to control (^aF>2.76, df 4,25; p <0.05).

Significantly different to Li (^bF>2.76, df 4,25; p <0.05).

Significantly different to Sal + Hal + Sal (^cF>2.76, df 4,25; p <0.05).

Significantly different to Li + Hal-Li + Li (^dF>2.76, df 4,25; p <0.05).

IV
DISCUSSION

A. LITHIUM TREATMENT AND WITHDRAWAL AND CENTRAL MONOAMINERGIC PROCESSES

1. Behavioural and Toxicological Considerations

As can be seen from the initial experiment carried out in this study, 1 mmol/kg/day of lithium carbonate administered chronically appears to be the highest amount of the salt tolerated by rats without the appearance of any overt toxic manifestations. This particular treatment regime gave relatively constant serum lithium levels (0.32-0.34 mEq/l) when measured 18 hours following the last drug administration. Since it has been shown that plasma and serum levels of lithium are 2-3 times greater when determined at 12 hours, as compared to measurements made 18 hours after the treatment (Frazer *et al.*, 1973; Mukherjee *et al.*, 1976; Plenge *et al.*, 1981), it is likely that serum lithium concentrations in rats maintained on a treatment schedule of 2 mEq lithium/kg/day would be in the human therapeutic range (0.6-1.2 mEq/l), as clinical assessments of serum lithium are generally made 12 hours after the last drug administration. This therefore implies that this dose level of lithium, which was used in all subsequent experiments, was appropriate not only for its apparent compatibility with the therapeutic level of lithium, but also for the fact that it was a sub-toxic dose. It also appears that studies which have utilized doses of lithium in excess of 2 mEq/kg/day may have been examining the toxic rather than the therapeutic effects of the cation. While it is possible that the changes in central monoamine function associated with the use of toxic levels of lithium may simply be a magnification of the effects responsible for its therapeutic effectiveness, this has not yet been conclusively demonstrated.

It has been suggested that the administration of lithium at sub-toxic doses exerts little or no effect on spontaneous locomotor activity (Smith, 1977), and that this lack of effect may be attributed, in part, to the use by many investigators of magnetic field recorders to measure movement (Smith, 1980). However, as can be seen from the data presented in this study, chronic lithium

administration at sub-toxic doses did cause a reduction in the spontaneous activity of rats, thus confirming the results of Cappeliez and White (1981), Smith (1981) and Staunton et al. (1982a). While these authors measured activity by means of photocell assemblies (Cappeliez and White, 1981; Staunton et al., 1982a) and open-field examination (Smith, 1981), the present study utilized a magnetic field recorder, showing that it is possible to demonstrate a reduction in spontaneous activity with this type of movement detector. The suggestion that the reduction in spontaneous activity may be due to a non-specific toxic effect of the cation (Smith, 1977) is not considered to be the case in the present study, since none of the animals displayed any overt toxic symptoms. Also, the possibility that there may be a local irritant action following lithium administration is discounted as the rats displayed no signs indicative of tissue irritation, e.g. licking the injection site. Any transient local irritation which may result following injection of the drug should have dissipated 18-24 hours following lithium administration, which was the time at which behavioural testing was carried out. Smith (1980) has also implied that there may be a degree of neurotoxicity associated with the administration of lithium, but it seems unlikely in view of the fact that spontaneous locomotor activity returned to control levels within 2 days of cessation of treatment. This therefore indicates that the reduction in spontaneous activity displayed by lithium-treated rats is a physiological consequence of chronic administration of the cation. The time course of this alteration in activity appears to correlate well with the clinical situation, since the full effect of lithium is not noted in manic patients until 6-8 days after the start of treatment (Schou, 1980), while the reduction in spontaneous activity in rats was noted after 9 days of lithium administration. The time-lag which is seen could be due to two factors: (a) the gradual accumulation of the cation in central neuronal tissue and (b) the development of adaptive response(s) to the presence of lithium in these tissues. Following cessation of lithium administration,

there was a rapid return to control levels of activity, with no evidence of a «rebound» phenomenon with regard to this particular behaviour. The fact that central tissue levels of lithium were significantly reduced following 2 days of withdrawal from the drug tend to suggest that the behavioural effects noted are directly related to the amount of lithium present in the brain.

The second behavioural experiment was designed to evaluate various animal models which have been suggested for the behavioural assessment of anti-manic drugs. Delini-Stula and Meier (1976) proposed the use of behavioural stimulation produced by reserpine in the presence of a monoamine oxidase inhibitor as a model for mania, since the excitation syndrome had been shown to be due to «excessive accumulation and subsequent liberation of biogenic amines in the central nervous system.» The present data agree with that of Delini-Stula and Meier (1976) in that there was an increase in locomotor activity of control animals following administration of pargyline-reserpine, although the enhancement of locomotion was not as pronounced as that reported by the previous authors. However, the finding that chronic lithium pre-treatment had no obvious effect on the behavioural stimulation would tend to cast doubt on the utility of this analogue of mania.

The concurrent administration of amphetamine and chlordiazepoxide has been said to produce «activity for activity's sake» (Davies et al., 1974) which is different to the stereotyped behaviour seen after administration of large doses of amphetamine alone. However, the fact that the animals which received either the low- or high-dose mixture were severely limited in their ability to perform any voluntary locomotion would tend to suggest that these behavioural stimulation paradigms are not suitable for the testing of anti-manic agents. Also, the finding that vastly elevated locomotor measurements were recorded despite the fact that the animals were restricted in their ability to walk would suggest that the type of instrumentation used in this experiment, i.e. a magnetic field recorder, is incapable of differentiating between an animal moving of its own

volition and one suffering from severe continuous tremor.

Since lithium treatment alone was found to reduce spontaneous activity in rats, it is evident that there is some change in the central systems which mediate behaviour. This depression of the behavioural response, however, was not observed in the animals which received some form of behavioural stimulation, implying that when the central monoaminergic neurotransmitter systems, which appear to be involved in the actions of both reserpine (Delini-Stula and Meier, 1976), and amphetamine (Davies *et al.*, 1974; Smith, 1981), are subject to a higher rate of synaptic stimulation than usual, the behavioural suppression effect of lithium is overcome. The fact that lithium treatment, at sub-toxic doses, was not able to ameliorate the behavioural excitation caused by either reserpine or amphetamine and yet is able to normalize mood and behaviour clinically, implies that behavioural stimulation induced by either of these agents does not resemble the clinical manic syndrome.

Following 2 days of withdrawal from lithium, spontaneous locomotor activity in «normal» animals returned essentially to control levels, and showed no obvious signs of any form of «rebound» phenomenon. It is possible that no «rebound» was seen since only gross locomotor activity was measured, whereas if some other behavioural parameter was to be examined, a more obvious effect may have been noted.

As can be seen, the analogues of mania which utilize behavioural stimulation do not appear to be appropriate models to study due to their lack of responsivity to chronic lithium treatment, and for this reason «normal» rats were used in all subsequent experiments to examine the effects of lithium on central monoaminergic neurotransmitter processes.

2. Metabolic Aspects of Monoamine Function

With regard to the neurochemical consequences of short- and long-term lithium administration and its subsequent withdrawal, the first point of

interest concerns the apparent discrepancy in the absolute values obtained for certain of the parameters measured. This is thought to be due to the fact that the animals obtained for the short-term experiment were of a different strain than those supplied for the long-term study. The difference in the rats was due to a change in the breeding stock utilized by the animal supply house.

The involvement of the synthetic processes for catecholamines in the mechanism of action of lithium has been the subject of much investigation over the years. Segal et al. (1975) found an increase in striatal tyrosine hydroxylase activity following lithium treatment, while Friedman and Gershon (1973) and Berggren et al. (1980) demonstrated decreases in striatal DA synthesis and DOPA accumulation, respectively, suggesting that there was a decrease in the activity of tyrosine hydroxylase. The present data however, would tend to support the finding of Hesketh et al. (1978) who noted no change in tyrosine hydroxylase activity after lithium administration for up to 3 weeks, and demonstrates that neither short- nor long-term lithium administration at sub-toxic doses affect catecholamine synthesis.

However, some investigators have found that lithium administration results in an enhancement of NA turnover (Schildkraut et al., 1969a; Stern et al., 1969; Greenspan et al., 1970). An increase in transmitter turnover in the absence of an elevated activity of the synthetic process should result in a decrease in tissue content of the transmitter. This may well be the case for NA in most regions of the rat brain following both short- and long-term lithium administration. However, this finding appears to be at variance with the data from Stern et al. (1969) and Greenspan et al. (1970) who found no change in steady state NA levels, and Leonard (1975) who noted only a slight decrease in NA content. This apparent discrepancy can be explained by the fact that these previous studies utilized whole brain for the estimation of endogenous NA, while in the present study estimations were carried out in discrete brain regions. It is therefore possible that the reduction in NA content in those regions affected by lithium treatment

could well be masked by the relatively high amount of NA in brain regions which were unaffected.

The fact that NA levels were found to decrease raises three possibilities regarding central noradrenergic function: (a) there is an increase in the activity of NA-containing neurones as a consequence of lithium treatment; (b) the ability of the neurones to store NA is impaired by lithium; or (c) a combination of the two. Any of these could explain the apparent increase in NA turnover noted previously (Schildkraut et al., 1969a; Stern et al., 1969; Greenspan et al., 1970). However, if there was an increase in the outflow of NA as a result of enhanced neuronal activity, a concomitant increase in the activity of the synthetic process would be expected (Euler, 1972), but no change was observed in tyrosine hydroxylase activity as a result of either short- or long-term lithium treatment. Also, increased noradrenergic neuronal activity would be expected to cause an elevation in the level of the major end-product of metabolism following NA release; i.e. MOPEG. This was not seen, and there was in fact a slight decrease in the tissue content of this metabolite following both short- and long-term lithium administration. MOPEG is the product of both deamination and O-methylation and so if either enzymatic process were inhibited, the level of MOPEG would be reduced. Schanberg et al. (1967) found that following chronic treatment with lithium there was a change in the metabolism of NA, and Schildkraut et al. (1969a) suggested that this metabolic alteration was due to an increase in the deamination of NA, accompanied by a decrease in the levels of the O-methylated metabolite, normetanephrine. These data indicate that the activity of monoamine oxidase is enhanced, while that of catechol O-methyltransferase is inhibited, a situation which should result in decreased MOPEG levels. Greenspan et al. (1970) found no change in the formation of normetanephrine following lithium administration, suggesting that there is, in fact, no inhibition of catechol O-methyltransferase. These authors also noticed an increase in O-methylated deaminated metabolites (Greenspan et al., 1970) which in addition to MOPEG included 3-methoxy-4-hydroxyphenylglycol

aldehyde and 3-methoxy-4-hydroxy mandelic acid. It is possible that a relative increase in the formation of these other metabolites following lithium treatment could cause a decrease in the amount of MOPEG present, as was seen in the present study.

However, the fact that tyrosine hydroxylase activity was not affected by either of the two lithium administration regimes used indicates that the decrease in NA content in certain brain regions, as noted in this study, and the enhanced NA turnover seen previously are not due to an increase in the activity of central noradrenergic neurones. This therefore leaves the possibility that lithium affects the storage capabilities of the noradrenergic nerve terminals. This theory was first proposed by Greenspan et al. (1970) and later elaborated by Slotkin et al. (1980) who found that chronic lithium administration results in a destabilization of NA storage in synaptic vesicles. If this is indeed the case, then there would be a reduction in the tissue content of NA, since it cannot be stored, and would also result in a decrease in the release of NA, as shown by Katz et al. (1968).

With regard to DA, there appears to be a time-dependent effect on tissue content of this neurotransmitter, since short-term lithium administration resulted in decreased hypothalamic content only, while after 26 days of treatment reduced levels were noted in hippocampus in addition to hypothalamus. This finding is contradictory to that of Ho et al. (1970) who found no change in hypothalamic DA content following chronic lithium treatment. The fact that the concentration of DA in most other brain regions is unaffected by lithium confirms the data of numerous other investigators (Corrodi et al., 1967; Ho et al., 1970; Hesketh et al., 1978; Eroglu et al., 1981). Striatal DOPAC content was enhanced following long-term lithium treatment, while HVA levels were not affected. It is possible that this is due to an increase in the activity of monoamine oxidase, as suggested by Schanberg et al. (1967) and Schildkraut et al. (1969a), without a concomitant increase in the activity of catechol O-methyltransferase. This, however, seems unlikely in view of the fact that Guerri (1982) has demonstrated that lithium

treatment does not alter the activity of monoamine oxidase. The alternative possibility is that there is an increase in the amount of substrate for the monoamine oxidase, i.e. DA, resulting in an elevation in the tissue content of DOPAC. The increase in intraneuronal DA could be due to either an enhancement in the uptake of the catecholamine from the synaptic cleft making more available for deamination, or due to a decrease in the storage of the neurotransmitter. The latter seems unlikely as neither short- nor long-term lithium treatment significantly altered striatal DA content.

Previous studies have provided conflicting reports on the effects of lithium treatment on the synthetic systems for 5-HT in rat brain. Rastogi and Singhal (1977) demonstrated an increase in tryptophan hydroxylase activity as a result of lithium administration, while Shaw and Ratcliffe (1976) reported an increase in the activity of 5-hydroxytryptophan decarboxylase. In contrast, Knapp and Mandell (1975) have shown that tryptophan hydroxylase activity decreases with lithium treatment, while Ho et al. (1970) found a decrease in 5-HT synthesis. The present data indicate that there is, in fact, no alteration in tryptophan hydroxylation, as determined by the activity of tryptophan hydroxylase, following both long- and short-term lithium administration, nor is there a change in the central concentration of the substrate for this enzyme, i.e. tryptophan. Despite the finding that the synthetic processes for this neurotransmitter were not affected by lithium treatment, 5-HT levels in midbrain (the region in which synthetic parameters were determined) declined after short-term lithium administration, while after 26 days of treatment, decreases were noted in hypothalamus and striatum in addition to midbrain. This suggests that there is an effect of lithium on the serotonergic system, which may be not only region-specific but also time-dependent. This reduction in midbrain neurotransmitter level following short-term lithium administration was not accompanied by a change in the regional concentration of the metabolite, 5-HIAA, while the hypothalamic concentration of 5-HIAA was reduced.

The decrease in 5-HT levels in hypothalamus and midbrain after 26 days of lithium treatment was accompanied by significant increases in 5-HIAA content of these brain regions. It is possible that these changes are due to increased utilization of the neurotransmitter in these areas, but if this were so, one would expect an elevated rate of synthesis of the indoleamine, which was not observed. Another possibility remains that lithium alters the ability of serotonergic neurones to store the neurotransmitter (Collard, 1978b). This would cause the decreased levels of 5-HT observed, which in turn could result in elevated levels of the metabolite. The decline in 5-HIAA content of hypothalamus following short-term lithium treatment is at present inexplicable.

Following lithium withdrawal, NA levels in both short- and long-term treated rats remained low, except in cortex, while the brain content of the noradrenergic metabolite, MOPEG, was found to decrease. These data would suggest that the activity of noradrenergic neurones is reduced following withdrawal of lithium, which appears to contradict the finding that the activity of the synthetic enzyme, tyrosine hydroxylase, is enhanced since an elevated rate of synthesis would tend to imply that there is an increased utilization of the neurotransmitter. This is particularly true in view of the fact that there was a further reduction in NA content in certain of the brain regions examined following lithium withdrawal. It is therefore possible that the observed reduction in MOPEG content is, in fact, an artifact, since the tissue content of this metabolite was measured in whole brain, where the amount of cortical tissue is proportionally greater than that for any other region; and cortical NA levels were found to increase after withdrawal of lithium.

Regional DA content was essentially unchanged as a result of lithium withdrawal relative to the lithium-treated animals, while HVA levels were found to increase. This was true of both treatment periods. This increase in HVA content, together with the elevated rate of catecholamine synthesis, may well be indicative

of an increase in the release of DA due to withdrawal of lithium. It has been suggested that this excessive release of DA may be due to a receptor sub-sensitivity during lithium withdrawal, which results in a failure of the pre-synaptic release regulation mechanism (R.H. Belmaker, Personal Communication). However, there was also a slight reduction in DOPAC content relative to both control and lithium-treated animals, particularly after long-term treatment. It is therefore possible that following lithium withdrawal there is a greater O-methylation of DOPAC than was previously observed, which would account for the increased levels of the deaminated O-methylated metabolite of DA, especially when one considers the elevated levels of substrate for catechol O-methyltransferase, i.e. DOPAC, which were present following lithium administration. In addition, if there were a decrease in the amount of primary substrate for the catabolic pathway, i.e. DA, possibly due to a reduction in neuronal uptake of the catecholamine, then there would not only be less DOPAC generated, but there could also be reduced competition for the methylating enzyme, so enabling the excess DOPAC synthesized as a result of lithium administration to be O-methylated. However, an argument against this possibility is derived from the work of Roffler-Tarlov et al. (1971) who have suggested that the formation of HVA from DOPAC is not a major route of synthesis of the DA metabolite, particularly in striatum, although at least a part of HVA present does originate from this metabolic pathway.

With regard to the serotonergic system, lithium withdrawal appears to result in a simple return towards control values in the parameters affected by either short- or long-term lithium administration.

These data therefore imply that alterations in various aspects of metabolism associated with all three monoaminergic neurotransmitter systems play a part in the therapeutic effect of lithium, whereas only those related to catecholamine function may be involved in any form of withdrawal phenomenon.

3. Synaptosomal Uptake Systems

The information regarding the effects of lithium on monoamine uptake is at present somewhat limited, with most studies having been concerned with the noradrenergic process (Colburn et al., 1967; Kuriyama and Speken, 1970; Cameron and Smith, 1980), although the serotonergic system has been briefly investigated (Kuriyama and Speken, 1970; Collard, 1978a), as has this inactivation mechanism for DA (Stefanini et al., 1976). The pilot study carried out to examine the effects of lithium on the uptake phenomenon resembled most of the previous investigations in that a single concentration of ligand was used. This experiment confirmed the results of Stefanini et al. (1976) who had observed an increase in the uptake of DA into synaptosomes prepared from the caudate nuclei of rats treated chronically with lithium. The present study further demonstrates that this alteration in the ability to take up DA is not restricted to a discrete nucleus, but may be a generalized phenomenon. The data from this investigation however, were not in accordance with those from Collard (1978a) who had reported that 5-HT uptake into synaptosomes prepared from rat forebrain was not affected by lithium administration. The present results showed that the synaptosomal uptake of this amine was reduced in all brain regions examined. It was with regard to the noradrenergic system however, that the greatest discrepancies were noted between the data from the present study and those published previously. Most of the previous authors who had examined NA uptake into synaptosomes from lithium-treated animals had shown that prior administration of the cation resulted in an enhancement of uptake (Colburn et al., 1967; Kuriyama and Speken, 1970; Cameron and Smith, 1980) whereas the data from this study demonstrated a regionally variable effect, with increased amounts of NA being accumulated in hypothalamus and striatum; and a reduction in uptake in midbrain and cortex. Reconciliation of these conflicting data is, however, possible since Colburn et al. (1967) and Kuriyama and Speken (1970) used synaptosomal preparations obtained from whole brain, and therefore brain regions which exhibited an increase in NA uptake, i.e. hypothalamus and striatum, may mask those which

displayed either no change or a decrease in uptake. In the case of the study of Cameron and Smith (1980), it may be that the results obtained using tissue slices reflect uptake of the amine into cell bodies or axonal varicosities, which would give a misleading picture, while when using synaptosomal preparations, these structures would either not be present or would be ruptured, so that the uptake examined would be primarily that of the amine into nerve terminals.

A simpler explanation is also possible. Synaptosomal uptake is known to be proportional to the amount of synaptosomal protein present (Horn, 1979), and in the present study no allowance was made for the protein content of the synaptosomal fraction. It therefore can not be ruled out that much of the data displaying changes in synaptosomal uptake of the monoamines following lithium treatment, and a general return towards control values after withdrawal, are in fact artifacts caused by differential amounts of protein being present in the sample.

The next experiment was therefore more rigorous in its design, in that the protein concentration of the sample was taken into consideration. This experiment also extended the previous work by examining monoamine uptake at a variety of substrate concentrations.

As can be seen, data from the kinetic study of NA uptake allowed for the calculation of dissociation constants for the low-capacity uptake system, and these were found to be of the same order of magnitude as values previously reported (Snyder and Coyle, 1969; Iversen et al., 1971). The V_{max} values are however, different to those calculated by these previous authors, but as Horn (1979) has stated, the V_{max} is dependent on the type and amount of tissue present, and the manner in which the synaptosomal fraction is prepared.

The most interesting aspect of this study was the discovery that there were in fact two uptake systems present in synaptosomes for NA, with the high-capacity process apparently coming into play at substrate concentrations greater than $1 \mu M$. It is possible that this second high-capacity system is involved with regulating

the inhibition of noradrenaline release mediated by the pre-synaptic α -adrenoceptors (the α_2 -adrenoceptor). There has been a considerable accumulation of evidence in recent years that stimulation of the α -adrenoceptors causes an inhibition of NA release (Langer, 1974; 1980) and the theory, simply stated, is that when NA released from the pre-synaptic terminals by stimulation reaches a threshold concentration in the synaptic cleft, it activates α_2 -receptors and so triggers a negative feedback system that inhibits further release of the transmitter (Langer, 1974). It should be stated at this point that the view that pre-synaptic α -receptors have an inhibitory role in NA release is not held by all investigators (Chan and Kalsner, 1979a; 1979b). However, if the hypothesis is accepted as being valid, then immediately following release, the local concentration of NA in the immediate vicinity of the pre-synaptic terminal would be expected to be high, which should cause activation of the pre-synaptic receptors. It may be that the high-capacity uptake process comes into operation at this time to reduce the local NA concentration to below the threshold level for α_2 -adrenoceptor activation, until diffusion away from the release site lowers the transmitter level to below the critical concentration. In this way, sufficient neurotransmitter would be released into the synaptic cleft to allow for propagation of the neuronal impulse prior to feedback inhibition. It has also been demonstrated that pre-synaptic inhibition of NA release by α_2 -adrenoceptors is more pronounced at low stimulation frequencies (Dubocovich and Langer, 1974), possibly because at high concentrations of NA the high-capacity uptake system is more efficient, so that there is less NA available in the immediate vicinity of the α_2 -adrenoceptors. In this way, high rates of neuronal traffic may be propagated.

After 12 days of lithium treatment, it was found that synaptosomal preparations no longer exhibited the high-capacity uptake process, and it is possible that this is the more important effect on the uptake systems than the increase in uptake noted at substrate concentrations below 1 μ M. If the hypothesis described above regarding the modulation of α_2 -adrenoceptor activation by this high-capacity

uptake system is correct, then inactivation of this removal process for NA would result in elevated levels of the neurotransmitter in the vicinity of the α_2 -adrenoceptors, allowing activation of the feedback inhibition of the release process. This would be particularly true during periods of high neuronal traffic, as these are times during which the high-capacity uptake process would be most effective in removing the neurotransmitter. If this is indeed the case, it would help to explain why lithium is able to alter behaviour in manic patients in whom neuronal activity is considered to be high, but not affect «normal» subjects, in whom neuronal activity is thought to be at a lower level. It is possible that alterations in the uptake parameters of the low-capacity system observed following lithium administration may be a purely adaptive response to the increased amounts of NA which would be present in the synaptic cleft following inactivation of the high-capacity process.

Two days after lithium withdrawal, it was found that there was a significant elevation in the activity of the high-capacity uptake system, although the low-capacity process in most regions had essentially returned to control levels. This increase in the activity of the high-capacity system could result in the passage of a higher rate of neuronal traffic than would normally be seen, as there would be a lesser likelihood of α_2 -receptor activation due to enhanced removal of NA from the pre-synaptic terminal region. This would obviously result in a reduction of the receptor-mediated inhibition of NA release. In a situation in which there was high activity in the noradrenergic system, e.g. in manic patients, the enhancement of NA release resulting from this increase in the high-capacity process would cause increased activation of post-synaptic receptor sites, due to the increased availability of NA in the synaptic cleft, and could well trigger the «rebound» mania noted in certain patients withdrawn from lithium therapy (Small et al., 1971; Lapierre et al., 1980).

The synaptosomal uptake of 5-HT was found to resemble that of NA in that two uptake processes again appear to be present, with characteristics similar to those noted for the catecholamine, i.e. a high-capacity system becoming apparent at substrate concentrations greater than 1 μ M. A similar situation had previously been observed by Snyder et al. (1973) using tissue slices from rat brain. These authors had determined that the high-affinity process which they detected had a K_m value of 2×10^{-7} M, which is comparable in magnitude to the low-capacity system found in the present study, while a dissociation constant of 8×10^{-6} M was displayed by the low-affinity process. Snyder et al. (1973) postulated that one of these uptake processes for 5-HT may reflect uptake into catecholaminergic neurones, and further determined that the low-affinity transport of 5-HT appears to be via a catecholaminergic mechanism.

If this is indeed so, then it would explain the present finding that the high-capacity uptake system for 5-HT into synaptosomes prepared from the brains of lithium-treated rats is markedly attenuated, as was the case with NA at high substrate concentrations. Kuriyama and Speken (1970) had previously observed a slight decrease in 5-HT uptake after lithium treatment, when synaptosomes prepared from the whole brains of mice were incubated with 2 μ M (3 H)5-HT. At low exogenous levels of 5-HT, synaptosomal uptake in most brain regions was found to be depressed relative to control values following lithium administration and this decreased uptake was associated with a reduced number of uptake sites. Certain areas also displayed a concomitant increase in affinity for the substrate. This reduction noted at low concentrations of 5-HT is at variance with the data of Collard (1978a), but this discrepancy can be explained by the fact that Collard (1978a) utilized synaptosomes prepared from rat forebrain, and the present study has shown that there was no difference in 5-HT uptake into cortical synaptosomes following lithium treatment.

However, the fact that withdrawal from lithium did not cause the same change in 5-HT uptake via the high-capacity system as was noted with NA would tend to suggest that the two monoamines do not utilize the same uptake process at high substrate concentrations, although it is tempting to speculate that there may be similarities in function of the two high-capacity systems. This is particularly true in light of reports describing the presence of 5-HT autoreceptors (Cox and Ennis, 1982) which appear to have a role in inhibiting 5-HT release (Gothert and Weinheimer, 1979). Unfortunately, the finding by Aghajanian et al. (1968) that LSD-responsive; and therefore presumably serotonergic, neurones in the midbrain raphe nuclei exhibit an extremely slow spontaneous rate of firing (20 to 40 spikes per minute) would tend to suggest that the fine modulation of transmitter release which could result from the combined effects of a high-capacity neuronal uptake system with autoreceptor stimulation would not be of physiological importance in serotonergic neurotransmission.

It is therefore possible that the decrease in 5-HT uptake noted following lithium treatment, particularly in the high-capacity system, is a facet of a situation in which there is a reduced release of transmitter (Katz et al., 1968) due to a decrease in the neuronal storage of 5-HT (Collard, 1978b). The situation is further complicated by the fact that following withdrawal of lithium, there was a decrease in synaptosomal uptake of the indoleamine at low substrate concentrations relative to both control and lithium-treated preparations. This change was characterized by a decrease in Vmax values of all brain regions examined, with an associated increase in affinity in most regions. The reason for this alteration after withdrawal of lithium is equivocal in view of the fact that the biochemical parameters associated with serotonergic function which were measured appear to demonstrate a return towards normal function at this time. However, the fact that there was a decrease in synaptosomal uptake of 5-HT consequent to withdrawal of lithium suggests that there may be an increase in neurotransmitter present in the synaptic cleft following the arrival of an impulse at the neuronal terminal,

resulting in an enhancement of the activation of post-synaptic 5-HT receptors. It may be that this over-activation of 5-HT receptors plays a part in the genesis of the «rebound» mania noted clinically following cessation of lithium therapy.

As with the synaptosomal uptake of NA and 5-HT, there appears to be more than one component to this inactivation mechanism for DA. In fact, Snyder and Coyle (1969) had previously suggested the presence of two systems for the accumulation of DA, which they termed Uptake_a (high-affinity) and Uptake_b (low-affinity), and which were present in all brain regions except striatum. In this area, they demonstrated the presence of Uptake_a only. As can be seen from the present study, it is possible in most brain regions, including striatum, to resolve the uptake curve for this catecholamine into two components, as suggested by Snyder and Coyle (1969). However, the fact that in the pons-medulla it was not possible to calculate kinetic constants for the low-affinity system, while the assumption of a single component did not explain the shape of the substrate-velocity curve obtained for this amine, tends to suggest the presence of a more complex uptake system. This view is supported by the finding that it was not possible to calculate kinetic constants for DA uptake following treatment with, and subsequent withdrawal from lithium when the presence of either one or two uptake components is assumed.

These data raise an interesting possibility regarding the DA uptake process. In order to be able to linearize substrate-velocity curves using the Lineweaver-Burk transformation, and hence calculate Km and Vmax values, the uptake of a compound must display hyperbolic saturation with respect to substrate concentration. However, as can be seen, the substrate-velocity curve for DA uptake does not display simple hyperbolic characteristics, but appears to show a sigmoid dependence on substrate concentration. This implies the presence of some form of multiple and co-operative process, in which the binding of the first substrate molecule to an active site enhances the binding of a second substrate molecule, and so enhances the activity (Lehninger, 1970). In this way, the uptake process can respond rapidly to changes in substrate concentration, and so provides fine

control for the amount of DA present in the synaptic cleft. It is possible that this process also provides a modulation mechanism for the activation of dopaminergic autoreceptors by controlling the concentration of DA in the vicinity of the pre-synaptic terminal, in a fashion similar to that postulated for NA.

Lithium administration for 12 days was found to enhance DA uptake into synaptosomal fractions prepared from all regions of rat brain. It therefore seems that the presence of the cation causes an adaptive response in the uptake process, such that increasing concentrations of the substrate result in the activation of greater numbers of uptake sites than is normal. The mechanism behind this enhancement of uptake activation is not known, but it seems likely that it is an important component in the therapeutic effect of lithium, although there are two possible consequences to this alteration in uptake. If the uptake process does play a modulating role in the activation of pre-synaptic receptors involved in the inhibition of DA release, then enhancement of uptake would result in an increased release of transmitter, as activation of the inhibitory autoreceptors would not occur as rapidly as normal. This implies that there may be a deficit in dopaminergic neurotransmission in the manic state, and that lithium normalizes the condition by allowing increased activation of post-synaptic structures. Alternatively, lithium may provide the means whereby excessive dopaminergic neuronal activity could be returned to a more normal state by the removal of DA from the synaptic cleft, possibly prior to activation of post-synaptic receptor sites.

In the case of lithium withdrawal, synaptosomal uptake of DA was found to be depressed. This is probably due to a reduced activation of uptake sites in response to increasing concentrations of the catecholamine, although again the mechanism is unknown. This attenuated inactivation of the neurotransmitter may also be a factor in the production of «rebound» mania following clinical withdrawal of lithium, the means by which it plays a part being dependent on the consequences of reduced DA uptake on dopaminergic neurotransmission.

Also, the fact that DA uptake was found to be altered as a result of both lithium administration and its subsequent withdrawal would help to explain some of the biochemical alterations observed previously. It was found that the level of the deaminated DA metabolite, DOPAC, was elevated after lithium administration, while the concentration of the deaminated O-methylated metabolite, HVA, was enhanced following withdrawal of lithium treatment. Since DA uptake was enhanced as a consequence of lithium administration, the concentration of DOPAC probably represents intra-neuronal metabolism of the amine, while HVA levels are probably indicative of extraneuronal metabolism, since they were found to increase when synaptosomal uptake of the catecholamine was reduced. These data lend support to the theory of Roffler-Tarlov et al. (1971) that catechol O-methyltransferase is located extraneuronally.

4. Central Catecholamine Receptor Populations

The effect of lithium treatment on central receptor populations has not been the subject of extensive investigation, primarily due to the fact that lithium has been considered to exert its therapeutic action through alterations in monoamine metabolism. The few studies which have examined this aspect of neurotransmitter function has been mainly concerned with the binding of β -adrenergic (dihydroalprenolol) and dopaminergic (spiroperidol) ligands to preparations of synaptic membranes following chronic lithium administration. Although the data from the present study should be viewed with a degree of caution, they do appear to be at variance with previously published reports. Treiser and Kellar (1979) have reported a small decrease in the binding of dihydroalprenolol to cortical membranes following incubation with 4 nM ligand, while Rosenblatt et al. (1979) found similar changes in binding in membrane preparations obtained from whole brain. These latter authors attributed this difference in binding to a decrease in the number of specific binding sites (Rosenblatt et al., 1979). Schultz et al. (1981) however, found that chronic lithium treatment did not affect the

binding of the ligand to cerebellar preparations. In support of the findings of Treiser and Kellar (1979) and Rosenblatt et al. (1979), the data from the present study indicate a decrease in the number of binding sites for dihydroalprenolol in pons-medulla and midbrain, but increased B_{max} values in hypothalamus, striatum and cortex. In certain of these brain regions (pons-medulla, hypothalamus and striatum), alterations in the dissociation constants were also observed. It is possible that the increase in the number of binding sites is a consequence of the decrease in NA release which has previously been observed following lithium treatment (Katz et al., 1968). This reduced neurotransmitter release could be due to either a disruption of NA storage, as proposed by Greenspan et al. (1970) and Slotkin et al. (1980), or due to enhanced inhibition of NA release resulting from alterations in the high capacity uptake process, as postulated earlier in this discussion, or a combination of both. The net effect of either or both of these changes would be to produce a denervation-like situation in which the absence of neurotransmitter in the synaptic cleft leads to a proliferation of receptor sites for the transmitter. The reason for the decrease in B_{max} observed in pons-medulla and cortex is not known. Following two days of lithium withdrawal, the number of binding sites for dihydroalprenolol appeared to return towards control values for most regions. The exceptions to this were the hippocampus and midbrain and the apparent increase in B_{max} values in these brain areas is, as yet, inexplicable.

Discrepancies also exist between previous investigations of spiroperidol binding and the present study. Pert et al. (1978), Rosenblatt et al. (1979) and Staunton et al. (1982b) did not observe any alteration in the binding of this dopaminergic ligand to striatal synaptic membranes following chronic lithium treatment, while Rosenblatt et al. (1980) found that there was reduced binding of spiroperidol to caudate membranes due to a decrease in binding site density. These data conflict with the present finding of an increase in the number of

binding sites for the ligand after 12 days of lithium treatment. This elevation in binding site density was also observed in pons-medulla and hippocampus. The validity of these observations must, however, be questioned in the light of our knowledge of other agents which affect dopaminergic receptors. Neuroleptics are known to cause an increase in the number of striatal dopaminergic binding sites (Burt et al., 1977; Hitri et al., 1978; Pert et al., 1978; Lai et al., 1981; Jenner et al., 1982), and it has been shown that administration of a DA agonist to previously neuroleptic-treated animals during the period in which an increased number of DA receptors are present results in an enhancement of stereotypical behaviour (Tarsy and Baldessarini, 1974; Smith and Davis, 1976; Barany and Gunne, 1979; Clow et al., 1979; Tye et al., 1979). Therefore, if an increased number of dopaminergic receptors were indeed present following lithium administration, then apomorphine administration should result in an elevation of stereotypical responses with respect to control animals. This has not been found to be the case in either this or previous investigations (Staunton et al., 1982a). In addition, if lithium were able to induce new dopaminergic receptors, it would be unlikely that co-administration of this anti-manic agent with a neuroleptic would prevent the appearance of new receptor sites, as determined both behaviourally (Klawans et al., 1977; Pert et al., 1978; Allikmets et al., 1979) and biochemically (Pert et al., 1978). These observations all tend to imply that the changes in spiroperidol binding constants noted following lithium treatment, and therefore in the withdrawal group also are artifactual and probably reflective of poor assay technique.

B. LITHIUM IN AN ANIMAL MODEL OF TARDIVE DYSKINESIA

The bucco-linguo-masticatory dyskinesia syndrome has been shown to develop after prolonged usage of neuroleptics or following their discontinuation (Faurbye et al., 1964; Klawans, 1973). At present, the most efficacious treatment for tardive dyskinesia is an increase in the dosage of, or a re-introduction of,

neuroleptic. The syndrome is considered to be caused by an induction of DA receptors followed by a late adaptive response of neurotransmitter mechanisms to the presence of the neuroleptic agent (Baldessarini and Tarsy, 1979). The most commonly used animal model of tardive dyskinesia has therefore been based on the administration of a neuroleptic drug followed by a withdrawal period. The rationale behind this analogue of the disease state is that neuroleptic treatment will cause a proliferation of dopaminergic receptor sites, while the withdrawal period results in a return towards normal functioning of the monoaminergic neurones. The aim of this phase of the investigation was to determine whether lithium, administered in conjunction with or following haloperidol, could modify the effects of the neuroleptic drug.

Previous authors have demonstrated that haloperidol administration followed by a withdrawal period results in an enhancement of apomorphine-induced stereotypical behaviour (Tarsy and Baldessarini, 1974; Smith and Davis, 1976; Tye *et al.*, 1979). However, while the present data using an objective scoring method based on animal movement indicates an increased stereotypical response to apomorphine following haloperidol withdrawal, no strong confirmation of the previous reports was found. For this reason, a more subjective scoring method based on observer rating, e.g. that used by Klawans *et al.* (1977b), may have been a more appropriate measure of behavioural response, since this scoring technique makes allowances for behaviours such as sniffing, licking and gnawing which cannot be detected by the electronic measuring system used. If this type of subjective rating method had been utilized, a more obvious enhancement of apomorphine-induced stereotypical behaviour would have been noted as the animals subjected to the Sal + Hal + Sal treatment regime exhibited a high degree of sniffing and gnawing behaviour. This was also observed in rats treated with Sal + Hal + Li, whereas the electronic scores for the animals in this group indicate that there may have been a reduction in stereotypical response when compared to both control and Sal + Hal + Sal.

In the case of rats treated with lithium alone, there appeared to be little difference to control with regard to these apomorphine-induced responses, while the Li + Hal-Li + Li group displayed a degree of behaviour which, while greater than that observed in the control and Li groups, was less than that in other animals which received haloperidol as part of their treatment regime. Therefore, if these observations are taken into account when examining the scores obtained from the apomorphine-induced stereotypical behaviour, they appear to be in agreement with the results of Klawans et al. (1977b) and Pert et al. (1978) which show an amelioration of the haloperidol-induced enhancement of stereotypy by concurrent treatment of lithium, but not when lithium is administered following haloperidol treatment. It was therefore decided to utilize these same treatment regimes to investigate the interaction of haloperidol and lithium on processes associated with monoaminergic neurotransmitter function.

Previous studies have shown that neuroleptic administration results in an increase in the activity of tyrosine hydroxylase (Casa et al., 1980; Engel et al., 1980), a situation which was also found in the present experiment, although it should be stated that the increase noted (132% of control) did not reach statistical significance. It is possible that this enhancement of tyrosine hydroxylase activity is indicative of an enhanced synthesis of catecholamine, accompanied by an increase in uptake of tyrosine since the tissue content of the precursor amino acid was not affected by haloperidol treatment. This increase in synthetic enzyme activity was only observed in the three groups which received haloperidol as part of their treatment schedules, and was approximately equal in these groups (126-132% of control) indicating that neither concurrent treatment with lithium nor administration of the anti-manic agent after haloperidol affects alterations in the catecholamine synthesis induced by the neuroleptic.

If the elevation in tyrosine hydroxylase activity suggested by the previous data is indicative of an increase in DA turnover, then an increase in the levels of DA metabolites should also be seen. As was noted previously, lithium admin-

istration alone resulted in a significant enhancement in the striatal concentration of DOPAC, probably due to the increased neuronal uptake which results from this treatment. In the Sal + Hal + Sal group, elevated levels of HVA were observed (139% of control), which could well suggest an increase in DA release since the O-methylated deaminated metabolite of this neurotransmitter is considered to be a reflection of the amount of DA liberated from neuronal terminals. Following concurrent lithium and haloperidol treatment (Li + Hal-Li + Li), the striatal concentration of HVA was reduced relative to the Sal + Hal + Sal group, while the amount of DOPAC present was equivalent to that seen in lithium-treated animals. Since the amount of the intra-neuronal metabolite of DA is enhanced when lithium is co-administered with haloperidol, it is possible that the increased activity of the high-capacity uptake system results in a decrease in the amount of neurotransmitter present in the synaptic cleft which may well result in a reduction in the activation of the post-synaptic receptor sites. However, if this were the case, an elevation in the number of DA receptors present would be expected in this situation, as it would exacerbate the «denervation» which resulted from the presence of the neuroleptic agent, but it has been shown that concurrent administration of lithium and haloperidol results in a normalization of the number of dopaminergic binding sites (Pert et al., 1978). Alternatively, it is possible that the Li + Hal-Li + Li treatment schedule results in an enhanced turnover of DA in the striatum which in some way limits the induction of new DA receptors. This is particularly true in view of the finding that striatal DA content in the Li + Hal-Li + Li group was higher than that seen following either Li or the Sal + Hal + Sal treatments.

If this is indeed the case, then it is likely to be a region-specific effect since the increase in DA content was only observed in striatum and hippocampus following the Li + Hal-Li + Li treatment schedule. In hypothalamus and cortex, decreased transmitter content was observed. Since the majority of the parameters

concerned with dopaminergic function were only measured in striatum, there is little basis for speculation regarding the changes in the other brain regions; however, they appear to be due to an effect of both lithium and haloperidol in the hypothalamus, but a lithium-specific effect in hippocampus.

With regard to the noradrenergic system, the situation is equally confusing. After administration of Sal + Hal + Sal, NA content was reduced in hippocampus (to 7% of control), but elevated in cortex (to 236% of control). This would tend to imply that there was an elevation in the turnover of this neurotransmitter in the hippocampus, but a reduction in the cortex. This could explain the finding that MOPEG was depleted following haloperidol administration since this metabolite was measured in whole brain only, and the cortex constitutes a major portion of the tissue used for the determination of this compound. Lithium treatment alone resulted in decreased transmitter content in most brain regions, which may well be due to a disruption of NA storage as mentioned previously. Again, an anomalous situation was observed in cortex when NA content was not altered by the Li administration schedule. Following combined treatment with the two drugs (Li + Hal-Li + Li), the major effects observed appeared to be related to the presence of the lithium, with the exception of cortex. In this brain region, transmitter content was elevated with respect to both the control and Li groups. As previously, the reduction in brain MOPEG content is likely to be related to this cortical effect. When lithium was administered following haloperidol (Sal + Hal + Li), a reduction in both cortical NA and whole brain MOPEG levels was observed.

It is unlikely that an alteration in noradrenergic function, as indicated by these data, is involved in the protective effect of lithium when administered concurrently with haloperidol, as the alterations observed when lithium was given after the neuroleptic (Sal + Hal + Li) are essentially the same as those found in the Li + Hal-Li + Li group, with the exception of the cortex. Klawans et al. (1977) had previously demonstrated that when rats are challenged with amphetamine,

which induces the release of NA as well as DA, lithium only provided protection against enhanced stereotypical responding when it was administered in conjunction with haloperidol.

Various parameters of serotonergic function were also investigated in these treatment groups, and it was found that none of the drug schedules caused any alteration in synthetic processes. Haloperidol treatment alone had no significant effect on regional 5-HT levels, while lithium caused a depletion of the amine in most brain regions examined. When lithium was added to the haloperidol schedules (Li + Hal-Li + Li and Sal + Hal + Li), the effects seen were the result of the presence of lithium. Administration of haloperidol alone caused a reduction of 5-HIAA levels in pons and midbrain, and it is possible that these decreases in metabolite concentration are indicative of a reduction in turnover of the monoamine. This however would be at variance with the findings of Rastogi et al. (1981) who have suggested that chronic neuroleptic treatment results in an enhanced turnover of 5-HT. The effects of long-term lithium were the same as those described previously in this study. When lithium was added to the haloperidol treatment, a return towards control levels of 5-HIAA was observed in pons and midbrain in both groups receiving the two drugs (Li + Hal-Li + Li and Sal + Hal + Li), while increased tissue concentration was found in hypothalamus. However, since these changes were seen when lithium was administered following haloperidol treatment (Sal + Hal + Li) in addition to being noted in the group receiving concurrent treatment (Li + Hal-Li + Li), it is considered to be an effect related to the presence of lithium alone, and probably plays little, if any, part in the ability of the drug to present supersensitive responses to either apomorphine- or amphetamine-induced behaviours following haloperidol treatment.

C. SUMMARY

As has been shown, the administration of lithium at a maximal sub-toxic dose produces a change in spontaneous locomotor activity. This decrease in

activity has a delayed onset, the magnitude of which seems comparable to the delayed onset of therapeutic effect which is observed clinically. The attenuation in behaviour, once established, appears to remain relatively constant, with the proviso that lithium administration is maintained, since discontinuation of drug treatment results in a rapid return towards normal spontaneous locomotor activity. It is likely that these effects are directly related to brain tissue levels of the cation. However, the reduction in spontaneous locomotor activity was only observed in «normal» animals, and not in those subjected to behavioural stimulants. The use of various behavioural paradigms was an attempt to produce animal analogues of mania, and the finding that lithium pretreatment did not ameliorate the observed behavioural stimulation tends to cast doubt on the utility of these particular animal models of the disorder. It was however, disappointing to find no obvious «rebound» effect on the behavioural parameter measured following cessation of lithium treatment, but it is possible that this was due to the fact that spontaneous locomotor activity is a very gross behaviour, and a more subtle test may have been able to uncover a «rebound» activity.

With regard to the metabolic processes associated with the monoaminergic neurotransmitters, it appears that the effects of short-term and long-term administration of lithium are qualitatively similar, although there are quantitative differences in some cases. These quantitative differences are probably unrelated to the tissue levels of the cation, as the lithium concentrations in brain detected after 12 days of drug treatment are essentially the same as those found following 26 days of lithium administration. Neither of these treatment regimes resulted in changes in the synthetic parameters measured for either catecholaminergic or the serotonergic systems. When tissue levels of the neurotransmitters were examined however, alterations in the regional contents of these substances were noted following lithium administration. NA levels in most brain regions were reduced following both short- and long-term lithium treatment. DA content was

found to be depressed in a time-dependent fashion, with the levels of this catecholamine being reduced in hypothalamus after 12 days of lithium administration, while after 26 days of treatment, reduced content was noted in hippocampus as well as hypothalamus. Lithium treatment also appears to exert a time-dependent effect on 5-HT levels, since short-term treatment caused reduced transmitter content in mid-brain, while long-term administration resulted in depressed levels in hypothalamus and striatum in addition to mid-brain. It would be expected that decreased neurotransmitter content in the presence of unaltered synthesis would result in an elevation in the levels of the metabolites of these substances. Short-term lithium treatment however, caused no change in the central levels of the major metabolites of the monoaminergic neurotransmitters, while 26 days of lithium administration resulted in an enhancement of the striatal content of DOPAC alone. These data would tend to suggest that the observed decreases in neurotransmitter levels were not a consequence of an increase in turnover of these substances. It is therefore possible that these alterations reflect decreases in the storage capabilities of the neurones which utilize these neurotransmitters.

Two days of withdrawal from either short-term or long-term lithium treatment resulted in a rapid decline in tissue and serum levels of the cation, the degree of reduction being approximately equal in both treatment groups. Associated with this decrease in tissue levels of lithium was an enhancement of tyrosine hydroxylase activity, although alterations in tyrosine content were only noticed following withdrawal from long-term lithium administration. Parameters of serotonergic synthesis were unaffected by abrupt cessation of drug treatment. NA levels were generally unchanged relative to lithium-treated animals, i.e. decreased content when compared to control. The exceptions to this were in the pons, in which a further decrease relative to that noted following lithium treatment was seen, and in the cortex, in which transmitter content was enhanced when compared to both control and lithium-treated animals. DA concentrations showed similar changes

following lithium withdrawal as were seen with NA, i.e. enhanced cortical levels and depressed pontine content, with other regions being comparable to those observed after lithium treatment. In contrast to the catecholamines, 5-HT levels returned essentially to control values following withdrawal from both short- and long-term lithium administration. The same was true for the major metabolite (5-HIAA) of this neurotransmitter. With regard to the catecholamine metabolites, HVA levels were enhanced relative to control values following withdrawal from both short- and long-term lithium treatment. The elevated DOPAC content which resulted from long-term administration of lithium was found to have been normalized after 2 days of withdrawal from the drug. At the same time, MOPEG levels were found to be significantly reduced relative to control. As can be seen, it is unlikely that serotonergic processes are involved in any form of «rebound» phenomenon since the parameters measured for this neurotransmitter system displayed a rapid return towards control values following cessation of lithium administration. In the case of NA and DA however, it appears that changes in the metabolic processes for the catecholamines may be involved in the «rebound» phenomenon since lithium withdrawal did not result in either a simple return towards control or a maintenance of the effects seen following lithium administration.

When the uptake of the monoamines into synaptosomal preparations was examined, the most interesting finding was that the uptake processes for all three substances examined displayed more than one system. In the case of NA and 5-HT, there appeared to be a low-capacity system which predominated at exogenous substrate concentrations of up to 1 μ M, and a high-capacity system which came into play at concentrations of substrate greater than 1 μ M. The uptake process for DA seems to be more complex than that for the other two monoamines, and may well involve some form of multiple and co-operative process. The uptake of NA into synaptosomes was found to be markedly altered by 12 days of lithium treatment. The previous observation that lithium administration enhances NA uptake at low substrate concentrations was confirmed in the present study, but more interesting was the

finding that the high-capacity system appears to be inactivated by lithium treatment. It is suggested that the high-capacity system may play a modulatory role in the presynaptic inhibition of NA release, and that lithium acts to reduce neuronal traffic in the noradrenergic system by abolishing this high-capacity uptake. There is also strong evidence for a neurochemical «rebound» phenomenon in this process, since following withdrawal of lithium treatment there is a significant increase in the activity of the high-capacity uptake system. It is possible that this alteration in uptake of the neurotransmitter could play a part in the clinical «rebound» mania which has been observed. The uptake of 5-HT into synaptosomal preparations resembled that of NA, since lithium treatment caused a marked decrease in monoamine uptake at high substrate concentrations. A difference however, was seen at low exogenous concentrations since lithium administration resulted in reduced activity of the low-capacity uptake system for 5-HT. This decrease in uptake of the indoleamine following lithium treatment may be a reflection of decreased transmitter release due to an impairment of 5-HT storage. Following the withdrawal of lithium, the activity of the high-capacity uptake system remained depressed relative to control. Uptake of 5-HT at low exogenous concentrations however, was further reduced relative to control by the cessation of lithium treatment, and this alteration may play a part in the genesis of «rebound» mania. When the uptake of DA into synaptosomal preparations was examined, it was found that lithium treatment resulted in enhanced uptake in all brain regions, while 2 days of withdrawal from lithium resulted in a depression of DA uptake at most substrate concentrations examined. These data show that the neuronal uptake processes represent a major site for lithium's action, and may well be an important factor in producing the «rebound mania» noted clinically. It should be noted however, that there is scope for further investigations of these processes to fully characterize the uptake systems, and to further examine the consequences of lithium treatment and withdrawal upon these processes.

With regard to the binding of a β -adrenergic ligand to synaptic plasma membranes, it appears that the effect of lithium administration on the number of binding sites present is regionally variable, causing an increase in certain brain areas but a decrease in others. Lithium withdrawal resulted in a return towards control values in most of the regions examined. The data obtained for the binding of spiroperidol to synaptic membrane preparations are highly equivocal, but it should be remembered that these were the results from a single, unreplicated study. However, these data do indicate that much remains to be done in order to elucidate fully the consequences of lithium treatment and its subsequent withdrawal on central receptor populations.

A second aspect of lithium's utility in psychiatric medicine was examined in the final study reported here, i.e. its potential usefulness in preventing the onset of tardive dyskinesia. The behavioural component of this study demonstrated that when lithium was administered in conjunction with haloperidol, it was possible to ameliorate the enhancement of apomorphine-induced stereotypical behaviour due to administration of haloperidol alone. This was not the case when lithium was administered after haloperidol treatment. When the biochemical parameters associated with monoaminergic neurotransmitter function were examined, it was found that lithium administration did not alter the enhanced tyrosine hydroxylase activity due to neuroleptic treatment. The presence of increased levels of striatal DA when lithium was co-administered with haloperidol (relative to the concentrations found when either lithium or haloperidol were given alone) together with the alterations observed in dopaminergic metabolites may well be indicative of an enhanced turnover of the neurotransmitter resulting from this treatment regime. It is possible that this altered turnover could in some way limit the appearance of new DA receptors, which are considered to be involved in the genesis of tardive dyskinesia. In the case of the noradrenergic system, the alterations in various parameters were qualitatively similar when lithium was administered in

conjunction with haloperidol as those observed when lithium was given following haloperidol treatment. This may imply that this neurotransmitter is not involved in the prevention of tardive dyskinesia induction. Since a similar situation was observed when parameters of serotonergic function were examined, it is unlikely that alterations in 5-HT metabolism play a part in the lithium-induced protection against the genesis of this dyskinetic syndrome. While it appears that alterations in the metabolism of DA (but not NA or 5-HT) are involved in the protective effect exerted by lithium, the possibility also remains that changes in metabolic functions associated with some other neurotransmitter substance(s) are involved in this aspect of lithium's action. Also, it may be that lithium causes alterations in receptor populations other than dopaminergic sites which may play a role in the prevention of neuroleptic-induced tardive dyskinesia.

° In conclusion, therefore, it appears that lithium exerts its therapeutic effect, at least in part, by causing alterations in various parameters of central monoamine function. However, it is not known whether all these changes are required for this anti-manic agent to exert its psychotherapeutic action, or whether the alteration in one particular parameter is the predominant effect. Also, it appears that there is strong evidence for a neurochemical basis for the «rebound mania» which has been observed clinically following withdrawal of lithium therapy. There is however, still the need for considerable future investigation of the biochemical consequences of lithium administration and withdrawal in order to establish the nature of the dysfunction underlying the genesis of mania.

V

REFERENCES

REFERENCES

- Achor, R.W.P., Hanson, N.O. and Gifford, R.W. Jr. (1955). Hypertension treated with Rauwolfia serpentina (whole root) and with reserpine. J. Am. Med. Assoc. 159, 841-845.
- Aghajanian, G.K., Foote, W.E. and Sheard, M.H. (1968). Lysergic acid diethylamide: sensitive neuronal units in the midbrain raphe. Science 161, 706-708.
- Allikmets, L.H., Stanley, M. and Gershon, S. (1979). The effect of lithium on chronic haloperidol enhanced apomorphine aggression in rats. Life Sci. 25, 165-170.
- Anden, N.-E., Butcher, S.G., Corrodi, H., Fuxe, K. and Ungerstedt, U. (1970). Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. Eur. J. Pharmacol. 11, 303-314.
- Ansell, G.B. (1981). The biochemical background to tardive dyskinesia. Neuropharmac. 20, 311-317.
- Arbilla, S., Briley, M.S., Dubocovich, M.L. and Langer, S.Z. (1978). Neuroleptic binding and their effects on the spontaneous and potassium-evoked release of ³H-dopamine from the striatum and of ³H-noradrenaline from the cerebral cortex. Life Sci. 23, 1775-1780.
- Arieti, S. and Bemporad, J. (1978). Critical review of the major concepts of depression. In: «Severe and Mild Depression: The Psychotherapeutic Approach.» Basic Books Inc., pp. 11-56.
- Atterwill, C.K. and Tordoff, A.F.C. (1982). Effects of repeated lithium administration on the subcellular distribution of 5-hydroxytryptamine in rat brain. Br. J. Pharmacol. 76, 413-421.
- Axelrod, J. and Tomchick, R. (1958). Enzymatic O-methylation of epinephrine and other catechols. J. Biol. Chem. 233, 702-705.
- Ayd, F.J. Jr. (1957). A preliminary report on Marsilid. Am. J. Psychiat. 114, 459.
- Baastrup, P.C. (1964). The use of lithium in manic-depressive psychosis. Compr. Psychiat. 5, 396-408.
- Baastrup, P.C., Poulsen, J.C., Schou, M., Thomsen, K. and Amdisen, A. (1970). Prophylactic lithium: double blind discontinuation in manic-depressive and recurrent depressive disorders. Lancet II, 326-330.
- Baastrup, P.C. and Schou, M. (1967). Lithium as a prophylactic agent. Arch. Gen. Psychiat. 16, 162-172.
- Baastrup, P.C. and Schou, M. (1968). Prophylactic lithium. Lancet I, 1419-1422.
- Bacopoulos, N.G., Bustos, G., Redmond, D.E. Jr. and Roth, R.H. (1982). Chronic treatment with haloperidol or fluphenazine decanoate: regional effects on dopamine and serotonin metabolism in primate brain. J. Pharmacol. Exp. Ther. 221, 22-28.
- Bakris, G.L., Taylor, M.A., Mulopulos, G.P. and Wawczk, S. (1981). Lithium prophylaxis and the kidney. J. Affect. Dis. 3, 37-42.

- Baldessarini, R.J. (1975). The basis for amine hypothesis in affective disorders. Arch. Gen. Psychiat. 32, 1087-1093.
- Baldessarini, R.J. and Tarsy, D. (1979). Relationship of the actions of neuroleptic drugs to the pathophysiology of tardive dyskinesia. Int. Rev. Neurobiol. 21, 1-45.
- Baldessarini, R.J. and Yorke, C. (1970). Effects of lithium and of pH on synaptosomal metabolism of noradrenaline. Nature 228, 1301-1303.
- Balsara, J.J., Jadhav, J.H. and Chandorkar, A.G. (1979). Effects of drugs influencing central serotonergic mechanisms on haloperidol-induced catalepsy. Psychopharmacology 62, 67-69.
- Barany, S. and Gunne, L.-M. (1979). Pharmacological modification of experimental tardive dyskinesia. Acta Pharmacol. et Toxicol. 45, 107-111.
- Barkai, A.I. (1979). Serotonin turnover in the intact rabbit brain: relationship to extracellular proteins and modification by pentobarbital or haloperidol. J. Pharmacol. Exp. Ther. 208, 44-48.
- Bartholini, G., Keller, H.H. and Pletscher, A. (1973). Effect of neuroleptics on endogenous norepinephrine in rat brain. Neuropharmacology 12, 751-756.
- Bateman, D.N., Dutta, D.K., McClelland, H.A. and Rawlins, M.D. (1979). Metoclopramide and haloperidol in tardive dyskinesia. Br. J. Psychiat. 135, 505-508.
- Berger, P.A. (1977). Neurotransmitters and affective disorders. In: «Neurotransmitter Function, Basic and Clinical Aspects,» (Fields, W.S., ed.), Symposia Specialists, Miami, pp. 305-335.
- Berger, P.A. and Barchas, J.D. (1977). Biochemical hypotheses of affective disorders. In: «Psychopharmacology: From Theory to Practice,» (Barchas, J.D., Berger, P.A., Ciaranello, R.D. and Elliott, G.R., eds.), Oxford Univ. Press, N.Y., pp. 151-173.
- Berggren, U., Ahlenius, S. and Engel, J. (1980). Effects of acute lithium administration on conditioned avoidance behaviour and monoamine synthesis in rats. J. Neural Trans. 47, 1-10.
- Berggren, U., Engel, J. and Liljequist, S. (1981). The effect of lithium on the locomotor stimulation induced by dependence-producing drugs. J. Neural Trans. 50, 157-164.
- Bertler, A. (1961). Effect of reserpine on the storage of catecholamines in brain and other tissues. Acta Physiol. Scand. 51, 75-83.
- Blackwell, B. and Shepherd, M. (1968). Prophylactic lithium: another therapeutic myth? Lancet I, 968-971.
- Boadle-Biber, M.C. (1982). Blockade by haloperidol of the increase in tryptophan hydroxylase activity induced by incubation of slices of brain stem with dibutyryl cyclic AMP. Biochem. Pharmacol. 31, 2203-2207.
- Bowman, W.C. and Rand, M.J. (1980). Peripheral adrenergic mechanisms. In: «Textbook of Pharmacology,» Blackwell Scientific Publications, Oxford. pp. 11.1-11.7.

- Brodie, B.B., Shore, P.A. and Pletscher, A. (1956). Serotonin-releasing activity limited to Rauwolfia alkaloids with tranquilizing action. Science 123, 992-993.
- Bunney, W.E. Jr. and Davis, J.M. (1965). Norepinephrine in depressive reactions. Arch. Gen. Psychiat. 13, 483-494.
- Bunney, W.E. Jr., Goodwin, F.K., Davis, J.M. and Fawcett, J.A. (1968). A behavioural-biochemical study of lithium treatment. Am. J. Psychiat. 125, 499-512.
- Burki, H.R., Ruch, W. and Asper, H. (1975). Effects of clozapine, thioridazine, perlapine and haloperidol on the metabolism of the biogenic amines in the brain of the rat. Psychopharmacologia (Berl.) 41, 27-33.
- Burt, D.R., Creese, I. and Snyder, S.H. (1977). Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196, 326-327.
- Cade, J.F.J. (1949). Lithium salts in the treatment of psychotic excitement. Med. J. Austr. 2, 349-352.
- Cade, J.F.J. (1978). Lithium - Past, present and future. In: «Lithium in Medical Practice» (Johnson, F.N. and Johnson, S., eds.), University Park Press, Baltimore, pp. 5-16.
- Cameron, O.G. and Smith, C.B. (1980). Comparison of acute and chronic lithium treatment on ³H-norepinephrine uptake by rat brain slices. Psychopharmacology 67, 81-85.
- Cappelliez, P. and White, N. (1981). Lithium induces dose-related increases and decreases in activity levels in the rat. Psychopharmacology 73, 34-38.
- Carlsson, A., Kehr, W. and Lindqvist, M. (1977). Agonist-antagonist interaction on dopamine receptors in brain, as reflected in the rates of tyrosine and tryptophan hydroxylation. J. Neural Trans. 40, 99-113.
- Carlsson, A. and Lindqvist, M. (1963). Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol. et. Toxicol. 20, 140-144.
- Carlsson, A. and Lindqvist, M. (1967). Metatyrosine as a tool for selective protection of catecholamine stores against reserpine. Eur. J. Pharmacol. 2, 187-192.
- Carroll, B.J., Curtis, G.C. and Kokmen, E. (1977). Paradoxical response to dopamine agonists in tardive dyskinesia. Am. J. Psychiat. 134, 785-789.
- Carruba, M.D., Nistico, G. and Mantegazza, P. (1979). Evidence for a receptor supersensitivity following impairment of a central serotonergic activity in the rabbit. Arch. Pharmacol. 309, 125-129.
- Carter, C.J. and Pycock, C.J. (1979). Potentiation of haloperidol-induced catalepsy by dopamine agonists: possible involvement of central 5-hydroxytryptamine. Pharmacol. Biochem. Behav. 10, 475-480.
- Casu, M., Klimek, V., Biggio, G. and Gessa, G.L. (1980). Tolerance to haloperidol effect on dopamine (DA) synthesis is independent from postsynaptic DA receptors. Pharmacol. Res. Comm. 12, 393-396.

- Chan, C.C. and Kalsner, S. (1979a). An examination of the negative feedback function of presynaptic adrenoceptors in a vascular tissue. Br. J. Pharmacol. 67, 401-407.
- Chan, C.C. and Kalsner, S. (1979b). Noradrenaline inhibition of transmitter efflux in a renal artery preparation and the presynaptic receptor theory. Can. J. Physiol. Pharmacol. 57, 1192-1195.
- Christensen, E., Moller, J.E. and Faurbye, A. (1970). Neuropathological investigation of 28 brains from patients with dyskinesia. Acta Psychiat. Scand. 46, 14-21.
- Ciaranello, R.D., Barchas, R.E., Byers, G.S., Stemmler, D.W. and Barchas, J.D. (1969). Enzymatic synthesis of adrenaline in mammalian brain. Nature 221, 368-369.
- Clow, A., Jenner, P., Theodorou, A. and Marsden, C.D. (1979). Striatal dopamine receptors become supersensitive while rats are given trifluoperazine for 6 months. Nature 278, 59-61.
- Colburn, R.W., Goodwin, F.K., Bunney, W.E. Jr. and Davis, J.M. (1967). Effect of lithium on the uptake of noradrenaline by synaptosomes. Nature 215, 1395-1397.
- Collard, K.J. (1978a). The effect of lithium on the increase in forebrain 5-hydroxyindoleacetic acid produced by raphe stimulation. Br. J. Pharmacol. 62, 137-142.
- Collard, K.J. (1978b). Lithium effects on brain 5-hydroxytryptamine metabolism. In: «Lithium in Medical Practice», (Johnson, F.J. and Johnson, S., eds.), University Park Press, Baltimore, pp. 123-133.
- Coppen, A., Noguera, R., Bailey, J., Burns, B.H., Swani, M.S., Hare, E.H., Gardner, R. and Maggs, R. (1971). Prophylactic lithium in affective disorders. Lancet II, 275-279.
- Corcoran, A.C., Taylor, R.D. and Page, I.H. (1949). Lithium poisoning from the use of salt substitutes. J. Am. Med. Assoc. 139, 685-688.
- Corrodi, H., Fuxe, K., Hokfelt, T. and Schou, M. (1967). The effect of lithium on cerebral monoamine neurones. Psychopharmacologia (Berl.) 11, 345-353.
- Corrodi, H., Fuxe, K. and Schou, M. (1969). The effect of prolonged lithium administration on cerebral monoamine neurons in the rat. Life Sci. 8 (Pt. 1), 643-651.
- Cox, B. and Ennis, C. (1982). Characterization of 5-hydroxytryptaminergic autoreceptors in the rat hypothalamus. J. Pharm. Pharmacol. 34, 438-441.
- Curzon, G. and Green, A.R. (1970). Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Br. J. Pharmacol. 39, 653-655.
- Dalen, P. (1973). Lithium therapy in Huntington's Chorea and tardive dyskinesia. Lancet I, 107-108.

- Dally, P.J. (1958). Indications for use of iproniazid in psychiatric practice. Br. Med. J. 1, 1338-1339.
- DaPrada, M. and Zurcher, G. (1976). Simultaneous radioenzymatic determination of plasma and tissue adrenaline, noradrenaline and dopamine within the femtomole range. Life Sci. 19, 1161-1174.
- Davies, C., Sanger, D.J., Steinberg, H., Tomkiewicz, M. and U'Prichard, D.C. (1974). Lithium and α -methyl-p-tyrosine prevent «manic» activity in rodents. Psychopharmacologia 36, 263-274.
- Delay, J., Deniker, P., Ropert, R., Beek, H., Barande, R. and Eurieult, M. (1959). Syndromes neurologiques experimentaux et therapeutique psychiatrique I. Effets neurologiques d'un nouveau neuroleptique majeur, le 7843 R.P. Presse Méd. 67, 123-126.
- Delini-Stula, A. and Meier, M. (1976). Inhibitory effects of propranolol and oxprenalol on excitation induced by a MAO inhibitor and reserpine in the mouse. Neuropharmacology 15, 383-388.
- de Montigny, C., Chouinard, G. and Annable, L. (1979). Ineffectiveness of Deanol in tardive dyskinesia: a placebo controlled study. Psychopharmacology 65, 219-223.
- DeVerteuil, R.L. and Lehmann, H.E. (1958). Therapeutic trial of iproniazid (Marsilid) in depressed and apathetic patients. Can. Med. Assoc. J. 78, 131-133.
- DiChiara, G., Onali, P.L., Tissari, A.H., Porceddu, M.L., Morelli, M. and Gessa, G.L. (1978). Destruction of post-synaptic dopamine receptors prevents neuroleptic-induced activation of striatal tyrosine hydroxylase but not dopamine synthesis stimulation. Life Sci. 23, 691-696.
- Dubocovich, M.L. and Langer, S.Z. (1974). Negative feedback regulation of nor-adrenaline release by nerve stimulation in the perfused cat's spleen: differences in potency of phenoxybenzamine in blocking the pre- and post-synaptic adrenergic receptors. J. Physiol. 237, 505-519.
- Dyson, W.L. and Mendelson, M. (1968). Recurrent depressions and the lithium ion. Am. J. Psychiat. 125, 544-548.
- Eayrs, J.T. (1960). Influence of the thyroid on the central nervous system. Br. Med. Bull. 16, 122-127.
- Ebstein, R.P., Eliashar, S., Belmaker, R.H., Ben-Uriah, Y. and Yehuda, S. (1980). Chronic lithium treatment and dopamine-mediated behaviour. Biol. Psychiat. 15, 459-467.
- Ehrlich, B.E., Diamond, J.M., Braun, L.D., Cornford, E.M. and Oldendorf, W.H. (1980). Effects of lithium on blood brain barrier transport of the neurotransmitter precursors choline, tyrosine and tryptophan. Brain Res. 193, 604-607.
- Elliott, G.R., Edelman, A.M., Renson, J.F. and Berger, P.A. (1977). Indoleamines and other neuroregulators. In: «Psychopharmacology: From Theory to Practice,» (Barchas, J.D., Berger, P.A., Ciaranello, R.D. and Elliott, G.R., eds.), Oxford University Press, New York, pp. 35-50.

- Engel, J. and Berggren, U. (1980). Effects of lithium on behaviour and central monoamines. Acta Psych. Scand., Suppl. 280, 61, 133-143.
- Engel, J., Liljequist, S. and Goldstein, M. (1980). Dyskinesia induced by long-term treatment with anti-psychotic drugs: involvement of pre- and post-synaptic dopaminergic mechanisms. In: «Ergot Compounds and Brain Function: Neuroendocrine and Neuropsychiatric Aspects,» (Goldstein, M., Calne, D.B., Lieberman, A. and Thorner, M.O., eds.), Raven Press, New York, pp. 387-395.
- Eroglu, L., Hizal, A. and Koyuncuoglu, H. (1981). The effect of long-term concurrent administration of chlorpromazine and lithium on the striatal and frontal cortical dopamine metabolism in rats. Psychopharmacology 73, 84-86.
- Euler, U.S.V. (1972). Synthesis, uptake and storage of catecholamines in adrenergic nerves. The effect of drugs. In: «Catecholamines. Handbook of Experimental Pharmacology, v. 33. (Blaschko, H. and Muscholl, E., eds.), Spring-Verlag, Berlin, pp. 186-230.
- Fang, V.S. and Shian, L. R. (1981). A serotonergic mechanism of the prolactin-stimulating action of metoclopramide. Endocrinology 108, 1622-1627.
- Faurbye, A., Rasch, P.-J., Petersen, P.B., Brandborg, G. and Pakkenberg, H. (1964). Neurological symptoms in pharmacotherapy of psychoses. Acta Psychiat. Scand. 40, 10-27.
- Feighner, J.P., Robins, E., Guze, S.B., Woodruff, R.A. Jr., Winokur, G. and Munoz, R. (1972). Diagnostic criteria for use in psychiatric research. Arch. Gen. Psychiat. 26, 57-68.
- Ferreira, A.J.D. and Freeman, H. (1958). A clinical trial of mersilid in psychotic depressed patients. Am. J. Psychiat. 114, 933-934.
- Fieve, R.R., Platman, S.R. and Platchik, R.R. (1968). The use of lithium in affective disorders I. Acute endogenous depression. Am. J. Psychiat. 125, 487-491.
- Fog, R., Pakkenberg, H., Juul, P., Bock, E., Jorgensen, O.S. and Andersen, J. (1976). High dose treatment of rats with perphenazine enanthate. Psychopharmacology 50, 305-307.
- Frazer, A., Mendels, J., Secunda, S.K., Cochrane, C.M. and Bianchi, C.P. (1973). The prediction of brain lithium concentrations from plasma or erythrocyte measures. J. Psychiat. Res. 10, 1-7.
- Friedman, E. and Gershon, S. (1973). Effect of lithium on brain dopamine. Nature 243, 520-521.
- Frohman, L.A. and Stachura, M. (1975). Neuropharmacologic control of neuroendocrine function in man. Metabolism 24, 211-234.
- Furukawa, T. and Yamada, K. (1979). Brain serotonin metabolism with relation to the head twitches elicited by lithium in combination with reserpine in mice. Pharmacol. Biochem. Behav. 10, 547-549.
- Gallager, D.W. and Bunney, W.E. (1979). Failure of chronic lithium treatment to block tricyclic antidepressant-induced 5-HT supersensitivity. Arch. Pharmacol. 307, 129-133.

- Gelenberg, A.J. (1976). Computerized tomography in patients with tardive dyskinesia. Am. J. Psychiat. 133, 578-579.
- Gerlach, J., Reisby, N. and Randrup, A. (1974). Dopaminergic hypersensitivity and cholinergic hypofunction in the pathophysiology of tardive dyskinesia. Psychopharmacologia (Berl.) 34, 21-35.
- Gershon, S. and Yuwiler, A. (1960). Lithium ion: a specific psychopharmacological approach to the treatment of mania. J. Neuropsychiat. 1, 229-241.
- Glowinski, J. and Iversen, L.L. (1966). Regional studies of catecholamines in the rat brain I. The disposition of (³H)norepinephrine, (³H)dopamine and (³H)DOPA in various regions of the brain. J. Neurochem. 13, 655-669.
- Goodwin, F.K., Murphy, D.L. and Bunney, W.E. Jr. (1969). Lithium carbonate treatment in depression and mania. Arch. Gen. Psychiat. 21, 486-496.
- Goodwin, F.K., Murphy, D.L., Dunner, D.L. and Bunney, W.E. Jr. (1972). Lithium response in unipolar versus bipolar depression. Am. J. Psychiat. 129, 44-47.
- Gothert, M. and Weiheimer, G. (1979). Extracellular 5-hydroxytryptamine inhibits 5-hydroxytryptamine release from rat brain cortex slices. Arch. Pharmacol. 310, 93-96.
- Grahame-Smith, D.G. and Green, A.R. (1974). The role of brain 5-hydroxytryptamine in the hyperactivity produced in rats by lithium and monoamine oxidase inhibition. Br. J. Pharmacol. 52, 19-26.
- Greenspan, K., Aronoff, M.S. and Bogdanski, D.F. (1970). Effects of lithium carbonate on turnover and metabolism of norepinephrine in the rat brain - correlation to gross behavioural effects. Pharmacology 3, 129-136.
- Gross, G. and Schumann, H.-J. (1980). Enhancement of noradrenaline release from rat cerebral cortex by neuroleptic drugs. Arch. Pharmacol. 315, 103-109.
- Guerra, C. (1982). Effect of lithium on synaptosomal brain enzymes. Biochem. Pharmacol. 31, 449-453.
- Hanlon, L.W., Romaine, M., Gilroy, F.J. and Deitrick, J.E. (1949). Lithium chloride as a substitute for sodium chloride in the diet. J. Am. Med. Assoc. 139, 688-692.
- Harrison-Read, P.E. (1978). Models of lithium action based on behavioural studies using animals. In: «Lithium in Medical Practice,» (Johnson, F.N. and Johnson, S., eds.), University Park Press, Baltimore, pp. 289-303.
- Hartigan, G.P. (1963). The use of lithium salts in affective disorders. Br. J. Psychiat. 109, 810-814.
- Hesketh, J.E., Nicolaou, N.M., Arbuthnott, G.W. and Wright, A.K. (1978). The effect of chronic lithium administration on dopamine metabolism in rat striatum. Psychopharmacology 56, 163-166.
- Hess, S.M. and Udenfriend, S. (1959). A fluorometric procedure for the measurement of tryptamine in tissues. J. Pharmacol. Exp. Ther. 127, 175-177.

- Himwich, H.E. and Alpers, H.S. (1970). Psychopharmacology. Ann. Rev. Pharmacol. 10, 313-334.
- Hitri, A., Weiner, W.J., Borison, R.L., Diamond, B.I., Nausieda, P.A. and Klawans, H.L. Jr. (1978). Dopamine binding following prolonged haloperidol pretreatment. Ann. Neurol. 3, 134-140.
- Ho, A.K.S., Loh, H.H., Craves, F., Hitzemann, R.J. and Gershon, S. (1970). The effect of prolonged lithium treatment on the synthesis, rate and turnover of monoamines in brain regions of rats. Eur. J. Pharmacol. 10, 72-78.
- Hokfelt, T., Fuxe, K., Goldstein, M. and Johansson, O. (1974). Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. Brain Res. 66, 235-251.
- Holzbauer, M. and Vogt, M. (1956). Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. J. Neurochem. 1, 8-11.
- Horn, A.S. (1979). Characteristics of dopamine uptake. In: «The Neurobiology of Dopamine,» (Horn, A.S., Korf, J. and Westerink, B.H.C., eds.), Academic Press, London, pp. 217-235.
- Hullin, R.P., McDonald, R. and Allsopp, M.N.E. (1972). Prophylactic lithium in recurrent affective disorders. Lancet I, 1044-1046.
- Iversen, L.L., Jarrott, B. and Simmonds, M.A. (1971). Differences in the uptake, storage and metabolism of (+) and (-)-noradrenaline. Br. J. Pharmacol. 43, 845-855.
- Jackson, D.M., Anden, N.-E., Engel, J. and Liljequist, S. (1975). The effect of long-term penfluridol treatment on the sensitivity of the dopamine receptors in the nucleus accumbens and in the corpus striatum. Psychopharmacologia (Berl.) 45, 151-155.
- Jenner, P., Hall, M.D., Murugaiah, K., Rupniak, N., Theodorou, A. and Marsden, C.D. (1982). Repeated administration of sulpiride for three weeks produces behavioural and biochemical evidence for cerebral dopamine receptor supersensitivity. Biochem. Pharmacol. 31, 325-328.
- Jeste, D.W. and Wyatt, R.J. (1979). In search of treatment for tardive dyskinesia: review of the literature. Schizophrenia Bull. 5, 251-293.
- Kane, J.M. and Smith, J.M. (1982). Tardive dyskinesia. Arch. Gen. Psychiat. 39, 473-481.
- Katz, R.I., Chase, T.N. and Kopin, I.J. (1968). Evoked release of norepinephrine and serotonin from brain slices: inhibition by lithium. Science 162, 466-467.
- Kazamatsuri, H., Chien, C.-P. and Cole, J.O. (1973). Long-term treatment of tardive dyskinesia with haloperidol and tetrabenazine. Am. J. Psychiat. 130, 479-483.
- Kingstone, E. (1960). The lithium treatment of hypomanic and manic states. Compr. Psychiat. 1, 317-320.
- Kizer, J.S., Humm, J., Nicholson, G., Greeley, G. and Youngblood, W. (1978). The effect of castration, thyroidectomy and haloperidol upon the turnover rates of dopamine and norepinephrine and the kinetic properties of tyrosine hydroxylase in discrete hypothalamic nuclei of the male rat. Brain Res. 146, 95-107.

- Kizer, J.S., Zivin, J.A., Saavedra, J.M. and Brownstein, M.J. (1975). A sensitive microassay for tryptophan hydroxylase in brain. J. Neurochem. 24, 779-785.
- Klawans, H.L. (1973). The pharmacology of tardive dyskinesia. Am. J. Psychiat. 130, 82-86.
- Klawans, H.L., Hitri, A., Nausieda, P.A. and Weiner, W.J. (1977a). Animal models of dyskinesia. In: «Animal Models in Psychiatry and Neurology», (Hanin, I. and Usdin, E., eds.), Pergamon Press, Oxford, pp. 351-363.
- Klawans, H.L., Weiner, W.J. and Nansieda, P.A. (1977b). The effect of lithium on an animal model of tardive dyskinesia. Prog. Neuro-Psychopharmacol. 1, 53-60.
- Klein, H.E., Broucek, B. and Greil, W. (1981). Lithium withdrawal triggers psychotic states. Br. J. Psychiat. 139, 255-256.
- Knapp, S. and Mandell, A.J. (1975). Effects of lithium chloride on parameters of biosynthetic capacity for 5-hydroxytryptamine in rat brain. J. Pharmacol. Exp. Ther. 193, 812-823.
- Koslow, S.H. and Schlumpf, M. (1974). Quantitation of adrenaline in rat brain nuclei and areas by mass fragmentation. Nature 251, 530-531.
- Kuriyama, K. and Speken, R. (1970). Effect of lithium on content and uptake of norepinephrine and 5-hydroxytryptamine in mouse brain synaptosomes and mitochondria. Life Sci. 9 (Pt. 1), 1213-1220.
- Lai, H., Carino, M.A. and Horita, A. (1981). Chronic treatments with zotepine, thioridazine and haloperidol affect apomorphine-elicited stereotypic behaviour and striatal ³H-spiroperidol binding sites in the rat. Psychopharmacology 75, 388-390.
- Langer, S.Z. (1974). Presynaptic regulation of catecholamine release. Biochem. Pharmacol. 23, 1793-1800.
- Langer, S.Z. (1980). Physiological and pharmacological role of presynaptic receptor systems in neurotransmission. In: «Presynaptic Receptors», (Langer, S.Z., Starke, K. and Dubocovich, M.L., eds.), Pergamon Press, Oxford, pp. 13-22.
- Lapierre, Y.D., Gagnon, A. and Kokkinidis, L. (1980). Rapid recurrence of mania following lithium withdrawal. Biol. Psychiat. 15, 859-864.
- Lehninger, A.L. (1970). Enzymes: mechanism, structure and regulation. In: «Biochemistry», Worth Publishers, New York, pp. 169-187.
- Lemieux, G., Davignon, A. and Genest, J. (1956). Depressive states during Rauwolfia therapy for arterial hypertension. Can. Med. Assoc. J. 74, 522-526.
- Leonard, B.E. (1975). Changes in rat brain monoamine metabolism following the acute administration of lithium chloride in combination with antidepressant drugs. Arch. Int. Pharmacodyn. Ther. 215, 202-207.
- Lerer, B., Ebstein, R.P., Felix, A. and Belmaker, R.H. (1980). Lithium amelioration of reserpine-induced hypoactivity in rats. Int. Pharmacopsychiat. 15, 338-343.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

- Maas, J.W. (1979). Catecholamines and the affective disorders. In: «Aromatic Amino Acid Hydroxylases and Mental Disease,» (Youdim, M.B.H., ed.), John Wiley and Sons, Chichester, pp. 191-231.
- Mackiewicz, J. and Gershon, S. (1964). An experimental study of the neuropathological and toxicological effects of chlorpromazine and reserpine. J. Neuropsychiat. 5, 159-169.
- Maggi, A. and Enna, S.J. (1980). Regional alterations in rat brain neurotransmitter systems following chronic lithium treatment. J. Neurochem. 34, 888-892.
- Maggs, R. (1963). Treatment of manic illness with lithium carbonate. Br. J. Psychiat. 109, 56-65.
- Maickel, R.P., Cox, R.H. Jr., Saillant, J. and Miller, F.P. (1968). A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. Int. J. Neuropharmacol. 7, 275-281.
- Maletzky, B. and Blachly, P.H. (1971). In: «The Use of Lithium in Psychiatry,» CRC Press, Cleveland, pp. 45-47.
- Mann, J. and Gershon, S. (1980). Absolute and relative contra-indications to lithium treatment. In «Handbook of Lithium Therapy,» (Johnson, F.N., ed.), University Park Press, Baltimore, pp. 265-278.
- Marwaha, J., Hoffer, B.J., Geller, H.M. and Freedman, R. (1981). Electrophysiologic interactions of antipsychotic drugs with central noradrenergic pathways. Psychopharmacology 73, 126-133.
- Matussek, N. and Muller, S. (1975). Effect of chronic lithium treatment on behaviour and on norepinephrine metabolism in rat brain in a norepinephrine-deficient state. In: «Neuropsychopharmacology,» (Boissier, J.R., Hippus, H. and Pichot, P., eds.), Excerpta Medica, Amsterdam, pp. 612-616.
- McCaman, R.E. (1965). Microdetermination of catechol-O-methyl transferase in brain. Life Sci. 4, 2353-2359.
- McGeer, E.G., Gibson, S. and McGeer, P.L. (1967). Some characteristics of brain tyrosine hydroxylase. Can. J. Biochem. 45, 1557-1563.
- Meek, J.L. and Neff, N.H. (1972). Fluorometric estimation of 4-hydroxy-3-methoxyphenylethyleneglycol sulphate in brain. Br. J. Pharmacol. 45, 435-441.
- Meller, E., Friedhoff, A.J. and Friedman, E. (1980). Differential effects of acute and chronic haloperidol treatment on striatal and nigral 3,4-dihydroxyphenylacetic acid (DOPAC) levels. Life Sci. 26, 541-547.
- Moskovitz, C. and Klawans, H.L. (1977). Theoretical aspects of the pharmacology of affective disorders. In: «Clinical Neuropharmacology,» Vol. 2 (Klawans, H.L., ed.), Raven Press, New York, pp. 55-86.
- Moskovitz, R., Springer, P. and Urquhart, M. (1981). Lithium-induced nephrotic syndrome. Am. J. Psychiat. 138, 382-383.
- Mukherjee, B.P., Bailey, P.T. and Pradhan, S.N. (1976). Temporal and regional differences in brain concentrations of lithium in rats. Psychopharmacology 48, 119-121.

- Muller, J.C., Pryor, W.W., Gibbons, J.E. and Orgain, E.S. (1955). Depression and anxiety occurring during Rauwolfia therapy. J. Am. Med. Assoc. 159, 836-839.
- Murphy, D.L. (1977). Animal models for mania. In: «Animal Models in Psychiatry and Neurology,» (Hanin, I. and Usdin, E., eds.), Pergamon Press, Oxford, pp. 211-222.
- Murphy, G.F., Robinson, D. and Sharman, D.F. (1969). The effect of tropolone on the formation of 3,4-dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid in the brain of the mouse. Br. J. Pharmacol. 36, 107-115.
- Nicolaou, N.M. (1980). Acute and chronic effects of neuroleptics and acute effects of apomorphine and amphetamine on dopamine turnover in corpus striatum and substantia nigra of the rat brain. Eur. J. Pharmacol. 64, 123-132.
- Noack, C.H. and Trautner, E.M. (1951). The lithium treatment of maniacal psychosis. Med. J. Austr. 38, 219-222.
- Nyback, H. and Sedvall, G. (1970). Further studies on the accumulation and disappearance of catecholamines formed from tyrosine-¹⁴C in mouse brain. Effect of some phenothiazine analogues. Eur. J. Pharmacol. 10, 193-205.
- Owen, F., Cross, A.J., Waddington, J.L., Poulter, M., Gamble, S.J. and Crow, T.J. (1980). Dopamine-mediated behaviour and ³H-spiperone binding to striatal membranes in rats after nine months haloperidol administration. Life Sci. 26, 55-59.
- Pakkenberg, H., Fog, R. and Nilakantan, B. (1973). The long-term effect of perphenazine enanthate on the rat brain. Some metabolic and anatomical observations. Psychopharmacologia (Berl.) 29, 329-336.
- Pare, C.M.B. and Sandler, M. (1959). A clinical and biochemical study of a trial of iproniazid in the treatment of depression. J. Neurol. Neurosurg. Psychiat. 22, 247-251.
- Perez-Cruet, J., Tagliamonte, A., Tagliamonte, P. and Gessa, G.L. (1971). Stimulation of serotonin synthesis by lithium. J. Pharmacol. Exp. Ther. 178, 325-330.
- Peroutka, S.J. and Snyder, S.H. (1980). Relationship of neuroleptic drug effects at brain dopamine, serotonin, α -adrenergic and histamine receptors to clinical potency. Am. J. Psychiat. 137, 1518-1522.
- Perris, C. (1966). A study of bipolar (manic-depressive) and unipolar recurrent depressive psychosis. Acta Psychiat. Scand. 42 (Suppl. 194), 1-188.
- Perry, P.J., Dunner, F.J., Hahn, R.L., Tsuang, M.T. and Bery, M.J. (1981). Lithium kinetics in single daily dosing. Acta Psychiat. Scand. 64, 281-294.
- Pert, A., Rosenblatt, J.E., Sivit, C., Pert, C.B. and Bunney, W.E. Jr. (1978). Long-term treatment with lithium prevents the development of dopamine receptor supersensitivity. Science 201, 171-173.
- Petty, F. and Sherman, A.D. (1981). A pharmacologically pertinent animal model of mania. J. Affect. Dis. 3, 381-387.

- Peuler, J.D. and Johnson, G.A. (1977). Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. Life Sci. 21, 625-636.
- Plenge, P., Møllerup, E.T., Bolwig, T.G., Brun, C., Hetmar, O., Ladefoged, J., Larsen, S. and Rafaelsen, O.J. (1982). Lithium treatment: does the kidney prefer one daily dose instead of two? Acta Psychiat. Scand. 66, 121-128.
- Plenge, P., Møllerup, E.T. and Norgaard, T. (1981). Functional and structural rat kidney changes caused by peroral or parenteral lithium treatment. Acta Psychiat. Scand. 63, 303-313.
- Pletscher, A., Shore, P.A. and Brodie, B.B. (1956). Serotonin as a mediator of reserpine action in brain. J. Pharmacol. Exp. Ther. 116, 84-89.
- Poitou, P. and Bohuon, C. (1975). Catecholamine metabolism in the rat brain after short- and long-term lithium administration. J. Neurochem. 25, 535-537.
- Poitou, P., Boulu, R. and Bohuon, C. (1975). Effect of lithium and other drugs on the amphetamine chlordiazepoxide hyperactivity in mice. Experientia 31, 99.
- Post, R.M. and Cutler, N.R. (1979). Pharmacology of acute mania. In: «Clinical Neuropharmacology», Vol. 4 (Klawans, H.L., ed.), Raven Press, New York, pp. 39-81.
- Prien, R.F. (1978). Lithium in the treatment of affective disorders. In: «Clinical Neuropharmacology», Vol. 3 (Klawans, H.L., ed.), Raven Press, New York, pp. 113-131.
- Prien, R.F., Caffey, E.M. and Klett, J. (1973). Prophylactic efficacy of lithium carbonate in manic-depressive illness. Arch. Gen. Psychiat. 28, 337-341.
- Quinn, G.P., Shore, P.A. and Brodie, B.B. (1959). Biochemical and pharmacological studies of RO 1-9569 (tetrabenazine), a non-indole tranquilizing agent with reserpine-like effects. J. Pharmacol. Exp. Ther. 127, 103-109.
- Ramsey, T.A. and Mendels, J. (1978). Lithium-responsive depressive illness: clinical and biological characteristics. In: «Neuro-Psychopharmacology», (Deniker, P., Radouco-Thomas, C. and Villeneuve, A., eds.), Pergamon Press, Oxford, pp. 1117-1124.
- Rastogi, R.B. and Singhal, R.L. (1976). Neonatal hyperthyroidism: alterations in behavioural activity and the metabolism of brain norepinephrine and dopamine. Life Sci. 18, 851-858.
- Rastogi, R.B. and Singhal, R.L. (1977). Lithium: modification of behavioural activity and brain biogenic amines in developing hyperthyroid rats. J. Pharmacol. Exp. Ther. 201, 92-102.
- Rastogi, R.B., Singhal, R.L. and Lapierre, Y.D. (1981). Effects of short- and long-term neuroleptic treatments on brain serotonin synthesis and turnover: focus on the serotonin hypothesis of schizophrenia. Life Sci. 29, 735-741.
- Reda, F.A., Scanlon, J.M., Kemp, K. and Escobar, J.I. (1974). Treatment of tardive dyskinesia with lithium carbonate. N. Eng. J. Med. 291, 850.
- Rice, D. (1956). The use of lithium salts in the treatment of manic states. J. Ment. Sci. 102, 604-611.

- Rifkin, A., Quitkin, F., Howard, A. and Klein, D.F. (1975). A study of abrupt lithium withdrawal. Psychopharmacologia (Berl.) 44, 157-158.
- Roffler-Tarlov, S., Sharman, D.F. and Tegerdine, P. (1971). 3,4-Dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid in the mouse striatum: a reflection of intra- and extra-neuronal metabolism of dopamine. Br. J. Pharmacol. 42, 343-351.
- Rosenblatt, J.E., Pert, A., Layton, B. and Bunney, W.E. Jr. (1980). Chronic lithium reduces (^3H)spiroperidol binding in rat striatum. Eur. J. Pharmacol. 67, 321-322.
- Rosenblatt, J.E., Pert, C.B., Tallman, J.F., Pert, A. and Bunney, W.E. Jr. (1979). The effect of imipramine and lithium on α - and β -receptor binding in rat brain. Brain Res. 160, 186-191.
- Rubovits, R., Patel, B.C. and Klawans, H.L. Jr. (1973). Effect of prolonged chlorpromazine pretreatment on the threshold for amphetamine stereotypy: a model for tardive dyskinesias. In: «Advances in Neurology», Vol. 1 (Barbeau, A., Chase, T.N. and Paulson, G.W., eds.), Raven Press, New York, pp. 671-679.
- Sangdee, C. and Franz, D.N. (1980). Lithium enhancement of central 5-HT transmission induced by 5-HT precursors. Biol. Psychiat. 15, 59-75.
- Schanberg, S.M., Schildkraut, J.J. and Kopin, I.J. (1967). The effects of psychoactive drugs on norepinephrine- H^3 metabolism in brain. Biochem. Pharmacol. 16, 393-399.
- Schildkraut, J.J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiat. 122, 509-522.
- Schildkraut, J.J., Logue, M.A. and Dodge, G.A. (1969a). The effects of lithium salts on the turnover and metabolism of norepinephrine in rat brain. Psychopharmacologia (Berl.) 14, 135-141.
- Schildkraut, J.J., Schanberg, S.M., Breese, G.R. and Kopin, I.J. (1969b). Effects of psychoactive drugs on the metabolism of intracisternally administered serotonin in rat brain. Biochem. Pharmacol. 18, 1971-1978.
- Schlagenhauf, G., Tupin, J. and White, R.B. (1966). The use of lithium carbonate in the treatment of manic psychoses. Am. J. Psychiat. 123, 201-207.
- Schou, M. (1957). Biology and pharmacology of the lithium ion. Pharmacol. Rev. 9, 17-58.
- Schou, M. (1963). Normothymotics, «mood-normalizers»: are lithium and imipramine drugs specific for affective disorders? Br. J. Psychiat. 109, 803-809.
- Schou, M. (1980). In: «Lithium Treatment of Manic-Depressive Illness: A Practical Guide», S. Karger, Basel.
- Schou, M., Juel-Nielsen, N., Stromgren, E. and Voldby, H. (1954). The treatment of manic psychoses by the administration of lithium salts. J. Neurol. Neurosurg. Psychiat. 17, 250-260.

- Schubert, J. (1973). Effect of chronic lithium treatment on monoamine metabolism in the rat brain. Psychopharmacologia (Berl.) 32, 301-311.
- Schultz, J.E., Siggins, G.R., Schocker, F.W., Turck, M. and Bloom, F.E. (1981). Effects of prolonged treatment with lithium and tricyclic antidepressants on discharge frequency, norepinephrine responses and Beta receptor binding in rat cerebellum: electrophysiological and biochemical comparison. J. Pharmacol. Exp. Ther. 216, 28-38.
- Segal, D.S., Callaghan, M. and Mandell, A.J. (1975). Alterations in behaviour and catecholamine biosynthesis induced by lithium. Nature (London) 254, 58-59.
- Selikoff, I.J., Robitzek, E.H. and Ornstein, G.G. (1952). Treatment of pulmonary tuberculosis with hydrazide derivatives of isonicotinic acid. J. Am. Med. Assoc. 150, 973-980.
- Shaw, J.P. and Ratcliffe, F. (1976). Effect of lithium on brain 5-HT metabolism in mice. Arch. Int. Pharmacodyn. 222, 116-124.
- Shiman, R., Akino, M. and Kaufman, S. (1971). Solubilization and partial purification of tyrosine hydroxylase from bovine adrenal medulla. J. Biol. Chem. 246, 1330-1340.
- Sigwald, J., Bouttier, D., Raymondeaud, C. and Piot, C. (1959). Quatre cas de dyskinésie facio-bucco-linguo-masticatrice à l'évolution prolongée secondaire à un traitement par les neuroleptiques. Rev. Neurol. 100, 751-755.
- Simpson, G.M. (1973). Tardive dyskinesia. Br. J. Psychiat. 122, 618.
- Singhal, R.L. and Rastogi, R.B. (1978). Neurotransmitter mechanisms during mental illness induced by alterations in thyroid function. Adv. Pharmacol. Chemother. 15, 203-262.
- Slotkin, T.A., Seidler, F.J., Whitmore, W.L., Salvaggio, M. and Bareis, D.L. (1980). Release of (³H)norepinephrine from synaptic vesicles isolated from rat brain after the intracisternal administration of (³H)norepinephrine: influence of nucleotides, ions and drugs, and destabilization of transmitter storage caused by acute or chronic lithium administration. Neuroscience 5, 753-762.
- Slotkin, T.A., Seidler, F.J., Whitmore, W.L., Salvaggio, M. and Lau, C. (1978). Ionic and nucleotide cofactor requirements for uptake of (³H)norepinephrine by rat brain synaptic vesicle preparations. Mol. Pharmacol. 14, 868-878.
- Small, J.G., Small, I.F. and Moore, D.F. (1971). Experimental withdrawal of lithium in recovered manic-depressive patients: a report of five cases. Am. J. Psychiat. 127, 1555-1558.
- Smith, D.F. (1977). Behaviour of rats given lithium salts. A review. Pharmako-psychiat. 10, 79-88.
- Smith, D.F. (1980). Lithium and motor activity of animals: effects and possible mechanism of action. Int. Pharmacopsychiat. 15, 197-217.
- Smith, D.F. (1981). Central and peripheral effects of lithium on amphetamine-induced hyperactivity in rats. Pharmacol. Biochem. Behav. 14, 439-442.

- Smith, R.C. and Davis, J.M. (1976). Behavioural evidence for supersensitivity after chronic administration of haloperidol, clozapine and thioridazine. Life Sci. 19, 725-732.
- Smith, R.C., Tamminga, C.A., Haraszti, J., Pandey, G.N. and Davis, J.M. (1977). Effects of dopamine agonists in tardive dyskinesia. Am. J. Psychiat. 134, 763-768.
- Snyder, S.H. and Coyle, J.T. (1969). Regional differences in ^3H -norepinephrine and ^3H -dopamine uptake into rat brain homogenates. J. Pharmacol. Exp. Ther. 165, 78-86.
- Snyder, S.H., Shaskan, E.G. and Kuhar, M.J. (1973). Serotonin uptake systems in brain tissue. In: «Serotonin and Behaviour» (Barchas, J. and Usdin, E., eds.), Academic Press, New York, pp. 97-108.
- Spector, S., Prockop, D., Shore, P.A. and Brodie, B.B. (1958). Effect of iproniazid on brain levels of norepinephrine and serotonin. Science 127, 704.
- Spector, S., Shore, P.A. and Brodie, B.B. (1960). Biochemical and pharmacological effects of the monoamine oxidase inhibitors, iproniazid, 1-phenyl-2-hydrazinopropane (JB516) and 1-phenyl-3-hydrazinobutane (JB835). J. Pharmacol. Exp. Ther. 128, 15-21.
- Stallone, F., Shelley, E., Mendlewicz, J. and Fieve, R.R. (1973). The use of lithium in affective disorders III: a double-blind study of prophylaxis in bipolar illness. Am. J. Psychiat. 130, 1006-1010.
- Stanley, M. and Wilk, S. (1980). Acute and chronic effects of haloperidol and clozapine on dopamine metabolism in two dopamine rich areas of the rat brain. Res. Comm. Psychol. Psychiat. Behav. 5, 37-47.
- Staunton, D.A., Magistretti, P.J., Shoemaker, W.J. and Bloom, F.E. (1982a). Effect of chronic lithium treatment on dopamine receptors in the rat corpus striatum I. Locomotor activity and behavioural supersensitivity. Brain Res. 232, 391-400.
- Staunton, D.A., Magistretti, P.J., Shoemaker, W.J., Deyo, S.N. and Bloom, F.E. (1982b). Effects of chronic lithium treatment on dopamine receptors in the rat corpus striatum II. No effect on denervation or neuroleptic-induced supersensitivity. Brain Res. 232, 401-412.
- Stefanini, E., Argiolas, A., Gessa, G.L. and Fadda, F. (1976). Effect of lithium on dopamine uptake by brain synaptosomes. J. Neurochem. 27, 1237-1239.
- Stern, D.N., Fieve, R.R., Neff, N.H. and Costa, E. (1969). The effect of lithium chloride administration on brain and heart norepinephrine turnover rates. Psychopharmacologia (Berl.) 14, 315-322.
- Stokes, P.E., Stoll, P.M., Shamoian, C.A. and Patton, M.J. (1971). Efficacy of lithium as acute treatment of manic-depressive illness. Lancet I, 1319-1325.
- Strombom, U. (1977). Antagonism by haloperidol of locomotor depression induced by small doses of apomorphine. J. Neural Trans. 40, 191-194.
- Swann, A.C., Heninger, G.R., Marini, J.L., Sheard, M.H. and Maas, J.W. (1980). Lithium effects on high-affinity tryptophan uptake: evidence against a stabilization mechanism. Brain Res. 194, 287-292.

- Swann, A.C., Heninger, G.R., Roth, R.H. and Maas, J.W. (1981). Differential effects of short- and long-term lithium on tryptophan uptake and serotonergic function in cat brain. Life Sci. 28, 347-354.
- Tamir, H. and Huang, Y.L. (1974). Binding of serotonin to soluble protein from synaptosomes. Life Sci. 14, 83-93.
- Tamir, H. and Rapport, M.M. (1976). Is the serotonin binding protein (SBP) a soluble storage form for serotonin? Res. Comm. Chem. Pathol. Pharmacol. 13, 225-235.
- Tarsy, D. and Baldessarini, R.J. (1974). Behavioural supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. Neuropharmacology 13, 927-940.
- Tarsy, D. and Baldessarini, R.J. (1976). The tardive dyskinesia syndrome. In: «Clinical Neuropharmacology», Vol. 1 (Klawans, H.L., ed.), Raven Press, New York, pp. 29-61.
- Teixeira, N.A. and Karniol, I.G. (1982). The influence of age and sex on weight variation in rats treated chronically with lithium chloride. Acta Pharmacol. Toxicol. 51, 1-5.
- Tepper, S.J. and Haas, J.F. (1979). Prevalence of tardive dyskinesia. J. Clin. Psychiat. 40, 508-516.
- Treiser, S. and Kellar, K.J. (1979). Lithium effects on adrenergic receptor supersensitivity in rat brain. Eur. J. Pharmacol. 58, 85-86.
- Treiser, S. and Kellar, K.J. (1980). Lithium: effects on serotonin receptors in rat brain. Eur. J. Pharmacol. 64, 183-185.
- Tye, N.C., Horsman, L., Wright, F.C. and Pullar, I.A. (1979). Differential enhancement of locomotor activity by dopamine agonists following chronic neuroleptic treatment: an animal model of tardive dyskinesia. Eur. J. Pharmacol. 55, 103-107.
- Uhrbrand, I. and Faurbye, A. (1960). Reversible and irreversible dyskinesia after treatment with perphenazine, chlorpromazine, reserpine and electroconvulsive therapy. Psychopharmacologia 1, 408-418.
- Waldmeier, P.C. (1980). Serotonergic modulation of mesolimbic and frontal cortical dopamine neurons. Experientia 36, 1092-1094.
- Waldmeier, P.C. and Delini-Stula, A.A. (1979). Serotonin-dopamine interactions in the nigrostriatal system. Eur. J. Pharmacol. 55, 363-373.
- Weilosz, M. and Kleinrok, Z. (1979). Lithium-induced head twitches in rats. J. Pharm. Pharmacol. 31, 410-411.
- Westerink, B.H.C. (1979). The effects of drugs on dopamine biosynthesis and metabolism in the brain. In: «The Neurobiology of Dopamine», (Horn, A.S., Korf, J. and Westerink, B.H.C., eds.), Academic Press, New York, pp. 255-291.
- Whybrow, P.C. and Ferrell, R. (1974). Thyroid state and human behaviour: contributions from a clinical perspective. In: «The Thyroid Axis, Drugs and Behaviour», (Prange, A.J. Jr., ed.), Raven Press, New York, pp. 5-28.

- Wilkinson, D.G. (1979). Difficulty in stopping lithium prophylaxis? Br. Med. J. 1, 235-236.
- Wolff, J. (1979). Lithium interactions with the thyroid gland. In: «Lithium: Controversies and Unresolved Issues,» (Cooper, T.B., Gershon, S., Kline, N.S. and Schou, M., eds.), Excerpta Medica, Amsterdam, pp. 552-564.
- Zall, H., Therman, P.-O.G. and Myers, M. (1968). Lithium carbonate: a clinical study. Am. J. Psychiat. 125, 549-555.
- Zeller, E.A. and Barsky, J. (1952). In vivo inhibition of liver and brain monoamine oxidase by 1-isonicotinyl-2-isopropyl hydrazine. Proc. Soc. Exp. Biol. Med. 52, 459-461.