INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in "sectioning" the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.

University
Microfilms
International

300 N. ZEEB ROAD, ANN ARBOR, MI 48106
18 BEDFORD ROW, LONDON WC1R 4EJ, ENGLAND

THE EFFECT OF ANTIPSYCHOTIC DRUGS ON REGIONAL DOPAMINE METABOLISM IN RAT BRAIN AND THE PREDICTION OF ANTIPSYCHOTIC EFFICACY

by

MICHAEL STANLEY

A dissertation submitted to the Graduate Faculty in Biomedical Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1979

This manuscript has been read and accepted for the Graduate Faculty in the Biomedical Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

Feb. 1, 1979
date

Chairman of the Examining Committee
Dr. S. Wilk

February 1, 1979
date

Executive Officer
Dr. T. A. Krulwich

Dr. M. Van Woert

Dr. S. Rosenblatt

Dr. B. Angrist

Dr. S. Maayani

Supervisory Committee

The City University of New York

ACKNOWLEDGEMENTS

To my wife, Barbara, who several years back told me to take organic chemistry: "It would be all right." She has been with me through it all, my successes and failures, and has loved and supported me always.

To my father, who had a kind and gentle way of appreciating life. I have come to appreciate him more and more.

To my mother, who taught me to look at the stars, trees and man with an appreciative eye.

To Sherwin, my advisor, who will apparently never lose the joy and enthusiasm of discovery, as well as the ability to pass it on.

To Ionel Rudic, for his help and friendship.

To my friend John Goodson, who ten years ago acquainted me with Flammonde.

To Gladys Ellenbogen, Maggie Garcia, George Rotter, Daniel Brower, Hank Hauer, Lewis Carroll, W.H. Auden, Shirley Irons, Laura Stelmach, N. Ned, Kate Beste-Chetwynde, Texas, Chun Wel Lin, Mike Bergamini, Sweetheart, John J. Rotrosen, Dianne Reingold, Burt Angrist, Tennessee Williams, Sam Gershon, Emily Dickinson, Edwin Arlington Robinson, Stephan Crane, Vachel Lindsay, e.e. cummings, Robinson Jeffers, William Blake, William Wordsworth,

Robert Browning, Gerard Manley Hopkins, A.E. Housman, H.L. Mencken, Evelyn Waugh, Ambrose Bierce, William Shakespeare, Andrew Marvel, Sir Phillip Sidney, Christopher Marlowe, Oscar Wilde and William Gaddis, who have all provided the differential reinforcement necessary to complete a task of this nature.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS (continued)

LIST OF ILLUSTRATIONS (continued)

DOPAMINE AS A NEUROTRANSMITTER IN THE CENTRAL NERVOUS SYSTEM

In 1958 Carlsson and his coworkers (Carlsson et al., 1958) determined that dopamine (DA) was a normal constituent in the rabbit brain. Subsequent investigations have revealed that DA is present in the central nervous system of all mammals studied. Bertler and Rosengren (1959) studied the brain distribution of DA in the cow, sheep, pig, dog, cat, guinea-pig and rat, and found it to be in highest concentration in the corpus striatum. They further noted that the distribution of DA was different from that of norepinephrine (NE). They suggested that because of distributional differences between DA and NE, DA may have a function of its own. Hithertofore, DA had generally been regarded only as an intermediate in the synthesis of NE.

Localization of Dopaminergic Pathways in Brain

In addition to the striatum, DA is also found in the tuberculum olfactorium (TO), nucleus accumbens (NA), globus pallidus and substantia nigra (Hornykiewicz, 1966; Lloyd et al., 1975).

Dopaminergic pathways have since been described using histofluorescence techniques developed by Falck and coworkers (Falck, 1962; Falck et al., 1962). These include the nigro-striatal, mesolimbic, tuberoinfundibular and cortical dopaminergic systems. The nigro-striatal pathway

has cell bodies which originate in the zona compacta of the substantia nigra and project forward to the striatum (the caudate-putamen complex in the rat). Ungerstedt's (1971) mapping of these fibers show that they ascend in the crux cerebri and internal capsule, fan out in the globus pallidus and terminate in the striatum. Another dopaminergic pathway considered to be of some importance in the mechanism of action of antipsychotic drugs is the mesolimbic system. The cell bodies for this system are found in the ventral tegmental area and project to the limbic structures, i.e., nucleus accumbens, tuberculum olfactorium, nucleus amygdaloideus centralis and the nucleus interstitialis striae terminalis.

Dopamine Synthesis and Metabolism

The synthesis of DA proceeds via the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (Dopa). The reaction is catalyzed by the enzyme tyrosine hydroxylase which is found in brain and believed to be the rate limiting step in the synthesis of DA (Levitt, 1965). Tyrosine hydroxylase is a mixed function oxidase enzyme and requires O_2, tetrahydropteridine and Fe^{++} for maximal activity (Nagatsu et al., 1964). Tyrosine hydroxylase is relatively specific in that it will not accept D-tyrosine, meta-tyrosine, L-tryptophan or tyramine as substrates. However, it will hydroxylate phenylalanine as well as L-tyrosine (Nagatsu,

et al., 1964). Alpha methylparatyrosine (αMPT) inhibits tyrosine hydroxylase competitively and can be converted to some extent to alpha methyldopa (Spector et al., 1965; Nagatsu et al., 1964). Mono- and diiodotyrosine are also potent inhibitors of this enzyme. It should also be noted that catechol compounds act as inhibitors of tyrosine hydroxylase. The nature of this inhibition has been described as a competition for the reduced pteridine cofactor and can be reversed by adding excess cofactor (Udenfriend et al., 1965). It is therefore possible that end-product inhibition may play an important physiological role in controlling the rate of dopamine synthesis. In this regard, Javoy et al. (1972) have shown a decrease in synthesis rate following the administration of a monoamine oxidase inhibitor, which, by inhibiting DA catabolism, increased DA levels.

Dopa is converted to DA by the decarboxylating enzyme alternately called dopa decarboxylase and aromatic L-amino acid decarboxylase. As the second name would imply, this enzyme accepts a wide variety of aromatic amino acids in addition to L-dopa as substrates. The enzyme has a requirement for pyridoxal phosphate as a cofactor. Dopa decarboxylase can be inhibited by α-methyl dopa or α-methyldopa hydrazine as well as a variety of other α-methyl amino acids.

The metabolism of DA can proceed via two pathways. Monoamine oxidase (MAO) can deaminate DA to form an aldehyde which is subsequently oxidized to the corresponding carboxylic acid - 3,4-dihydroxyphenylacetic acid (DOPAC). Aldehydes lacking an α-hydroxyl group are preferentially oxidized to acids (Wilk and Zimmerberg, 1973). MAO is a flavoprotein that is found both intra- and extraneuronally and is principally found in the outer mitochondrial membrane (Schnaitmian et al., 1967). A number of compounds have been found to inhibit MAO.

Catechol-O-methyltransferase (COMT) methylates dopamine or DOPAC predominately in the meta position. The products of this reaction are 3-methoxytyramine and homovanillic acid (HVA) respectively. COMT in brain is more uniformly distributed than the catecholamines. COMT requires S-adenosylmethionine, which serves as a methyl donor, and Mg^{++} ion for activity and tropolones act as effective inhibitors of COMT (Axelrod and Thomchick, 1958).

Measurement of Dopamine Turnover

While it is sometimes helpful to know whether a given drug has caused an increase or decrease in the content of a specific neurotransmitter, it is frequently more informative to learn what effect a compound may have on the synthesis and metabolism of the transmitter, i.e., its turnover. The importance of measurement of turnover is derived

from the fact that it is more indicative of the functional activity of a system. Under physiological conditions, the turnover of a neurotransmitter such as DA, is frequently thought of as the maintenance of a given concentration or pool size by the simultaneous processes of synthesis and metabolism. This process of renewal under physiological or steady-state conditions is believed to be an equal balance between synthesis and metabolism. A balance must also be maintained between the formation of metabolites and their elimination. As previously mentioned, catechol compounds are capable of exerting an inhibitory effect on tyrosine hydroxylase which would easily affect steady-state conditions.

Methods for Estimating Turnover:

Labeled precursors, such as ^{3}H-tyrosine and ^{3}H-Dopa can be administered systemically and will penetrate the blood-brain barrier unlike DA itself. There are, however, several drawbacks in utilizing this technique to estimate turnover. The use of labeled Dopa to estimate the turnover of DA is compromised by the relatively wide distribution of aromatic L-amino acid decarboxylase. Thus DA would be formed at sites where DA synthesis does not normally occur. Labeled tyrosine is more useful in the determination of DA turnover. There are, however, some problems encountered using labeled tyrosine to estimate DA turnover. Tyrosine

participates in several different metabolic pathways, which creates the problem of separating the compound one is interested in measuring from other labeled compounds. Moreover, since a relatively low amount of labeled tyrosine is incorporated into DA, the measurement of the exponential decline in specific activity of DA is far from routine. Finally, Udenfriend and Zaltzman-Nirenberg (1963) have shown that labeled tyrosine tends to remain in the brain for a long time, thus it is continually adding to the synthesis and turnover is underestimated.

It is possible to bypass the blood brain barrier by injecting DA directly into either a specific brain area or into the CSF via the ventricles. While this technique is capable of labeling a pool of transmitter large enough to follow over time, it is not without its drawbacks. It has been found that there can be a relatively high amount of non-specific uptake which could influence turnover measurements (Snyder and Coyle, 1969). It is also possible for a transmitter to be metabolized before being taken up by the neuron which would make physiological estimates of turnover even more difficult.

Another method frequently employed to measure turnover is inhibition of synthesis. By inhibiting tyrosine hydroxylase with α-MPT, it would be expected that the rate at which DA disappeared can be related to its rate of utilization.

The principal difficulty with this approach is that by inhibiting synthesis, the pool size is constantly changing which itself could influence synthesis. Also, with increasing time, the pool size is exponentially reduced making measurements of small quantities difficult.

Other methods for estimating turnover are inhibition of metabolism and blockade of metabolite transport. Inhibition of metabolism can be a useful technique when dealing with a non-complex system that has few metabolites and metabolic pathways, e.g., serotonin (5-HT). DA can be metabolized to a number of compounds via a number of routes which can complicate interpretation of the results. Blockade of metabolite transport by using a compound such as probenecid has been of value in measurement of 5-HT turnover, however it is not useful for estimation of DA turnover. In the rat the inhibition of transport of DA metabolites by probenecid is restricted to HVA while DOPAC levels remain relatively unaffected. Moreover, in the rat, HVA transport is not totally blocked by probenecid (Wilk et al., 1975c; Westerink and Korf, 1976b; Karoum et al., 1977).

The Use of Dopamine Metabolite Levels to Estimate Dopamine Turnover

The measurement of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), the principal metabolites of DA, is particularly useful in evaluating the

properties of drugs thought to exert their effects through central dopaminergic systems. Studies by Roth et al. (1976) have shown that stimulation of either the nigro-striatal or the mesolimbic pathways results in a stimulus-dependent increase in the accumulation of DOPAC in the striatum and TO, respectively. The accumulation of DOPAC seen following stimulation was related to the number of impulses delivered up to a maximal frequency. Lesions of these dopaminergic pathways effectively blocked impulse flow and caused a decrease in the steady state levels of DOPAC.

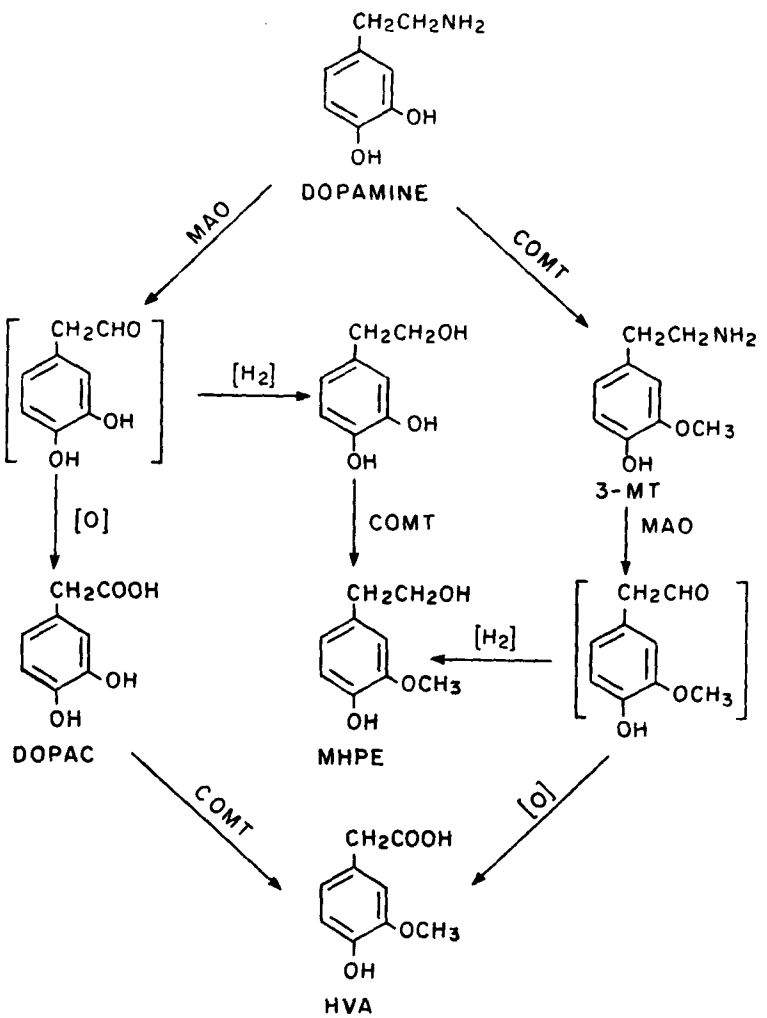
Bunney and Aghajanian (1974) have reported that chlorpromazine causes an increase in firing of the dopaminergic cells of these systems and that amphetamine causes a decrease (Bunney and Aghajanian, 1973). Roth et al. (1976) found that DOPAC levels increased with the administration of chlorpromazine and decreased with the administration of amphetamine. Thus, they concluded that the changes seen in DOPAC levels following either electrical stimulation or various drug treatments serves as a good indicator of the functional activity of the two dopaminergic systems.

Wilk et al. (1975_c) investigated DA metabolism in the striatum and in the TO of the rat (Wilk et al., 1975_a). They found the concentration of DOPAC to be greater than that of HVA for both structures. The DOPAC/HVA ratios they

reported for the striatum and TO were 1.22 and 2.01 respectively. Karoum et al. (1977) reported an identical ratio for the striatum (i.e., 1.2). Also in their studies of DA metabolism in striatum, Wilk et al. (1975c) measured the rate of disappearance of DOPAC and HVA following administration of the monoamine oxidase inhibitor pargyline. They estimated that the rate of formation of DOPAC was considerably greater than that of HVA. On the basis of their findings, i.e., that the concentration (at steady state) as well as the formation of DOPAC is greater than that of HVA; they concluded that the principal metabolite of DA in the rat striatum is DOPAC, not HVA. The higher DOPAC/HVA ratio in the TO suggests that in this structure DOPAC is even more quantitatively important than in the striatum.

Westerink and Korf (1976b) also estimated DA turnover in the rat striatum and in the mesolimbic area, a block dissection which included the TO and the NA. These investigators also found the steady state levels, as well as the estimated rate of formation of DOPAC to be significantly greater than HVA for both brain regions studied. Westerink and Korf also calculated that the rate of DOPAC formation approximated the rate of DA turnover. They also reported that virtually all of the HVA formed in both areas was the result of O-methylation of DOPAC and that practically no HVA was formed by the oxidation of 3 methoxytyramine (Fig.

FIG. 1 Possible routes for the metabolism of dopamine


CH2CH2NH2
OH
OH
DOPAMINE
MAO
COMT
CH2CHO
OH
OH
[H2]
CH2CH2OH
OH
OH
CH2CH2NH2
OCH3
OH
3-MT
[O]
COMT
MAO
CH2COOH
OH
OH
DOPAC
CH2CH2OH
OCH3
OH
MHPE
[H2]
CH2CHO
OCH3
OH
COMT
[O]
CH2COOH
OCH3
OH
HVA

1). In support of this hypothesis they noted that the levels reported for 3-methoxytyramine were very low (Carlsson et al., 1974) relative to DOPAC and HVA. Also they reported that the turnover of HVA in the striatum calculated from the HVA decline resulting from the combined treatment with pargyline and tropolone yielded a value very similar to the one estimated for the remaining DOPAC once transport out of the brain had been accounted for. They therefore concluded that DA turnover is best estimated by DOPAC turnover.

Karoum et al. (1977) also studied the effects of pargyline and probenecid on DA metabolism. As noted previously in other studies, they found the concentration of DOPAC in the striatum to be greater than that of HVA. Following treatment with pargyline, they found that both DOPAC and HVA declined exponentially. Striatal DOPAC declined at a faster rate than HVA following pargyline administration. The rate of loss of DOPAC and HVA is thought to equal their rate of formation (assuming steady state conditions exist). Thus they estimated striatal DOPAC to be formed about twice as fast as HVA. They further noted that striatal DOPAC levels were essentially unchanged by probenecid administration and that HVA transport was blocked only to a small degree. On the basis of their findings, Karoum et al. also concluded that DOPAC is apparently the major DA metabolite in the rat brain.

The Effect of Probenecid on Dopamine Metabolite Levels

Guldberg and Broch (1971) found that the level of HVA in the rat striatum had significantly increased following the administration of 200 mg/kg of probenecid. In contrast to probenecid's effect on HVA, no significant increase in DOPAC levels was observed. Wilk et al. (1975c) also found 200 mg/kg of probenecid to cause a doubling of HVA in the rat striatum while DOPAC levels were not significantly altered. Westerink and Korf (1976b) and Karoum et al. (1977) reported that DOPAC levels remained essentially unchanged following probenecid treatment. Roffler-Tarlov et al. (1971) found that a 200 mg/kg dose of probenecid caused a linear increase in HVA levels with time (up to 2 hours) while DOPAC levels remained unchanged. Spano and Neff (1972) found it was possible to block the transport of DOPAC in the striatum of the guinea pig following a dose of 600 mg/kg of probenecid. This finding, while at variance with the foregoing experiments, may be reconciled by differences in species and/or the high dose (600 mg/kg) used. Because of the high toxicity associated with this dose of probenecid, the significance of these findings is questionable. Thus, since probenecid's effect on the concentration of HVA is only that of a doubling, the rate of formation of HVA calculated using this technique is considerably less than that found by inhibiting HVA formation (Wilk et al., 1975c; Westerink and Korf, 1976b; Karoum et al., 1977).

The Effects of Antipsychotic Drugs on Dopamine Metabolism

The importance of dopamine and its relationship to the mechanism of action of antipsychotic drugs and hence its implication in schizophrenia emerged when in 1963 Carlsson and Lindqvist postulated that the clinically useful antipsychotic drugs caused an increase in the turnover of central catecholamines (Carlsson and Lindqvist, 1963). They noted an increase in the O-methylated metabolites 3-methoxytyramine and normetanephrine, following the administration of chlorpromazine or haloperidol in animals pretreated with the monoamine oxidase inhibitor nialamide compared to the increase seen when only nialamide was administered. Phenoxybenzamine (an α-adrenergic blocker) and promethazine (an antihistamine) were ineffective in increasing the levels of either 3-methoxytyramine or normetanephrine. Carlsson and Lindqvist suggested that the increase in the concentration of these metabolites might be due to a compensatory activation of monoaminergic neurons following receptor blockade. Since this initial observation a number of other investigators, using a variety of techniques, have shown that neuroleptic drugs increase dopamine turnover.

Nybäck et al. (1967) found that when rats were treated with chlorpromazine, following a slow infusion of 1-C^{14}-

tyrosine, the formation of labeled DA in brain was significantly increased. The concentration of labeled NE, however, was not altered significantly. The authors concluded that chlorpromazine accelerates the synthesis of DA. In another study using labeled tyrosine, Gey and Pletscher (1968) also found an increased synthesis of DA but not NE following the administration of chlorpromazine. In a later experiment using the same techniques, Nybäck (1972) demonstrated that promethazine, an antihistamine of the phenothiazine class, had no effect on dopamine synthesis.

Andén et al. (1964) found that treatment with chlorpromazine or haloperidol caused an increase in the accumulation of both of the acidic dopamine metabolites, DOPAC and HVA in the striatum of the rabbit. The same investigators found that treatment with phenoxybenzamine, promethazine and phenobarbital had no significant effect on DA metabolite levels. Da Prada and Pletscher (1966a,b) also measured changes in DA metabolism and noted a marked increase in HVA concentration following the administration of chlorpromazine, haloperidol and chlorprothixene.

The increase in the accumulation of DA metabolites seen following the administration of antipsychotic drugs is not regarded as a non-specific effect, such as that seen following treatment with the organic acid transport inhibitor probenecid.

Bunney et al. (1973) measured the firing rate of single neurons in the substantia nigra and ventral tegmental area of the rat midbrain. They found that haloperidol and several antipsychotic phenothiazines (including chlorpromazine) could cause an increase in the firing rate of these cells and cause a reversal of the decrease in firing rate brought about by amphetamine as well. The non-antipsychotic phenothiazine promethazine failed to exert any effect in either instance.

All the foregoing experiments mentioned had confirmed using a variety of different techniques, that DA turnover is increased following the administration of clinically active antipsychotic drugs. These observations have also served as partial evidence in support of what has come to be known as the DA hypothesis of schizophrenia (described fully in a later section).

Compensatory Feedback Loop

Since the early study by Carlsson and Lindqvist (1963) in which they suggested that the drug-induced increase in catecholamine metabolites might be due to a compensatory feedback mechanism, several investigators have attempted to elucidate the means by which these effects are brought about. Nybäck and Sedvall (1971) reported that when rats were subjected to unilateral lesions of the nigro-striatal pathway, the *in vivo* formation of ^{14}C-dopamine from ^{14}C-

tyrosine was reduced to approximately 15% in the striatum ipsilateral to the lesion. The levels of endogenous dopamine were approximately equal in the striata of both sides twenty-four hours following lesioning. Chlorpromazine induced a marked increase in the formation of ^{14}C-dopamine from ^{14}C-tyrosine in the striatum contralateral to the side which had received the lesion. However, chlorpromazine did not increase dopamine accumulation in the striatum on the lesioned side. The authors concluded that while it is possible that the lesion might have disrupted other neurons which could exert an influence on dopamine synthesis in the striatum, it was more likely that the transection of the nigro-striatal pathway was responsible for the abolition of the chlorpromazine effect. Further, they suggested that chlorpromazine increased dopamine synthesis in the striatum by an indirect mechanism which in turn activated the nigro-striatal pathway.

The neuronal feedback loop, proposed by Carlsson and Lindqvist (1963), has been evoked to explain many of the changes seen in dopaminergic transmission in the nigro-striatal pathway following the administration of drugs known to alter or interact with this dopaminergic system. When administered systemically, amphetamine produced inhibition of cell firing in the striatum and the substantia nigra (Bunney et al., 1973; Aghajanian and Bunney, 1973;

Siggins et al., 1974). In contrast, haloperidol and other dopaminergic blockers caused an increase in neuronal activity in both the striatum and the substantia nigra (Bunney et al., 1973), and as might be expected, dopaminergic blockers also have been shown to block the depressant effect of amphetamine (Bunney and Aghajanian, 1975b).

Bunney and Aghajanian (1976) conducted a series of experiments to test whether a feedback loop was relevant to the drug effects observed. They measured changes in the firing rate of single cells in either the pars compacta of the substantia nigra or the striatum following the i.v. infusion of amphetamine, apomorphine or haloperidol. The effects of these compounds were also followed in animals which had received discrete lesions in the vicinity of the tail of the caudate and the crus cerebri. Both of these sites were chosen based on prior studies by the same authors which revealed several inputs to the substantia nigra from, among other areas, the globus pallidus, the head of the caudate and the tail of the caudate. They proposed that several of these inputs might be involved in the hypothesized feedback loop.

They found that after lesions of either the crus cerebri or the tail of the caudate there was an increase in substantia nigra cell firing. Also the normally pronounced

depressant effect of i.v. amphetamine on the firing rate of cells was markedly reduced in rats receiving lesions in the same areas. In fact, lethal doses of amphetamine failed to produce more than a 50% decrease in cell firing (as recorded in the zona compacta, A-9, of the substantia nigra). The direct dopamine receptor agonist, apomorphine, continued to exert its usual depressant effect (i.v.) which could be easily reversed by i.v. haloperidol. Bunney and Aghajanian conclude that the attenuation of the normally potent depressant effect of amphetamine, brought about by the placement of discrete lesions in the vicinity of the crus cerebri and the tail of the caudate, strongly suggests that these effects are mediated via "a neuronal feedback pathway rather than as a direct or indirect action on dopamine receptors located on A-9 dopamine cell bodies or dendrites."

Groves et al. (1975) recorded cell firing in a small number of neurons in the pars compacta of the substantia nigra in the rat. In this series of experiments the investigators infused either haloperidol or amphetamine directly onto this structure. They found that when amphetamine or haloperidol were infused directly onto the cells of the substantia nigra, cell firing decreased and increased respectively. They noted that the effects of amphetamine, which caused a decrease in firing in the substantia nigra (Bunney and Aghajanian, 1976), and the effects of haloperidol which caused an increase in the firing rate in this area, were generally regarded as being mediated

through a neuronal feedback loop (Corrodi et al., 1967). They proposed that these drug effects on firing rate might be due to independent effects of these compounds acting directly on dopamine receptors in the substantia nigra and striatum rather than via the proposed feedback loop.

In another study designed to test the presence and significance of the proposed neuronal feedback loop, Garcia-Munoz et al. (1977) conducted a series of lesion studies in rats. They first identified striato-nigral fibers autoradiographically and then made discrete unilateral lesions of these tracts without disrupting the nigrostriatal pathway. Following a two-to-three week recovery period, several animals were treated with 1 mg/kg of haloperidol and killed 30 minutes later. A comparison of drug-induced increases in DOPAC levels in the striatum (lesioned and unlesioned side were analyzed separately) revealed no significant differences between the lesioned and unlesioned sides. They conclude that the effects of haloperidol on dopaminergic systems is not dependent on a feedback pathway.

Differences in methodologies may account for many of the apparent discrepancies reported by Bunney and Aghajanian (1976), Groves et al. (1975) and Garcia-Munoz et al. (1977). Thus, Groves et al. (1975) used iontophoretic application of drugs and recorded firing rates from clusters of cells while Bunney and Aghajanian (1976) administered

their drugs intravenously and recorded changes in firing of single cells. The lesions of Garcia-Munoz et al. (1977) apparently did not lesion to the same extent or the identical area as those of Bunney and Aghajanian (1976). Therefore, the existence, or significance, of a feedback loop remains unresolved.

Dopamine Agonist Effects in Animals

The DA hypothesis of schizophrenia was expanded to include the dopaminergic agonists as their pharmacology became better understood. It had been noted that rats given amphetamine display an increase in locomotor activity (Weissmann et al., 1966) and at higher doses exhibit what is referred to as stereotyped behavior (Randrup et al., 1963). In the rat stereotyped behavior generally takes the form of increased sniffing or compulsive gnawing (Ernst, 1967). Stereotyped behavior can also be seen in rats given apomorphine, a drug thought to act directly at the dopamine receptor (Andén et al., 1967). Classical neuroleptic drugs, such as chlorpromazine and haloperidol, act as effective antagonists of amphetamine and apomorphine as demonstrated by their ability to suppress the abnormal behaviors induced by agonists (Randrup et al., 1963; Janssen et al., 1967). It should also be noted that drugs such as promethazine are ineffective antagonists of drug-induced stereotypies (Janssen et al., 1967).

Dopamine Agonists and Their Effects on Human Behavior

A finding which has contributed greatly to the overall development of the dopamine hypothesis of schizophrenia is the observation that amphetamine abusers can develop a psychosis which appears to be very closely related to paranoid schizophrenia (Connell, 1958; Angrist and Gershon, 1970; Davis and Janowsky, 1973). In fact Connell has reported that frequently this drug-induced psychosis has been misdiagnosed as paranoid schizophrenia (Connell, 1958). Several laboratories have now reported similar results noting that amphetamine can induce a paranoid schizophreniform psychosis in non-schizophrenics and cause a worsening of symptoms in schizophrenics (Angrist and Gershon, 1970; Janowsky and Davis, 1974; Ellinwood et al., 1972).

In addition to amphetamine a variety of other substances have been shown to either induce psychosis in non-schizophrenics or to exacerbate the symptoms in schizophrenics. L-Dopa, the precursor in the synthesis of DA and commonly prescribed in Parkinsonism, represents such a compound. L-Dopa has been reported to produce psychosis in Parkinsonian patients (Calne et al., 1969), cause behavioral deterioration in schizophrenics (Angrist et al., 1973) and induce psychosis in non-schizophrenics (Sathananthan et al., 1973). Methylphenidate has also been shown to cause a psychosis in normals and an exacerbation of schizophrenic symptoms (Janowsky and Davis, 1974).

A criticism that has been raised in regard to all three drugs (amphetamine, l-Dopa and methylphenidate) inducing or worsening a psychosis, is that they not only affect dopaminergic systems but noradrenergic systems as well. Amphetamine and methylphenidate appear to have a similar mechanism of action, i.e., both are known to release NE and DA as well as block their reuptake into catecholamine neurons (Randrup and Munkvad, 1966; Glowinski and Axelrod, 1965). L-Dopa is converted enzymatically into DA which in turn can be converted into NE. Thus, it is possible for l-Dopa to serve as a precursor for both catecholamines.

In an effort to resolve the question of which system (i.e., noradrenergic versus dopaminergic) was most likely involved in the psychotogenic properties of these compounds, Angrist et al. (1975) found it was possible to induce a paranoid state in non-schizophrenics using the direct acting DA agonist piribedil (ET-495). The same investigators also found it was possible to cause a worsening in the psychiatric status of schizophrenics treated with piribedil (Angrist et al., 1975). However, Angrist et al. (1975) found that they were unable to produce a psychosis in either schizophrenics or non-schizophrenics given the DA agonist apomorphine. The failure of this drug to elicit a psychosis might be related to its potent emetic properties (Hill

and Tedeschi, 1971) which may have precluded the use of higher doses.

As previously mentioned haloperidol was found to be an effective antagonist of amphetamine-induced stereotypies in animals. It should also be noted that haloperidol is also an effective treatment in man for amphetamine-induced psychosis (Angrist et al., 1974).

Dopamine Hypothesis of Schizophrenia

In 1963 Randrup et al. described the abnormal behavior exhibited by rats injected with amphetamine. They observed that a dose of 3 mg/kg of amphetamine affected animal behavior from about one half hour to two hours following injection. Rats treated in this manner exhibited constant sniffing, licking and/or biting of the bottom of the wire cages and tended to remain crouched with their backs pressed against the corner of their cages. This behavior is representative of what is called amphetamine-induced stereotypy and has been frequently used as a preclinical screening test for the selection of potential antipsychotic agents. In the same study it was noted that neither α- or β-adrenergic drugs, nor the 5-HT antagonist methysergide reversed this behavior. However, chlorpromazine and haloperidol were very effective in blocking the amphetamine-induced abnormal behavior. They concluded that the stereotyped behavior induced by amphetamine was not due to NE or

5-HT and that another receptor (unnamed) mediated this behavior.

In 1964 van Rossum and Hurkmans postulated that the "psychomotor stimulant" drugs may exert their effect by interacting with DA receptors located within the extra-pyramidal system.

In 1965 Randrup and Munkvad reported that the blockade of amphetamine-induced stereotypy by neuroleptic drugs might have clinical implications. They noted that amphetamine had been found to produce a psychotic state in man closely resembling schizophrenia. They further noted that the clinical effect of neuroleptics on schizophrenic symptoms appeared to coincide with their ability to antagonize amphetamine-induced stereotypy.

Randrup and Munkvad in 1972 summarized the current evidence concerning the association among schizophrenia, DA, amphetamine, and neuroleptic drugs. At the same time they put forth what has come to be known as the DA hypothesis of schizophrenia. Evidence for the DA hypothesis of schizophrenia can be summarized as follows: 1) Neuroleptics have therapeutic effects in treating schizophrenics; 2) they also effectively antagonize amphetamine and apomorphine-induced behavior in animals; 3) the specific nature by which neuroleptics antagonize stereotyped behaviors in animals suggests that their action is probably via blockade of

the nigro-striatal system; 4) the neuroleptic treatment of schizophrenia is frequently accompanied by neurological side effects reminiscent of Parkinson's disease; 5) there is strong evidence to support the contention that Parkinson's disease is accompanied by a reduction of brain DA in the nigro-striatal system (which adds additional support for the idea that neuroleptics reduce or block dopaminergic activity in brain); 6) Parkinson's disease can frequently be treated with l-Dopa which effectively elevates brain DA and has among its side effects psychosis and paranoid delusions. Taking this evidence together, Randrup and Munkvad hypothesized "that in schizophrenic patients (particularly those who benefit best from neuroleptics) there is hyperactivity of the nigro-striatal DA system in the brain." Thus the theory of DA and schizophrenia was based largely on pharmacologic evidence rather than on the presence of any demonstrable lesion.

Dopamine Receptor Models

Dopamine-Stimulated Adenylate Cyclase

Evidence that antipsychotic drugs interact with DA receptors had largely been indirect in nature. Antipsychotic compounds have been found to block or antagonize various behaviors, such as emesis and stereotypy which can be elicited by DA agonists. Antipsychotic drugs have also been shown to increase the rate of firing of cells within known

dopaminergic pathways. Additionally they have been shown to cause increases in DA turnover as measured by various techniques. It was not until 1972 when Kebabian et al. (1972) described an adenylate cyclase isolated from rat striatum that could be specifically activated by low concentrations of DA (ED-50 - 4μM for DA vs. 28μM for NE). Low concentrations of the dopaminergic agonist apomorphine were found to stimulate the adenylate cyclase activity of homogenates. Chlorpromazine and haloperidol, DA antagonists, blocked the stimulatory effect of DA. The DA-stimulated adenylate cyclase also shows stereospecificity with regard to the action of DA antagonists. It has been demonstrated that the pharmacologically active cis isomer α-fluphenthixol acts as a potent inhibitor of the DA-stimulated adenylate cyclase, while the trans isomer β-fluphenthixol has virtually no activity (Miller et al., 1974). Similarly, it has also been shown that the (+) but not the (-) isomer of butaclamol effectively inhibits the dopamine-stimulated cyclase in both the striatum and the limbic structure, the TO (Lippmann et al., 1975). The respective activities reported for both isomers of butaclamol in the DA-stimulated adenylate cyclase also coincide with their pharmacological (Meltzer et al., 1977c) and clinical activities (Mielke, et al., 1975). On the basis of these findings it has been concluded that the DA-stimulated

adenylate cyclase may serve as the DA receptor in the brain (Clement-Cormier et al., 1974).

Displacement of ^{3}H-Haloperidol from Dopamine-Rich Areas

A second DA receptor model (not linked to an adenylate cyclase) was proposed and independently developed by Creese et al. (1975) and Seeman et al.(1976a,b). Both groups found that there was specific binding for ^{3}H-haloperidol and ^{3}H-DA to a membrane fraction derived from either calf or rat striatum. They further noted that the binding for both ^{3}H-haloperidol and ^{3}H-DA is saturable. Thus, they found that ^{3}H-haloperidol and ^{3}H-DA binding is specific for areas rich in DA, such as the striatum, and essentially undetectable for areas such as the cerebellum. It has also been possible to demonstrate selective stereospecific binding by using active and inactive stereoisomers of neuroleptic drugs (e.g., α- vs. β-flupentixol).

Creese et al. (1975) postulate that both haloperidol and DA bind to the same postsynaptic receptor since lesions of the nigro-striatal pathway (which would cause an extensive depletion of presynaptic receptors) did not decrease the binding for either ligand. Creese et al. (1975) propose that haloperidol and DA respectively label the antagonist and agonist states of the dopamine receptor.

As noted previously, the DA receptor model linked to an adenylate cyclase was also located preferentially in DA-

rich areas. Positive correlations between pharmacological potencies of phenothiazines and their inhibition of DA-stimulated adenylate cyclase were found; however, major discrepancies were noted for butyrophenones (Iversen, et al., 1976). Thus several butyrophenones are found to be much weaker than chlorpromazine in inhibiting the DA-stimulated adenylate cyclase while both their animal and clinical pharmacologic potencies are considerably more potent than chlorpromazine. However, in the DA receptor model proposed by Creese et al. (1976) and Seeman et al. (1976a,b), this discrepancy does not appear to exist. Both groups have examined an extensive number of neuroleptic compounds (including several butyrophenones) and have reported high correlations when their potencies for displacing ^{3}H-haloperidol are compared to their potencies in the clinic (Creese et al., 1976; Seeman et al., 1976a,b).

Norepinephrine-Stimulated Adenylate Cyclase

It should be noted at this point that several investigators have also described a noradrenergic cyclic AMP generating system in mammalian brain (Horn and Phillipson, 1976; Skolnick et al., 1976). Clinically effective antipsychotic drugs have been found to block the norepinephrine-stimulated increase in cAMP without altering the basal activity of the enzyme (Blumberg et al., 1976), whereas compounds believed to be clinically ineffective, such as

promethazine, -butaclamol and metoclopramide, are inactive in blocking the NE-induced increase in cAMP formation (Blumberg et al., 1976). Blumberg et al. (1976) and Horn and Phillipson (1976) have studied this noradrenergic system in slices of limbic forebrain and in homogenates of limbic forebrain, respectively. Blumberg et al. (1976) found 1) stereospecificity, the -isomer of butaclamol was ineffective while the +isomer was effective; 2) haloperidol was of very low potency (perhaps due to solubility problems); 3) clozapine was relatively potent in blocking the response in the limbic system. Horn and Phillipson (1976) reported 1) several neuroleptics block NE stimulation; clozapine, chlorpromazine, thioridazine, promazine; 2)clinically potent drugs, Pimozide, trifluperazine and α-flupenthixol were less potent than the preceeding drugs; 3) no stereospecificity was found in the ability of α- and β- isomers of flupenthixol to antagonize the NE-stimulated cyclase system.

It has been pointed out (Greengard, 1976) that while NE can stimulate cyclases in rat brain, the concentration of NE required to achieve a maximal stimulation is seven times greater than the amount of DA required to reach the same maxima. Kebabian et al. (1972) have suggested that because the same maxima could be reached by greater concentrations of NE, it might be that NE was stimulating the DA-stimulated cyclase. Evidence in support of this assumption is that in

the presence of optimal amounts of DA, no further stimulation can be elicited by the addition of NE.

Regional Selectivity

It had previously been thought that the antipsychotic effects of neuroleptic drugs could not be separated from the extrapyramidal side effects which were frequently associated with them (Freyhan, 1957; Denham, 1961). In fact some investigators suggested that the dose of a given neuroleptic should be increased until the appearance of extrapyramidal symptoms to insure an adequate clinical effect (Haase, 1959; 1961). There have however been studies that have tested and rejected this hypothesis (Chien and DiMascio, 1967). Thioridazine provides an example of a compound that has been found to be clinically efficacious in the treatment of schizophrenia and yet produces a very low incidence of extrapyramidal symptoms (Sandoz Pharmaceuticals, 1969). However, because patients treated with antipsychotic drugs frequently develop "Parkinson-like" symptoms, and because a degeneration of the nigro striatal system is frequently observed in patients with Parkinsonism (Calne et al., 1969), it is believed that it is the action of antipsychotic drugs on this dopaminergic system which is responsible for these side effects (van Rossum, 1967). While the extrapyramidal side effects have been attributed to the action of these drugs on the nigro-striatal system,

it has been suggested that their antipsychotic efficacy may be based on their action in the limbic system (Andén, 1974; Matthysse, 1973; Snyder et al., 1974).

Therefore, in theory, a drug which blocked dopaminergic receptors only in the nigro-striatal system should produce extrapyramidal symptoms without displaying any antipsychotic efficacy. Conversely, a compound which exerted its dopaminergic blocking effects solely in the limbic system and not in the nigro-striatal system would be expected to be clinically effective without inducing extrapyramidal signs. Clozapine is a drug with such a clinical profile. Although some studies support this hypothesis of regional selectivity by demonstrating a preferential effect on dopamine turnover in either the limbic system (for drugs with a low incidence of extrapyramidal symptoms) or the nigro-striatal system (for drugs with a high incidence of extrapyramidal symptoms) (Crow et al., 1976; Andén, 1974; Andén and Stock, 1973; Zivkovic et al., 1975; Bowers and Rozitis, 1974), other studies indicate that areas of the limbic system (e.g., the tuberculum olfactorium) are no more sensitive to the effects of these drugs and in some instances less sensitive than the striatum (Wilk et al., 1975b; Bowers and Rozitis, 1976; Bartholini et al., 1975; Wiesel and Sedvall, 1975; Westerink and Korf, 1975).

Andén and Stock (1973) examined the effects of clozapine and haloperidol on dopamine metabolism in the limbic area and striatum of the rabbit. Clozapine is considered a novel antipsychotic in that it is clinically efficacious without producing any clear cut extrapyramidal symptoms (Gross and Langer, 1966; 1970; DeMaio, 1972). Haloperidol, on the other hand, is clinically effective but with a relatively high incidence of extrapyramidal symptoms (Carlsson, 1978). When the concentration of homovanillic acid was determined in each area, it was found that clozapine had caused a significantly greater increase in the limbic area than in the striatum. Haloperidol was found to be equipotent for both areas. The authors concluded that the reported anticholinergic properties of clozapine (Stille et al., 1971) might account for the differential effect seen in the limbic area. This line of reasoning was based on an observation from a previous study where haloperidol was given concommittantly with the anticholinergic drug trihexyphenidyl and an attenuation of the drug-induced increase in HVA was noted (Andén, 1972). The significance of this finding is derived from the fact that anticholinergic drugs (such as trihexyphenidyl) are frequently used to treat the extrapyramidal symptoms which can arise during treatment with antipsychotic drugs. They also mentioned the possibility that the DA receptors of the limbic area might be blocked to a greater extent by clozapine than by haloperidol.

Wilk et al. (1975b) also measured the effects of clozapine and haloperidol in the rat striatum and TO acutely. Andén and Stock (1973) found that clozapine produced a greater percent increase in the limbic system of the rabbit than in the striatum. In contrast to the findings of Andén and Stock (1973), Wilk et al. (1975b) reported that the percent rise in DOPAC in the striatum exceeded that seen in the TO for all doses tested. It should be noted, however, that the two studies are not comparable in all respects. Andén and Stock used rabbits and a block dissection of the limbic area in their study; Wilk et al. used rats and the specific limbic structure, the TO, for their experiments. Additionally, Wilk et al. conducted a careful analysis of these drug-effects by studying their effects over time as well as dose-response. The dose-response curves for haloperidol and clozapine were so similar (except in potency) that Wilk et al. (1975b) concluded that both of these compounds utilize the same mechanism of action with regard to DA metabolism. Moreover, Wilk et al. (1975b) point out that the differential sensitivity noted for both haloperidol and clozapine revealed that both compounds increased DOPAC and HVA to a greater extent and at a lower dose in the striatum as compared to the TO.

Antagonism of Dopaminergic Systems and the Prediction of Antipsychotic Efficacy

The chemical property of dopaminergic antagonism or receptor blockade represents a feature common to clinically active antipsychotic drugs (Carlsson and Lindqvist, 1963; Bunney and Aghajanian, 1974; Janssen et al., 1967). This action has been exploited in a variety of ways in an effort to add new drugs to the armamentarium of the clinician.

Behavioral Measures Used to Predict Antipsychotic Efficacy

Behavioral methods were among the first and simplest forms of screening tests to be developed. Tests such as the inhibition of exploratory behavior, the induction of catalepsy (catalepsy in rats generally refers to that drug-induced state of an animal that allows it to be placed or positioned and maintained in awkward or bizarre postures (Munkvad et al., 1968; Stanley and Glick, 1976)), reduction of aggressive behavior and inhibition of conditioned avoidance were initially thought to be "sensitive and selective" (Hill and Tedeschi, 1971). However, a number of compounds, other than antipsychotics, have been reported to yield positive results in many of these procedures, e.g., chlordiazepoxide, barbiturates, morphine and other narcotic analgesics, etc. (Hill and Tedeschi, 1971). In some of these procedures, such as anti-aggressive tests and exploratory

behavior, it is frequently difficult to discriminate between reduction of these and a general reduction in activity due to ataxia. In this regard Cook and Kelleher (1961) point out the potential for peripheral effects caused by psychopharmacological agents to influence these behaviors.

One of the classical procedures used extensively in the past and to a lesser degree today is the effect of drugs on the conditioned avoidance-escape response in rats. In this procedure rats are conditioned to avoid an electric foot shock (an unconditioned stimulus - UCS) by climbing a pole (conditioned response - CR), jumping across a gate (CR), pressing a level (CR), etc. Typically three types of responses can be seen following drug administration: (1) the animal responds to both the UCS and the CR, which would indicate a lack of drug effect since this behavior is commonly seen in control rats receiving saline; (2) a response to the UCS (e.g., foot shock) without a response to the CR, typical of antipsychotic drugs, is taken as an indication of neuroleptic specificity; and (3) the complete lack of response to either the UCS or the CR; indicative of a nonspecific blockade of both avoidance responses. The latter response is seen with higher doses of barbiturates. Cook and Kelleher have reported (1961) that compounds such as serotonin and morphine, as well as some known antipsychotic drugs, produce a specific block of the conditioned avoidance response, i.e.,

response #2 described above. This lack of specificity has also been noted for tranylcypromine (a monoamine oxidase inhibitor), and LSD which are capable of blocking the CR without blocking the USC at the same dose. Thus, it would appear that the conditioned avoidance task lacks the desired selectivity necessary for a useful screening procedure.

Behavioral tests applied more recently include the antagonism of amphetamine and apomorphine-induced stereotypies. Amphetamine and apomorphine-induced stereotypies, typically studied in rats, have been characterized as a dose-dependent phenomena which starts with a mild increase in grooming behavior proceeding to a marked increase in grooming accompanied by intense sniffing and licking. At higher doses the rat will exhibit intense biting of objects within the cage and finally culminating in the biting and chewing of the animal's own paws and body (Ungerstedt, 1971). While this procedure appears to have some specificity for compounds which block DA receptors (as do classical antipsychotic drugs) the potencies of several known antipsychotic drugs in this test are not consistent with their clinical potencies. An example of two antipsychotic compounds that fail to antagonize amphetamine and apomorphine-induced stereotypies at clinically relevant doses are thioridazine and clozapine (Bürki et al., 1975a; Stille et al., 1971). Sulpiride, an antipsychotic drug with a low incidence of EPS, also fails to

antagonize either apomorphine or amphetamine-induced behaviors (Honda et al., 1977).

A major factor that detracts from the use of behavioral tests is the ambiguity which can arise when evaluating animal behavior. A relevant note of caution regarding the subjectiveness of behavioral tests is provided by van Rossum and his co-workers (1970): "Animal experiments may give reproducible results only when circumstances are carefully controlled and standardized. The same test procedure may lead to quantitative or even qualitative differences in the hands of different experimenters."

Use of the Dopamine-Stimulated Adenylate Cyclase System to Predict Antipsychotic Properties

Karobath and Leitich (1974) and Clement-Cormier et al. (1974) both examined an extensive number of antipsychotic compounds in an effort to determine their relative potencies in the dopamine-sensitive adenylate cyclase system and compare them with their clinical potencies. Both groups of investigators found that clinically effective compounds were capable of inhibiting the dopaminergic stimulation of this system but the relative potencies of some of the compounds tested failed to correspond to their known clinical potency. Notable examples included the clinically potent butyrophenones haloperidol and droperidol both of which

inhibited this system with a potency similar to that of chlorpromazine and clozapine.

Roufogalis et al. (1976) investigated the finding that haloperidol and chlorpromazine were equipotent inhibitors of the dopamine-sensitive adenylate cyclase. By altering the placement of the chloro group on chlorpromazine from the 2 position to the 1, 3 and 4 positions, they found a 5 to 50 fold decrease in inhibition potency. Because the chlorpromazine analogues did not differ in lipid solubility but did show potency differences in inhibiting the formation of cAMP, the authors concluded that the inhibition of adenylate cyclase appeared to be related more to changes in structure rather than solubility, at least for drugs of the phenothiazine class.

Use of the Labeled Haloperidol Binding Assay to Predict Antipsychotic Activity

A predictive system for neuroleptic drugs that has gained attention in recent years is the ^{3}H-haloperidol binding assay (Creese et al., 1976; Seeman et al., 1976a,b). Antipsychotic drugs displace ^{3}H-haloperidol from its binding to a membrane fraction of calf or rat striatum. While it has been reported that binding displacement (Kd) and clinical potencies yield a significant positive correlation, it would be improbable that drugs which rely on their metabolism to an active species would display any significant binding. Similarly, drugs

which may not have access to the brain because of poor lipid solubility may give false positive results in this system.

Neuroleptic-Induced Prolactin Release

There is a growing amount of evidence that prolactin (PRL) secretion in large part is under inhibitory control of DA. Using the pituitary incubated with radioactive leucine, investigators (Koch et al., 1970) have found that DA inhibits the secretion of PRL. Shaar and Clemens provided additional supportive evidence that catecholamines (probably DA) are involved in the inhibition of PRL release (Shaar and Clemens, 1974). They found that extracts from the hypothalamus which were known to inhibit the release of PRL could be rendered inactive by either enzymatic digestion with MAO or treatment with alumina. Treatment of the hypothalamic extracts with peptic digests resulted in no loss of PRL releasing activity.

Galactorrhea (inappropriate lactation) has been frequently reported in patients treated with antipsychotic medication. Because prolactin plays an important role in milk secretion, investigators thought it would be of interest to determine the effects of antipsychotics on its circulating levels. Subsequently, investigations into the effects of a variety of compounds on circulating prolactin levels has gone forth in an effort to better understand

the pharmacology of psychoactive drugs and the underlying physiology of the PRL response.

Clemens et al. (1974) found that antipsychotic drugs markedly stimulated the release of prolactin in rats. They compared single dose effects of known antipsychotic drugs (e.g., haloperidol, chlorpromazine, thioridazine, fluphenazine, sulpiride) with those of drugs judged not to have antipsychotic properties (e.g., the antihistamines promethazine, pyrathiazine, methdilazine, and the anti-emetic thiethylperazine).

All of the antipsychotic compounds and thiethylperazine caused a significant elevation in prolactin levels. The non-antipsychotic antihistamines failed to induce a significant increase in serum prolactin. The authors suggested that because thiethylperazine had a significant effect on elevating serum prolactin, perhaps it should be reevaluated in the clinic. Another finding of interest in this study is that of the relative elevations of prolactin observed following the administration of various antipsychotic drugs. Sulpiride, for example, has an effect that is three times greater than that of chlorpromazine, while thioridazine's effect is almost twice as great as haloperidol. All of the foregoing prolactin effects are in disagreement with their relative clinical potencies, i.e., haloperidol >> thioridazine and chlorpromazine >> sulpiride.

The marked increase in prolactin response following the administration of neuroleptics has been studied by Sachar and his coworkers (Sachar et al., 1976) to determine if a dose-response effect can be seen. They found a dose-response effect following i.m. doses of thiothixene, haloperidol and prochlorperazine in man. A dose of 1 mg or 1.5 mg of haloperidol gave a maximal response in prolactin release (a dose well below that which is used clinically (Sachar et al., 1976). The authors also note that no further increases or decreases in prolactin levels can be seen in patients on chronic antipsychotic medication following an acute daily dose. Thus, it appears that the prolactin response to antipsychotics is not subject to the development of tolerance (Sachar et al., 1976; Meltzer and Fang, 1976).

Langer et al. (1977b) have used the prolactin response as the basis for devising a system whereby antipsychotic potency can be determined. They found a relatively high correlation between a drug's potency (relative to haloperidol) in increasing prolactin and in the clinic. The authors point out that a maximal increase in prolactin is achieved at doses of 1-2 mg I.M. of haloperidol while schizophrenic patients typically require much higher doses to control their symptoms (Langer et al., 1977b).

While there is considerable evidence that DA and dopaminergic drugs can exert effects on circulating prolactin levels, it is less clear as to whether peripheral actions, central actions and/or a combination of both are involved in the various responses noted. DA has been shown to inhibit the in vitro secretion of prolactin from pituitary glands (Shaar and Clemens, 1974). It has also been reported that i.v. DA can lower serum prolactin in normal subjects (Meltzer et al., 1978). Because DA does not pass the blood-brain barrier, the latter experiment would support the hypothesis that prolactin is released at the level of the pituitary rather than a central site (hypothalamic). Meltzer and his coworkers also studied the effects of i.v. DA on prolactin release in two psychiatric patients treated with neuroleptics. They found that a saline infusion had no effect on the increased steady state prolactin levels. However, an infusion of DA caused a marked decrease of prolactin levels which was readily reversed upon termination of the infusion (Meltzer et al., 1978).

Langer et al. (1977a) studied the effects of three neuroleptics and their quaternary counterparts on the secretion of prolactin in man and their ability to inhibit DA-stimulated adenylate cyclase of striatal homogenates from the Cebus monkey. They found that the tertiary neuroleptics caused a prompt release of prolactin while equimolar doses

of their quaternary derivatives had no effect. The quaternary drugs were inactive in causing an inhibition of the DA-stimulated cyclase while the tertiary compounds were active. Because it has been shown that some quaternary neuroleptics are behaviorally active when injected directly into sites in the brain, it might be inferred that the effects exerted by neuroleptics on circulating prolactin are centrally mediated. Nonetheless, the strong supportive evidence for a DA-mediated peripheral control should not be discounted (Honda et al., 1977; Meltzer et al., 1978).

The specificity of the increase of prolactin is an area of research that has received some study. Tolis et al., (1975) reported that morphine, administered to preoperative women, caused a prompt and marked increase in prolactin levels. It was possible to block the rise in prolactin by administering apomorphine.

Turkington (1972) has reported increases in serum prolactin following treatment with the tricyclic antidepressants imipramine and amitriptyline, as well as the antihypertension medication α-methyldopa. In the same study he found that several phenothiazines also increased prolactin while drugs such as lithium carbonate, propranolol, isoniazid, aminosalicyclic acid, digoxin and chlordiazepoxide were ineffective.

Thus while there is strong supportive evidence that DA is involved in the regulation of prolactin secretion, the effects of compounds regarded as weak or nonspecific DA blockers warrants further study.

Organization of Experiments

As pointed out earlier, several studies of DA metabolism in rat brain reveal that DOPAC is the major DA metabolite. It has also been reported that measurement of DOPAC serves as a good indicator of the functional activity of dopaminergic neurons (Roth et al., 1976). Thus, Roth et al. (1976) demonstrated that DOPAC levels increase as a direct result of stimulation of dopaminergic neurons. Bunney and Aghajanian (1975a) find that antipsychotic drugs, such as chlorpromazine, cause an increase in the firing rate of dopaminergic cells. The same antipsychotic compounds also cause an increase in DA metabolite levels (Wilk et al., 1975b). DOPAC has been determined to be the major DA metabolite in specific brain regions such as the striatum (Wilk et al., 1975c; Westerink and Korf, 1976b; Karoum et al., 1977) and meso-limbic areas (Westerink and Korf, 1976). Additionally, Wilk et al. (1975c) and Westerink and Korf (1976b) reported that DOPAC formation approximated DA turnover. Therefore, measurement of DOPAC appears to be a valid method for determining changes in DA turnover.

This thesis was designed to further test the hypothesis that antipsychotic drugs owe their clinical efficacy

to their interaction with dopamine neurons in meso-limbic areas (Andén, 1974; Andén and Stock, 1973; Matthysse, 1973; 1974). The studies proposed herein used the measurement of DOPAC as an indicator of functional neuronal activity and an index of DA turnover. Another aspect explored in this thesis was to determine whether antipsychotic drugs exert a unique effect on DA metabolism which can be distinguished from other compounds that alter DA metabolism yet are devoid of antipsychotic activity.

The study by Wilk et al. (1975b) which compared the effects of haloperidol and clozapine on DA turnover in the striatum and TO did not uphold the findings previously reported by Andén and Stock (1973). Andén and Stock had reported that clozapine had a regionally selective effect in the meso-limbic area as opposed to the striatum. In contrast to Andén and Stock (1973), Wilk et al. (1975b) reported that both clozapine and haloperidol preferentially increased DOPAC levels in the striatum (at all doses tested) rather than the TO. Additionally, a study by Wilk and Glick (1976) found that clozapine did not induce a preferential effect in the NA compared with its effect in the striatum.

The possibility that the antipsychotic effects of neuroleptic drugs are mediated by their action in the mesolimbic dopaminergic system has been suggested by a number of

investigators (Andén, 1974; Snyder et al., 1974; Matthysse, 1973). The TO and NA have been difficult structures to study biochemically because of the limited amount of tissue available and the limitations of standard fluorometric techniques used in the analysis of DA metabolite levels. As a result of these limitations, investigators have had to use less circumscribed dissection techniques in order to yield sufficient tissue for assay. However, with the development of a highly sensitive gas chromatographic procedure for the determination of dopamine metabolites (Watson et al., 1974), the feasibility of a study of regional effects of neuroleptic drugs in specific areas such as the TO was realized.

Experiment 1 compared the effects of acute vs. chronic treatment of antipsychotic compounds on DA turnover in the striatum and TO. In this experiment a comparison was made between a maximal-supramaximal dose and a half-maximal dose of both haloperidol and clozapine over time. It has previously been reported by Bowers and Rozitis (1974) that tolerance to the rise in HVA initially seen in the striatum developed, but no tolerance was seen in the limbic area. As in the study by Andén and Stock (1973) a block dissection rather than a specific area of the limbic system was used for analysis which may have influenced the outcome of this experiment. We were interested to learn whether

results different from those obtained by Bowers and Rozitis (1974) would occur if a different species, the rat, and the specific mesolimbic structure, the TO, were studied.

In experiment 2 the concept of regional selectivity was further explored. Several investigators have proposed that the extrapyramidal side effects frequently seen with antipsychotic drugs are due to their dopaminergic blocking properties in the striatum (Andén, 1974; Andén and Stock, 1973; Matthysse, 1974). Conversely, antipsychotic efficacy has been ascribed to dopaminergic blocking properties in the meso-limbic area (Andén, 1974; Matthysse, 1974). Previous studies in the rat have indicated that the striatum is more sensitive to the DA metabolite-elevating effects of clozapine than the TO (Wiesel and Sedvall, 1975; Wilk et al., 1975b). We thought more information might be gained by measuring the concentration of clozapine in various areas of the brain and relate the concentration to DOPAC levels in the same areas.

In experiment 3 we again considered the question of dopaminergic regional selectivity using a series of compounds which have been shown to be clinically inactive. These compounds were chosen because they were found to be active in a variety of behavioral screening tests, and we considered that these findings might relate to effects on DA metabolism in the striatum. Thus, if antipsychotic

efficacy is mediated through the limbic system as suggested by Andén (1974), Matthysse (1974) and Andén and Stock (1973), we would not expect to see any effects on DA metabolism in this region as a result of treatment with clinically ineffective drugs.

Because the classical neuroleptic drugs cause a characteristic increase in DOPAC levels, we considered that it would be of interest to determine the similarities and differences between these compounds and drugs which also increase DOPAC levels such as morphine and oxotremorine. In this experiment (No. 4) we constructed time-action and dose-response curves for both morphine and oxotremorine and compared them to those of haloperidol, chlorpromazine and clozapine.

Experiment 5: The antagonism of dopaminergic systems in the brain by antipsychotic drugs has been used by many investigators to predict efficacy and potency in the clinic (Janssen et al., 1967; van Rossum et al., 1970, Creese et al., 1976; Bunney and Aghajanian, 1975a; Meltzer et al., 1977b). In this experiment we utilized the dose-dependent increase in DOPAC following the administration of perlapine, a sedative agent structurally related to clozapine and regarded as a non-antipsychotic, in an effort to establish a new predictive system.

In experiment 6 we examine the effects of thiethylperazine, a phenothiazine regarded as lacking antipsychotic efficacy, on DOPAC levels. The results of our animal experiments with this drug are compared to those obtained in the clinic and potency and efficacy are determined.

Experiment 7 examines the effect of the structurally-related compounds metoclopramide and sulpiride on DOPAC levels in the striatum and the TO. Sulpiride is an antipsychotic with a reported low incidence of EPS and metoclopramide is reported to have a high incidence of EPS without antipsychotic efficacy. Because of their reported clinical differences, it might be expected that these compounds would have regionally different responses with regard to their effects on DA turnover in the striatum and the TO.

General Methods

Animal Procedures

Because of the low water solubility of several of the drugs used in this study, it was necessary to prepare acidified solutions using acetic acid. Acidified stock solutions of clozapine, haloperidol, U-25,927, A1-499 and AHR-1,900 with a pH ranging from 4.2 to 4.7 were prepared. Dilutions of the stock solutions were made with saline. Control rats were injected with an acidified saline solution of comparable pH. All drugs were administered i.p. to male Sprague-Dawley rats, weighing from 175 to 200 grams, on a milligram per kilogram basis. Animals were kept at room temperature until killed by decapitation.

Prior to their being used in experiments, all animals were kept in the animal facility in the Basic Science Building. Animals were kept at the constant temperature of 24^{o}C and maintained on a 10 hour light-14 hour dark cycle. The animals used in these studies were supplied by Perfection Breeders of New York State.

Dissection and tissue preparation

A transverse cut was made through the ventral surface of the brain at the level of the optic chiasm, which exposed the transected anterior commissure. The tuberculum olfactorium was removed from the rostral half of the brain by cutting vertically downward from the medial part of the anterior commissure and then by making a tangential cut from the anterior commissure to the lateral olfactory tract. This technique minimizes the inclusion of non-dopaminergic tissue which could otherwise compromise the determined dopaminergic values. The mean weight of the tuberculum olfactorium samples was 8.4 mg $\pm$ 0.5 SEM.

The striatum was removed from both halves of the brain by using the lateral ventricles as the medial limit and the corpus callosum as the lateral limit. Using this method it is relatively simple to remove striatal tissue without fear of contamination. Additionally, a visual inspection can reveal the presence of non-striatal tissue which is then discarded. The mean weight of the striatal samples was 47.3 mg $\pm$ 1.9 SEM.

Following their dissection each sample is homogenized in 1 ml of cold 1N HCl and centrifuged for 15 min at 10,000 RPM in a Sorvall RC-2B refrigerated centrifuge. The supernatants are transferred to glass test tubes and stored at -80^{o}C in a Revco ultrafreezer until assayed for either dopamine or its metabolites DOPAC and HVA.

Chemical Procedures

HVA and, less frequently, DOPAC are generally measured in DA-rich areas by fluorometric assays. There are, however, several disadvantages associated with the use of fluorometry. Kirschberg et al. (1972) have reported that several of the acidic metabolites associated with catecholamine metabolism can be extracted under the same conditions used for the extraction of HVA and DOPAC. They have shown that under these conditions profound and nonlinear reductions in the fluorescence of HVA, which can be the cause of significant analytical errors, can occur.

A second disadvantage associated with fluorometric procedures is that samples must frequently be pooled in order to obtain concentrations high enough to allow for reliable analysis. Two major difficulties arise as a result of pooling for concentration: first, larger numbers of animals are required (this problem is especially acute for biochemical determinations carried out in the small limbic structures of the rat brain) for each experiment; second, the ability to do a repeat analysis of a sample is not possible (this is especially troublesome when an unsatisfactory blank is obtained or a contaminant is inadvertently introduced into the sample). A corollary to the problem of concentration in fluorometry is that it is not possible to analyze HVA, DOPAC and DA in the same sample.

Thus while pooling may permit a more accurate analysis of the compound under investigation, it can also serve to obscure individual differences and always increases the sample size.

The concentrations of both DA and HVA reported by investigators using fluorometric procedures are frequently lower than those found by gas-liquid chromatography, gas chromatography-mass spectrometry and radioisotopic methods. Therefore, the possibility exists that with an underestimated baseline value, changes brought about by drug treatment could be erroneously amplified.

We chose to use the sensitive and selective gas-liquid chromatographic technique developed in this laboratory for the analysis of DOPAC and HVA. This procedure requires only a fraction of the total sample for analysis thereby allowing for multiple determinations should they be required.

Procedure for the determination of 3,4-dihydroxyphenyl-acetic acid and homovanillic acid

Reagents

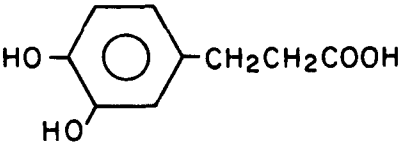
1. Pentafluoropropionic anhydride (Pierce Chemicals)
2. 1-chloro-1,1,3,3,3-pentafluoro-2-propanol (PCR Co.)
3. homovanillic acid (Sigma Chemical Co.)
4. 3,4-dihydroxyphenylacetic acid (Sigma Chemical Co.)
5. 4-hydroxy-3-methoxyphenylpropionic acid (ICN Co.)
6. 3,4-dihydroxyphenylpropionic acid (Aldrich Chemical Co.)
7. Ether: Electronic grade E-138 (Fisher Chemical Co.)
   The ether was prepared as follows: to a 1 lb. can 1 mg of sodium diethyldithiocarbamate was added. This procedure was used to retard the formation of peroxides and thereby increase the recovery of the procedure. The ether was stored cold in an explosion-proof refrigerator.

Method

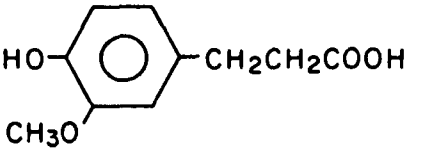
For striatal samples 100 λ of the supernatant was added to a 15 ml ground glass stoppered centrifuge tube containing 16 ng of both internal standards (4-hydroxy-3-methoxyphenylpropionic acid and 3,4-dihydroxyphenyl-propionic acid) (Fig. 2). This mixture was then extracted once with 1 ml of toluene by vortexing for 5 seconds. After centrifuging at 2,000 RPM for 5 min., the toluene extract was discarded. The sample tubes were then immersed in ice cold water for approximately 2 min. and extracted once with 1 ml of cold ether. The samples were vortexed and centrifuged at 2,000 RPM for 1 min. and the ether extract was transferred to a 3 ml. silanized glass stoppered centrifuge tube. The extract was placed under a gentle stream of nitrogen and evaporated to dryness. The residue was reacted with 10 λ of 1-chloro-1,1,3,3,3-pentafluoro-2-propanol and 50 λ of pentafluoropropionic anhydride by stoppering the tubes and placing them in a heated sandbath (kept at 75°C) for 15 min. The tube was then cooled to room temperature under running water and the excess reagents removed under a gentle nitrogen flow. An additional 50 λ of pentafluoropropionic anhydride was added to the tube which was reacted again at 75°C. After 5 min. had elapsed the tube was cooled to room temperature and the excess pentafluoropropionic anhydride was

FIG. 2 Structural formulas of the internal standards used in the DOPAC-HVA assay

INTERNAL STANDARDS FOR DOPAC AND HVA ASSAY



3,4-Dihydroxyphenylpropionic acid
(DOPPA)



4-hydroxy-3-methoxyphenylpropionic acid
(HMPPA)

FIG. 3 A flow chart outlining the major steps in the preparation and derivatization of DOPAC and HVA

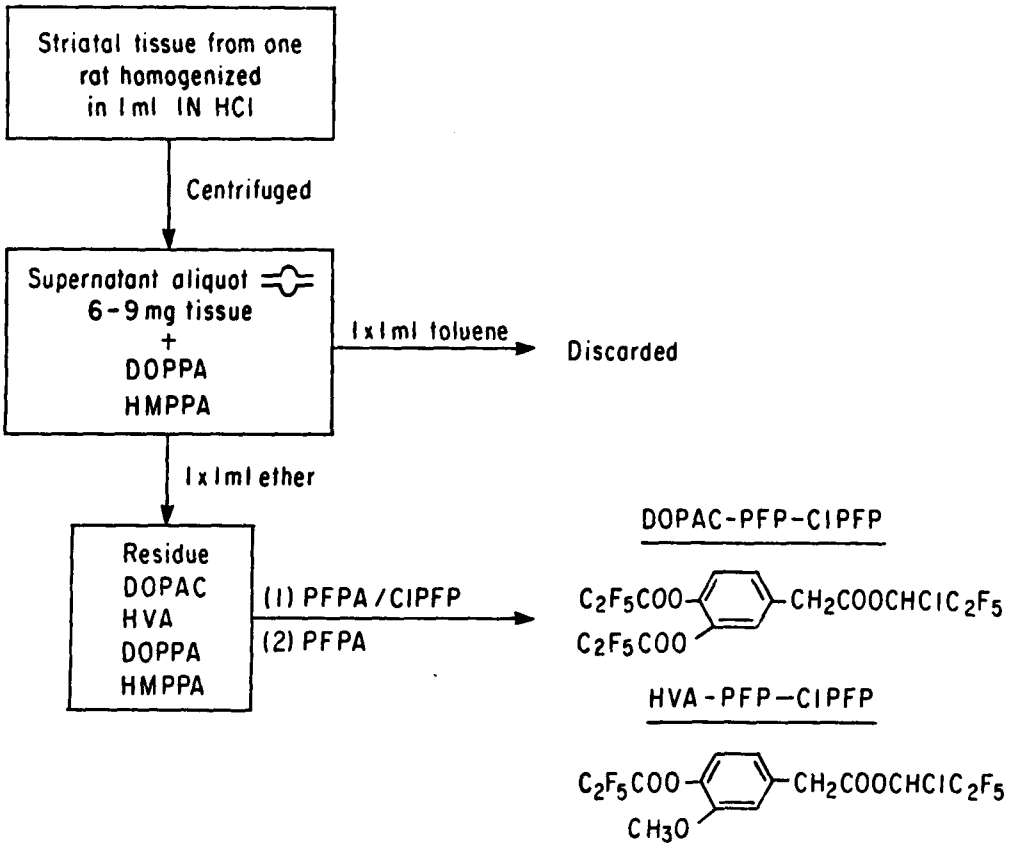
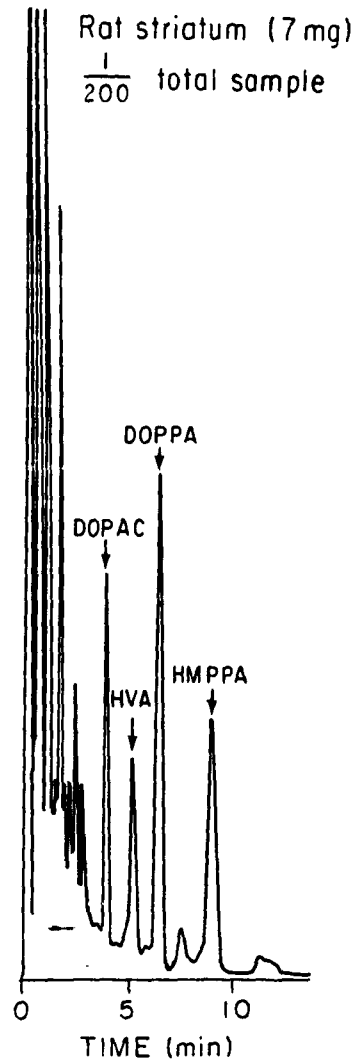

Striatal tissue from one rat homogenized in 1 ml 1N HCl
Centrifuged
Supernatant aliquot
6-9 mg tissue
+
DOPPA
HMPPA
1 x 1 ml toluene
Discarded
1 x 1 ml ether
Residue
DOPAC
HVA
DOPPA
HMPPA
(1) PFPA / ClPFP
(2) PFPA
DOPAC-PFP-ClPFP
HVA-PFP-ClPFP

FIG. 4 A sample chromatogram illustrating the peaks and retention times of DOPAC and HVA as well as their internal standards


Rat striatum (7 mg)
1/200 total sample
DOPPA
DOPAC
HVA
HMPPA
0
5
10
TIME (min)

removed under nitrogen. The overall procedure is illustrated in Fig. 3. The derivatives were resuspended in toluene (100 λ for tuberculum olfactorium and 200 λ for striatum). 3 λ was injected onto a 3% JXR column (100/120 mesh coated on gas chrom Q) with an inlet and detector temperature of 175°C and a column temperature of 130° C (Fig. 4).

Analysis of dopamine

A new gas-liquid chromatographic technique for the measurement of dopamine was developed during the course of this thesis. The principal advantage and reason for the development of this procedure was that it permitted the determination of DA from an aliquot of the same homogenate from which DOPAC and HVA concentrations are determined. Thus from the initial 1 ml homogenate, 0.1 ml can be taken for the analysis of DA. Enough of the sample remains so as to allow for additional assays of the same or different compounds (e.g., drug levels).

Procedure for the determination of dopamine

Reagents

1. Pentafluoropropionic anhydride (Pierce Chemical Co.)
2. Dopamine (Sigma Chemical Co.)
3. α-methyldopamine hydrobromide (Hoffman-La Roche)
4. Ether: Electronic grade #-138 (Fisher Chemical Co.) (treated as described above)
5. 0.5M tris-chloride buffer pH 8.5.
6. 10% sclution of Disodium Ethylenediaminetetra-acetate pH 7.2
7. 0.25N acetic acid in methanol
8. Aluminum oxide Woelm basic activity grade I

   The alumina was prepared by boiling it three times with 6 N HCl with vigorous stirring. The alumina was then washed and stirred with distilled water. Following each wash with distilled water fine alumina particles were poured off after the larger ones had settled, thus eliminating a potential source of contamination. The rinsing process was repeated until the pH of the rinse water equalled the pH of the distilled water. The alumina was then filtered under suction and dried for 12 hours at 300°C. The dried alumina was then placed in a tightly closed container and stored in a dessicator at room temperature until used.

9. Sodium diethyldithiocarbamate
10. Toluene

Method

100 mg of purified alumina was added to a 15 ml glass stoppered centrifuged tube. 4 ml of 0.5M tris-chloride buffer pH 8.5, 0.1 ml 10% disodium ethylene-diaminetetraacetate pH 7.2, 50 ng of α-methyldopamine as an internal standard and 0.2 ml of the sample super-natant were also added to the same tube. The tube was then stoppered and gently agitated by hand for 3 min. to adsorb the catecholamines. Catechol compounds adsorb maximally onto alumina at a pH range from 8.0 to 8.5. After shaking, the tubes were stoppered and centrifuged at 2,000 RPM for 2 min. The supernatant was then removed by aspiration. The alumina was then washed 3 times with 5 ml of distilled water by shaking gently by hand for 1 min. each time, centrifuging as before and removing the water by aspiration. The catecholamines were then eluted by gently shaking for 3 min. with 1 ml of 0.25 N acetic acid in methanol. After centrifugation the eluate was trans-ferred to a 3 ml ground glass stoppered centrifuge tube. 10 λ of a 1.25 mg/ml solution of sodium diethyldithiocar-bamate was added to the eluate as an antioxidant. The contents were evaporated to dryness under a vigorous nitro-gen flow and the residue reacted with 50 λ of pentafluoro-propionic anhydride in 100 λ of ether for 5 min. at room temperature. The reagents were then evaporated under a

gentle stream of nitrogen and the derivatives dissolved in 0.5 ml of toluene for chromatographic analysis. Standards containing 50 ng α-methyldopamine and 100 ng of dopamine were processed in parallel with the samples. The derivatives could be resolved on either a 3% SE-30 or a 3% OV-17 column (coated on gas chrom Q 100/120 mesh). Conditions for resolution by the SE-30 column were as follows: 175°C inlet temperature, 130°C column temperature, 175°C detector temperature, nitrogen flow 35 ml/min. Conditions for the OV-17 were the same as those used for the SE-30 except that the nitrogen flow was reduced to 17 ml/min. In both cases 3 λ of the standards or samples were injected onto the columns.

Dopamine in the tuberculum olfactorium was assayed using 0.25 ml of the sample supernatant. 10 ng of α-methyldopamine was added as the internal standard. Standards containing 10 ng of α-methyldopamine plus 20 ng dopamine were processed in parallel with the samples. Chromatographic conditions were the same as those used for striatal samples however, 4 λ was generally injected onto the columns. Figures 5 and 6 illustrate typical chromatograms of DA standards and samples in striatum and TO, respectively.

FIG. 5 I - Chromatogram of 50 ng of α-methyldopamine + 100 ng of dopamine carried through the procedure.

II - Chromatogram of striatum (7.4 mg) containing 50 ng of α-methyldopamine as internal standard. Dopamine = 11.8 ng/g. 3% SE-30, 140°, N_2 = 15 ml/min.

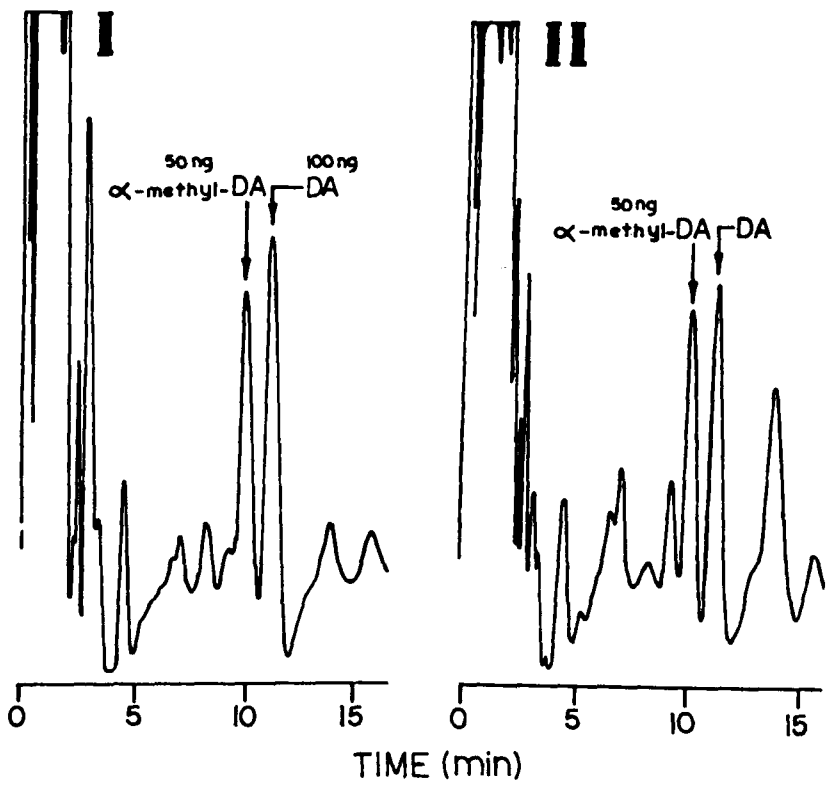
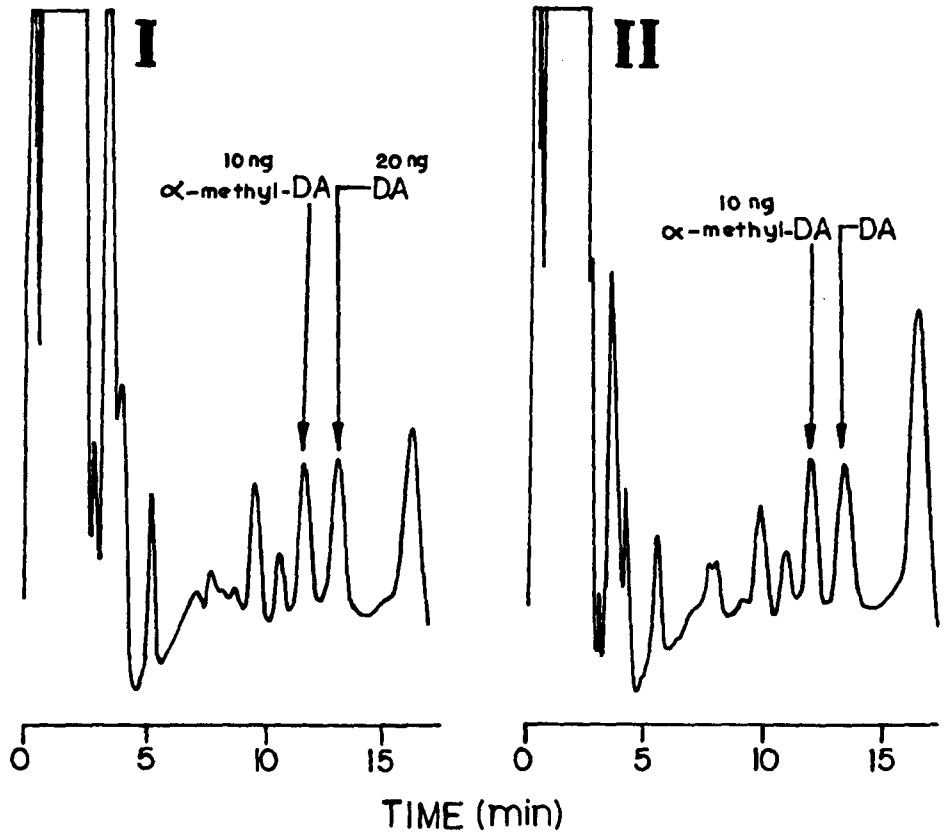

I
50 ng
α-methyl-DA
100 ng
DA
II
50 ng
α-methyl-DA
DA
0
5
10
15
0
5
10
15
TIME (min)

FIG. 6 I - Chromatogram of 10 ng α-methyldopamine + 20 ng dopamine carried through the procedure.

II - Chromatogram of TO (2.1 mg) containing 10 ng α-methyldopamine as internal standard. Dopamine = 6.1 ng/g 3% SE-30, 140°, N_2 = 15 ml/min.


I
10 ng
α-methyl-DA
20 ng
DA
II
10 ng
α-methyl-DA
DA
0
5
10
15
0
5
10
15
TIME (min)

A new gas chromatographic procedure for the measurement of clozapine was developed during the course of this thesis. A major advantage of this procedure is that it required only a small fraction (100λ) of the same sample from which DOPAC, HVA and DA are determined. Thus, it is possible to not only quantitate DOPAC, HVA and DA levels, but to also measure the concentration of clozapine in the same sample.

Procedure for the determination of clozapine

Reagents

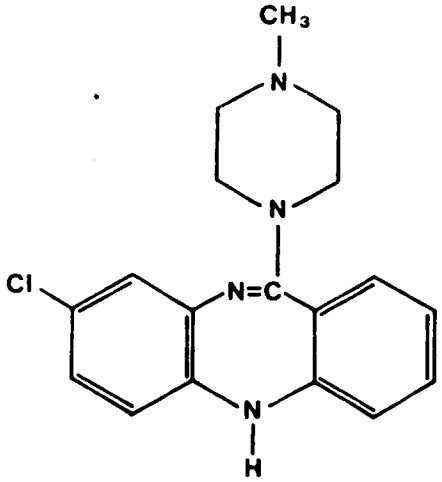
1. Pentafluoropropionic anhydride (Pierce Chemical Co.)
2. Clozapine (Sandoz-Wander, Inc., N.J.) (Fig. 7)
3. HF-2046 (Research Institute Wander, Bern, Switzerland) (Fig. 7)
4. Ether: Electronic grade #-138 (Fisher Chemical Co.)
5. 1 N NaOH
6. Toluene

Method

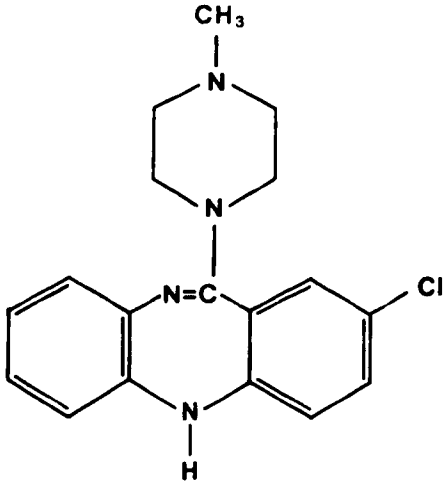
Tissue samples were prepared in 1 ml of 1 N HCl as described for DOPAC/HVA and DA assays.

To a 13 ml ground glass stoppered centrifuge tube was added 100 µl of the tissue supernatant, 100 ng HF-2046 (internal standard) and 130 µl 1 N NaOH. Clozapine and the internal standard were extracted into 1.8 ml ether by vortexing for 1 min. The tubes were centrifuged, the ether layer transferred to 3 ml ground glass stoppered centrifuge tubes and the ether removed by evaporation under a gentle stream of nitrogen. Derivatives were prepared by adding 50 µl pentafluoropropionic anhydride and 100 µl ether to the tubes, stoppering and allowing the reaction to proceed for 15 min. at room temperature. The anhydride was then evaporated under a gentle stream of nitrogen and the derivatives redissolved in 100 µl toluene for gas chromatographic analysis. 3 µl were injected onto a 3% JXR column at 200°C at a flow rate of 60 ml/min. The inlet and detector temperatures were maintained at 215°C. Quantitation was based on the peak height ratios of the derivatives of clozapine and HF-2046 and was based on the ratios obtained by processing standard solutions containing 100 ng of each compound through the procedure. Fig. 8 shows a typical chromatogram of clozapine extracted and derivatized according to the procedure described above.

FIG. 7 Structural formula of clozapine and its internal standard HF-2046.

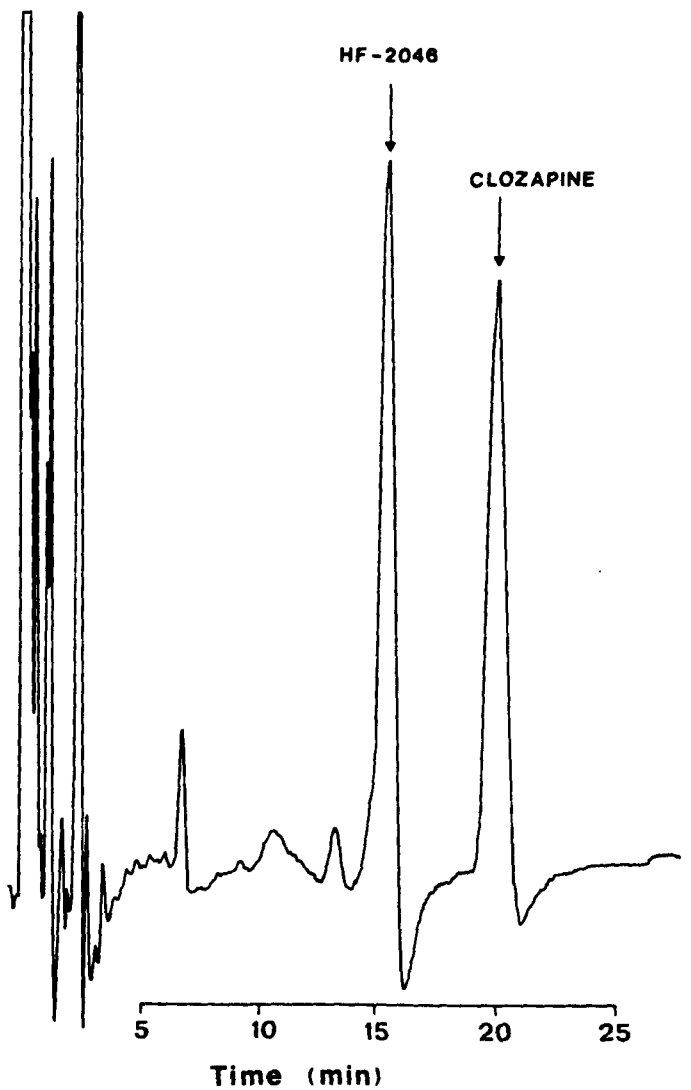


CLOZAPINE



HF - 2046

FIG. 8 Chromatogram of clozapine in the tuberculum olfactorium of a rat which received 40 mg/kg clozapine i.p. and was killed one hour later. 2.5 mg of tissue plus 100 ng of HF-2046 (internal standard) was treated as described in methods. 3% JXR, 200°C, N_2 = 60 ml/min. Clozapine concentration = 126 nm/g.


HF-2046
CLOZAPINE
5
10
15
20
25
Time (min)

Specific Methods and Results

Experiment One: Chronic Effects of Clozapine and Haloperidol on Dopamine Metabolism

Because the acute experiments of Wilk et al. (1975b) had failed to demonstrate differences between haloperidol and clozapine in either the TO or striatum, we decided to examine their effect on dopamine metabolism in the same areas following chronic treatment.

Groups of 8 male Sprague-Dawley rats (175-200 g) were treated in the following manner: Group I received daily injections for 14 days of either a maximal dose of clozapine (40 mg/kg) or a supra-maximal dose of haloperidol (1.0 mg/kg). Because of the high toxicity of clozapine when given on a chronic basis, a dose yielding a maximal increase in DOPAC levels had to be employed. A dose of 60 mg/kg/day of clozapine killed two separate groups of 8 rats each within 3 days. Even at a dose of 40 mg/kg there was a 50% attrition rate.

Group II received daily injections of half-maximal (ED50) doses of either clozapine (20 mg/kg) or haloperidol (0.15 mg/kg). Groups III and IV received daily injections of saline until the last day of treatment when they were given a maximal, supra-maximal or half-maximal dose of either clozapine or haloperidol. Thus both groups of rats received either high or low doses on a chronic or acute basis. A fifth group (V) was given acidified saline injections chronically.

FIG. 9 The effects of acute (diagonal stripe) and chronic (horizontal stripe) treatments by haloperidol on DOPAC levels in rat striatum. A) 1 mg/Kg haloperidol; B) 0.15 mg/Kg haloperidol. Solid colored histogram indicates the mean ± SEM of eight saline-treated rats. **p > 0.005

DR. SHERWIN WILKC5100,51183 478HIL.PLT

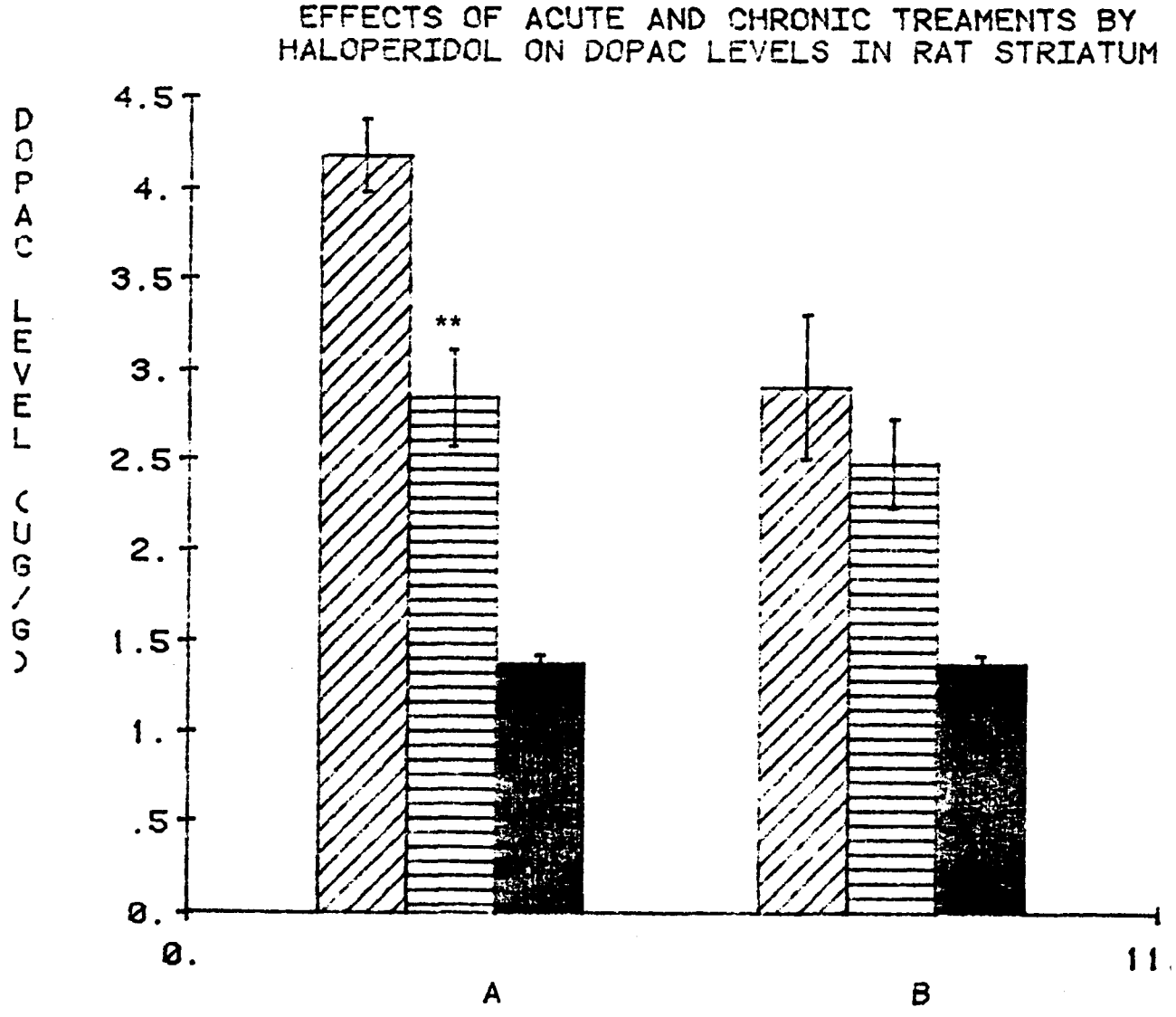
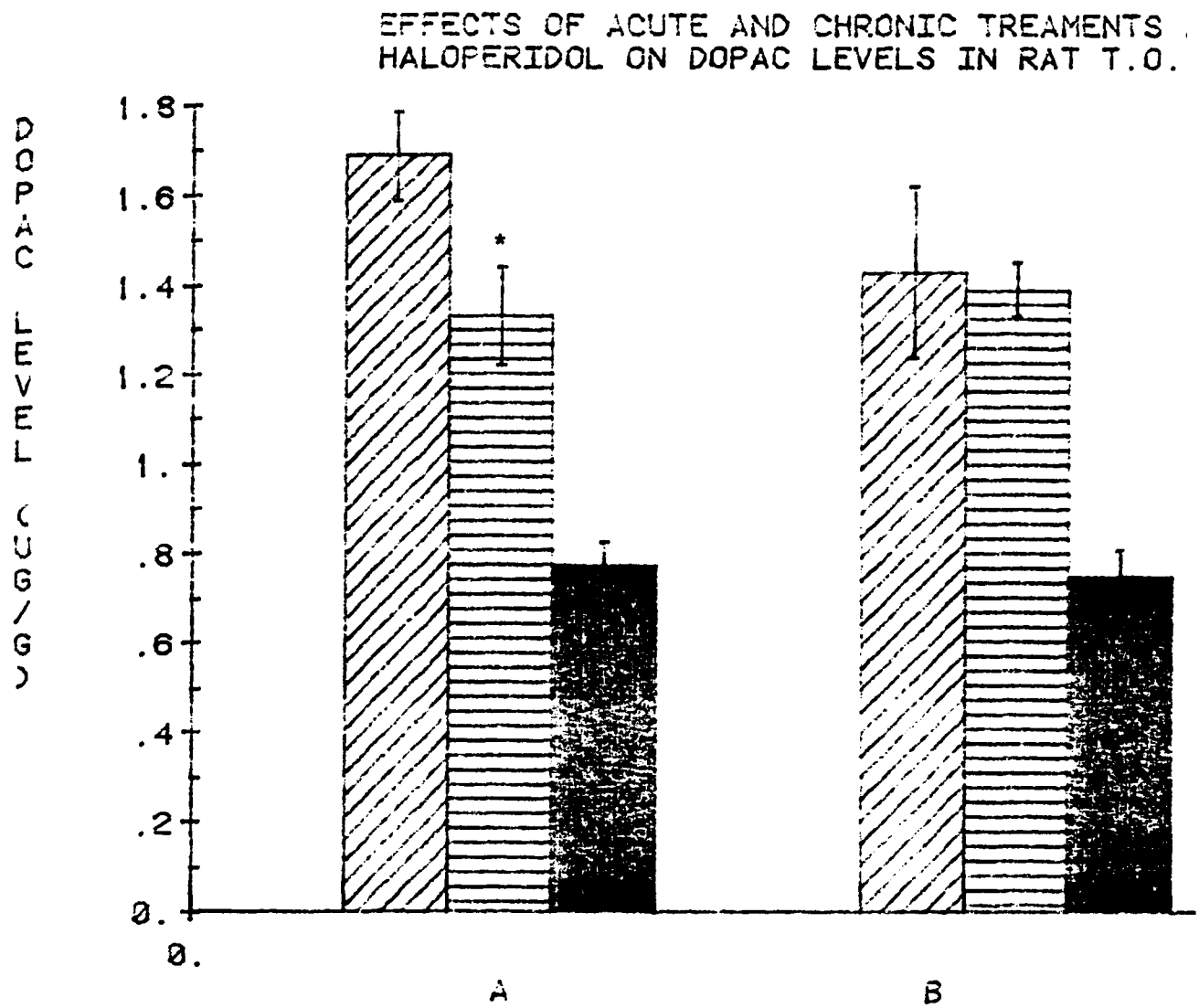


FIG. 10 The effects of acute (diagonal stripe) and chronic (horizontal stripe) treatments by haloperidol on DOPAC levels in rat TO. A) 1 mg/Kg of haloperidol; B) 0.15 mg/Kg haloperidol. Solid colored histogram indicates the mean ± SEM of eight saline-treated rats. *$p > 0.05$

DR. SHERWIN WILK[5180,51183 75SWIL.PLT



On the last day of treatment each animal was killed 1 hr. after receiving his final injection of either drug or vehicle. The brains were rapidly removed and the TO and striatum were dissected out. Biochemical analysis of DA and its major metabolites DOPAC and HVA were performed as described in the General Methods section.

Figures 9 and 10 illustrate that following chronic treatment with a supra-maximal dose of haloperidol (1 mg/kg) there was a significant reduction in the level of DOPAC in both the striatum and the TO from the values obtained after acute treatment with the same dose. However, no significant reduction in DOPAC levels could be seen in either region following chronic treatment with a half-maximal dose (ED-50) (0.15 mg/kg) of haloperidol compared to the DOPAC values after acute treatment (Figures 9 and 10).

Figure 11 shows that no significant reduction in DOPAC levels could be seen between chronic and acute treatment with a maximal dose (40 mg/kg) of clozapine. This finding was true for both the striatum as well as the TO (Fig. 12). No significant differences in striatal or tubercular DOPAC levels were seen between acute and chronic treatment with a half-maximal dose (20 mg/kg) of clozapine (Figs. 11 and 12).

FIG. 11 Effects of acute (diagonal stripe) and chronic (horizontal stripe) treatments by clozapine on DOPAC levels in rat striatum. A) 40mg/Kg clozapine; B) 20 mg/Kg clozapine. Solid colored histogram indicates the mean ± SEM of eight saline-treated rats.

DR. CHERKIN WOLKCS100,51103 DOZWOL.PLT

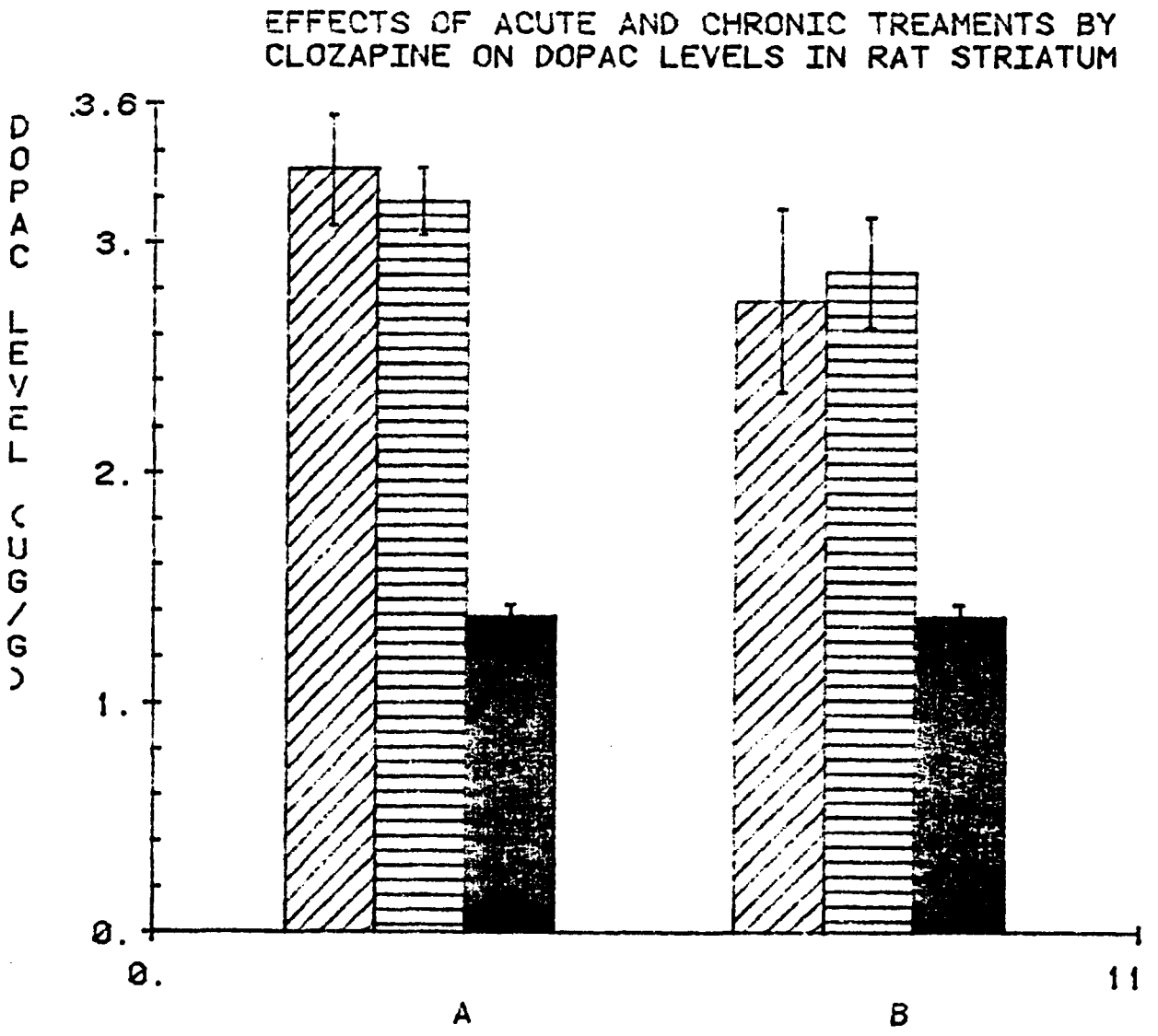
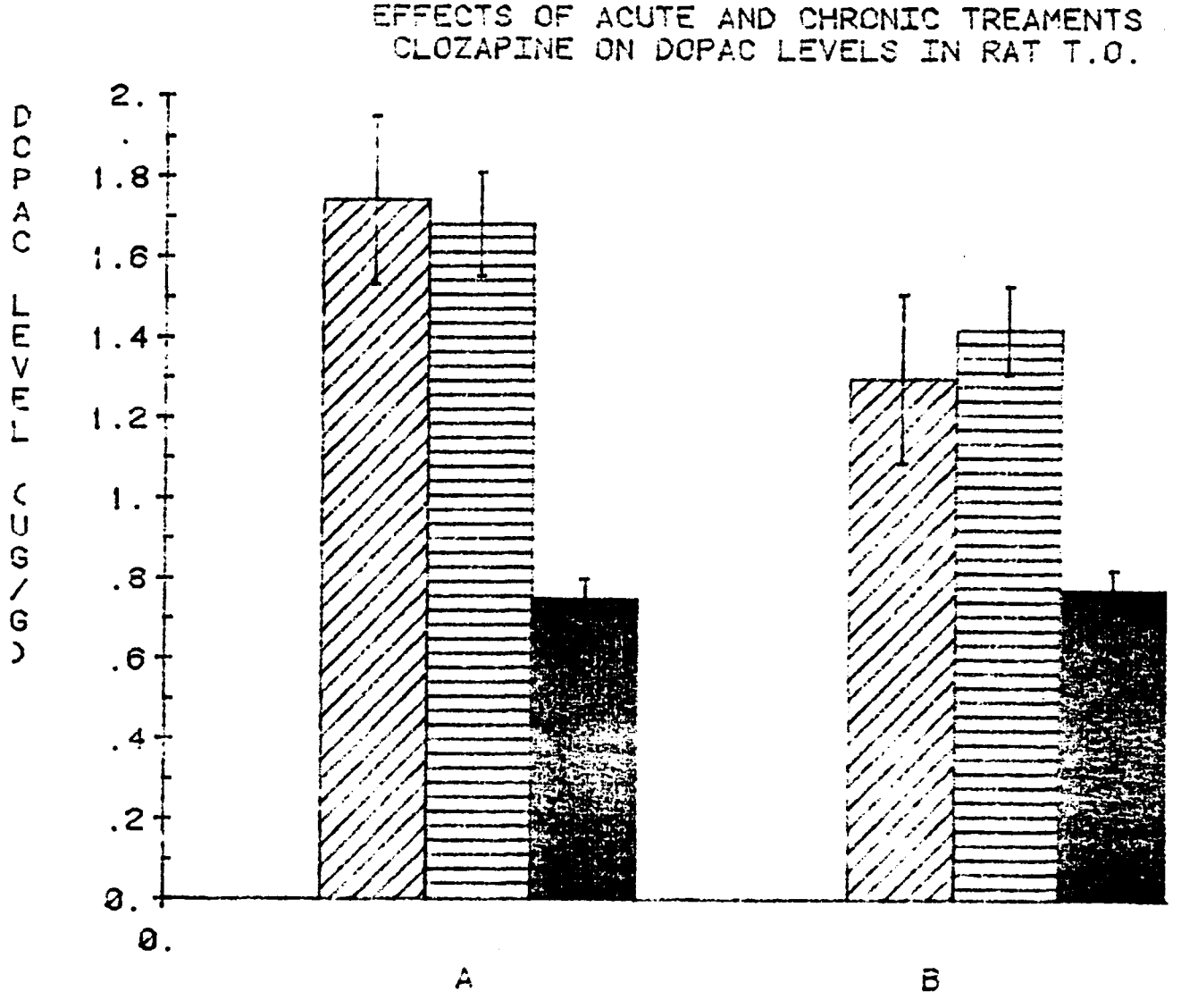


FIG. 12 Effects of acute (diagonal stripe) and chronic (horizontal stripe) treatments by clozapine on DOPAC levels in rat TO. A) 40 mg/Kg clozapine; B) 20 mg/Kg clozapine. Solid colored histogram indicates the mean ± SEM of eight saline-treated rats.

DR. SHERWIN WILK[5180,51187 SWILK.PLT



Experiment Two: Clozapine concentrations in brain regions and its relationship to dopamine metabolite increase

In this experiment we ascertained whether the increased concentration of DOPAC induced by clozapine was related to the amount of clozapine in brain. We developed a simple and sensitive gas chromatographic method for the quantitation of clozapine in milligram amounts of tissue. This method has been applied to study in particular the relationship between clozapine concentrations in striatum and TO and the elevation of DOPAC.

Male Sprague-Dawley rats weighing 175-200 grams were used in these studies. Clozapine was injected i.p. and animals allowed to remain at room temperature until decapitation. Brains were rapidly removed and the striatum and TO dissected as described in the General Methods section. Tissue samples were immediately homogenized in 1 ml of cold 1N HCl, centrifuged and the supernatants were transferred to glass tubes and stored at -80^{o}C until assayed.

One hour after i.p. administration of clozapine at doses of 10, 20 or 40 mg/kg, the levels of drug in striatum and TO were approximately equivalent (Table 1). At the highest dose injected, however, (60 mg/kg) the concentration of clozapine in the TO was significantly greater than the concentration in the striatum ($P<0.001$).

TABLE 1

Comparison of clozapine levels in striatum and tuberculum olfactorium 1 h following i.p. administration

Clozapine dose (mg/kg)	Clozapine concentration (nm/g)[1]	
	Tuberculum olfactorium	Striatum
10	17 ± 2(5)	20 ± 2(6)
20	31 ± 10(7)	33 ± 7(4)
40	111 ± 12(4)	104 ± 24(4)
60	206[2] ± 13(8)	132 ± 19(9)

[1]Values are given as mean ± S.E.M. Numbers in parentheses equal number of samples.

[2]Significantly greater than the striatal level $P<0.001$.

The increase in DOPAC evoked by clozapine was related to the clozapine concentration in striatum and in the TO in a typical dose-response fashion. Thus assuming the system obeys Michaelis-Menten kinetics, curves were fitted to the data points by the Prophet computer system (Fig. 13). The resulting curves resembled the dose-response curves for DOPAC elevation previously obtained 2 h after drug administration (Wilk et al., 1975b).

The effect of chronic clozapine treatment on clozapine concentrations in brain was studied in another experiment. Rats were treated once daily for twelve days with doses of 20 or 40 mg/kg. They were then decapitated one hour after the final injection. 20 mg/kg is the ED-50 of this drug for elevation of DOPAC (Wilk et al., 1975b) and 40 mg/kg is a near-maximal dose. Higher levels of clozapine caused significant mortality. Clozapine concentrations were compared to those found in rats receiving a single injection of these doses. The concentrations of clozapine in acutely and chronically treated animals did not vary significantly (Table 2).

FIG. 13 Percent increase in DOPAC in striatum (▼) and tuberculum olfactorium (●) as a function of clozapine concentration in these regions. Curves are computer drawn by the Prophet system assuming Michaelis-Menten kinetics. Control DOPAC : striatum 7.20 ± 0.3 (SEM) nmoles/g, N = 15; tuberculum olfactorium 5.42 ± 0.3 (SEM) nmoles/g, N = 15.

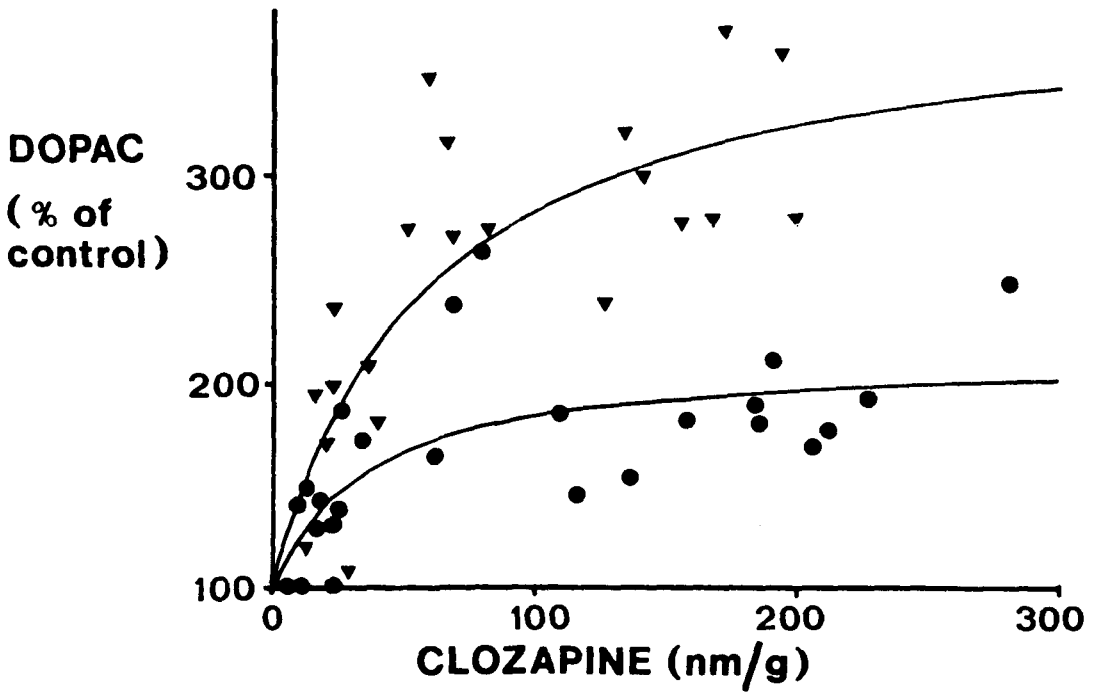

DOPAC
(% of
control)
300
200
100
0
100
200
300
CLOZAPINE (nm/g)

TABLE 2

Comparison of acute and chronic clozapine treatment on levels of clozapine and DOPAC in striatum[1].

Clozapine treatment	Striatal concentration (nm/g)	
	Clozapine	DOPAC
20 mg/kg acute	33 ± 7	16.0 ± 1.0
20 mg/kg chronic	40 ± 8	17.1 ± 1.0
40 mg/kg acute	104 ± 24	21.8 ± 0.8
40 mg/kg chronic	101 ± 16	18.9 ± 0.9

[1]Acute treatment: rats were decapitated 1 h after i.p. administration of drug. Chronic treatment: rats received a single i.p. injection of drug daily for 12 days and were decapitated 1 h following the last injection. Values are expressed as mean ± S.E.M. for 4 determinations. Control DOPAC = 7.2 nmoles/g.

Experiment Three: The effect of non-antipsychotic drugs on dopamine metabolism

In this experiment, the effects of the non-antipsychotic drugs AL-499, AHR-1900, SCH-12,679 and U-25,927 (Fig. 14) on dopamine metabolism were compared to the effects of the known antipsychotic drugs haloperidol, chlorpromazine and clozapine. We considered the possibility that regional selectivity might be demonstrated by examining the effects of the clinically ineffective analogs of antipsychotics on the metabolism of DA. Because these analogs were active in many behavioral screening tests (Table 3) we considered that these findings might possibly relate to effects in the striatum. Also, if antipsychotic efficacy is mediated through the limbic system, we would not expect to see any effect on dopamine metabolism in this region following treatment with these clinically inactive compounds.

Male Sprague-Dawley rats weighing 175-200 g were used for this study. Rats were injected i.p. with clozapine (10, 20, 40, 60 mg/kg), haloperidol (0.075, 0.15, 0.30, 0.60 mg/kg), chlorpromazine (1.25, 2.50, 5.0, 10.0 mg/kg), AHR-1900 (2.5, 5.0, 10.0, 20.0, 40.0 mg/kg), AL-499 (5.0, 10.0, 20.0, 40.0 mg/kg), U-25,927 (5.0, 10.0, 20.0, 40.0 mg/kg) and SCH-12,679 (5, 10, 20, 40 mg/kg). Animals were kept at room temperature until killed by

TABLE 3. The activity of the non-antipsychotic agents in behavioral screening tests

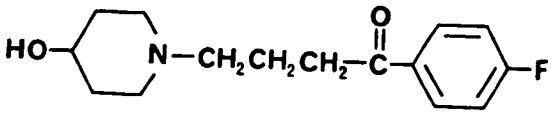
Screening Test	(a,b)* AL-499	(c) AHR-1,900	(d,e) SCH-12,679	(f) U-25,927
Catalepsy	+ +	+		
Conditioned avoidance	+ +	+ +	+	+
Inhibition of d-amphetamine aggregate toxicity	+ +		+ +	+ +
α-Adrenergic blockade	+ +	+ +		
Antagonism of isolation-induced fighting in mice		+ +		
Reduction of increased hyper-activity	+ +			+ +
Antagonism of apomorphine-induced emesis in dogs	+			+ +
Muscle tremors	+ +			
Rigidity	+ +			
Antagonism of aggression in Rhesus monkeys			+ +	
Decrease of spontaneous motility	+ +			+ +
Sedation		+ +		
Ptosis			-	
Hypothermia			-	
Antagonism of hypothalamic self-stimulation in rats			+++	
Depression of locomotor activity			+ +	
Antagonism of foot shock-induced fighting in mice			+ +	

- None + Weak + + Moderate +++ Potent

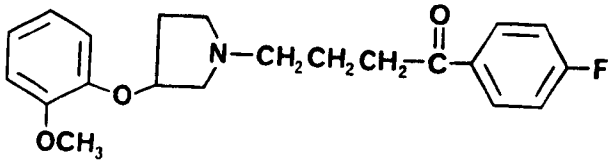
*Letters in parenthesis indicate the references from which the screening test data were derived.

(a) D.M. Gallant et al. 1968.
(b) A.A. Sugerman 1968.
(c) D.M. Gallant and M.P. Bishop 1969.
(d) S. Park et al. 1972.
(e) A. Barnett et al. 1974
(f) L. O'Meallie et al. 1969

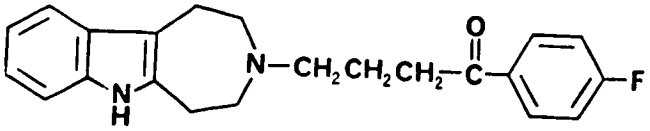
FIG. 14 Structural formulas of the non-antipsychotic drugs used in this study.



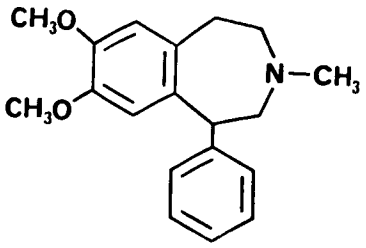
AL-499



AHR-1900



U-25,927



SCH-12,679

decapitation. Brains were removed and the striatum and the TO were dissected out and subjected to the biochemical analysis described in the general methods section.

A dose-dependent increase in the level of striatal and tubercular DOPAC was observed 1 hour after the administration of haloperidol, chlorpromazine and clozapine (Figures 15 and 16). With the exception of AHR-1900, the non-antipsychotic compounds had no effect on dopamine or its metabolites in either the striatum or the TO (Figures 15 and 16). While AHR-1900 did exert a significant effect on DOPAC levels, neither the slope nor the ceiling effect bore any similarity to the slope and ceiling effect of the antipsychotic drugs tested. Comparable results were also obtained when HVA levels were measured.

The elevation of DOPAC in both the striatum and the TO following the administration of AHR-1900 was also studied as a function of time. A dose of 20 mg/kg of AHR-1900, which produces a near maximal effect, was given to rats which were subsequently killed at 30 min., 1, 2 and 4 hour intervals. A time-action curve was constructed from the data which revealed that the 1 hour point reflected this compound's peak on dopamine metabolism in both the striatum and the TO (Figs. 17 and 18).

DA was assayed by the gas chromatographic technique described in the General Methods section. AHR-1900 did

FIG. 15 Dose-response curves for the increase in striatal DOPAC 1 hour following i.p. administration of haloperidol (●——●), chlorpromazine (▲——▲), clozapine (■——■), AL-499 (□ ++--- □), AHR-1900 (▽----▽), SCH-12,679 (△-----△) and U-25,927 (○——○). Each point represents the mean ± SE of 4 rats. Hatched area represents mean ± SE of 13 saline-treated rats (1.13 µg/g ± 0.06).

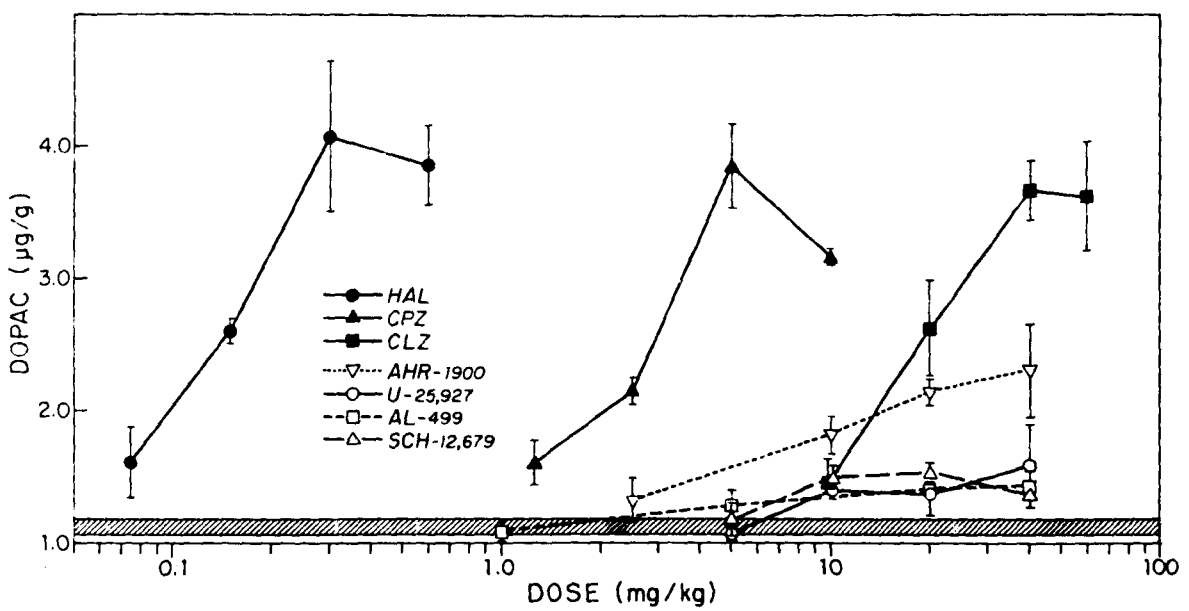

DOPAC (µg/g)
4.0
3.0
2.0
1.0
0.1
1.0
10
100
DOSE (mg/kg)
HAL
CPZ
CLZ
AHR-1900
U-25,927
AL-499
SCH-12,679

FIG. 16 Dose-response curves for the increase in DOPAC in the tuberculum olfactorium 1 hour following i.p. administration of haloperidol (●——●), chlorpromazine (▲——▲), clozapine (■——■), AL-499 (□++---□), AHR-1900 (▽ ----▽), SCH-12,679 (△----△) and U-25,927 (○——○). Each point represents the mean ± SE of 4 rats. Hatched area represents mean ± SE of 13 saline treated rats (0.80 µg/g ± 0.05).

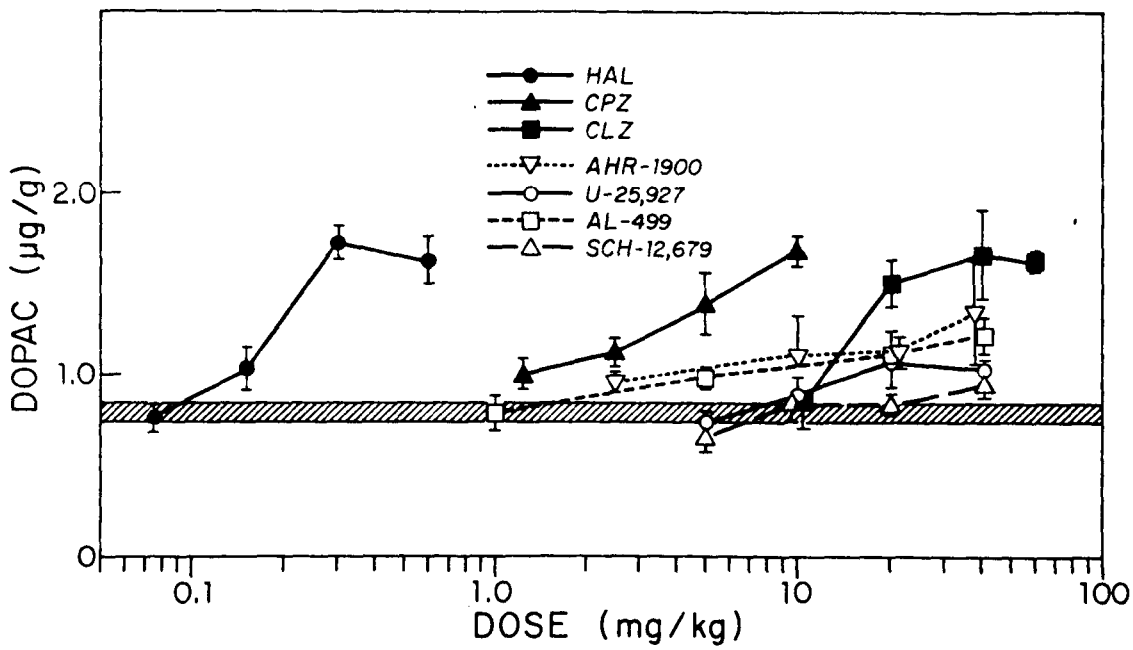

HAL
CPZ
CLZ
AHR-1900
U-25,927
AL-499
SCH-12,679
DOPAC (µg/g)
2.0
1.0
0
0.1
1.0
10
100
DOSE (mg/kg)

FIG. 17 Time-action curve for the increase in striatal DOPAC following i.p. administration of 20 mg/kg of AHR-1900. Each point represents the mean ± SE of 4 rats.

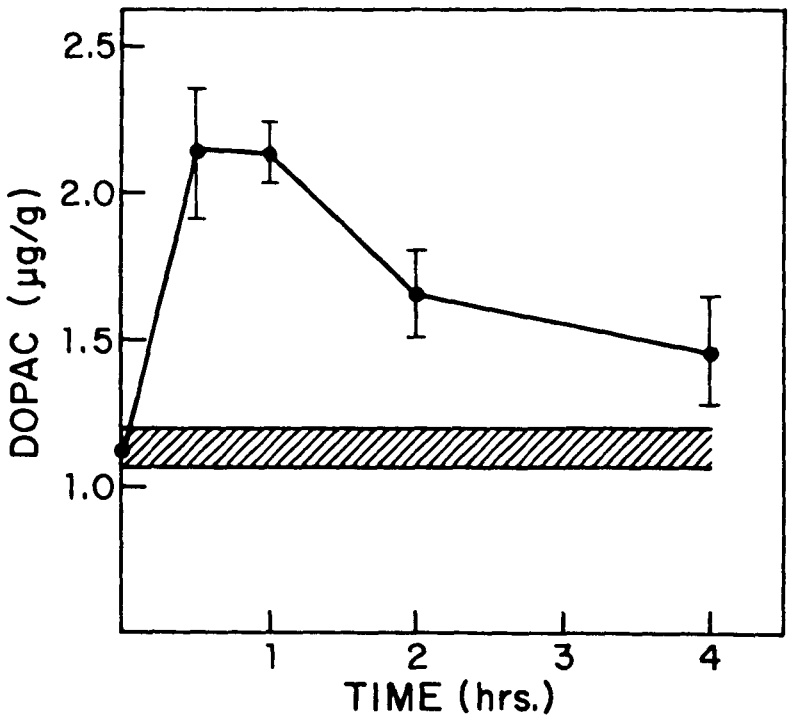
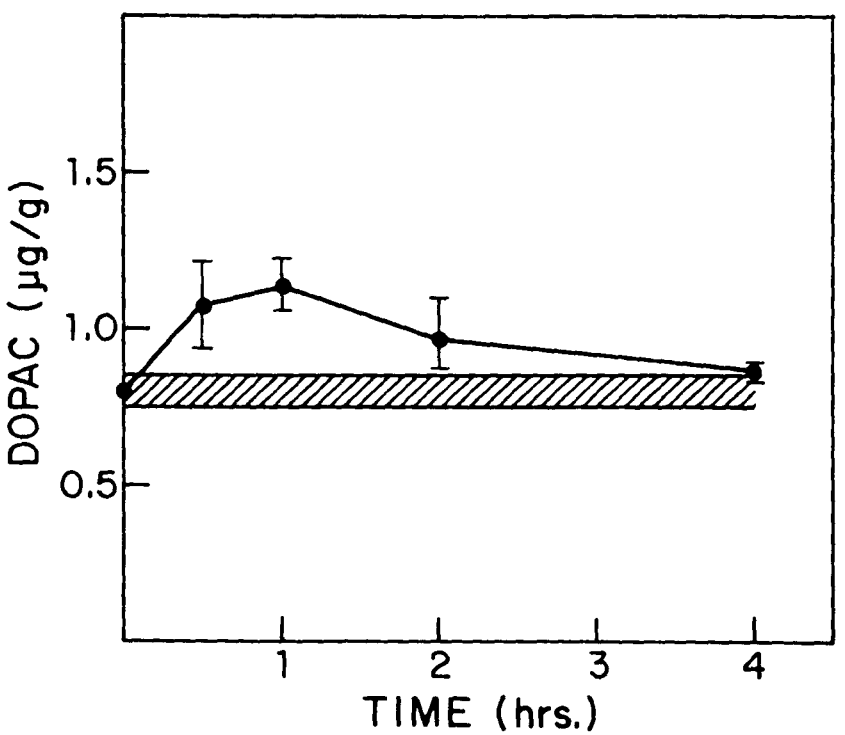

2.5
2.0
1.5
1.0
DOPAC (µg/g)
1
2
3
4
TIME (hrs.)

FIG. 18 Time-action curve for the increase in DOPAC in the tuberculum olfactorium following i.p. administration of 20 mg/kg of AHR-1900. Each point represents the mean ± SE of 4 rats.


DOPAC (µg/g)
1.5
1.0
0.5
1
2
3
4
TIME (hrs.)

not significantly alter the levels of DA in either the striatum or the TO (Table 4).

TABLE 4

The effect of AHR-1900 on dopamine concentration in the striatum and tuberculum olfactorium of the rat.

Treatment	Dopamine (µg/g ± S.E.M.)[1]	
	Striatum	Tuberculum olfactorium
Control	10.5 ± 0.30(13)	7.2 ± 0.50(10)
AHR-1900 2.5 mg/kg	9.8 ± 0.07(3)	7.1 (2)
AHR-1900 10 mg/kg	10.0 ± 0.66(4)	7.5 ± 0.29(4)
AHR-1900 20 mg/kg	9.8 ± 0.27(5)	6.2 ± 0.52(3)
AHR-1900 40 mg/kg	9.3 ± 0.61(4)	6.6 (2)

[1]Dopamine was determined by gas chromatography 1 h following i.p. administration of saline or AHR-1900. The number of experimental animals is given in parentheses. None of the mean values differed significantly from control ($p>0.05$).

Experiment Four: The effect of oxotremorine and morphine on dopamine metabolism

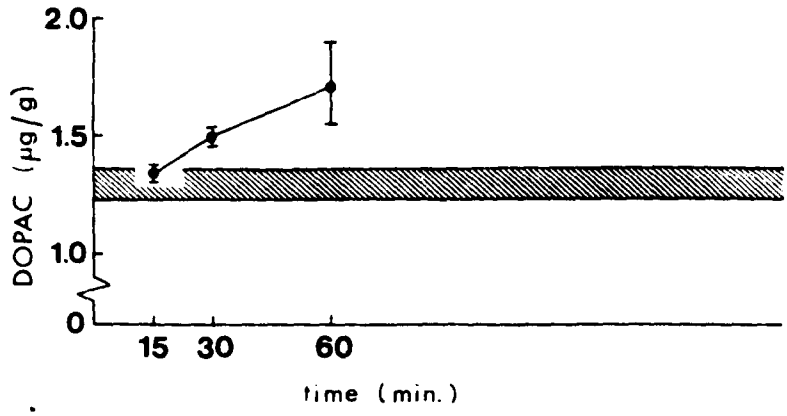
The results of Experiment Three made it possible for us to clearly distinguish differences between groups of clinically active and inactive compounds. We considered the possiblity that a distinction could also be made between typical antipsychotic drugs and compounds such as oxotremorine and morphine which are known to effect dopamine metabolism in the same direction as neuroleptic compounds.

Groups of rats (N = 4 per group) were injected i.p. with solutions of either morphine sulphate or oxotremorine. The groups treated with oxotremorine were also given an injection of methylatropine (10 mg/kg) 15 min. prior to their receiving the cholinergic agonist. The purpose of this pretreatment with a peripheral anticholinergic was to lessen the peripheral toxic effects seen following oxotremorine.

Male Sprague-Dawley rats weighing 175-200 grams were injected with the following doses of morphine and oxotremorine respectively: 10, 20 and 40 mg/kg; 0.25, 0.50 and 1.0 mg/kg.

All animals were kept at room temperature following their injection until killed by decapitation one hour

FIG. 19 Time-action curves for the increase in DOPAC in the TO (bottom graph) and striatum (upper graph) following i.p. administration of 1 mg/Kg of oxotremorine. Hatched area represents the mean ± SEM of 20 saline-treated rats. (0.79 µg/g ± 0.03 TO; 1.15 µg/g ± 0.06 striatum.)


2.0
1.5
1.0
0
DOPAC (µg/g)
15 30 60
time (min.)

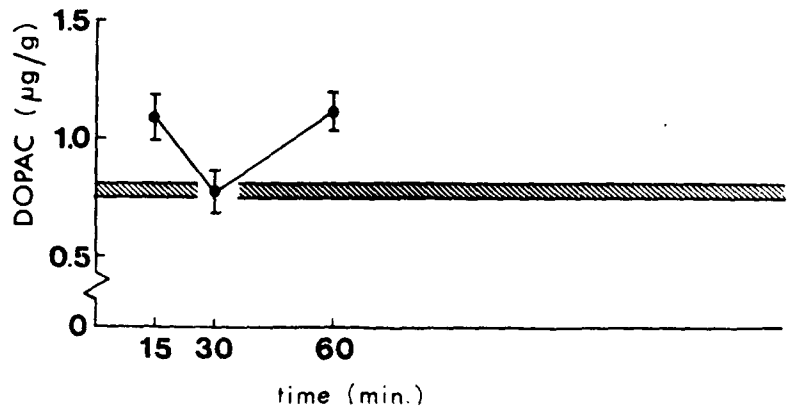
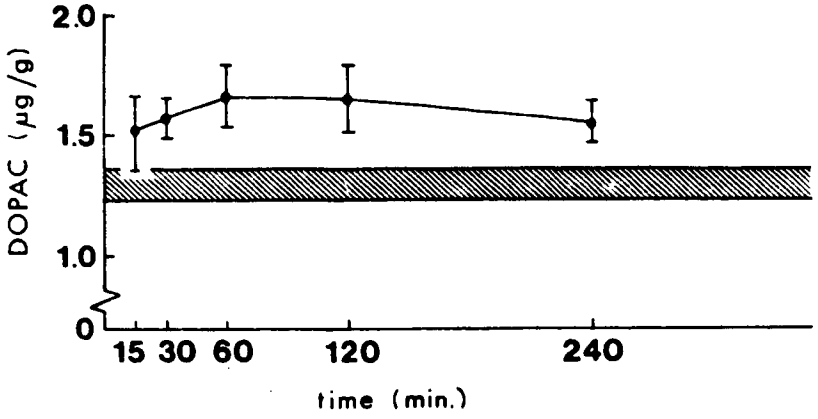
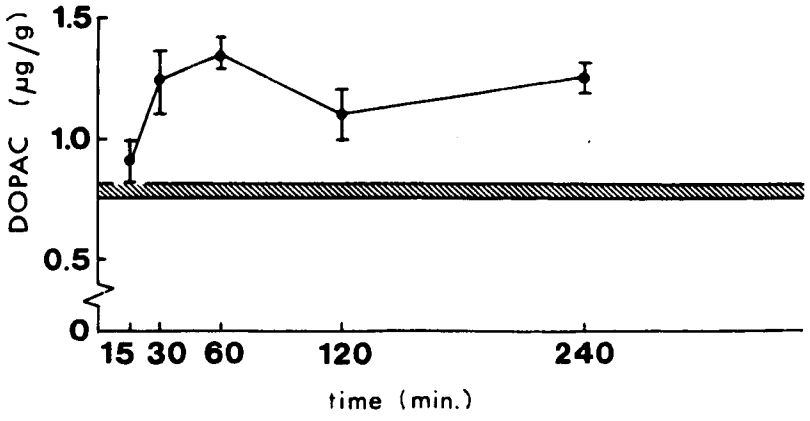

1.5
1.0
0.5
0
DOPAC (µg/g)
15 30 60
time (min.)

FIG. 20 Time-action curves for the increase in DOPAC in the TO (bottom graph) and striatum (upper graph) following i.p. administration of 10 mg/Kg of morphine. Hatched area represents the mean ± SEM of 20 saline-treated rats. (0.79 µg/g ± 0.03 TO; 1.15 µg/g ± 0.06 striatum.)


2.0
1.5
1.0
0
DOPAC (μg/g)
15 30 60 120 240
time (min.)


1.5
1.0
0.5
0
DOPAC (μg/g)
15 30 60 120 240
time (min.)

later. DOPAC and HVA were analyzed in both the TO and striatum according to the methods described in the General Methods section.

The results of a fixed dose of either oxotremorine (1 mg/kg) or morphine (10 mg/kg), administered over time, indicated that both compounds had their peak effect on DOPAC levels in the striatum and TO at about 1 hour (Figs. 19 and 20). Dose-response effects were therefore studied at the one-hour time point.

The dose-response curves of both morphine and oxotremorine on DOPAC accumulation in the striatum shows that the maximal effect elicited by both compounds is much less than that which is seen following known antipsychotic compounds (Fig. 21). It should also be noted that no strict dose-response relationship was found, thus the shape of the dose-response curves for the striatum, following treatment with either morphine or oxotremorine, is considerably different from classic neuroleptics (Fig. 21). The same findings were also true for the dose-response curve constructed for oxotremorine in the TO (Fig. 22). As in the striatum, Figure 22 reveals a low maximal response and an atypically flat slope in comparison to the responses of known antipsychotic drugs. The effect of morphine on DOPAC accumulation in the TO, however, more closely resembles the response seen after neuroleptic treatment (Fig. 22).

FIG. 21 Dose-response curves for the increase in striatal DOPAC following i.p. administration of haloperidol (HAL), chlorpromazine (CPZ), clozapine (CLZ), oxotremorine (OXO) and morphine (MOR). Each point represents the mean ± SE of 4 rats. Hatched area represents mean ± SE of 20 saline-treated rats (1.15 µg/g ± 0.06).

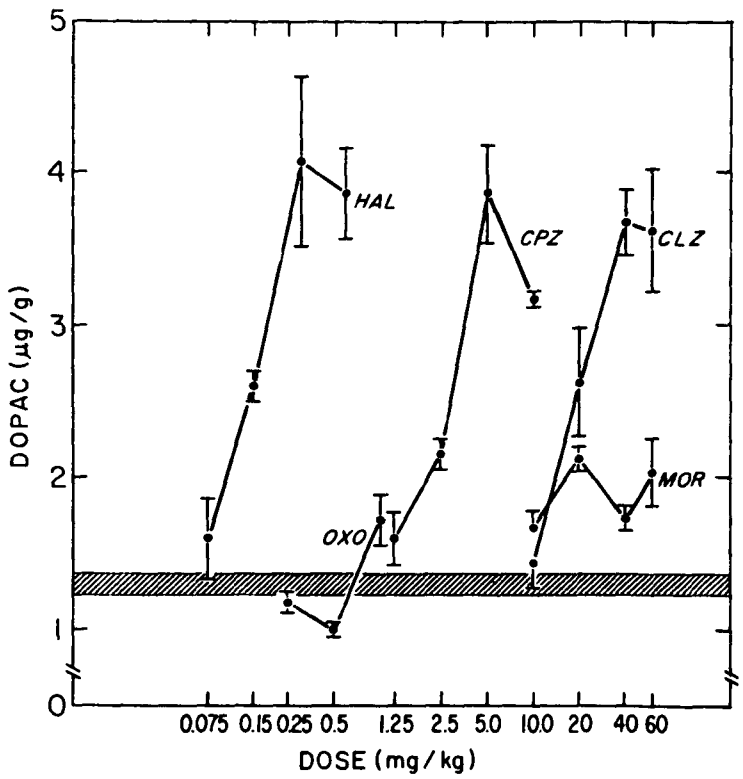
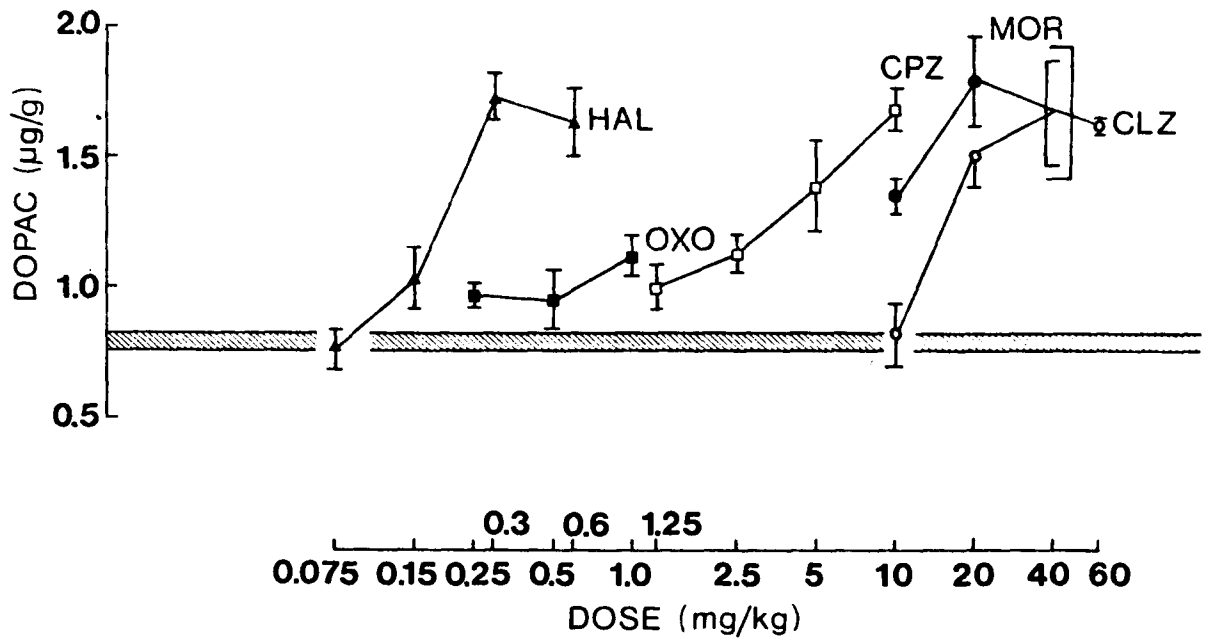

5
4
3
2
1
0
DOPAC (µg/g)
HAL
CPZ
CLZ
MOR
OXO
0.075 0.15 0.25 0.5 1.25 2.5 5.0 10.0 20 40 60
DOSE (mg/kg)

FIG. 22 Dose-response curves for the increase in DOPAC in the TO following i.p. administration of haloperidol (HAL), chlorpromazine (CPZ), clozapine (CLZ), oxotremorine (OXO) and morphine (MOR). Each point represents the mean ± SE of 4 rats. Hatched area represents the mean ± of 20 saline-treated rats (0.79 µg/g ± 0.03).


2.0
1.5
1.0
0.5
DOPAC (µg/g)
HAL
OXO
CPZ
MOR
CLZ
0.3
0.6
1.25
0.075
0.15
0.25
0.5
1.0
2.5
5
10
20
40
60
DOSE (mg/kg)

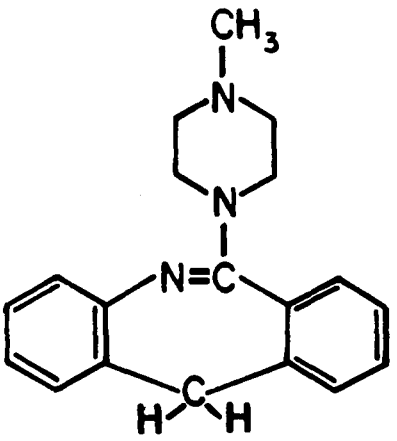
Experiment Five: The effect of perlapine on dopamine metabolism

On the basis of the results of the foregoing experiments, we found that the effects of antipsychotics on DA metabolism (increases in the concentration of DOPAC) could be easily differentiated from non-antipsychotic drugs and compounds such as morphine and oxotremorine. We decided to test the effects of perlapine on the accumulation of DOPAC in the striatum and the TO. Perlapine is a close analog of clozapine (Fig. 23), but is generally considered to be without antipsychotic effect (Wander Ltd., Berne, personal communication).

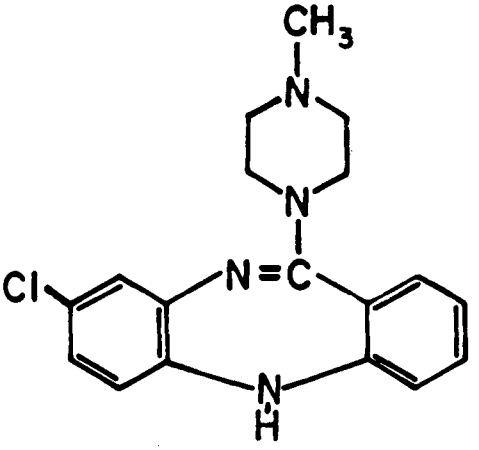
Male Sprague-Dawley rats weighing 175-200 grams were given i.p. injections of clozapine (10, 20, 40 and 60 mg/kg), perlapine (1.25, 2.5, 5.0, and 10.0 mg/kg), haloperidol (0.075, 0.15, 0.30 and 0.60 mg/kg) and chlorpromazine (1.25, 2.5, 5.0 and 10.0 mg/kg). Animals were kept at room temperature until killed by decapitation. Brains were rapidly removed and the striatum and TO dissected out, homogenized in cold 1N HCl and stored at -80^{o}C until biochemical analysis.

A dose-dependent increase in the level of striatal DOPAC was observed 1 hour following the administration of perlapine, haloperidol, chlorpromazine and clozapine (Fig. 24). The dose-response curve for perlapine and its ED-50

FIG. 23 Structural formulas of perlapine and clozapine



PERLAPINE



CLOZAPINE

FIG. 24 Dose-response curves for the increase in striatal DOPAC 1 hour following i.p. administration of haloperidol (HAL), chlorpromazine (CPZ), perlapine (PERL) and clozapine (CLOZ). Each point represents the mean ± SE of 4 rats. Hatched area represents mean ± SE of 13 saline-treated rats (1.13 μg/g ± 0.06).

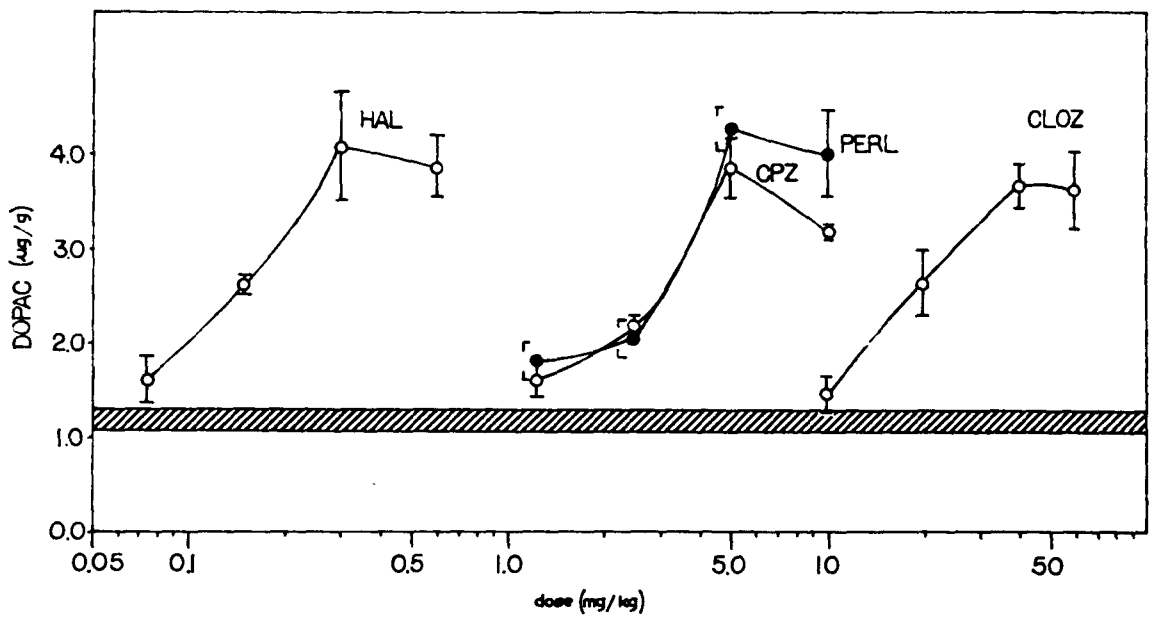
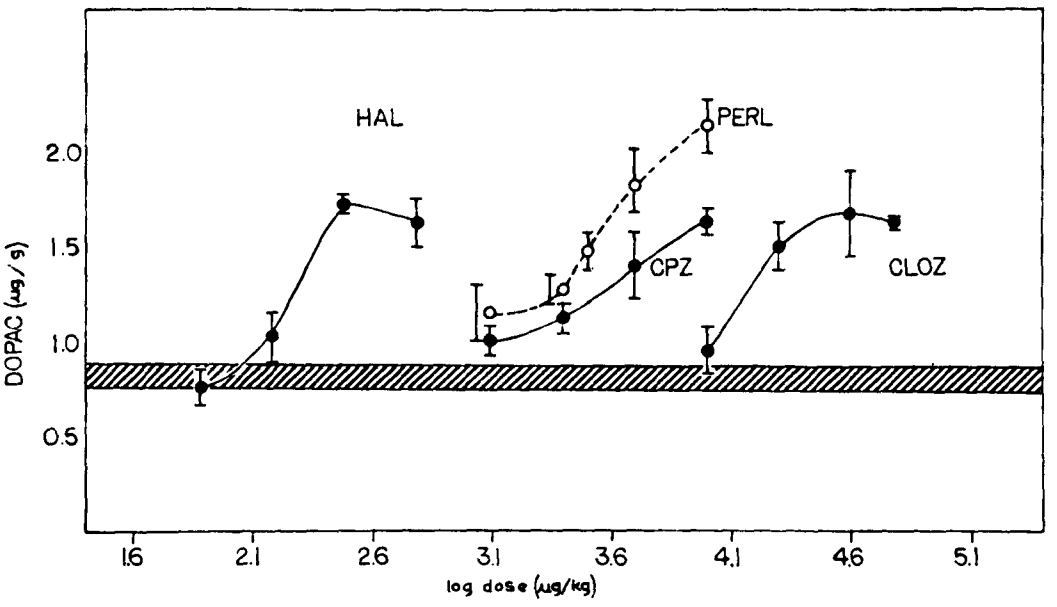

HAL
CPZ
PERL
CLOZ
DOPAC (µg/g)
4.0
3.0
2.0
1.0
0.0
0.05
0.1
0.5
1.0
5.0
10
50
dose (mg/kg)

FIG. 25 Dose-response curves for the increase in DOPAC in the tuberculum olfactorium 1 hour following i.p. administration of haloperidol (HAL), chlorpromazine (CPZ), perlapine (PERL) and clozapine (CLOZ). The number of experimental animals is the same as in Fig. 24. Hatched area represents mean ± SE of 13 saline-treated rats (0.80 µg/g ± 0.05).


HAL
PERL
CPZ
CLOZ
2.0
1.5
1.0
0.5
DOPAC (µg/g)
1.6
2.1
2.6
3.1
3.6
4.1
4.6
5.1
log dose (µg/kg)

(that dose which produced a half-maximal increase in DOPAC) was similar to that of chlorpromazine. The ED-50's for both perlapine and chlorpromazine were intermediate between haloperidol and clozapine. All four drugs used in this experiment produced a similar dose-dependent increase in the level of DOPAC in the TO (Fig. 25). The same order of potency was observed for both the striatum and the TO. While the ED-50's for perlapine and chlorpromazine in the TO were similar, perlapine evoked a significantly greater increase in the level of DOPAC at the 10 mg/kg dose ($p < 0.05$).

The elevation of DOPAC in the striatum and TO following perlapine was studied as a function of time after drug administration. A dose (3.2 mg/kg) which is close to the ED-50 for this drug was administered to rats which were subsequently killed at the following intervals: 30 min., 1, 2 and 4 hours. The resulting time-action curves for both the striatum and TO following perlapine were compared to those previously constructed using the ED-50 dose of clozapine (20 mg/kg). The time-action curves for both of these drugs are virtually superimposable both in the striatum and in the TO (Figs. 26 and 27).

The percent increase in striatal DOPAC was compared to the percent increase in striatal HVA for each dose of all drugs used in this experiment. The percent increase

FIG. 26 Time-action curves for the increase in striatal DOPAC following perlapine (3.2 mg/kg) and clozapine (20 mg/kg). Each point represents the mean value ± SE of 4 rats.

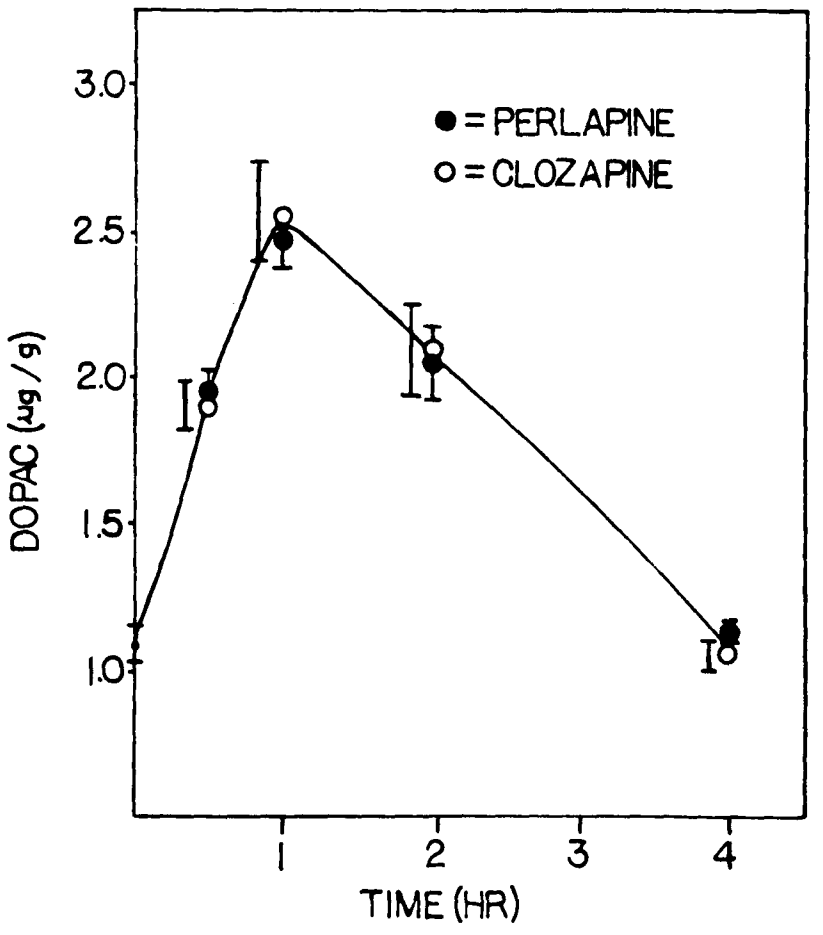
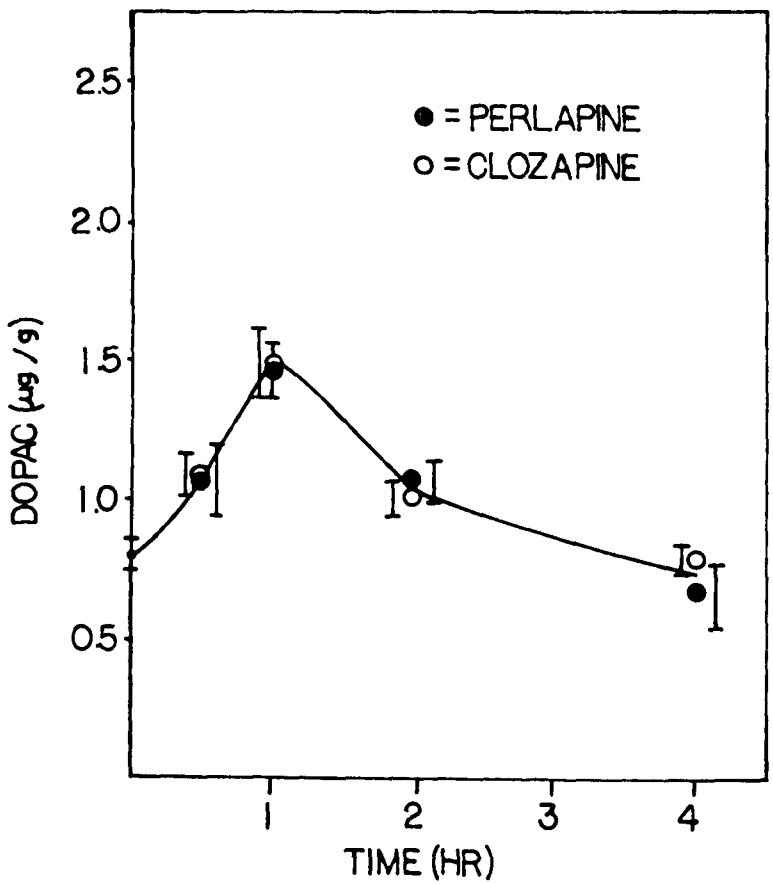

3.0
2.5
2.0
1.5
1.0
DOPAC (µg/g)
● = PERLAPINE
○ = CLOZAPINE
1
2
3
4
TIME (HR)

FIG. 27 Time-action curves for the increase in DOPAC in the tuberculum olfactorium following perlapine (3.2 mg/kg) and clozapine (20 mg/kg). Each point represents the mean ± SE of 4 rats.


2.5
2.0
1.5
1.0
0.5
DOPAC (µg/g)
● = PERLAPINE
○ = CLOZAPINE
1
2
3
4
TIME (HR)

in DOPAC was found to be similar to that of HVA one hour after administration, irrespective of the drug or dose used. Thus the ratio: (percent increase in striatal DOPAC divided by the percent increase in striatal HVA) was close to unity (Table 5).

DA was assayed according to the new gas chromotographic procedure fully described in the General Methods section. Quantitative results from both OV-17 and SE-30 columns were in close agreement. Perlapine did not significantly alter the level of DA in the striatum or in the TO (Table 6).

TABLE 5

Percent increase of striatal dopamine metabolites following neuroleptic treatment[1].

	Percent of control		
	DOPAC	HVA	DOPAC/HVA
Perlapine (mg/kg)			
1.25	160	167	0.96
2.5	180	178	1.01
5.0	379	390	0.97
10.0	353	351	1.00
Chlorpromazine (mg/kg)			
1.25	142	174	0.82
2.5	190	226	0.84
5.0	342	328	1.04
10.0	281	274	1.03
Haloperidol (mg/kg)			
0.075	142	120	1.18
0.15	230	212	1.08
0.30	360	320	1.13
0.60	342	370	0.92
Clozapine (mg/kg)			
10	128	135	0.95
20	232	272	0.85
40	325	274	1.19
60	320	381	0.84

[1]Metabolite levels were quantitated 1 h after drug administration. Control DOPAC = 1.13 µg/g, HVA = 0.81 µg/g.

TABLE 6

Effect of perlapine on dopamine levels in the striatum and tuberculum olfactorium of the rat.

Perlapine dose (mg/kg)	Dopamine (µg/g ± S.E.)[1]	
	Striatum	Tuberculum olfactorium
0 (control)	10.5 ± 0.3(13)	7.7 ± 0.7(7)
1.25	11.9 ± 1.3(4)	9.2 ± 1.0(4)
2.5	11.1 ± 0.3(4)	8.8 ± 0.9(4)
3.2	12.6 (2)	7.7 ± 0.6(4)
5.0	11.1 ± 0.7(8)	7.1 ± 0.6(4)
10.0	11.3 ± 1.0(4)	-

[1] Dopamine was determined by gas chromatography 1 h following i.p. administration of perlapine. Number of experimental animals is given in parentheses. None of the mean values differed significantly from control ($p>0.05$).

Experiment 6: Thiethylperazine and dopamine metabolism.

On the basis of the results of the previous experiments, we were able to formalize a predictive system for antipsychotic drugs based on the dose-dependent increase in striatal DOPAC. In this experiment we were afforded the opportunity to correlate our findings in the rat with a clinical trial testing the efficacy of thiethylperazine in ten recently admitted schizophrenics.

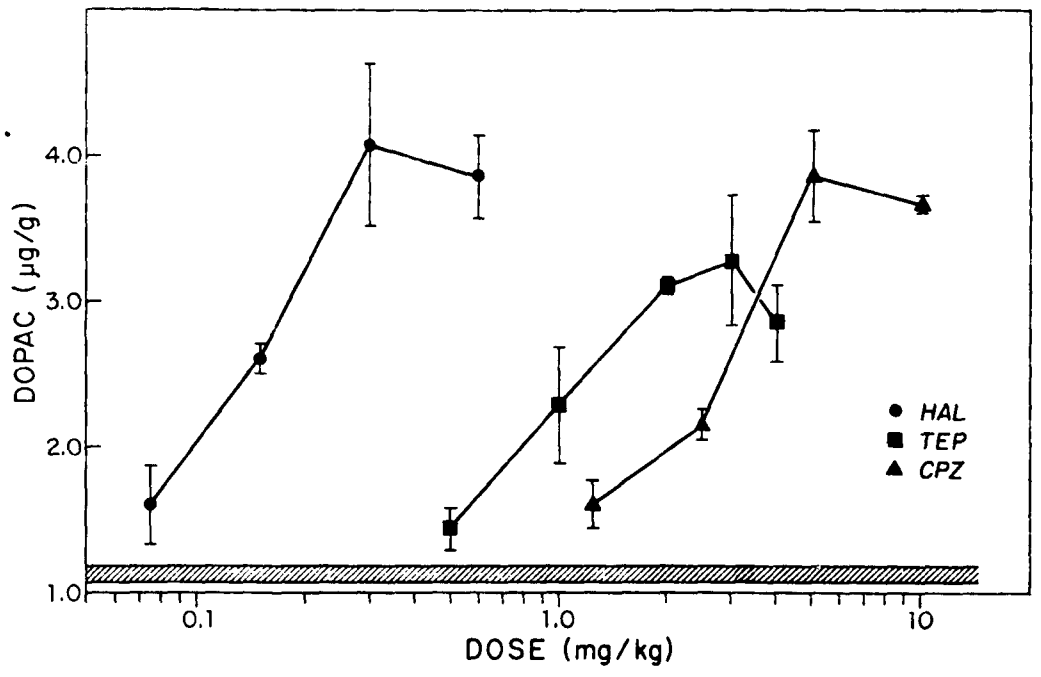
Groups of four male Sprague-Dawley rats weighing 175-200 grams were injected with the following doses of thiethylperazine: 0.5, 1.0, 2.0, 3.0 and 4.0 mg/kg i.p. Animals were kept at room temperature until killed by decapitation. Brains were removed and the striatum and TO were dissected out and assayed for DOPAC and HVA as described in the General Methods section.

Dose-dependent elevations in DOPAC levels were observed in both the striatum and TO following the administration of thiethylperazine. Dose-response curves, comparing the increase in striatal DOPAC one hour after drug administration, are shown for haloperidol, thiethylperazine and chlorpromazine in figure 28. The ED50 for thiethylperazine shows it to be approximately three times as potent as chlorpromazine, but only 1/7 as potent as haloperidol.

The clinical phase of this experiment was conducted at Bellevue Psychiatric Hospital under the direction of Drs. Samuel Gershon, Burton Angrist and John Rotrosen.

Ten recently admitted patients entered and concluded the study. All had histories of multiple prior hospitalizations for acute

FIG. 28 Dose-response curves for the increase in striatal DOPAC level one hour following intraperitoneal administration of haloperidol (HAL), thiethylperazine (TEP) and chlorpromazine (CPZ). Each point represents mean value ± SE of four rats. Hatched area represents mean ± SE of 13 saline-treated rats (1.13 μg/g ± 0.06).


DOPAC (µg/g)
4.0
3.0
2.0
1.0
0.1
1.0
10
DOSE (mg/kg)
HAL
TEP
CPZ

exacerbations; all except one patient were acutely psychotic upon entrance into the study.

Following a washout period (mean duration 6.7 days) the entire group received thiethylperazine (mean duration 18.5 days and the mean maximum daily dose administered was 324 mg). None of the ten patients improved during the washout period.

Eight of the ten patients showed a marked therapeutic response with complete or nearly complete remission of psychotic symptomatology. One patient showed minimal improvement without improving further after 40 mg/day of haloperidol. One patient did not show any change associated with administration of thiethylperazine.

Table No. 7 shows the relative potency of thiethylperazine in our predictive system (which compares favorably with its' clinical potency) as well as the results of other systems.

TABLE 7 - Clinical Potency of Neuroleptics vs Potency in Predictive Test Systems

Test (Species)	Chlorpromazine	Haloperidol	Thiethylperazine
Clinical potency (man)	1	20-50	~3
Prolactin elevation (man)	1	30	>2.5
CSF HVA elevation (monkey)	1	20	>3
Striatal DOPAC elevation (rat)	1	20	3
Blockade of stereotypy (rats) induced by			
Apomorphine	1	62.5	8
Amphetamine	1	20	1.2
Inhibition of dopamine stimulation of striatal adenyl cyclase (rat, *in vitro*)	1	0.3	<1

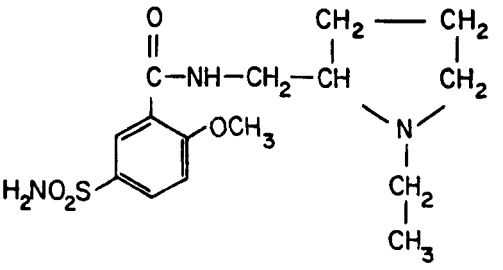
Experiment 7: The effects of metoclopramide and sulpiride on dopamine metabolism in the rat brain.

In this experiment we chose to examine the effects of two structurally unusual compounds, metoclopramide and sulpiride (Fig. 29) on the accumulation of DOPAC in the striatum and TO. Sulpiride has been shown to be an effective antipsychotic with a low incidence of extrapyramidal side effects. However, unlike most antipsychotic drugs, sulpiride has been reported to be non-cataleptic and ineffective in antagonizing apomorphine- and amphetamine-induced stereotypies. In contrast to sulpiride, metoclopramide has been found to cause catalepsy and effectively antagonize apomorphine- and amphetamine-induced stereotypies. However, in spite of this classical profile, metoclopramide is regarded as being devoid of antipsychotic and tranquilizing properties.

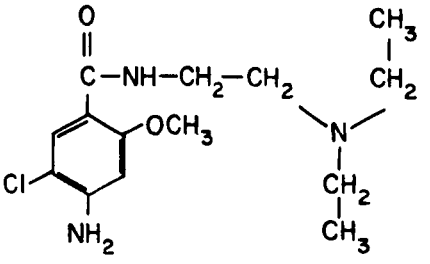
Male Sprague-Dawley rats weighing 175-200 grams were given i.p. injections of metoclopramide (1.25, 2.5, 5.0 and 10.0 mg/kg) or sulpiride (25, 50, 75 and 100 mg/kg). Animals were kept at room temperature until killed by decapitation. Brains were rapidly removed and the striatum and TO dissected out, homogenized in cold IN HCl and stored at -80^{o}C until biochemical analysis.

The accumulation of DOPAC in the striatum following metoclopramide or sulpiride was studied as a function of time after drug administration. A dose of 50 mg/kg of sulpiride was found to produce a significant elevation of DOPAC. Thus rats were given 50 mg/kg

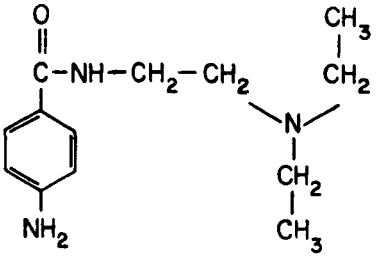
FIG. 29 Structural formulas of metoclopramide, sulpiride and procainamide.



SULPIRIDE



METOCLOPRAMIDE



PROCAINAMIDE

of sulpiride and killed at 1, 2, 4, 6 and 8 hours. The results of this time-action study revealed that sulpiride had an unusually long onset to peak action. It was not until the 4 hr point had been reached that an asymptotic response was achieved (Fig. 30). Most of the neuroleptics we have tested have had their peak effect on dopamine metabolites at 1 or 2 hours and the effect is significantly decreased by 4 hours. DOPAC levels were elevated by sulpiride even after 8 hours.

In contrast to sulpiride, metoclopramide had its peak effect on DOPAC levels at 1 hour (Fig. 31).

A dose-dependent increase in the level of striatal DOPAC was observed 4 hours following the administration of sulpiride (Fig. 30). One hour after metoclopramide was given, a dose-dependent increase in striatal DOPAC was found (Fig. 32). These data were compared with those obtained for chlorpromazine and clozapine (Fig. 33). The ED-50 doses for metoclopramide and chlorpromazine were found to be similar and their dose-response curves were virtually superimposable. The ED-50 for sulpiride was found to be about three times greater than that of clozapine (60 mg/kg vs. 20 mg/kg).

While the striatal dose response curves of metoclopramide and sulpiride were found to be similar to those of known antipsychotics, the effect of sulpiride in the TO was atypical. Sulpiride displayed an exaggerated increase in DOPAC levels in the TO as compared with the three other compounds tested (Fig. 33). Metoclopramide's effect on tubercular DOPAC levels was, as in the striatum, indistinguishable from that of chlorpromazine (Figs. 32 and 33).

FIG. 30 Time-action curve for the increase in striatal DOPAC following sulpiride (50 mg/kg). Each point represents the mean value ± SE of 4 to 8 rats.

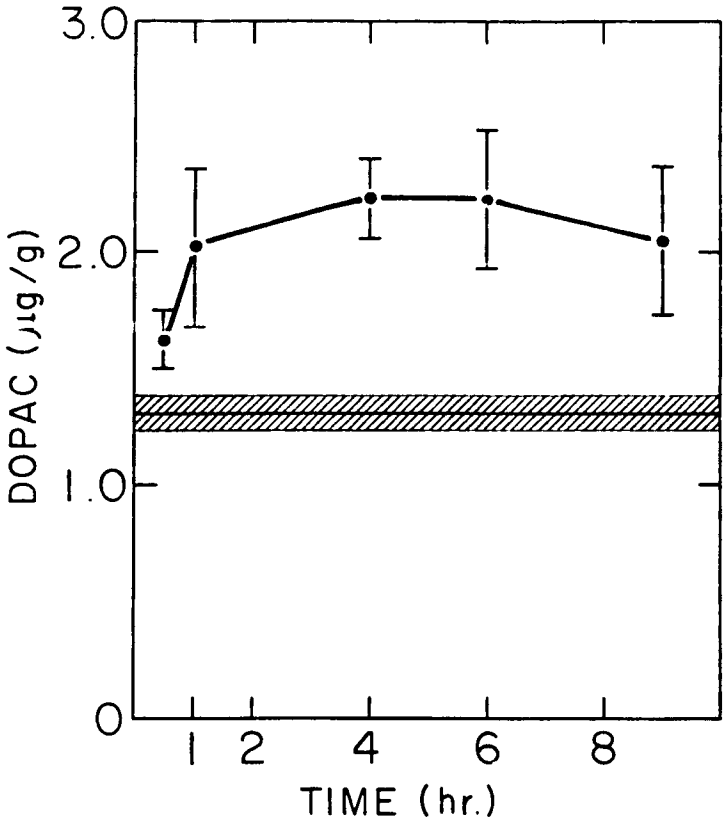

3.0
2.0
1.0
0
DOPAC (µg/g)
1
2
4
6
8
TIME (hr.)

FIG. 31 Time-action curve for the increase in striatal DOPAC following metoclopramide (5 mg/kg). Each point represents the mean value ± SE of 4 rats.

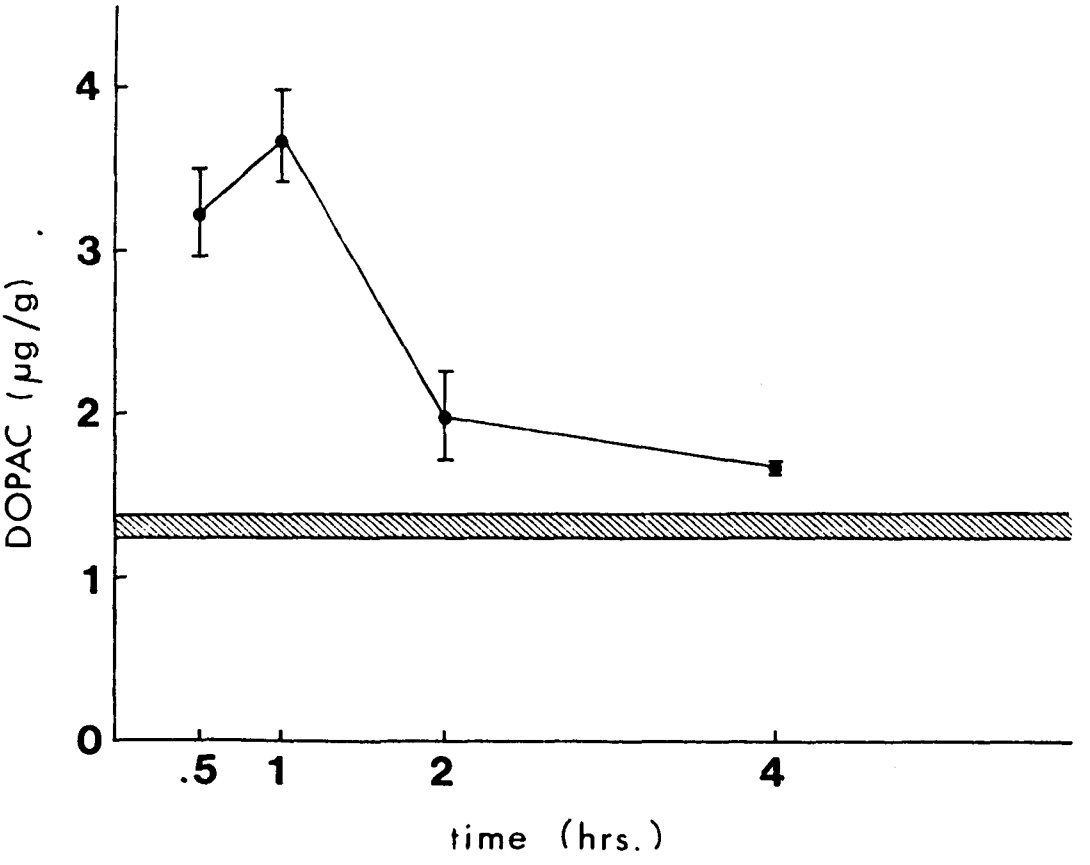

DOPAC (µg/g)
4
3
2
1
0
.5
1
2
4
time (hrs.)

FIG. 32 Dose-response curves for the increase in striatal DOPAC one hour following the i.p. administration of chlorpromazine (CPZ), metoclopramide (MET) and clozapine (CLZ) and four hours following the i.p. administration of sulpiride. Each point represents the mean ± SE of four to eight rats. Hatched area represents mean ± SE of 10 saline-treated rats (1.37 µg/g ± 0.06).

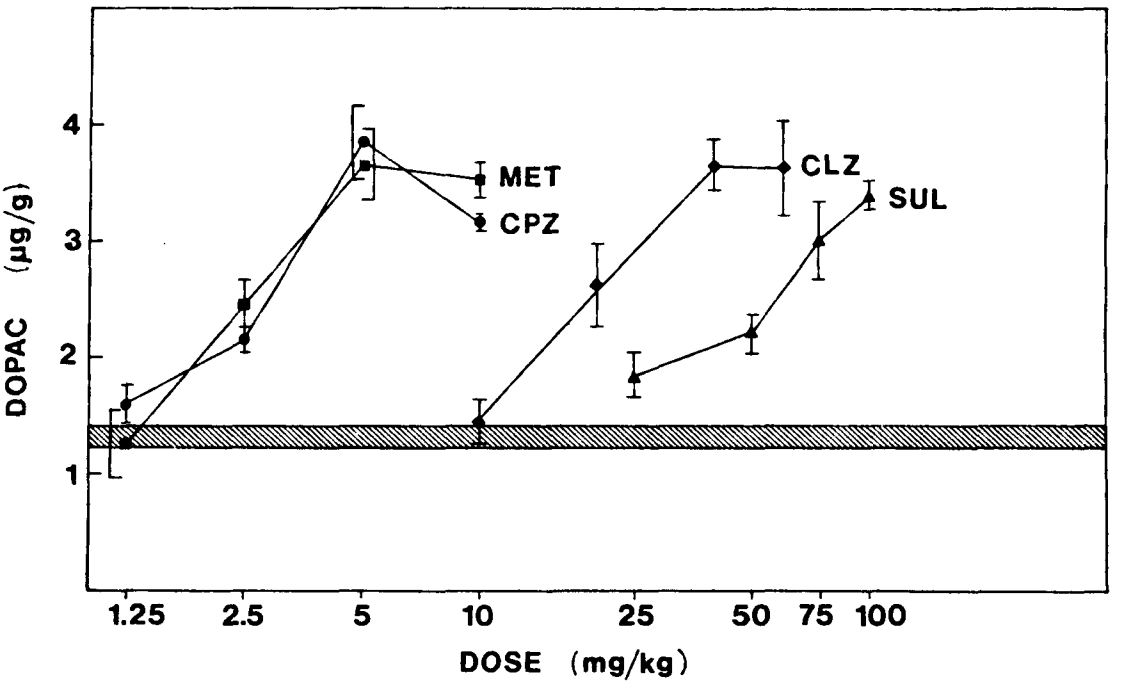
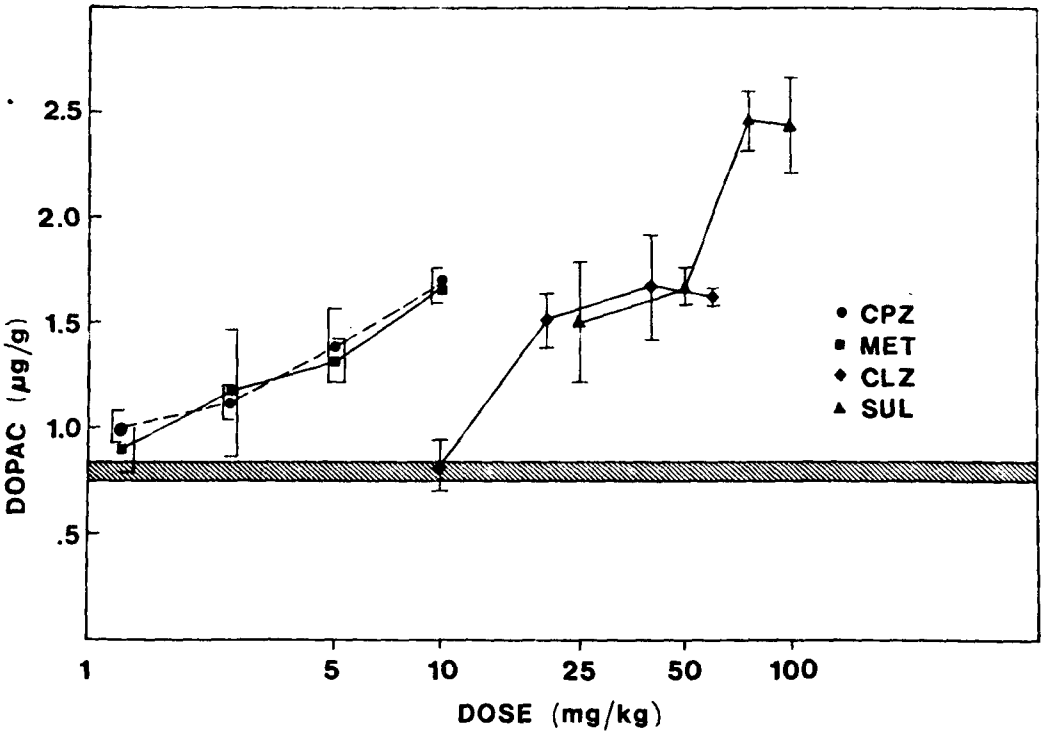

DOPAC (µg/g)
4
3
2
1
MET
CPZ
CLZ
SUL
1.25
2.5
5
10
25
50
75
100
DOSE (mg/kg)

FIG. 33 Dose-response curves for the increase in DOPAC in the tuberculum olfactorium one hour following the i.p. administration of chlorpromazine (CPZ), metoclopramide (MET) and clozapine (CLZ) and four hours following the i.p. administration of sulpiride. Each point represents the mean ± SE of 11 saline-treated rats (0.77 µg/g ± 0.05).


2.5
2.0
1.5
1.0
.5
DOPAC (µg/g)
1
5
10
25
50
100
DOSE (mg/kg)
CPZ
MET
CLZ
SUL

DISCUSSION

Regional Selectivity

The concept of regional selectivity is one of considerable importance in that it may have a direct bearing on the pathophysiology of schizophrenia. Several investigators have proposed that antipsychotic drugs owe their clinical efficacy to their action in the meso-limbic dopaminergic structures in the brain (Andén and Stock, 1973; Andén, 1972; Bowers and Rozitis, 1974; Matthysse, 1973). It has also been suggested that many of the adverse side effects seen with these compounds are due to their dopaminergic blocking properties in the striatum (van Rossum, 1967; Crow et al., 1976). The reasoning for the latter point is derived in part from the findings in patients with Parkinson's disease. It has been shown that patients with Parkinson's disease suffer a loss of DA typically in the nigro-striatal tract (Hornykiewicz, 1966; Lloyd et al., 1975). It has been further shown that patients treated with antipsychotic compounds frequently develop a pseudo-Parkinsonism in the course of their treatment (Alpert et al., 1978). Antipsychotic drugs are believed to act by blocking dopaminergic receptors in various areas of the brain (Andén and Stock, 1973; Wiesel and Sedvall, 1975). Therefore, because Parkinsonian patients suffer from a known DA deficiency

and because antipsychotic compounds are thought to create a functional DA deficit presumably by blocking dopaminergic receptors, the similarity of symptoms brought about by both conditions is noteworthy.

In an early study Andén and Stock (1973) reported that haloperidol, an antipsychotic compound producing a high incidence of extrapyramidal symptoms, caused a greater percent increase in DA turnover in the striatum as opposed to the limbic area. In contrast to haloperidol, clozapine, a drug with proven antipsychotic efficacy and virtually free of extrapyramidal symptoms (Matz et al., 1974), induced a greater increase in DA turnover in the limbic area vs. the striatum (Andén and Stock, 1973). This was the first report to offer experimental support for the theory of regional selectivity.

Wilk et al. (1975b) conducted a similar experiment where the effects of clozapine and haloperidol on DA turnover were determined for the TO (a meso-limbic structure) as well as the striatum. They reported that with the exception of duration of action, both compounds responded similarly and had a more profound effect on DA turnover in the striatum as opposed to the TO. The authors concluded that their results did not offer any support for the theory of regional selectivity as proposed by Andén and Stock (1973).

The differences noted between the findings of Andén and Stock and Wilk et al. led us to speculate as to their

possible cause. Although Wilk et al. (1975b) had failed to show a regionally selective response in the direction that the theory of regional selectivity would predict, we thought a more detailed examination of regional selectivity might yield more information.

We chose to study regional selectivity in a number of ways. First we studied the effects of chronic haloperidol and clozapine in both the striatum and TO; second, we developed a gas chromatographic method for the determination of the levels of clozapine in the TO and striatum; third, we looked at the effects on DA turnover of a series of compounds that had been shown to be active in a variety of preclinical tests, yet they were found to be inactive in the clinic; and fourth, we measured the changes in DA turnover caused by sulpiride, a clinically efficacious compound with a low incidence of extrapyramidal side effects, and metoclopramide, a drug reported to lack antipsychotic activity and to have a high incidence of extrapyramidal side effects.

The majority of experiments examining the effects of antipsychotic drugs on dopaminergic systems are done on an acute basis. Because antipsychotic drugs are almost always given on a chronic basis, we thought it would be of interest to see if the changes in DA turnover brought about by acute administration of these compounds would be modified by chronic treatment. In man, extrapyramidal side effects are usually seen

early in treatment and they frequently subside spontaneously with repeated administration (Goodman and Gillman, 1975). In contrast to the side-effects brought about by neuroleptics, the antipsychotic effects of these compounds usually require two to three weeks of treatment before an adequate clinical response is seen (National Institute of Mental Health, 1964). Thus, if tolerance occurs only to the extrapyramidal effects with this class of drugs, the theory of regional selectivity would predict that tolerance would occur only in the striatum. Chronic neuroleptic treatment should not modify the response in the limbic area.

We chose to examine the effects of chronic treatment with either haloperidol or clozapine on DA turnover in the striatum and TO. The doses chosen for this study were based on the findings of Wilk et al. (1975b) in their acute experiment. We found that a maximal dose of haloperidol, administered chronically, caused a significant decrease in DA turnover relative to that seen acutely in both the striatum and TO. An ED-50 dose of haloperidol failed to modify DA turnover from that which is seen following acute treatment. Neither a maximal nor ED-50 dose of clozapine produced a significant change in DA turnover as compared with the same doses given acutely. We believe the reason clozapine did not decrease DOPAC levels after chronic

treatment may be related, in part, to the fact that we were unable to use a dose higher than 40 mg/kg because of its toxicity and therefore were unable to maintain concentrations sufficient for the development of tolerance. For example, we found that when 60 mg/kg of clozapine was administered on a chronic basis, the attrition rate was 100% within two days for two separate groups of eight rats each. Similar difficulties regarding the toxicity of clozapine in rats have also been noted by Smith and Davis (1976) and P. Waldmeier (personal communication). The mechanism underlying the tolerance to the increase in DA-turnover seen following chronic haloperidol is not known. Some possible effects which might account for the tolerance seen include: an actual reduction in drug concentration at its site of action due to an enhanced metabolism; a compensatory readjustment of the rate of DA-turnover; or a change in receptor sensitivity or proliferation following prolonged drug exposure. A more general cause for the development of tolerance seen following chronic haloperidol and not seen following chronic clozapine might be related to the dose and duration of action of both compounds. With regard to the duration of action of both compounds, Wilk et al (1975b) have previously noted that four hours after an injection of an ED-50 dose of haloperidol, the levels of DOPAC and HVA were still significantly elevated while four hours

after an ED-50 dose of clozapine, they had returned to normal or control levels. If equipotent doses (i.e., ED-50) of haloperidol and clozapine have different durations of action and the one having the longest duration of action (haloperidol) is the compound we have found capable of causing tolerance, it is likely that the persistence of a drug effect is a requirement for the development of tolerance. A similar conclusion was reached by Waldmeier and Maitre (1976) and is discussed below. Therefore, it appears that dose is an important factor in the establishment of tolerance. An ED-50 dose of haloperidol may have an insufficient duration of action relative to the concentration achieved when a maximal dose is used.

While acute studies have failed to provide a definite answer regarding regional selectivity, chronic studies have yielded equally mixed results. For example, in one of the earliest studies of regional selectivity and chronic treatment, Bowers and Rozitis (1974) measured the effect of 21 days of neuroleptic treatment on HVA levels in both the limbic and striatal regions. They reported that this treatment resulted in a significant decrease in striatal HVA from levels seen acutely at the same dose. The limbic system, however, showed no such effect as the acute and chronic HVA values did not vary significantly. Bowers and Rozitis believed their data suggested that the striatum of

the rabbit responds to chronic antipsychotic drug administration differently from limbic forebrain. Further they indicated that their results add another treatment parameter whereby regional differences in dopamine metabolism after antipsychotic drug treatment may be observed. However, in a subsequent study which was designed to retest the regionally selective effect noted after chronic neuroleptic treatment, Bowers and Rozitis (1976) were unable to replicate their previous findings. Thus, their earlier findings are in disagreement with the results of their later study which found a significant decrease in HVA in both the striatum and limbic area following chronic administration of antipsychotic drugs.

Findings similar to ours regarding the lack of tolerance following chronic clozapine administration were reported by Bürki et al. (1974). They reported that when rats received 80 mg/kg p.o. of clozapine for seven days, their HVA levels were not significantly lower than those rats receiving the same dose of clozapine acutely. However, they did report that tolerance to the increase in HVA, following chronic treatment with 3 mg/kg p.o. of haloperidol, did occur.

Waldmeier and Maitre (1976), however, reported the development of tolerance in rats treated with clozapine or haloperidol as measured by a significant reduction in the levels of DOPAC and HVA from those seen following acute

treatment. Because clozapine has been found to have a short duration of action relative to haloperidol (Wilk et al., 1975b), Waldmeier and Maitre reasoned that they would require a dose of clozapine that would exert an effect that would last for 24 hours. It is for this reason the authors decided to use the relatively high dose of 100 mg/kg p.o. clozapine. They concluded that because tolerance was seen with clozapine, as well as with classical neuroleptics such as haloperidol, that clozapine's effects on dopaminergic systems does not appear to be qualitatively different from those of classical antipsychotic drugs. However, we have been informed (P. Waldmeier, personal communication) that the animals in the clozapine group were in very poor health by the end of this study which may have influenced the biochemical findings.

Scatton (1977) recently investigated the development of tolerance to the increase in DA turnover following repeated neuroleptic treatment. He chose haloperidol and sulpiride as examples of classical and atypical antipsychotic compounds, respectively. He compared the acute and chronic effects of both compounds in the striatum, TO, NA and frontal cortex. While Scatton found that tolerance to the effect of both compounds developed in all of the four areas studied, he reported that the dose of neuroleptic required to produce tolerance was lower in the striatum than in the mesolimbic

areas. We found that tolerance did not occur preferentially in either the striatum or TO and in this respect our results are in partial disagreement.

As previously indicated, the effect of antipsychotic drugs on dopaminergic systems in the brain has been the subject of numerous investigations. Almost all of these studies have related the drug effect to the dose administered. However, by relating the drug-induced change to the actual drug concentration in the specific brain region of interest rather than to the dose, problems introduced by the blood-brain barrier, metabolism or by possible differential distribution within the brain itself can be avoided. For example, Wilk et al. (1975b) previously reported that the rat striatum was more sensitive than the TO to the DA metabolite elevating properties of clozapine. This finding could be interpreted as due to intrinsic regional differences or merely due to a difference in drug distribution.

In experiment 2, clozapine was quantitated in discrete brain regions by gas chromatography. We found that the increase in DOPAC produced by clozapine was related to the clozapine concentration in both the striatum and the TO by assuming that the system obeys Michaelis-Menten kinetics (Fig. 13). The dose-response curves are similar to those previously obtained by Wilk et al. (1975b) where the percent increase in DOPAC was plotted against the dose administered and the animals

were killed at a later time (2 hours after drug administration). Although the ED-50's of the curves shown in Fig. 13 appear similar, the maximal response in the striatum clearly exceeds that of the TO. The data presented in Table 1 demonstrate that following 10, 20 or 40 mg/kg of clozapine, its distribution is similar in striatum and TO. Therefore, regional differences in response to clozapine cannot be ascribed to differences in drug distribution. Only at the highest dose administered was a difference in drug distribution observed. Following 60 mg/kg the concentration of clozapine was greater in the TO than in the striatum. This may represent saturation of a transport system or saturation of drug metabolizing enzymes in the TO at this high dose.

The measurement of drug concentration in striatal and limbic tissues presented itself as a means whereby regionally different responses in DA turnover might be accounted for. The rather high concentrations of clozapine required to produce a half-maximal increase in DOPAC (40 nmoles/g) is consistent with the relatively high doses of this drug which are necessary in <u>in vivo</u> studies to obtain an increase in DA turnover. Thus we have previously found ED-50's for striatal DOPAC elevation of 18 mg/kg for clozapine, 3 mg/kg for chlorpromazine and 0.15 mg/kg for haloperidol (experiment 3). This separation of doses compares favorably to the brain concentrations of these drugs. Thus, Wiesel and

Alfredsson (1976) report a brain level of about 9 nmoles/g for chlorpromazine 1 hour following 7.5 mg/kg of this drug, and Öhman et al. (1977) report a brain level of about 0.7 nmoles/g haloperidol 1 hour after 0.25 mg/kg of this drug. Our studies therefore demonstrate that clozapine penetrates the brain as well as chlorpromazine and haloperidol. Analysis of clozapine concentrations in brain indicate that clozapine is indeed much less potent than chlorpromazine in elevating DOPAC levels. Since the two drugs have similar clinical potencies, these findings are at variance with the DA hypothesis of schizophrenia. Bürki et al. (1975a) have concluded that clozapine does not block DA receptors at pharmacologically relevant doses. However, Wiesel and Sedvall (1975) have indicated that the data on clinical potencies may be misleading. Although clozapine and chlorpromazine are employed at similar doses, the plasma levels of clozapine appear to be much higher than those of chlorpromazine. It is therefore possible that in this particular instance plasma levels correlate well with data derived from animal studies. Certainly additional studies on the plasma levels of these two drugs at therapeutically active doses are required to resolve this issue. The concentration of DOPAC appears to be better correlated to the concentration of clozapine in brain than to the dose of drug injected. Thus, we have observed that apparently anomalous DOPAC levels

could be accounted for by the concentration of drug in brain. This suggests that more precise in vivo data can be obtained by using drug concentrations in brain rather than doses injected to construct dose-response curves.

A study by Wiesel et al. (1975) demonstrated that chronic treatment with chlorpromazine produced a form of pharmacodynamic tolerance to the elevation of homovanillic acid (HVA) evoked by this drug. Thus a higher brain concentration of chlorpromazine was required in chronic studies than in acute studies to produce a given elevation in HVA. We treated rats chronically with 20 mg/kg and 40 mg/kg clozapine (experiment 1). Both the elevation in DOPAC and the concentration of clozapine were similar to that found in rats receiving only a single injection of clozapine (Table 2). Therefore, a pharmacodynamic tolerance to clozapine was not observed. These findings are consistent with our results in our first experiment, where dose injected was compared to DOPAC levels.

It is of interest that the concentration of clozapine in striatum which produced a half maximal elevation of DOPAC (40 μM) is much greater than the IC-50 of clozapine in antagonizing the DA-stimulated adenylate cyclase (3.5 μM) (Miller et al., 1974) or the Ki for inhibiting the binding of ^{3}H-haloperidol to calf striatal membranes (0.1 μM) (Creese et al., 1976). Since antipsychotic drug concentrations are reported not to be greater in DA-rich areas of

the brain after pharmacologically relevant doses (Wiesel et al., 1975; Öhman et al., 1977), lipid solubility probably plays the major role in determination of brain levels. The concentration of drug at the receptor biophase is perhaps more clearly reflected by the in vitro studies.

In experiment 3 we considered another method whereby the concept of regional selectivity could be tested. We were interested in testing compounds known to have a preclinical antipsychotic profile in screening tests, yet were found to be inactive in the clinic. Our reasoning was that if antipsychotic efficacy is related to DA receptor blockade in limbic areas, these non-antipsychotic compounds should have no effect on DA metabolism in the TO. However, because the same compounds did display activity in a variety of preclinical screening tests (Table 3), we considered that it might be possible to demonstrate a regionally selective effect in the striatum, i.e., a change in DA turnover in the striatum with no change in the TO. This might be particularly true for compounds reported as active in such tests as catalepsy which some feel is directly related to the production of extrapyramidal side effects in man (Hill and Tedeschi, 1971).

Because the non-antipsychotic compounds used in this study did yield positive results in no fewer than five separate screening tests, we had anticipated an effect on DA turnover in the striatum. However, we were unable to demonstrate

any selective effect on DA metabolism. Although one of the non-antipsychotic drugs (AHR-1900) did cause a significant increase in DA metabolites, without altering the concentration of DA, its dose-response curve was atypically flat when compared to the curves of haloperidol, chlorpromazine and clozapine (Figs. 15 and 16). Moreover, DOPAC levels at 40 mg/kg, the maximally effective dose tested for AHR-1900, were still lower than those resulting from the administration of the ED-50 dose of haloperidol, 0.15 mg/kg. The response of AHR-1900 is also uncharacteristic for a drug belonging to the potent butyrophenone class. These findings lead us to conclude that AHR-1900 is probably affecting DA metabolism by a mechanism different from drugs with proven antipsychotic efficacy.

Other investigators had measured the effects of non-antipsychotic compounds (such as promethazine) in behavioral (Randrup et al., 1963), electrophysiological (Bunney and Aghajanian, 1975a) and biochemical systems (Carlsson and Lindqvist, 1963; Nybäck, 1972). However, unlike the compounds we chose to study, none of the compounds used in their studies had been reported to have any behavioral, electrophysiological or biochemical activity of a similar nature to known antipsychotics. Therefore, it would be likely to assume or expect their failure when they were compared with neuroleptics in these various tests.

In experiment 7 the concept of regional selectivity was further explored.

Metoclopramide and sulpiride are structurally related to the antiarrhythmic compound procainamide (Fig. 29). Sulpiride has been shown to be an effective antipsychotic with a low incidence of EPS (Mielke et al., 1977a; 1977b). However, unlike most antipsychotics, sulpiride has been reported to be non-cataleptic and ineffective in antagonizing apomorphine- and amphetamine-induced behaviors (Tagliamonte et al. 1975; Honda et al., 1977). In contrast to sulpiride, metoclopramide has been found to cause catalepsy (Costall and Naylor, 1973; Worms and Lloyd, 1978) and effectively antagonize apomorphine and amphetamine stereotypies (Hackman et al., 1973; Janssen et al., 1967; Dolphin et al., 1975). However, in spite of this classical neuroleptic profile, metoclopramide is regarded as being devoid of antipsychotic (Borenstein and Bles, 1965) and tranquilizing properties (Nakra et al., 1975). According to their preclinical and clinical profiles, metoclopramide and sulpiride should be at opposite ends of the regional selectivity spectrum. That is, we would anticipate great disparities between striatal and tubercular ED-50's for both compounds. Metoclopramide would be expected to be more potent in the striatum than in the TO, while the opposite results would be expected for sulpiride.

The results of our study show that both compounds produce a dose-dependent increase in DOPAC levels in both the TO and the striatum. While sulpiride did cause a supramaximal increase in DOPAC accumulation in the TO, the ED-50's for both areas were virtually identical (approximately 60 mg/kg). Metoclopramide's effect on DA turnover in both the striatum and TO was practically indistinguishable from that of chlorpromazine. As with sulpiride, we found metoclopramide to be of equal potency in both areas (i.e., ED-50 in striatum was equal to ED-50 in TO).

The concept of regional selectivity has been the subject of numerous investigations (Andén, 1974; Wilk et al., 1975b). Although experiments designed to test this theory have ranged from acute and chronic to *in vivo* and *in vitro*, no definitive answer has been reached.

Some of the *in vivo* DA turnover studies have reported results which offer support for the concept of regional selectivity were performed using multiple drugs, but single doses and times (Andén and Stock, 1973; Crow et al., 1976; Bowers and Rozitis, 1974). It has previously been pointed out by Wilk et al (1975b) that apparent differential sensitivities can sometimes be explained by differences in onset and duration of action. In this regard, it is of interest to note that in those studies where careful dose-response investigations were carried out, no support for the theory

of regional selectivity was found (Wilk et al., 1975b; Wiesel and Sedvall, 1975; Stawarz et al., 1975).

The in vitro DA receptor studies have provided no support for regionally selective drug effects. Similar potencies have been noted in both striatal and meso-limbic preparations for the DA-stimulated adenylate cyclase (Clement-Cormier et al., 1974) and the radioreceptor assays (Creese et al., 1976).

In summary, our results do not offer support for the theory of regional selectivity. The chronic administration of neuroleptics did not result in the differential development of tolerance in a meso-limbic structure as opposed to the striatum. A study of drug concentrations in brain versus increased DA turnover also failed to reveal regionally selective differences. Clinically inactive compounds failed to produce characteristic effects on DA turnover in either the striatum or the TO in spite of their active preclinical antipsychotic profile. Finally, the potency of sulpiride in increasing DA turnover did not differ from TO to striatum, although it is reported to be an antipsychotic drug with a low incidence of extrapyramidal side effects. Metoclopramide was also found to be equipotent in both anatomical regions studied in spite of claims that it is devoid of antipsychotic activity and produces a high incidence of extrapyramidal side effects.

Predictability in Psychopharmacology

Since their introduction, antipsychotic drugs have been shown to have a number of pharmacological actions. Many of these actions have been used by a number of investigators to classify and identify currently used and potentially useful antipsychotic compounds. An ideal animal testing procedure should be designed so as to evaluate both the potential of a neuroleptic to have a favorable clinical profile as well as its liability to produce side effects. The ability of a particular animal test to predict potential therapeutic and side effects will depend to a large extent on the sensitivity and selectivity of the test. The relative potencies of known antipsychotics in a given test should also be considered. Thus a useful screening test should have a reasonable amount of sensitivity so that proven antipsychotics are detected at a reasonable dose range (i.e., test doses should ideally parallel doses used in the clinic). Sensitivity alone is not sufficient for determining neuroleptic potential. A test which lacks sensitivity will tend to be less selective, since large doses of neuroleptics will exert influences on organ systems not related to neuroleptic activity.

It should be recognized, therefore, that selectivity is as important as sensitivity in animal screening tests.

If a screening test fails to discriminate between antipsychotic and non-antipsychotic compounds, its predictive utility is severely compromised.

The relative potencies of proven antipsychotic drugs in a particular test serve as a useful indicator whereby the potency of new drugs can be meaningfully assessed. Not only does such a test serve to predict relative clinical potencies, but it can also be important when therapeutic indices are being considered. Only when the relative potencies of both therapeutic and toxic effects are parallel in animals and man can a valid therapeutic index be determined.

The results of our initial experiments suggested to us that antipsychotic drugs might display a characteristic effect on DA turnover, as reflected by DOPAC accumulation. Thus, when we compared the effects of known antipsychotic compounds on DA turnover with those of clinically inactive compounds, we found that the dose-response curves of the former to be quite similar (differing only in potency). On the other hand, the effect the non-antipsychotic compounds exerted on DA turnover appeared to be clearly different from the antipsychotics. In fact only one compound, AHR-1900, demonstrated an appreciable effect on DOPAC levels. As previously noted, AHR-1900's dose-response curve was atypically flat in comparison to those of haloperidol,

chlorpromazine and clozapine. Aside from its shallow slope, relative to those of the antipsychotics, AHR-1900 had a low maximal response. The maximal elevation in DOPAC levels brought about by the highest dose of AHR-1900 (40 mg/kg) was less than the increase induced by the ED-50 dose of haloperidol (0.15 mg/kg). Thus, the lack of potency for AHR-1900 in elevating DOPAC levels (i.e., a dose 250 times greater than that of haloperidol is required to achieve a similar elevation in DOPAC) is not consistent with the fact that it is a member of the potent butyrophenone class.

Subsequent to our studies of the non-antipsychotic compounds, we examined the effects of morphine and oxotremorine on DA turnover. Although both compounds had been reported to elevate DA metabolites in the rat brain (Kuschinsky and Hornykiewicz, 1974; Nose and Takemoto, 1974; Westerink and Korf, 1976a), we wondered if complete time action and dose-response effects would differentiate these compounds from those with proven antipsychotic activity. The results of this experiment indicate the effects of morphine and oxotremorine to be clearly different from those of haloperidol, chlorpromazine and clozapine. The only exceptional finding in this study could be attributed to morphine. Morphine's effect of DA metabolism in the TO closely resembled the effects seen following typical neuroleptic

compounds. This latter finding is of particular interest in that it has been suggested that the limbic area may be the site involved in the antipsychotic action of drugs which are used in the treatment of schizophrenia (Andén and Stock, 1973; Matthysse, 1973; Andén, 1974).

Andén and Stock (1973) and Carlsson (1978) have suggested that the greater the response difference between striatal and mesolimbic structures (i.e., striatal DOPAC > TO DOPAC) the greater the propensity of a drug to cause extrapyramidal symptoms. Thus, Carlsson points out that clozapine and thioridazine, drugs with a reported low incidence of extrapyramidal side effects, exhibited less of a response difference between these structures, whereas haloperidol and chlorpromazine, compounds with a reported high and moderate incidence of extrapyramidal symptoms respectively, show greater differences in DA turnover in these areas. Therefore, the results of our experiment are particularly striking in that morphine has long been known as a compound that induces profound catalepsy in animals (Kuschinsky and Hornykiewicz, 1972), a prototypical behavioral indicator of extrapyramidal side effects (Hill and Tedeschi, 1971). Kuschinsky and Hornykiewicz (1972) have reported that a dose of 10 mg/kg of morphine causes catalepsy in 100% of all animals tested.

The results of this experiment which show morphine to have a more characteristic effect on DA turnover in the TO than in the striatum are also at odds with the concept of regional selectivity in two respects. Firstly, morphine has not been shown to have a spectrum of action similar to that of known antipsychotic compounds. In a clinical trial where morphine was used to treat schizophrenic patients, it was shown to be without antipsychotic activity (Wikler et al., 1952). A second point in conflict with the concept of regional selectivity has to do with morphine's effect in the striata. The induction of catalepsy has most frequently been ascribed to the blockade of dopaminergic receptors in the striatum and yet our results indicate the principal effect of morphine is in the TO. To summarize these points we find morphine to be a non-antipsychotic compound that produces marked catalepsy in animals and exerts its main influence on DA metabolism in a limbic structure rather than the striatum.

The mechanism of action by which morphine increases DOPAC and HVA was initially thought to be similar to that of neuroleptics. However, a number of differences have been noted. Morphine-induced catalepsy is antagonized by apomorphine or l-Dopa whereas chlorpromazine-induced catalepsy is not (Kuschinsky and Hornykiewicz, 1972). The increase in HVA seen following morphine administration is

antagonized by naloxone, while the chlorpromazine-induced increase in HVA is not (Kuschinsky and Hornykiewicz, 1972). Similar results have also been reported for the narcotic fentanyl and the neuroleptic haloperidol (Freye and Kuschinsky, 1976). These findings suggest that narcotics and neuroleptics act at different sites in the rat brain.

More direct evidence of distinct sites of action of narcotics and neuroleptics come from studies on DA-stimulated adenylate cyclase and from studies on the neuroleptic and opiate receptors. Morphine's in vitro effects in the DA-stimulated adenylate cyclase model have yielded conflicting results. Morphine has been reported to stimulate basal activity by some investigators (Puri et al., 1975) and to have no consistent effect on basal activity as reported by others (van Inwegen et al., 1975). Iwatsubo and Clouet (1975) found that 3 to 3000 μM of morphine did not block DA-stimulation while only 3 μM of haloperidol totally blocked DA-stimulation.

Morphine's effect on the DA-stimulated adenylate cyclase in vivo has also been of an equally conflicting nature. Van Inwegen et al. (1975) found that morphine had no effect on basal or DA-stimulated adenylate cyclase in naive, addicted or withdrawn rats. By contrast, Clouet et al. (1975) found acute treatment with morphine to produce an increased adenylate cyclase activity. Iwatsubo and Clouet (1975) found no change in basal adenylate cyclase in morphine-dependent rats whereas

both Merali et al. (1975) and Puri et al. (1976) reported an increase. To further confound the issue, Iwatsubo and Clouet (1975) found chronic treatment with morphine to increase the stimulation of adenylate cyclase by DA whereas Puri et al. (1976) found this treatment to result in a decrease in the DA-stimulation. Thus, while it appears that the effects of morphine on the DA-stimulated adenylate cyclase are inconclusive, the effects of neuroleptics in this system can be clearly distinguished by exhibiting such properties as high potency and stereospecificity.

More consistent results on differences between narcotics and neuroleptics come from studies on the non-adenylate cyclase linked DA receptor model and the opiate receptor model. Stereospecific binding sites have been identified in brain for both opiates and neuroleptics (Goldstein et al., 1971; Pert and Snyder, 1973; Creese et al., 1976; Seeman et al., 1976). It is of interest to note that the highest concentration of both opiate and neuroleptic binding sites occur in the striatum (Pert and Snyder, 1973; Creese et al., 1975). However, in spite of their regional proximity, they can be clearly differentiated. For example, (+) and (-) butaclamol display no stereospecific effect with regard to opiate binding whereas the pharmacologically active (+) isomer exhibits a 1,000 fold greater potency in displacing ^{3}H-haloperidol from its binding site than the inactive (-) isomer (Enna et al., 1976).

In general, the effects of both morphine and oxotremorine on DA metabolism do not appear similar to the effects of antipsychotic drugs which are known to block DA-receptors, but rather they cause a non-specific elevation of DA metabolites. A number of investigators have shown that both morphine and oxotremorine cause an increase in DA metabolites (Kuschinsky and Hornykiewicz, 1974; Nose and Takemoto, 1974; Westerink and Korf, 1976a). However, when the dose-response curves of these compounds are compared to those of drugs with known antipsychotic efficacy, differences in magnitude and shape (Fig. 21) of their response becomes apparent.

In another study we decided to examine the effects of perlapine, a sedative and sleep-promoting compound that has been reported to lack antipsychotic efficacy (Wander, Ltd., personal communication). This drug has been shown to be much less active than chlorpromazine in disrupting conditioned avoidance behavior in rats (Take et al., 1970), but is more potent than thioridazine in inducing catalepsy in the rat (Bürki et al., 1975b). Perlapine has also been shown to increase DA metabolites in rat striata without altering the levels of DA itself (Bürki et al., 1975b). Perlapine has also been shown to produce a dose-dependent increase in serum prolactin levels (Meltzer et al., 1977b).

Because our previous studies indicated that antipsychotic drugs display a characteristic effect on DA metabolism, we thought this response might be useful in predicting antipsychotic efficacy.

We compared the effect of perlapine in this system with three representatives of structurally distinct classes of antipsychotic drugs: a butyrophenone (haloperidol), a phenothiazine (chlorpromazine) and a dibenzodiazepine (clozapine). Perlapine was shown to produce a dose-dependent increase in the levels of DOPAC (and HVA) in striatum and TO similar to the antipsychotics. Perlapine apparently does not elevate DA metabolites by a reserpine-like effect since DA levels are not lowered (Table 6). It is also unlikely that metabolite levels are elevated by a probenecid-like effect. As previously mentioned, probenecid does not increase DOPAC in either striatum or TO and the maximal increase in HVA is much less than is seen with perlapine or other neuroleptics (Wilk et al., 1975c).

Moreover, perlapine has been reported not to elevate whole brain 5-hydroxyindoleacetic acid levels (Bürki et al., 1975b). One hour after administration of either haloperidol, chlorpromazine or clozapine the percent elevation in striatal DOPAC approximated that of HVA. This was also observed with perlapine (Table 5).

The time-action effect of perlapine in the striatum and TO was virtually identical to the antipsychotic clozapine. It therefore appears likely that all four drugs increase DA metabolite levels by the same mechanism. The increase in DA metabolites in view of the unchanged level of DA is indicative of an increased turnover of DA. The increased turnover of DA produced by neuroleptics is believed to be secondary to receptor blockade (Carlsson and Lindqvist, 1963). It therefore seems reasonable to conclude that perlapine blocks DA receptors in the striatum and TO.

On the basis of unpublished clinical trials in three psychiatric hospitals, perlapine was judged to exhibit practically no antipsychotic activity (Wander, Ltd., Berne, personal communication). If these studies could be confirmed, the hypothesized relationship between DA and antipsychotic drugs would be seriously compromised. It is, however, possible that the marked sedative properties of perlapine may have complicated the clinical trials. On the basis of our biochemical data, we feel that perlapine should be re-evaluated clinically at dose levels similar to chlorpromazine. We predict that these studies will demonstrate that perlapine posseses antipsychotic efficacy.

Subsequent to our animal studies we were informed that a clinical reevaluation of perlapine would be impossible as it had been found to have a high incidence of hepatotoxicity (S. Gershon, personal communication). In our search for a compound to test in our predictive system, we decided to examine the effects of thiethylperazine on DA turnover. Thiethylperazine is a member of the phenothiazine class and is currently marketed as an antiemetic. This compound has been shown to be more potent than chlorpromazine in elevating CSF HVA levels in monkeys (Matthysse, 1973), in antagonizing stereotypies in rats (Janssen et al., 1967), and in stimulating prolactin release in rats (Clemens et al., 1974). In spite of this seemingly classical preclinical profile, thiethylperazine has come to be regarded as being devoid of antipsychotic activity (Matthysse, 1973; Clemens et al., 1974; Karobath and Leitch, 1974; De Jaramillo and Guth, 1963).

Drs. Rotrosen, Angrist and Gershon of New York University felt that a reevaluation of thiethylperazine was in order. They obtained approval to conduct this study in schizophrenic patients admitted to their clinical research ward in Bellevue Psychiatric Hospital. All participants in the study had diagnoses of "definite" schizophrenia by the criteria of Spitzer et al. (1977). Eight of ten patients showed a marked therapeutic response with complete or nearly complete remission of psychotic symptomatology. One patient showed

minimal improvement and one showed no change. The clinical potency of thiethylperazine was judged to be approximately three times greater than chlorpromazine and twenty to fifty times less potent than haloperidol (Table 7).

We found thiethylperazine's effect on the accumulation of striatal DOPAC to be comparable to that of haloperidol and chlorpromazine (Fig. 28). We determined thiethylperazine to have a potency three times greater than chlorpromazine and approximately twenty times less than that of haloperidol. As shown in Table 7, the relative potencies we determined are in close agreement with clinical findings.

In experiment 7 we investigated the effects of metoclopramide and sulpiride on DOPAC accumulation in the TO and striatum. Both metoclopramide and sulpiride were of interest to us in that they have been reported to display what appear to be anomolous properties when their animal pharmacology is compared with their effects in the clinic. Classical antipsychotic drugs, such as chlorpromazine and haloperidol, elevate serum prolactin, inhibit apomorphine and amphetamine stereotypies, increase DA turnover and cause catalepsy (Goodman and Gillman, 1975). Sulpiride has been shown to be an effective antipsychotic with a low incidence of extrapyramidal side effects (Mielke, 1977a; 1977b). However, unlike most antipsychotic drugs, sulpiride has been reported to be non-cataleptic and ineffective in antagonizing apomorphine- and

amphetamine-induced stereotypies (Tagliamonte et al., 1975; Honda et al., 1977). In contrast to sulpiride, metoclopramide has been found to cause catalepsy (Costall and Naylor, 1973; Worms and Lloyd, 1978) and effectively antagonize apomorphine and amphetamine stereotypies (Hackman et al., 1973; Janssen et al., 1967; Dolphin et al., 1975). However, in spite of this classical profile, metoclopramide is regarded as being devoid of antipsychotic (Borenstein and Bles, 1965) and tranquilizing properties (Nakra et al., 1975). Some of the common properties shared by sulpiride and metoclopramide include their ability to elevate serum prolactin levels in man (Healy and Burger, 1978; Mielke et al., 1977b) and animals (Clemens et al., 1974; Yamauchi et al., 1977), increase DA turnover (Scatton, 1977; Peringer et al., 1976) and an inability to antagonize the DA-stimulated adenylate cyclase (Peringer et al., 1976; Roufogalis et al., 1976) as well as a relative inactivity in displacing ^{3}H-spiroperidol from striatal binding sites (Howard et al., 1978).

As previously mentioned, metoclopramide has come to be known as a compound devoid of antipsychotic activity and is frequently referred to as such in the literature (Borenstein and Bles, 1965; Nakra et al., 1975). We believe it is important to examine the findings of the studies which have contributed to the belief that metoclopramide has no antipsychotic efficacy.

The only published study we know of that was conducted in a psychiatric population was carried out by Borenstein and Bles in 1965. In their study, metoclopramide was given to eight schizophrenic patients for approximately three weeks. Each patient received 40 mg/day of metoclopramide for the first twelve days, and then 100 mg/day. At this dose, two out of eight patients showed some improvement by the time the study was completed. We feel the dose used in the study of Borenstein and Bles was much less than we would anticipate one to need to treat schizophrenia. Based on our findings, we would expect metoclopramide to have a clinical potency, similar to chlorpromazine (200-800 ng/day). This dose-range is based on a comparison of the ED-50's (that dose which is required to cause a half-maximal increase in striatal DOPAC) of chlorpromazine and metoclopramide. Other behavioral studies, such as the antagonism of apomorphine-induced stereotypy and catalepsy, also indicate metoclopramide to have a potency similar to that of chlorpromazine (Worms and Lloyd, 1978). Therefore, we do not find it strange that metoclopramide's effect in this study was less than complete. The dose-range employed by Borenstein and Bles was roughly equivalent to that which is recommended for metoclopramide's use as an anti-emetic. An analogous situation can be easily envisioned if, for example, thiethylperazine were to be administered to schizophrenic patients in its recommended anti-emetic dose; 10-30 mgs/day (JAMA, council

on drugs, 1963). The clinically efficacious dose of thiethylperazine in the treatment of schizophrenia is ten times greater (300 mg/day) than its anti-emetic dose (Rotrosen et al., 1978).

A second clinical study, designed to determine whether metoclopramide had tranquilizing properties, was conducted by Nakra et al. (1975). They chose to compare the effects of metoclopramide with those of prochlorperazine (a major tranquilizer) in normal volunteers. Metoclopramide or prochlorperazine were given p.o. and subjects were tested for changes in EEG, pulse rate, blood pressure, pupil size and a number of psychological measures. They reported that metoclopramide had no tranquilizing properties. However, it should also be noted that they found prochlorpromazine to have no tranquilizing properties as well. Prochlorpromazine is a phenothiazine with known antipsychotic and tranquilizing efficacy (Goodman and Gillman, 1975). It would seem, therefore, that if the testing procedures utilized by Nakra et al. (1975), were unable to identify a compound with proven antipsychotic activity, their study should be considered invalid. Our results indicate that metoclopramide should display antipsychotic activity at a dose-range similar to that of chlorpromazine (200-800 mg/day).

It was of interest that sulpiride had an unusually long onset and duration of action. Where we usually find that antipsychotic drugs reach a maximal effect on DA turnover in the vicinity of one or two hours, we found sulpiride to reach its maximal effect between four and six hours. Two possibilities that might account for this anomolous behavior are that sulpiride might be metabolized to an active compound or that its physicochemical properties hinder its entry into the brain. Both possibilities were considered in a study by Honda et al. (1977). They found that when sulpiride was administered i.p., it was relatively ineffective in antagonizing apomorphine- and amphetamine-induced behaviors and was non-cataleptic. However, when sulpiride was administered intraventricularly, it was found to be active in all of the aforementioned tests and its potency was similar to that of haloperidol. Thus, Honda et al. (1977) concluded that their results offered support for the contention that sulpiride has difficulty in gaining access to the brain. Additionally, it has been reported that there have been no known metabolites of sulpiride found in any mammalian species tested (Mielke, 1977b).

The results of our study show that the striatal DOPAC dose-response curves for both metoclopramide and sulpiride are similar to the dose-response curves of known antipsychotic drugs. The low potency and delayed time-action

curve seen following sulpiride administration can probably be attributed to the relatively poor lipophilicity of this compound. Based on these findings, we believe that both sulpiride and metoclopramide should display antipsychotic activity.

Our predictive system is based on the dose-dependent increase in striatal DOPAC caused by neuroleptic drugs. We find that antipsychotic drugs produce dose-response curves that have similar slopes and maxima. By determining a compound's potency in our system, we are able to judge its relative potency in the clinic. We use the dose that produces a half-maximal elevation in striatal DOPAC (the ED-50) for potency comparisons.

As we were developing this predictive system, we began to find instances in which the TO dose-response curves yielded unusual results. For example, we noted supramaximal increases in TO DOPAC levels following treatment with either perlapine or sulpiride. We are, as yet, unable to explain this finding other than to suggest it may reflect differences between TO and striatal metabolite transport systems.

Morphine produced a significant elevation in TO DOPAC levels without causing a clear incremental dose-response effect on striatal DOPAC. While this response is indicative of an increase in DA turnover, it should be noted that

morphine was without antipsychotic activity when tested in schizophrenic patients (Wikler et al., 1952).

Because of the unusual responses we found in TO DA metabolism, we have come to regard drug effects in this region as unreliable for predictive purposes. Our conclusions regarding drug effects in this meso-limbic structure are in agreement with those of Westerink and Korf (1976a). They compared the effects of antipsychotic drugs with such non-antipsychotic compounds as halothane, chlorimipramine, ether, etc. by dividing the percent increase in striatal DOPAC by the percent increase in the TO. They found that when the ratios of antipsychotics were compared with the ratios of non-antipsychotics, no differences could be seen. They concluded that regionally distinct differences in drug response seen in the meso-limbic areas are not specific and therefore may not be related to antipsychotic activity.

On the basis of this work and a thorough examination of the literature, we believe our predictive system provides a relatively high degree of reliability in comparison to other available predictive systems. A number of behavioral methods have been shown to yield false - positive and false - negative results. For example, non-antipsychotic compounds such as morphine, tranylcypromine and LSD yield

false - positive results in conditioned avoidance responding (Cook and Catania, 1964; Cook and Kelleher, 1961). That is, they behave as clinically active antipsychotic compounds. Compounds such as clozapine and sulpiride provide examples of the false - negative variety, as both drugs fail to antagonize amphetamine- or apomorphine-induced stereotypies (Bürki et al., 1975a; Worms and Lloyd, 1978). Thus, while clozapine and sulpiride are both clinically active, they behave as non-antipsychotic compounds in these tests. Morphine is without antipsychotic efficacy (Wikler et al., 1952), and yet, it is a potent inducer of catalepsy (Kuschinsky and Hornykiewicz, 1972), a frequently used test for prediction of antipsychotic potential (Hill and Tedeschi, 1971; Worms and Lloyd, 1978).

The use of the DA-stimulated adenylate cyclase as a predictive system has not been without difficulties in clearly identifying clinically active antipsychotic drugs and inactive compounds. Aside from the generally poor potency for the butyrophenone compounds, a clinically active compound, such as sulpiride, is totally without effect (Roufogalis et al., 1976) in this system.

The ^{3}H-haloperidol binding assay has been proposed as a quick and efficient way in which to predict antipsychotic activity (Creese et al., 1976). However, this

system is not without its problems. Compounds that rely on their metabolism to an active species are not going to be judged useful. In contrast, a compound, such as domperidone, which displaces ^{3}H-haloperidol from its binding sites with a potency similar to haloperidol itself (P. Janssen, personal communication), would likely be called active, and yet in vivo studies have indicated that domperidone does not penetrate the blood-brain barrier (P. Janssen, personal communication). And as might be anticipated, domperidone has also been reported to be without antipsychotic efficacy even at doses up to 300 mg/day (P. Janssen, personal communication). These problems might be considered to be part of all in vitro tests, however, a more significant discrepancy has been noted for this predictive system. Compounds such as perlapine and metoclopramide have been shown to elevate DOPAC levels with a potency similar to that of chlorpromazine (see results of experiments 5 and 7). Metoclopramide has also been reported to be approximately equivalent to chlorpromazine in the antagonism of apomorphine-induced stereotypies and the induction of catalepsy. In spite of their in vivo potency in antidopaminergic tests, both compounds are reported to have little or no binding potency in the ^{3}H-haloperidol

assay (Jenner et al., 1978; Howard et al., 1978). The same lack of potency in displacing ^{3}H-haloperidol was noted for the clinically active sulpiride as well as other substituted benzamides (Jenner et al., 1978). It would seem, therefore, that while the in vivo antidopaminergic potency and behavior of these compounds is similar to known antipsychotics, it is most unlikely that they would have been chosen as potentially useful clinical agents based on their ability to displace ^{3}H-haloperidol.

Antipsychotic drugs have been shown to cause a dose-dependent increase in the circulating levels of prolactin in animals and man (Meltzer et al., 1977b Langer et al., 1977b). Langer et al. (1977b) have used this drug-induced increase in prolactin as a means of detecting clinical potency. It is important to note that the novel antipsychotic drug, clozapine, was omitted from their study for what the authors referred to as "inadequate prolactin-releasing activity"(Langer et al., 1977b). Clozapine has been shown to increase prolactin release in rats (Meltzer et al., 1978), however, its effect in man is weak and transitory (Meltzer et al., 1978). Questions concerning the specificity of this response have also emerged. Prolactin increases have been reported following treatment with such diverse compounds as morphine (Tolis et al., 1975), imipramine, amitriptyline

and α-methyl dopa (Turkington, 1972). Domperidone causes an increase in serum prolactin while failing to enter the brain (P. Janssen, personal communication).

In summary, we have tried to develop a system for the prediction of potentially useful antipsychotic compounds. Our system is based on the dose-dependent increase in striatal DOPAC in the rat. Using this system we have been able to characterize compounds with known antipsychotic efficacy and to predict the antipsychotic activity for three separate drugs (perlapine, thiethylperazine and metoclopramide). One of these compounds, thiethylperazine, has already been reevaluated in the clinic and determined to have antipsychotic activity at the approximate dose-range we had predicted. A clinical trial of metoclopramide in schizophrenic patients, at the dose-range we predicted, is currently in progress. We feel that our predictive system offers the advantages of sensitivity and selectivity over existing systems and has thus far been proven to be valid.

Future research suggested by these studies should include the search for new and atypical antipsychotic compounds that offer either a new spectrum of action or fewer adverse side effects and to determine how they perform in some of the preclinical screening tests. An area that should be investigated further concerns the effects of compounds such as metoclopramide and perlapine and their relative

lack of potency in the brain DA receptor models. It might be useful to determine if it is possible to isolate a different receptor with properties that compare favorably with the in vivo potencies of the atypical as well as typical neuroleptics.

Because a number of studies have indicated that there is a degree of non-specificity involved in the prolactin response, it might be of interest to determine whether differences in drug response among classes of compounds known to elevate prolactin can be found. To determine differences between slopes and maxima requires careful time-action and dose-response studies. Also, with regard to the prolactin response, it may be useful to learn how much of the prolactin released is due to peripheral and central actions. For example, domperidone appears to elevate prolactin without entering the brain. It would be of interest to determine if an equipotent dose of haloperidol would result in a supramaximal response in animals previously treated with domperidone by stimulating prolactin secretion via central mechanisms.

References

Aghajanian, G.K., Bunney, B.S. and Kuhar, M.J. (1973). in: New Concepts in Neurotransmitter Regulation, ed. A.J. Mandel (Plenum Press, New York) p. 115.

Alpert, M., Diamond, F., Weisenfreund, J., Taleporos, E. and Friedhoff, A.J. (1978) The neuroleptic hypothesis: Study of the covariation of extrapyramidal and therapeutic drug effects. Brit. J. Psychiat. 133:169.

Andén, N.E. (1972) Dopamine turnover in the corpus striatum and the limbic system after treatment with neuroleptic and anti-acetylcholine drugs. J. Pharm. Pharmacol. 24:905.

Andén, N.E. (1974) Antipsychotic drugs and catecholamine synapses. J. Psychiat. Res. 11:97.

Andén, N.E., Roos, B.E., and Werdinius, B. (1964) Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. Life Sci. 3:149.

Andén, N.E., Rubenson, A., Fuxe, K., and Hökfelt, T. (1967) Evidence for dopamine receptor stimulation by apomorphine. J. Pharm. Pharmacol. 19:627.

Andén, N.E. and Stock, G. (1973) Effect of clozapine on the turnover of dopamine in the corpus striatum and in the limbic system. J. Pharm. Pharmacol. 25:346.

Angrist, B.M. and Gershon, S. (1970) The phenomenology of experimentally-induced amphetamine psychosis: Preliminary observations. Biol. Psychiat. 2:95.

Angrist, B.M., Sathananthan, G.S. and Gershon, S. (1973) Behavioral effects of L-Dopa in schizophrenic patients. Psychopharmacologia 31:1.

Angrist, B.M., Lee, H.K. and Gershon, S. (1974) The antagonism of amphetamine-induced symptomatology by a neuroleptic. Amer. J. Psychiatry 131:817.

Angrist, B.M., Thompson, H., Shopsin, B. and Gershon, S. (1975) Clinical studies with dopamine-receptor stimulants. Psychopharmacologia 44:273.

Axelrod, J. and Tomchick, R. (1958) Enzymatic O-methylation of epinephrine and other catechols. J. Biol. Chem. 233:702.

Barnett, A., Taber, R.I. and Steiner, S.S. (1974) The behavioral pharmacology of SCH-12,679, a new psychoactive agent. Psychopharmacologia 36:281.

Bartholini, G., Keller, H. and Pletscher, A. (1975) Drug-induced changes in dopamine turnover in striatum and limbic system of the rat. J. Pharm. Pharmacol. 27:439.

Bertler, A. and Rosengren, E. (1959) Occurrence and distribution of dopamine in brain and other tissues. Experientia 15:10.

Berzewski, H., Helmchen, H., Hippius, H., Hoffman, H. and Kanowski, S. (1969) Das klinische wirkungsspektrum eines neuen dibenzodiazepin-derivates. Arzneim. Forsch. 19:496.

Blumberg, J.B., Vetulani, J., Stawarz, R.J. and Sulser, F. (1976) The noradrenergic cyclic AMP generating system in the limbic forebrain: Pharmacological characterization in vitro and possible role of limbic noradrenergic mechanisms in the mode of action of antipsychotics. European J. Pharmacol. 37:357.

Borenstein, P. and Bles, G. (1965) Effets cliniques et électroencéphalographiques du métoclopramide en psychiatrie. Thérapie 20:975.

Bowers, M. and Rozitis, A. (1974) Regional differences in homovanillic acid concentrations after acute and chronic administration of antipsychotic drugs. J. Pharm. Pharmacol. 26:743.

Bowers, M. and Rozitis, A. (1976) Brain homovanillic acid: Regional changes over time with antipsychotic drugs. European J. Pharmacol. 39:109.

Bunney, B.S. and Aghajanian, G.K. (1973) Electrophysiological effects of amphetamine on dopaminergic neurons. in: Frontiers in Catecholamine Research, eds. S.H. Snyder and E. Usdin (Pergamon Press, New York) p. 957.

Bunney, B.S. and Aghajanian, G.K. (1974) A comparison of the effects of chlorpromazine, 7-hydroxychlorpromazine and chlorpromazine sulfoxide on the activity of central dopaminergic neurons. Life Sci. 15:309.

Bunney, B.S. and Aghajanian, G.K. (1975a) Antipsychotic drugs and central dopaminergic neurons: A model for predicting therapeutic efficacy and incidence of extrapyramidal side effects. in: Predictability in Psychopharmacology, eds. A. Sudilovsky, S. Gershon and B. Beer (Raven Press, New York) p. 225.

Bunney, B.S. and Aghajanian, G.K. (1975b) in: Pre- and Post-synaptic Receptors, eds. E. Usdin and W.E. Bunney, Jr. (Marcel Dekker, Inc., New York) p. 89.

Bunney, B.S. and Aghajanian, G.K. (1976) d-Amphetamine-induced inhibition of central dopaminergic neurons: Mediation by a striato-nigral feedback pathway. Science 192:391.

Bunney, B.S., Walters, J.R., Roth, R.H. and Aghajanian, G.K. (1973) Dopaminergic neurons: Effect of antipsychotic drugs and amphetamine on single cell activity. J. Pharmacol. Exptl. Therap. 185:560.

Bürki, H.R., Eichenberger, E., Sayers, A.C. and White, T.G. (1975a) Clozapine and the dopamine hypothesis of schizophrenia, a critical appraisal. Pharmakopsychiat. 8:115.

Bürki, H.R., Ruch, W. and Asper, H. (1975b) Effects of clozapine, thioridazine, perlapine and haloperidol on the metabolism of the biogenic amines in the brain of the rat. Psychopharmacologia 41:27.

Bürki, H.R., Ruch, W., Asper, H., Baggiolini, M. and Stille, G. (1974) Effect of single and repeated administration of clozapine on the metabolism of dopamine and noradrenaline in brain of the rat. European J. Pharmacol. 27:180.

Calne, D.B., Stern, G.M., Laurence, D.R., Sharkey, J. and Armitage, P. (1969) L-Dopa in postencephalitic Parkinsonism. Lancet 1:744.

Carlsson, A. (1978) Antipsychotic drugs, neurotransmitters, and schizophrenia. Amer. J. Psychiat. 135:164.

Carlsson, A. and Lindqvist, M. (1963) Effects of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normethanephrine in mouse brain. Acta Pharmacol. Toxicol. 20:140.

Carlsson, A., Lindqvist, M. and Kehr, W. (1974) Postmortal accumulation of 3-methoxytyramine in brain. Naunyn-Schmiedeb, Arch. Pharmacol. 284:365.

Carlsson, A., Lindqvist, M., Magnusson, M. and Waldeck, B. (1958) On the presence of 3-hydroxytyramine in brain. Science 127:471.

Chien, C.P. and DiMascio, A. (1967) Drug-induced extrapyramidal symptoms and their relations to clinical efficacy. Amer. J. Psychiat. 123:1490.

Clemens, J.A., Smalstig, E.G. and Sawyer, B.D. (1974) Antipsychotic drugs stimulate prolactin release. Psychopharmacologia 40:123.

Clement-Cormier, Y.C.C., Kebabian, J.W., Petzold, G.L., and Greengard, P. (1974) Dopamine-sensitive adenylate cyclase in mammalian brain: A possible site of action of antipsychotic drugs. Proc. Nat. Acad. Sci. 71:1113.

Clouet, D.H., Gold, G.J. and Iwatsubo, K. (1975) Effects of narcotic analgesic drugs on the cyclic adenosine 3',5'-monophosphate-adenylate cyclase system in rat brain. Brit. J. Pharmacol. 54:541.

Connell, P.H. (1958) Amphetamine psychosis. Maudsley Monographs (University Press, Oxford) p. 5.

Cook, L. and Catania, A.C. (1964) Effects of drugs on avoidance and escape behavior. Fed. Proc. 23:818.

Cook, L. and Kelleher, R. (1961) Drug effects on the behavior of animals. New York Acad. Sci. 70:315.

Corrodi, H., Fuxe, K. and Hokfelt, T. (1967) The effect of some psychoactive drugs on central monoamine neurons. European J. Pharmacol. 1:363.

Costall, B. and Naylor, R.J. (1973) Is there a relationship between the involvement of extrapyramidal and mesolimbic brain areas with the cataleptic action of neuroleptic agents and their clinical antipsychotic effect? Psychopharmacologia 41:133.

Council on Drugs (1963) Evaluation of thiethylperazine maleate. J. Amer. Med. Assn. 136:167.

Creese, I., Burt, D.R. and Snyder, S.H. (1975) Dopamine receptor binding: Differentiation of agonist and antagonist states with ^{3}H-haloperidol. Life Sci. 17: 993.

Creese, I., Burt, D.R., and Snyder, S.H. (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192:481.

Crow, T.J., Deakin, J.F.W., Johnstone, E.C. and Longden, A. (1976) Dopamine and schizophrenia. Lancet 2:563.

Da Prada, M. and Pletscher, A. (1966a) Acceleration of cerebral dopamine turnover by chlorpromazine. Experientia, 22:465.

Da Prada, M. and Pletscher, A. (1966b) On the mechanism of chlorpromazine-induced changes of cerebral homovanillic acid. J. Pharm. Pharmacol. 18:628.

Davis, J.M. and Janowsky, D.S. (1973) Amphetamine and methylphenidate psychosis. in: Frontiers in Catecholamine Research (Pergamon Press, London) p. 977.

De Jaramillo, G.A.V. and Guth, P.S. (1963) Study of the localization of phenothiazines in dog brain. Biochem. Pharmacol. 12:525.

De Maio, D. (1972) Clozapine, a novel major tranquilizer. Arzneim Forsch. 22:919.

Denham, J. (1961) The implications of extrapyramidal symptoms in treatment of schizophrenia. Revue Cannal. Biol. 20:545.

Dolphin, A., Jenner, P., Marsden, C.D., Pycock, C. and Tarsy, D. (1975) Pharmacological evidence for cerebral dopamine receptor blockade by metoclopramide in rodents. Psychopharmacologia 41:133.

Ellinwood, E.H., Sudilowsky, A. and Nelson, L. (1972) Behavioral analysis of chronic amphetamine intoxication. Biol. Psychiat. 4:215.

Enna, S.J., Bennett, J.P., Burt, D.R., Creese, I. and Snyder, S.H. (1976) Stereospecificity of interaction of neuroleptic drugs with neurotransmitter and correlation with clinical potency. Nature 263, 338.

Ernst, A.M. (1967) Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. Psychopharmacologia 10:316.

Falck, B. (1962) Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. Acta Physiol. Scand. 56, Suppl. 197:1.

Falck, B., Hallarp, N.A., Thieme, G. and Torp. A. (1962) Fluorescence of catecholamines and related compounds condensed with formaldehyde. Histochem. Cytochem. 10:348.

Freye, E. and Kuschinsky, K. (1976) Effects of fentanyl and droperidol on the dopamine metabolism of the rat striatum. Pharmacology 14:1.

Freyham, F.A. (1957) Psychomotility and parkinsonism in treatment with neuroleptic drugs. Archives of Neurol. Psychiat. 78:465.

Gallant, D.M., Bishop, M.P. and Figueroa, R.G. (1968) AL499: A preliminary evaluation of a new butyrophenone derivative in chronic schizophrenic patients. Curr. Therap. Res. 10:244.

Gallant, D.M. and Bishop, M.D. (1969) AHR-1900: A butyrophenone derivative. Curr. Therap. Res. 11:793.

Garcia-Munoz, M., Nicolaou, N.M., Tulloch, I.F., Wright, A.K. and Arbuthnott, G.W. (1977) Feedback loop or output pathway in striato-nigral fibres? Nature 265:363.

Gey, K.F. and Pletscher, A. (1968) Acceleration of turnover of ^{14}C-catecholamines in rat brain by chlorpromazine. Experientia 24:335.

Glowinski, J. and Axelrod, J. (1965) Effects of drugs on the uptake, release and metabolism of H^3-norepinephrine in the rat brain. J. Pharmacol. Exper. Ther. 149:43.

Goldstein, A., Lowney, L.I. and Pal, B.K. (1971) Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. Proc. Natl. Acad. Sci. USA 68:1742.

Goodman, L.S. and Gilman, A. (1975) The Pharmacological Basis of Therapeutics (MacMillan Publishing Co., New York) p. 152.

Greengard, P. (1976) in: Antipsychotic Drugs: Pharmacodynamics and Pharmacokinetics, eds. G. Sedvall, B. Uvnas and Y. Zotterman (Pergamon Press, New York) p. 271.

Gross, H. and Langner, E. (1966) Das Wirkungsprofil eines chemisch neuratigen breitband neuroleptikums der dibenzodiazepingruppe. Wein. Med. Wochenschr. 116:814.

Gross, H. and Langner, E. (1970) Das neuroleptikum 100-129/HF-1854 (clozapin) in der psychiatrie. Intern. Pharmacopsychiat. 4:220.

Groves, P.M., Wilson, C.J., Young, S.J. and Rebec, G.V. (1975) Self-inhibition by dopaminergic neurons. Science 190:522.

Gudelsky, G.A. and Moore, K.E. (1976) Differential drug effects on dopamine concentrations and rates of turnover in median eminence, olfactory tubercle and corpus striatum. J. Neural. Trans. 38:95.

Guldberg, H.C. and Broch, O.J. (1971) On the mode of action of reserpine on dopamine metabolism in the rat striatum. European J. Pharmacol. 13:155.

Haase, H.J. (1959) The role of drug-induced extrapyramidal syndromes. in: Psychopharmacology Frontiers, ed. N.S. Kline (Little, Brown, Boston) p. 201.

Haase, H.J. (1961) Extrapyramidal modifications of fine movements, a "condition sine qua non" of fundamental therapeutic action of neuroleptic drugs. Rev. Canad. Biol. 20:425.

Hackman, R., Pentikainen, P., Neuvonen, P.J. and Vapaatalo, H. (1973) Inhibition of the apomorphine gnawing compulsion by amphetamine. Experientia 34:1524.

Healy, D.L. and Burger, H.G. (1978) Sustained elevation of serum prolactin by metoclopramide: A clinical model of idiopathic hyperprolactinemia. J. Clin. Endocrinol. Met. 46:709.

Hill, R.T. and Tedeschi, D.H. (1971) in: An Introduction to Psychopharmacology, eds. R. Rech and K. Moore (Raven Press, New York) p. 237.

Honda, F., Satok, Y., Shimomura, K., Satok, H., Noguchi, H., Uchida, S. and Katol, R. (1977) Dopamine receptor blocking activity of sulpiride in the central nervous system. Japan. J. Pharmacol. 27:397.

Horn, A.S. and Phillipson, D.T. (1976) A noradrenaline sensitive adenylate cyclase in the rat limbic forebrain: Preparation, properties and the effects of agonists, adrenolytics and neuroleptic drugs. European J. Pharmacol. 37:1.

Hornykiewicz, D. (1966) Dopamine (3-hydroxytyramine) and brain function. Pharm. Rev. 18:725.

Howard, J.L., Large, B.T., Wedley, S. and Pullar, I.A. (1978) The effects of standard neuroleptic compounds on the binding of ^{3}H-spiroperidol in the striatum and mesolimbic system of the rat in vitro. Life Sci. 23:599.

Iversen, L.L. (1975) Dopamine receptors in the brain. Science 188:1084.

Iversen, L.L., Rogawski, M.A. and Miller, R.J. (1976) Comparison of the effects of neuroleptic drugs on pre- and post-synaptic dopaminergic mechanisms in the rat striatum. Mol. Pharmacol. 12:251.

Iwatsubo, K. and Clouet, D. (1975) Dopamine-sensitive adenylate cyclase of the caudate nucleus of rats treated with morphine or haloperidol. Biochem. Pharmacol. 24:1499.

Janowsky, D.S. and Davis, J.M. (1974) Dopamine, psychomotor stimulants and schizophrenia: Effects of methylphenidate and the stereoisomers of amphetamine in schizophrenics. in: Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes, ed. E. Usdin (Raven Press, New York) p. 317.

Janssen, P.A.J., Niemegeers, C.J.E., Schellenkens, K.H.L. and Lenaerts, F.M. (1967) Is it possible to predict the clinical effects of neuroleptic drugs (major tranquilizers) from animal data? Part IV. Arzneim. Forsch. 17:841.

Javoy, F., Agid, Y., Bouvet, D. and Glowinski, J. (1972) Feedback control of dopamine synthesis in dopaminergic terminals of the rat striatum. J. Pharmacol. Exper. Ther. 182:454.

Jenner, P., Elliott, P.N.C., Clow, A., Reavill, C. and Marsden, C.D. (1978) A comparison of in vitro and in vivo dopamine receptor antagonism produced by substituted benzamide drugs. J. Pharm. Pharmacol. 30:46.

Karobath, M. and Leitich, H. (1974) Antipsychotic drugs and dopamine-stimulated adenylate cyclase prepared from corpus striatum of rat brain. Proc. Natl. Acad. Sci. 71:2915.

Karoum, F., Neff, N.H. and Wyatt, R.J. (1977) The dynamics of dopamine metabolism in various regions of rat brain. European J. Pharmacol. 44:311.

Kebabian, J.W., Petzold, G.L. and Greengard, P. (1972) Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain and its similarity to the "dopamine receptor." Proc. Natl. Acad. Sci. 69:2145.

Kirschberg, G.J., Côté, L.J., Lowe, Y.H. and Ginsburg, S. (1972) Interference with the fluorometric assay for homovanillic acid caused by acid metabolites of catecholamines. J. Neurochem. 19:2873.

Koch, Y., Lu, K.H. and Meites, J. (1970) Biphasic effects of catecholamines on pituitary prolactin release in vitro. Endocrinology 87:673.

Korf, J., Zieleman, M. and Westerink, B.H.C. (1976) Dopamine release in the substantia nigra? Nature 260:257.

Koslow, S.H., Cattabeni, F. and Costa, E. (1972) Norepinephrine and dopamine: Assay by mass fragmentography in the picomole range. Science 176:177.

Kuschinsky, K. and Hornykiewicz, O. (1972) Morphine catalepsy in the rat: relation to striatal dopamine metabolism. European J. Pharmacol. 19:119.

Kuschinsky, K. and Hornykiewicz, O. (1974) Effects of morphine on striatal dopamine metabolism: possible mechanism of its opposite effect on locomotor activity in rats and mice. European J. Pharmacol. 26:41.

Langer, G., Ahn, H.S., Perel, J.M. Makman, M.H. and Sachar, E.J. (1977a) No effects of quarternary neuroleptics on human prolactin and adenyl cyclase. Lancet 1:493.

Langer, G., Sachar, E.J., Gruen, P.H. and Halpern, F.S. (1977b) Human prolactin responses to neuroleptic drugs correlate with antischizophrenic potency. Nature 266:639.

Levitt, M., Spector, S. Sjoerdsma, A. and Udenfriend, S. (1965) Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. J. Pharmacol. Exp. Ther. 148:1.

Lippmann, W., Pugsley, T. and Merker, J. (1975) Effect of butaclamol and its enantiomers upon striatal homovanillic acid and adenyl cyclase of olfactory tubercle in rats. Life Sci. 16:213.

Lloyd, K.G., Davidson, L. and Hornykiewicz, O. (1975) The neurochemistry of Parkinson's disease: Effect of L-Dopa therapy. J. Pharmacol. Exper. Ther. 195:453.

Matthysse, S. (1973) Antipsychotic drug actions: A clue to the neuropathology of schizophrenia? Federation Proc. 32: 200.

Matthysse, S. (1974) Dopamine and the pharmacology of schizophrenia: The state of the evidence. J. Psychiat. Res. 11: 107.

Matz, R., Rick, W., Oh, D., Thompson, H. and Gershon, S. (1974) Clozapine--a potential antipsychotic agent without extrapyramidal manifestations. Curr. Therap. Res. 16:687.

Meltzer, H.Y. and Fang, V.S. (1976) The effect of neuroleptics on serum prolactin in schizophrenic patients. Arch. Gen. Psychiat. 33:379.

Meltzer, H.Y., Fang, V.S., Simonovic, M. and Paul, S.M. (1977a) Effect of metabolites of chlorpromazine on plasma prolactin levels in male rats. Eur. J. Pharmacol. 41:431.

Meltzer, H.Y., Fessler, R.G. and Fang, V.S. (1977b) Perlapine: Relationship between stimulation of prolactin secretion and antipsychotic activity. Psychopharmacology 54:183.

Meltzer, H.Y., Paul, S.M. and Fang, V.S. (1977c) Effect of fluphenthixol and butaclamol isomers on prolactin secretion in rats. Psychopharmacology 51:181.

Meltzer, H.Y., Goode, D.J. and Fang, V.S. (1978) The effect of psychotropic drugs on endocrine function. in: A Review of Psychopharmacology: A Decade of Progress, eds. A. DiMascio and M. Lipton (Raven Press, New York).

Merali, Z., Singhal, R.L., Hrdina, P.D. and Ling, G.M. (1975) Changes in brain cyclic AMP metabolism and acetylcholine and dopamine during narcotic dependence and withdrawal. Life Sci. 16:1889.

Mielke, D.H., Gallant, D.M., Oelsner, T., Kessler, C.M., Tomlinson, W.K. and Cohen, G.H. (1975) Butaclamol hydrochloride (AY 23,028): An early evaluation in severely ill schizophrenics. Dis. Nerv. Syst. 36:7.

Mielke, D.H., Gallant, D.M., Roniger, J.J., Kessler, C, and Kessler, L.R. (1977a) Sulpiride: Evaluation of antipsychotic activity in schizophrenic patients. Dis. Nerv. Syst. 38:569.

Mielke, D.H., Gallant, D.M. and Kessler, C. (1977b) An evaluation of a unique new antipsychotic agent, Sulpiride: Effects on serum prolactin and growth hormone levels. Am. J. Psychiatry 134:1371.

Miller, R.J. and Hiley, C.R. (1976) Antidopaminergic and antimuscarinic effects of dibenzodiazepines. Relationship to drug induced parkinsonism. Naunyn-Schmiedeb. Arch. Pharmacol. 292:289.

Miller, R.J., Horn, A.S. and Iversen, L.L. (1974) The action of neuroleptic drugs on dopamine-stimulated adenosine cyclic 3',5'-monophosphate production in rat neostriatum and limbic forebrain. Mol. Pharmacol. 10:759.

Munkvad, I., Pahkenberg, H. and Randrup, A. (1968) Aminergic systems in the basal ganglia associated with stereotyped hyperactive behaviour and catalepsy. Brain Behav. Evol. 1:89.

Nagatsu, T., Levitt, M. and Udenfriend, S. (1964) Tyrosine hydroxylase: The initial step in norepinephrine biosynthesis. J. Biol. Chem. 239:2910.

Nakra, B.R.S., Bond, A.J. and Lader, M.D. (1975) Comparative psychotropic effects of metoclopramide and prochlorperazine in normal subjects. J. Clin. Pharmacol. 15:449.

National Institute of Mental Health (1964) Psychopharmacology service center collaborative study group. Phenothiazine treatment in acute schizophrenia. Arch. Gen. Psychiat. 10:246.

Nose, T. and Takemoto, H. (1974) Effect of oxo-tremorine on homovanillic acid concentration in the striatum of the rat. European J. Pharmacol. 25:51.

Nybäck, H. (1972) Effect of brain lesions and chlorpromazine on accumulation and disappearance of catecholamines formed in vivo from ^{14}C-tyrosine. Acta. Physiol. Scand. 84:54.

Nybäck, H. and Sedvall, G. (1971) Effect of nigral lesion on chlorpromazine-induced acceleration of dopamine synthesis from (^{14}C)-tyrosine. J. Pharm. Pharmacol. 23:322.

Nybäck, H., Sedvall, G. and Kopin, I.J. (1967) Accelerated synthesis of dopamine-C^{14} from tyrosine-C^{14} in rat brain after chlorpromazine. Life Sci. 6:2037.

Öhman, R., Larsson, M., Nilsson, I.M., Engel, J. and Carlsson, A. (1977) Neurometabolic and behavioral effects of haloperidol in relation to drug levels in serum and brain. Naunyn-Schmiedeb. Arch. Pharmacol. 299:105.

O'Meallie, L., Gallant, D.M., Bishop, M.P., Bishop, G. and Steele, C.A. (1969) Cardiotoxic effects of a new butyrophenone compound: U-25,927. Curr. Therap. Res. 11:460.

Park, S, Gershon, S. and Floyd, A. (1972) A clinical trial of a benzazepine (SCH-12,679) in acute schizophrenic patients. Curr. Therap. Res. 14:298.

Peringer, E., Jenner, P., Donaldson, I.M., Marsden, C.D. and Miller, R. (1976) Metoclopramide and dopamine receptor blockade. Neuropharmacology 15:463.

Pert, C.B. and Snyder, S.H. (1973) Opiate receptor: Demonstration in nervous tissue. Science 179:1011.

Puri, S.K., Cochin, J. and Volicer, L. (1975) Effect of morphine sulfate on adenylate cyclase and phosphodiesterase activities in rat corpus striatum. Life Sci. 16:759.

Puri, S.K., Volicer, L. and Cochin, J. (1976) Changes in the striatal adenylate cyclase activity following acute and chronic morphine treatment and during withdrawal. J. Neurochem. 27:1551.

Randrup, A. and Munkvad, I. (1965) Special antagonism of amphetamine-induced stereotyped behavior. Psychopharmacologia 7:416.

Randrup, A. and Munkvad, I. (1966) Role of catecholamines in the amphetamine excitatory response. Nature 211:540.

Randrup, A. and Munkvad, I. (1972) Evidence indicating an association between schizophrenia and dopaminergic hyperactivity in the brain. Orthomolec. Psychiat. 1:2.

Randrup, A., Munkvad I. and Udsen, P. (1963) Adrenergic mechanisms and amphetamine-induced abnormal behavior. Acta Pharmacol. 20:145.

Roffler-Tarlov, S., Sharman, D.F. and Tergerdine, P. (1971) 3,4-dihydroxyphenylacetic acid in the mouse striatum: A reflection of intra- and extra-neuronal metabolism of dopamine? Brit. J. Pharmacol. 42:343.

Rollema, H., Westerink, B.H.C. and Grol, C.J. (1976) Correlation between neuroleptic-induced suppression of stereotyped behavior and HVA concentrations in rat brain. J. Pharm. Pharmacol. 28:321.

Roth, R.H., Murrin, L.C. and Walters, J.R. (1976) Central dopaminergic neurons: Effects of alterations in impulse flow on the accumulation of dihydroxyphenylacetic acid. European J. Pharmacol. 36:163.

Rotrosen, J., Angrist, B., Aronson, M., Gershon, S., Gruen, P. Sachar, E., Denning, R.K., Matthysse, S., Stanley, M. and Wilk, S. (1978) Thiethylperazine: Clinical antipsychotic efficacy and correlation with potency in predictive systems. Arch. Gen. Psychiat. 35:1112.

Roufogalis, B.D., Thornton, M. and Wade, D.N. (1976) Specificity of the dopamine sensitive adenylate cyclase for antipsychotic antagonists. Life Sci. 19:927.

Sachar, F.J., Gruen, P.H., Altman, W., Halpern, F.S. and Frantz, A.G. (1976) Uses of neuroendocrine techniques in psychopharmacological research. in: Hormones, Behavior and Psychopathology, ed. E.J. Sachar (Raven Press, New York) p. 161.

Sandoz Pharmaceuticals (1969) Mellaril (thioridazine): A 10-year Review of Clinical Experience in the United States. Sandoz Pharmaceuticals, Hanover, N.J.

Sathananthan, G., Angrist, B.M. and Gershon, S. (1973) Response thershold to L-Dopa in psychiatric patients. Biological Psychiat. 7:139.

Scatton, B. (1977) Differential regional development of tolerance to increase in dopamine turnover upon repeated neuroleptic administration. European J. Pharmacol. 46:363.

Schnaitman, C., Erwin, V.G. and Greenawalt, J.W. (1967) The submitochondrial localization of monoamine oxidase. An enzymatic marker for the outer membrane of rat liver mitochondria. J. Cell Biol. 32:719.

Seeman, P., Lee, T., Chau-Wong, M. and Wong, K. (1976a) Antipsychotic drug doses and neuroleptic dopamine receptors. Nature 261:717.

Seeman, P., Lee, M., Chau-Wong, M. and Wong, K. (1976b) Correlation of antipsychotic drug potency and neuroleptic receptor block. Proc. Soc. Neurosci. Abstract No. 1274.

Shaar, C.J. and Clemens, J.A. (1974) The role of catecholamines in the release of anterior pituitary prolactin in vitro. Endocrinology 95:1202.

Siggins, G.R., Hoffer, B.J. and Ungerstedt, U. (1974) Electrophysiological evidence for involvement of cyclic adenosine monophosphate in dopamine responses of caudate neurons. Life Sci. 15:779.

Skolnick, P., Daly, J.W., Freedman, R. and Hoffer, B.J. (1976) Interrelationship between catecholamine-stimulated formation of adenosine 3',5'-monophosphate in cerebral slices and inhibitory effects on cerebellar purkinje cells: Antagonism by neuroleptic compounds. J. Pharmacol. Exper. Ther. 197:280.

Smith, R.C. and Davis, J.M. (1976) Behavioral evidence for supersensitivity after chronic administration of haloperidol, clozapine and thioridazine. Life Sci. 19:725.

Snyder, S.H., Banerjee, S.P., Yamamura, H.I. and Greenberg, D. (1974) Drugs, neurotransmitters and schizophrenia. Science 184:1243.

Snyder, S.H. and Coyle, J.T. (1969) Regional differences in H^3-norepinephrine and H^3-dopamine uptake into rat brain homogenates. J. Pharmacol. Exper. Ther. 165:78.

Spano, P.F. and Neff, N.H. (1972) Metabolic fate of caudate nucleus dopamine. Brain Res. 42:139.

Spector, S., Sjoerdsma, A. and Udenfriend, S. (1965) Blockade of endogenous norepinephrine synthesis by α-methyltyrosine, an inhibitor of tyrosine hydroxylase. J. Pharmacol. Exper. Ther. 147:86.

Spitzer, R.L., Endicott, J. and Robbins, E. (1977) Research diagnostic criteria; NIMH collaborative study, 3rd Ed., 1.

Stanley, M.E. and Glick, S.D. (1976) Interaction of drug effects with testing procedures in the measurement of catalepsy. Neuropharmacol. 15:393.

Stawarz, R.J., Hill, H., Robinson, S.E., Setler, P., Dingell, J.V. and Sulser, F. (1975) On the significance of the increase in homovanillic acid (HVA) caused by antipsychotic drugs in corpus striatum and limbic forebrain. Psychopharmacologia 43:125.

Stille, G., Lauener, H. and Eichenberger, E. (1971) The pharmacology of 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo (b,E) (1,4) diazepine (Clozapine) II. Farmaco. 26:603.

Sugerman, A.A. (1968) A pilot study of AL-499 in chronic schizophrenic patients. Curr. Therap. Res. 10:533.

Tagliamonte, A., de Montis, G., Olianas, M., Vargin, L., Corsini, G.U. and Gessa, G.L. (1975) Selective increase of brain dopamine synthesis by sulpiride. J. Neurochem. 24:707.

Take, Y., Mikoda, T., Nalkajima, R., Chiba, S., Saji, Y. and Nagawa, Y. (1970) Pharmacological studies of 6-(4-methyl-1-piperazinyl)-morphanthridine (MP-11). J. Takeda Res. Lab. 29:416.

Tolis, G., Hickey, J. and Guyda, H. (1975) Effects of morphine on serum growth hormone, cortisol, prolactin and thyroid stimulating hormone in man. J. Clin. Endocrinol. Metab. 41:797.

Turkington, R.W. (1972) Prolactin secretion in patients treated with various drugs. Arch. Intern. Med. 130:349.

Udenfriend, S. and Zaltzman-Nirenberg, P. (1963) Norepinephrine and 3,4-dihydroxyphenethylamine turnover in guinea pig brain in vivo. Science 142:394.

Udenfriend, S., Zaltzman-Nirenberg, P. and Nagatsu, T. (1965) Inhibitors of purified beef adrenal tyrosine hydroxylase. Biochem. Pharmacol. 14:837.

Ungerstedt, U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand., Suppl. 361:1.

van Inwegen, R.G., Strada, S.J. and Robison, G.A. (1975) Effects of prostaglandins and morphine on brain adenyl cyclase. Life Sci. 16:1875.

van Rossum, J.M. and Hurkmans, J.A. (1964) Mechanism of action of psychomotor stimulant drugs. Int. J.Neuropharmacol. 3:227.

van Rossum, J.M. (1967) The significance of dopamine receptor blockade for the action of neuroleptic drugs. in: Neuropsychopharmacology, ed. H. Brill (The Hague: Excerpta Medica Foundation) p. 321.

van Rossum, J.M., Janssen, P.A.J., Boissier, J.L., Julou, L., Loew, D.M., Moller Nielsen, I., Munkvad, I., Randrup, A., Stille, G. and Tedeschi, D.H. (1970) The Neuroleptics Vol. 5, eds. D.P. Bobon, P.A.J. Janssen and J. Bobon (Karger, New York) p. 23.

Waldmeier, P.C. and Maitre, L. (1976) Clozapine: Reduction of the initial dopamine turnover increase by repeated treatment. European J. Pharmacol. 38:197.

Watson, E., Travis, B. and Wilk, S. (1974) Simultaneous determination of 3,4-dihydroxyphenylacetic acid and homovanillic acid in milligram amounts of rat striatal tissue by gas-liquid chromatography. Life Sci. 15:2167.

Weissman, A., Koe, B.K., and Tenen, S. (1966) Antiamphetamine effects following inhibition of tyrosine hydroxylase. J. Pharmacol. Exper. Ther. 151:335.

Westerink, B.H.C. and Korf, J. (1975) Influence of drugs on striatal and limbic homovanillic acid concentrations in the rat brain. European J. Pharmacol. 33:31.

Westerink, B.H.C. and Korf, J. (1976a) Regional rat brain levels of 3,4-dihydroxyphenylacetic acid and homovanillic acid: Concurrent flurometric measurement and influence of drugs. European J. Pharmacol. 38:281.

Westerink, B.H.C. and Korf, J. (1976b) Turnover of acid dopamine metabolites in striatal and mesolimbic tissue of the rat brain. European J. Pharmacol. 37:249.

Wiesel, F.A. and Alfredsson, G. (1976) The distribution and metabolism of chlorpromazine in rats and the relationship to effects on cerebral monoamine metabolism. European J. Pharmacol. 40:263.

Wiesel, F.A., Alfredsson, G., Likwornik, V. and Sedvall, G. (1975) A relation between drug concentrations in brain and striatal homovanillic acid levels in chlorpromazine treated rats. Life Sci. 16:1145.

Wiesel, F.A. and Sedvall, G. (1975) Effects of antipsychotic drugs on homovanillic acid levels in striatum and olfactory tubercle of the rat. European J. Pharmacol. 30:203.

Wikler, A., Pescor, M.J., Kalbaugh, E.P. and Angelucci, R.J. (1952) Effects of frontal lobotomy on the morphine abstinence syndrome in man. Arch. Neurol. Psychiat. 67:510.

Wilk, S. and Glick, S.D. (1976) Dopamine metabolism in the nucleus accumbens: The effect of clozapine. European J. Pharmacol. 37:203.

Wilk, S., Watson, E. and Glick, S.D. (1975a) Dopamine metabolism in the tuberculum olfactorium. European J. Pharmacol. 30:117.

Wilk, S., Watson, E. and Stanley, M.E. (1975b) Differential sensitivity of two dopaminergic structures in rat brain to haloperidol and to clozapine. J. Pharmacol. Exptl. Therap. 195:265.

Wilk, S., Watson, E. and Travis, B. (1975c) Evaluation of dopamine metabolism in rat striatum by a gas chromatographic technique. European J. Pharmacol. 30:238.

Wilk, S. and Zimmerberg, B. (1973) Absence of 3-methoxy-4-hydroxyphenyl ethanol in rat brain. Biochem. Pharmacol. 22:623.

Worms, P. and Lloyd, K.G. (1978) Predictability and specificity of behavioural screening tests for neuroleptics. in: Pharmacological Methods in Toxicology, ed. G. Zbinden and F. Gross (Pergamon Press, London).

Yamauchi, J., Takahara, J. and Ofuji, T. (1977) Effect of metoclopramide on rat prolactin secretion in vivo. Life Sci. 20:1581.

Zivkovic, B., Guidotti, A., Revuelta, A. and Costa, E. (1975) Effect of thioridazine, clozapine and other antipsychotics on the kinetic state of tyrosine hydroxylase and on the turnover rate of dopamine in striatum and nucleus accumbens. J. Pharmacol. Exper. Ther. 194:37.