LOCAL CONTROL OF DOPAMINE SYNTHESIS IN THE BRAIN

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ABSTRACT

Administration of γ-hydroxybutyrate (GOBA) has been shown to block firing of dopaminergic cells in brain, with a concomittant increase in dopamine (DA). The increase of DA after GOBA was employed as an index of tyrosine hydroxylase activity in vivo. The reputed DA agonist apomorphine inhibited the rise of DA after GOBA, an inhibition which was antagonized by haloperidol. The action of apomorphine was found not to be due to inhibition of monoamine oxidase or of catechol-O-methyl-transferase, but was similar to that of amphetamine. The DA agonists piribedil, apocodeine, M7, ergocornine and 2-Br-α-ergocryptine inhibited the rise of DA after GOBA. The last four, however, were not antagonized in their inhibition by haloperidol. Ergocornine, M7 and piribedil also differed from apomorphine in that they elevated DA levels in controls. These results provide strong evidence for the presence of a dopamine receptor which acts locally to inhibit dopamine synthesis. In addition, the results suggest that DA agonists do not have a common mode of action.

C. ADRIAN HANDFORTH

CONTROLE LOCAL DE LA SYNTHÈSE DE LA DOPAMINE DANS LE CERVEAU

L'Administration de γ-hydroxybutyrate (GOBA) bloque la décharge des cellules dopaminergique dans le cerveau, avec une augmentation concomittante de dopamine (DA). L'Augmentation de dopamine après GOBA fut utilisée comme un index de l'activité de la tyrosine hydroxylase in vivo. L'Apomorphine, generalement considerée comme agoniste de la dopamine, inhibe l'augmentation de dopamine après GOBA, inhibition antagonisée par le Haloperidol. L'Action de l'apomorphine n'est pas due à l'inhibition de la MAO ni de la COMT mais est similaire à celle de l'amphetamine. Les agonistes de la dopamine, piribedil, apocodeine, M7, ergocornine et 2-Br-α-ergocryptine inhibent l'augmentation de dopamine après GOBA. Les quatres derniers cependent, ne sont pas antagonisés dans leur inhibition par le haloperidol. L'Ergocornine, le M7, et le piribedil diffèrent également de l'apomorphine en ceci qu'ils élèvent le niveau de dopamine chez les contrôles. Ces résultats fournissent sérieuse évidence pour la présence d'un recepteur pour la dopamine qui agit localement pour inhiber la synthèse de la dopamine. De plus, ces résultats suggèrent que les agonistes de la dopamine n'ont pas un mode d'action commun.

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PREFACE

Dopamine is a neurotransmitter found in brain. First found in the striatum, dopamine is now known to be widely distributed throughout the brain, being present in low concentrations throughout the limbic system and parts of the cortex. Particular attention has been focussed on dopamine in view of its involvement in clinical syndromes. A deficiency of dopamine is involved in Parkinson's disease, which is treated by administration of a dopamine precursor. Drugs employed in the treatment of psychosis have been found experimentally to block the dopamine receptor. Animal studies have shown dopamine to be implicated in a wide variety of behaviours, including motor activity, learning, aggression and sexual behaviour.

In view of the clinical relevance of this neurotransmitter, the study of factors controlling the metabolism of dopamine holds particular interest. The rate-limiting enzyme in dopamine synthesis is tyrosine hydroxylase. The regulation of tyrosine hydroxylase activity is complex and involves pterin cofactor concentration, oxygen level, end-product inhibition by dopamine and the rate of firing of the dopaminergic neuron. Apomorphine, which reputedly stimulates dopamine receptors, decreases dopamine turnover and inhibits dopaminergic neuronal firing. Agents, such as haloperidol, which block dopamine receptors, have the reverse effect. The activity of the neuron is believed to influence tyrosine hydroxylation by a mechanism which is as yet unknown.

Apomorphine has also been shown to decrease dopamine metabolism in the absence of neuronal firing. However, doubts have been raised as to whether apomorphine decreases dopamine metabolism by acting on the receptor. The aim of this thesis was to resolve these doubts and to

study the effects of other agents that activate dopamine receptors.

The results of these investigations provide strong evidence for the existence of a receptor that inhibits dopamine synthesis by a mechanism which does not depend on dopaminergic neuronal firing for its action.

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ABBREVIATIONS

DA	Dopamine
NE	Norepinephrine
AMPT	α-Methyl-p-tyrosine
DBH	Dopamine ß-hydroxylase
MAO	Monoamine oxidase
COMT	Catechol O-methyl transferase
HVA	Homovanillic acid
DOPAC	3, 4-dihydroxyphenylacetic acid
BH_4	Tetrahydrobiopterin
EGTA	Ethylenebis (oxyethylenenitrilo) tetra-acetic acid
GOBA	γ-Hydroxybutyric acid
ET 495	Piribedil
CB 154	2-Bromo-α-ergocryptine
M7	5, 6-Dihydroxy-2-dimethylaminotetralin
EDTA	Disodium ethylenediamine tetraacetate
$DMPH_4$	6, 7-Dimethyl-5, 6, 7, 8-tetrahydropterin
PPO	2, 5-Diphenyloxazole

I. INTRODUCTION

A. Dopamine

Dopamine, 3,4-dihydroxyphenylethylamine, is one of several physiologically important 3,4-dihydroxy derivatives of phenylethylamine. The others are norepinephrine and epinephrine. Together they are termed catecholamines and they are widely distributed throughout the animal kingdom, usually in nerve cells. They are found in adrenal medulla, the peripheral and central nervous system, and in small amounts in chromaffin cells throughout the body (1,2,3). Epinephrine (also known as adrenaline) serves mainly as a hormone but small amounts have been found in brain (3) and in heart (4). The first evidence for involvement of catecholamines in neurochemical transmission was obtained in 1921 by Loewi (5) who found an active principle released by heart after stimulation of the vagus nerve. The action of adrenaline was shown to be similar to that of the active principle. Von Euler finally isolated norepinephrine from splenic nerves (6) and norepinephrine was shown to be released on stimulation (7). Sensitive fluorescence-histochemical methods (8) have shown norepinephrine (NE) to be present throughout the sympathetic nervous system. Gradient Centrifugation,

autoradiographic and electromicroscopic techniques (9,10,11) showed that NE is localized within vesicles inside the nerve endings. Synthesis occurs in the synaptic terminal and the vesicles serve as storage sites. After the contents of vesicles are released by the action potential, the catecholamine acts on the receptor, leading to depolarization or hyperpolarization. Some is metabolized, but most is taken up by the releasing neuron and stored in vesicles until used again. In the brain, dopamine (DA) and norepinephrine are found in separate nerve tracts. Dopamine is present in noradrenergic neurons only as a precursor of NE.

(i) Biosynthesis and Metabolism

The general scheme for the synthesis and metabolism of dopamine is shown in Table 1. The dashed lines refer to minor reactions. The metabolism and alternate pathways of synthesis and metabolism of norepinephrine and epinephrine are not shown. The first reaction in the sequence to be studied was decarboxylation (12), which was shown to occur in tissue extracts with the substrates phenylalanine and tyrosine (13). Hallé then proposed in 1906 that tyrosine is the precursor of epinephrine (14), which is correct, but through the intermediate epinine, which is incorrect. Hallé's proposal was disputed by Ewins and Laidlaw (15). In 1939 Blaschko proposed the correct pathway, from tyrosine to epinephrine (16). The pathway was confirmed finally with the demonstration in 1964 of tyrosine hydroxylase (17).

Tyrosine hydroxylase (EC 1.14.3-), also known as tyrosine 3-monooxygenase, hydroxylates the 3 or 5 position of the tyrosine phenyl ring, and thereby forms L-dopa. Isotopic studies demonstrated this *in vivo* in 1953 (18,19), and it was directly shown by Nagatsu et al. (17). The enzyme is found in spleen, heart, adrenal medulla, vas deferens and brain (18,19). Reduced pteridine

TABLE I

PATHWAYS OF DOPAMINE METABOLISM

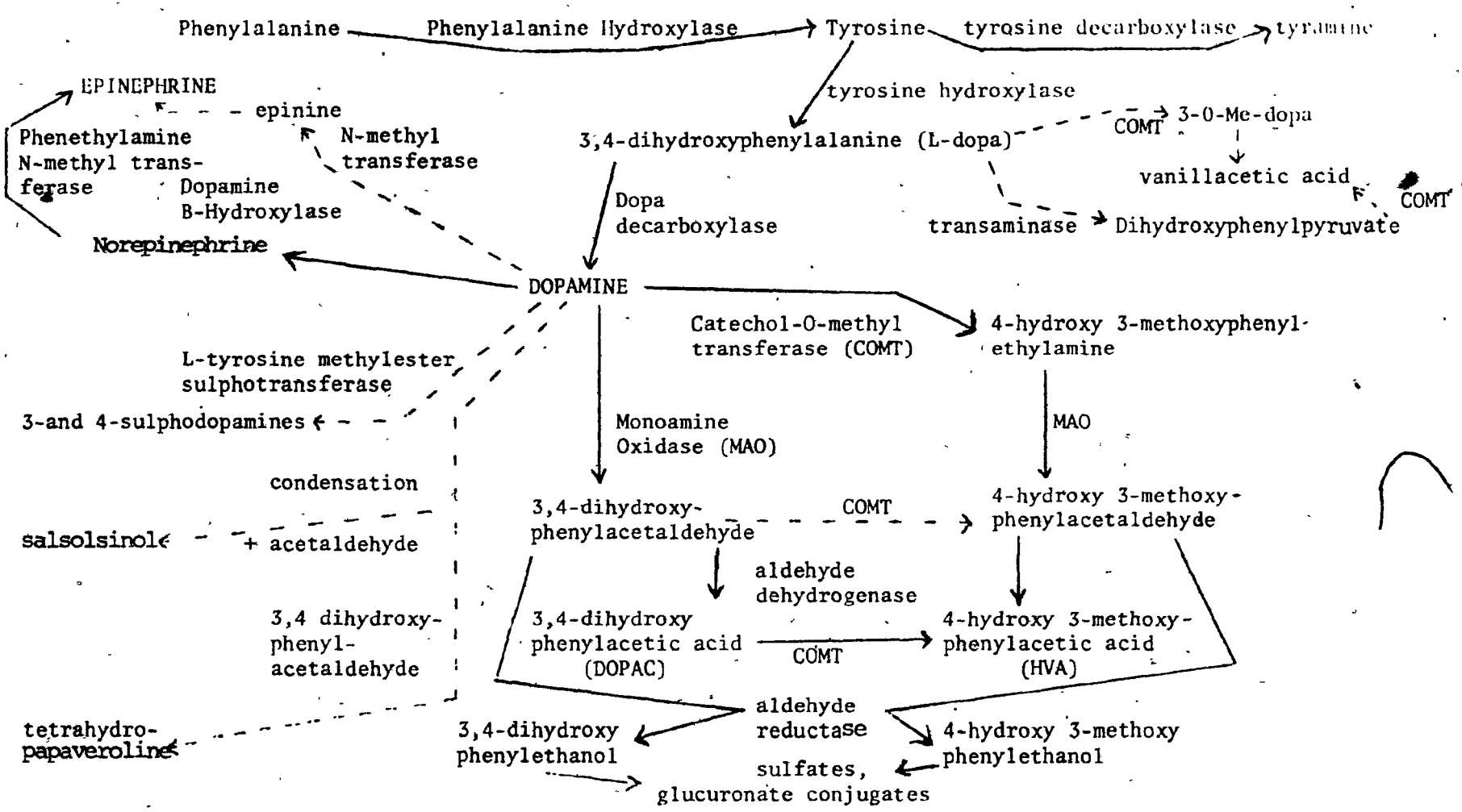


TABLE II

Drugs commonly used to affect dopamine (DA) metabolism

Drug	Action
Apomorphine	Stimulates DA receptor (agonist)
Haloperidol	Blocks DA receptor
Amphetamine	Blocks uptake, releases DA
Pargyline	Inhibits monoamine oxidase
6-Hydroxydopamine	Destroys DA neuron
α-methyl-p-tyrosine	Inhibits tyrosine hydroxylase
Reserpine	Depletes DA from vesicles

is required as a cofactor (20), Fe^{++} and O_2 are required for maximal activity (17). The enzyme is usually assayed with 3,5-^{3}H-tyrosine as substrate. The tritiated water released by the hydroxylation is separated from the tyrosine and dopa by an ion-exchange column (17). In 1956 Udenfriend and Wyngaarden (19) suggested that tyrosine hydroxylase is the rate-limiting enzyme in the pathway. Much evidence has been adduced to support this statement (21,22,23,24, 25,26,17). A commonly used inhibitor of tyrosine hydroxylase is α-methyl-p-tyrosine (AMPT), which competitively inhibits the enzyme. Following administration of this drug, catecholamines decline throughout the body, the rate of decline related to the rate of utilization (27).

Tyrosine hydroxylase is thought to be mainly soluble, despite the original report to the contrary (17). Particulate fractions do not contain much activity (26) but the supernatant fractions of adrenal and brain do (30, 31,29). The enzyme tends to adsorb and become sedimentable (32). In the brain tyrosine hydroxylase and dopa decarboxylase are localized completely intraneuronally (33,34,35) and are found in high concentrations in the synaptosomes.

Dopa decarboxylase (EC 4.1.1.26), which converts

L-dopa to dopamine, was first discovered in 1938 by Holtz (36). It acts on a wide variety of substrates, such as tyramine, histamine and serotonin, leading to the suggestion that it be called L-aromatic amino acid decarboxylase (37). Dopa decarboxylase is widely distributed; in the central nervous system the distribution of activity is similar to that of the catecholamines. The product of the reaction is found in low amounts if it is simply going to serve as the precursor of norepinephrine in, for example, the adrenal. Where dopamine is itself the end of the biosynthetic pathway it is found in high amounts such as in the striatum (38,39,40).

Dopamine-β-hydroxylase (EC 1.14.2.1) converts dopamine to norepinephrine. The reaction was first shown in vivo by Hagen (41) in 1496. It has been shown to be present throughout the sympathetic nervous system, in brain (42,43) and especially in adrenal (44,45) which is the major site of norepinephrine synthesis. Here the enzyme is found to be associated with the chromaffin granule of the adrenal medulla (46). DBH is also associated with noradrenergic vesicular elements in other parts of the body (47,48,49). It does not, however, occur in dopamine-containing granules (50,51).

In the adrenal and in small amounts in the brain (52,53,54) the enzyme phenylethanolamine N-methyltransferase (EC 2.1.1.-) transfers a methyl group from S-adenosyl methionine (55) to norepinephrine to form epinephrine.

In the metabolism of dopamine, two pairs of enzyme are involved. The first pair consists of monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). Each can act on the product of MAO or on the product of both MAO and COMT. This pair consists of aldehyde dehydrogenase and aldehyde reductase. The main metabolic products of dopamine are homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC). These pathways are outlined in Table 1. The corresponding pathways for norepinephrine and epinephrine are similar.

MAO (EC 1.4.3.4) was first described in 1928 by Hare (56). It acts on all the catecholamines, converting the amine moiety to an aldehyde (57). Multiple forms exist, as shown by electrophoresis (58,59) and inhibitor studies (60). Type A and Type B enzymes, as they are called, both act on dopamine. At the subcellular level, MAO is localized on the outer mitochondrial membrane (61). Some have reported a decrease in striatum of MAO after lesions of the

nigrostriatal tract (the main dopaminergic tract in brain, see section A iii)(62), but others have not (63,64). MAO is most probably localized on mitochondria both within and without catecholamine nerve endings in the striatum. MAO is the predominant enzyme catabolizing dopamine (DA) in brain (65,66) and when MAO inhibitors such as pargyline or pheniprazine are administered to rats, DA levels in brain rise rapidly (67,68).

COMT (EC 2.1.1.6) was first described in 1957 in rat liver (69), although its existence had been inferred previously from the existence of 3-0-methylcatecholamines (70,71). It transfers a methyl group from S-adenosyl-methionine to the meta position of catechols (71). Tropolones inhibit COMT (72). COMT is present mainly outside the sympathetic neuron, in contrast to MAO (73). COMT does not decline in the striatum after nigrostriatal lesions (74).

The aldehyde of deaminated dopamine is mainly oxidized by aldehyde dehydrogenase (EC 1.2.1.3) rather than reduced (75,76). Only a small amount of the aldehyde of dopamine is reduced <u>in vivo</u> to alcohols (77,78) by aldehyde reductase (EC 1.1.1.2). Neither aldehyde dehydrogenase

(79,80) nor aldehyde reductase (81) appear to be highly concentrated in synaptosomal fractions. Aldehyde dehydrogenase decreased in the striatum after nigrostriatal lesions of an electrolytic nature in cats (64) or chemical nature in the rat (62). However, electrolytic lesions in rats did not have this effect (80). Aldehyde reduction did not diminish after nigrostriatal lesions (80). Duncan et al. (80) suggested that dopamine is not necessarily taken up by the nerve terminal prior to catabolism.

In addition to the major pathways outlined here, other minor reactions have been discovered which may be important under certain conditions. These are shown by dotted lines in Table 1. When L-dopa is administered in conjunction with a peripheral decarboxylase inhibitor (as in treatment for Parkinson's disease) some of the L-dopa is O-methylated (82), but much is diverted to a pathway which is normally trivial, transamination (83). Thus 4-hydroxy-3 methoxyphenyl lactic acid (vanillactic acid) is formed.

An apparently new pathway which has been reported is the conversion of dopamine to epinine by an N-methytransferase in rat brain which uses 5-methyltetrahydrofolic acid

as the methyl donor (84,85,86). Sulfo-conjugates of dopamine have also been found recently and have been suggested to represent metabolic intermediate, or convenient forms for transport (87). The amine moiety of dopamine may condense with aldehydes, such as acetaldehyde or 3,4-dihydroxyphenylacetaldehyde to form salsolsinol and tetrahydropaveroline respectively (88,89,90).

These two products have been found in man by Sandler (91,100).

(ii) Storage and Uptake of Dopamine

The Falck-Hillarp technique reveals that monoamines are present in low concentrations in the cell body of the neuron (92), very low in the axons, and very high in the so-called varicosities (93) especially in the caudate nucleus (94) and certain other regions of the brain. The varicosities have been identified as true synaptic terminals (95). Where the terminals are present, small granular vesicles are also present. Administration of AMPT abolishes the small granular vesicles. Addition of catecholamine to brain slices will cause appearance of granules in areas known to be monoaminergic, but not if reserpine, which is known to deplete monoaminergic,

is present (96). These observations indicate that monoamines in general, and dopamine in particular, are stored in vesicles, and released from vesicles as neurotransmitters. Vesicles are found in all parts of the dopamine neuron and are considered to be synthesized in the cell body and transported down the axon, along with the enzymes involved in DA metabolism, to the nerve terminal. The small granules seen in various preparations are the synaptic vesicles, which release transmitter into the synaptic cleft. After release, dopamine is mostly taken up again into the neuron and into the vesicle. Some may be metabolized, with formation of HVA and DOPAC as the main metabolites.

In addition to the well established presence of dopamine granules in the terminal boutons of the nigrostriatal pathway, it has recently been shown that the dendritic arborizations of the substantia nigral cells store granular DA. Dopamine may be a neurotransmitter in dendro-dendritic synapses (97).

The postsynaptic receptor may be associated with adenyl cyclase. A dopamine-sensitive adenyl cyclase has been located in the caudate nucleus which is activated by agents that activate dopamine receptors, and is blocked

by agents which block dopamine receptors (98). Like the dopamine receptor, it becomes "supersensitive" under conditions of chronic lack of dopamine (99).

(iii) Localization of Dopamine in the Central Nervous System

The introduction of the Falck-Hillarp fluorescence histochemical method made possible detailed mapping of monoamine neurons in the brain (101,102). Essentially the method is based on the fact that when tissue slices from brain are treated with hot formaldehyde gas, neuronal monoamines are converted to high-intensity fluorophores. Examination of dopamine neurons in brain reveals that the DA cell bodies are exclusively located in the mesencephalon and the hypothalamus. DA fibres, unlike NE fibres, do not possess collaterals; unlike the diffuse innervation of NE systems, where a single cell can project to the cerebellum and cerebral cortex, the projection of DA fibres is orderly. DA terminals form a meshwork of very fine terminals (103). One dopamine cell body can give rise to 500,000 terminals in the striatum (104). Classically there are three main DA systems in brain.

(1) Large amounts of dopamine were discovered

by Carlsson to be located in the striatum (40). Most of the DA in brain is located here. The striatum is a collective term that refers to the caudate nucleus and putamen. An associated basal ganglion is the globus pallidus. The main input to striatum is from the cerebral cortex, parts of the thalamus and substantia nigra (in the mesencephalon). The output from striatum is to the substantia nigra and to the globus pallidus, which sends most of its efferents to parts of the thalamus. Initially the existence of an ascending DA pathway was inferred from the reductions in striatal DA seen after lesions in substantia nigra at points in between (105-111). Attempts to demonstrate fibers from substantia nigra to striatum using conventional staining techniques failed (112,113). The Falck-Hillarp technique confirmed the existence of the pathway. The axon fibres stain very poorly, but the axons may be traced from the substantia nigra to the striatum under certain conditions, such as early on in development (103,114), after injection of DA into substantia nigra (115) or after dopa treatment (116). Another way of demonstrating the nigrostriatal pathway utilizes axoplasmic transport. Following the injection of labeled leucine or dopa into the substantia nigra, selective accumulation

of radioactive protein or dopamine can be observed in the striatum: This type of study has also revealed that there may be a parallel, nondopaminergic pathway (117,118). Further evidence for the existence of a dopaminergic nigrostriatal tract is provided by the finding that electrical stimulation of the substantia nigra causes release of DA or its metabolites from the striatum (119,120). The substantia nigral DA nuclei are known as A8 and A9. A8 is actually located in the lateral reticular formation.

(2) The mesolimbic DA nerve tracts arise from cell bodies of group A10 near the nucleus interpeduncularis in the mesencephalon and innervate the nucleus accumbens and tuberculum olfactorium (121,122,123). The limbic nuclei known as nucleus amygdaloideus centralis and interstitialis striae terminalis are innervated partly by group A8.

(3) A third system is the tuberoinfundibular system which courses from the arcuate and anterior periventricular nucleus of the hypothalamus to terminals in the primary capillary plexus which supplies the pituitary gland (124,125,126).

More recently other dopaminergic nerve terminal

sites have been found in the brain. Dopamine is likely to be of great importance at these sites despite its low concentration. Dopamine is present in all limbic nuclei (127). Dopamine was shown by Thierry et al. to be present in cerebral cortex (128,129). There DA terminals occur in the limbic cortex, e.g. in the anteriorcingulate gyrus, the entorhinal cortex and the amygdaloid cortex, as well as the frontal cortex (130). Thus dopamine appears to be involved in the limbic system. Lesion studies suggest that the frontal cortex is supplied by the A10 cell group (mesolimbic) whereas the anterior cingulate is supplied by the substantia nigra (131).

(iv) Actions of Administered L-DOPA and DA

The systemic administration of L-dopa, the precursor of dopamine, causes in rats and mice aggression, hypertension, pilo-erection, transpiration and catatonic postures (132,133). Reserpine, which depletes the monoamines, causes akinesia and rigidity and this is antagonized by L-dopa. The administration of L-3,4-dihydroxyphenylserine, which forms NE, has no effect (133-135).

Implantation of dopa into the neostriatum causes a compulsive gnawing behaviour, similar to that seen when

amphetamine or apomorphine is given (136). Intracaudatal injection of dopamine antagonizes carbachol-induced tremor (137).

If a rat is subjected to acute ablation of one striatum, the administration of L-dopa will cause the animal to turn its head and tail to the operated side, become excited and rotate vigorously to the operated side (138). This is interpreted as stimulation of DA receptors in the intact neostriatum, a reasonable assumption, as injection of DA into the neostriatum of normal rats causes turning to the contralateral side (139). If amphetamine or apomorphine are given in this nucleus they result in an action similar to that of L-dopa (140). The asymmetry produced by amphetamine can be abolished by AMPT, but not that produced by apomorphine. This indicates that amphetamine acts by releasing endogenous DA, while apomorphine acts directly onto the receptor.

If these drugs are given to a rat whose nigrostriatal tract has been interrupted a few days before, a different situation arises. The difference stems from the fact that in chronic axotomy, the receptors are still present on the operated side. L-Dopa and apomorphine cause

turning to the unoperated side. This effect has been interpreted as due to denervation supersensitivity. The receptors on the operated side become more sensitive, so that when apomorphine or L-dopa is given, they are more effective at producing turning than the normal receptors on the unoperated side (141). Amphetamine does not act like apomorphine in the chronically axotomized animal but causes turning to the operated side (142). Haloperidol prevents the effects of apomorphine, L-dopa or amphetamine, probably by blocking the dopamine receptor (143). These observations are important as they provide evidence for dopamine receptor stimulation (agonism), blocking actions or releasing actions by drugs.

When dopamine is applied microiontophoretically, it is generally found to inhibit unit firing. Krnjevic and Phillis first showed this with cortical neurons (144). Inhibition has also been seen in thalamus (145), hippocampus (146) and especially the striatum (147,148,149). Stimulation of the substantia nigra can inhibit firing of caudate cell (150). However, these findings that dopamine has an inhibitory nature are contradicted by other evidence. Lesions of the nigrostriatal pathway do not alter the rate of spontaneous neuronal firing in the caudate (151); they result

in increased firing in the putamen. Hull et al. reported that there is no correlation between caudate unit firing and the level of dopamine, as altered by lesions (152). However these lesions may have altered other pathways. L-Dopa increases evoked potentials elicited by click stimuli in the caudate pathway, globus pallidus and substantia nigra, instead of decreasing potential as expected of an inhibitory neurotransmitter (153,154). Thus at the cellular level, the action of dopamine is not well understood.

(v) The Role of Dopamine in Pathological States

In Parkinson's disease there is a severe deficiency of dopamine in all parts of the nigrostriatal complex (155-157). Norepinephrine and serotonin (a biogenic amine neurotransmitter) are also low, but these two can be elevated by MAO inhibitors, unlike dopamine (157). The loss of dopamine in the disease is related to the loss of cells from the substantia nigra (158). In animals lesions of the ventromedial tegmental area result in experimental Parkinsonism, with loss of cells in substantia nigra and of dopamine in the striatum (106,159-161). Drugs which deplete or block dopamine produce in man reversible Parkinsonian symptoms (162, 163). The syndrome is treated by administration of L-dopa,

the biosynthetic precursor of dopamine (164,165). HVA and dopa decarboxylase are low in Parkinson's disease (166,167). These deficiencies are seen after nigrostriatal lesions in animals.

Thus there is very good evidence that a deficiency of dopamine is implicated in Parkinson's disease. There are a number of other syndromes where dopamine pathology is implicated, but in these the evidence is suggestive rather than conclusive.

The most important of these is schizophrenia. Amphetamine, which releases DA, can elicit a condition in man resembling paranoid schizophrenia (168). Antipsychotic drugs, such as the phenothiazines and butyrophenones increase DA turnover (169-171), probably by increasing DA neuronal firing (see section B (ii) b). Among the many biochemical actions of antipsychotic drugs, the most potent is the blockade of dopamine-sensitive adenyl cyclase (98). Antipsychotic drugs block the inhibitory effect of iontophoresed dopamine on unit firing (172) and block the release of dopamine from the dopamine neuron (173). It has been suggested that dopamine in the limbic cortex is involved in schizophrenia (174).

The primary deficiency in Huntington's chorea appears to be a lack of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the substantia nigra, putamen, globus pallidus and caudate (175). Glutamic acid decarboxylase, which catalyses the synthesis of GABA, is also deficient in these areas (176,177); the dopamine system remains intact (158). In view of evidence that GABA neurons inhibit the activity of dopamine neurons (see section C (iii)) and the loss of GABA in the nigrostriatal system, it has been postulated that there is an imbalance in Huntington's chorea in favour of dopamine, leading to hyperkinesia and choreiform activity, i.e., the reverse of Parkinson's disease. L-Dopa exacerbates choreiform movements, while phenothiazines and butyrophenones suppress them (178).

The most effective treatment for Gilles de la Tourette (multiple tic) syndrome is treatment with the butyrophenone haloperidol (179). The similarity of Tourette's syndrome to stereotyped movements seen in animals after amphetamine or in Parkinsonian patients after L-dopa therapy has led to the proposal by Snyder of involvement of dopamine in the striata of patients with multiple tic syndrome (180). Agents that inhibit DA synthesis or

disrupt DA stores also alleviate the syndrome (181).

The treatment of hyperactivity in children by amphetamine has led to speculation that this syndrome is mediated partly by deficiencies in dopamine metabolism (182).

Abnormally high HVA (indicative of DA turnover) in cerebrospinal fluid of patients with personality disorders has recently been reported (183) in a preliminary report.

(vi) The Role of Dopamine in the Functioning Brain.

Dopamine appears to be involved in many of the activities engaged in by the brain as it reacts with the external or internal environment.

Dopamine probably plays a role in affective aggression, as apomorphine, which is believed to be a DA agonist (184) causes fighting in rats (185). Similarly, L-dopa (186), amphetamine and MAO inhibitors induce affective aggression (187). These same drugs block predatory aggression (188,189). Amphetamine-induced aggression can be blocked by neuroleptics (190).

The ability of an animal to learn a conditioned avoidance response (where it has to act to avoid noxious stimulation when a warning bell is heard) apparently depends on the presence of a normal functioning dopamine system (191,192). Electrical stimulation of the substantia nigra does not affect learning, but impairs the memory retention of a passive avoidance response (where an animal has to learn not to step off a platform) (193).

Dopamine may play an important role in endocrine function. L-Dopa increases growth hormone release from the pituitary (194) as does apomorphine (195,196). L-Dopa lowers prolactin in serum (197). Infusion of dopamine into portal hypophyseal vessels inhibits prolactin secretion (198). The finding that agents which inhibit prolactin secretion, such as 2-Br-α-ergocryptine (199), are also dopamine agonists (200) has led to the use of inhibition of prolactin secretion as a screening test for drugs of anti-parkinsonian efficacy (201).

Dopamine appears to be important in the regulation of food intake. Lesions of the nigrostriatal pathway (202) or of the substantia nigra (203) produce aphagia (lack of eating) and adipsia (lack of drinking) identical to that

seen in the classical lateral hypothalamic syndrome as described by Teitelbaum and Stellar (204). The lateral hypothalamic lesion also cuts the nigrostriatal pathway, and the subsequent effect on consummatory behaviour may largely reflect the loss of dopamine (205). Food deprivation increases dopamine and dopamine turnover in the hypothalamus (206).

The dopamine system in brain plays a crucial role in the control of motor activity in laboratory animals. The motor activity is a direct measure of the stimulatory or depressant effects of a drug. Apomorphine (184,207), amphetamine (208,209) and L-dopa (208) stimulate motor activity by activating dopaminergic mechanisms. Dopamine itself, injected intraventricularly(368) or into the nucleus accumbens (210) increases motor activity. Lesions of the nigrostriatral pathway block the locomotor response due to amphetamine (211).

Apomorphine and L-dopa increase male sexual behaviour in rats, these effects can be antagonized by haloperidol (212,213).

Dopamine may play a minor role in sleep (214) and in self-stimulation (215). Dopamine neurons in the caudate

nucleus may play a modulatory role in thermoregulation (216,217).

It should be emphasized that dopamine is only one of many neurotransmitters and that each of the brain functions surveyed here requires the complex interaction of several transmitters.

B. Regulation of Dopamine Synthesis

Given that the machinery of the dopamine synapse consists of:

1) a rate-limiting enzyme in dopamine synthesis, tyrosine hydroxylase,
2) Vesicles which release DA into the synaptic cleft under the influence of an action potential,
3) a receptor on the dendrite or cell body of the post-synaptic cell, and,
4) mechanisms for reuptake and for metabolism of dopamine,

one can readily see that there are a number of theoretical ways in which the machine can be controlled, so that not too much or too little dopamine is received at the receptor,

not too much or too little is synthesized.

If too much dopamine is released into the synaptic cleft, the hyperpolarized receptor may respond by inhibiting the firing of the dopamine cell through a neuronal loop; or the released dopamine may inhibit its own release; or inhibit its own synthesis; or there may be a combination of these mechanisms. If too little dopamine is released, the reverse may occur. If there is a chronic insufficiency, the post-synaptic receptor may become more sensitive. There has to be a balance between the rate of release, as moderated by neuronal firing, and the rate of synthesis, so that if the neuron is firing rapidly, more dopamine is synthesized to keep up with demand. If the situation is chronic, more tyrosine hydroxylase may be synthesized. On the other hand, the released dopamine may inhibit dopamine synthesis or release. If the neuron is not firing; less DA synthesis is needed, but there also may be a release from inhibition of synthesis.

The following sections will show that the regulation of dopamine synthesis is fully as complex as these considerations permit.

(i) Control of Tyrosine Hydroxylase

a) Activity

Tyrosine hydroxylase is an enzyme which requires molecular oxygen, a reduced pterin factor and either iron or catalase for optimal activity (17). The affinity for oxygen is low, and hypoxia can inhibit tyrosine hydroxylase *in vivo* (218,219). The enzyme exists in two more or less interconvertible states: particulate (membrane) and soluble (cytoplasmic).

In the adrenal, the Km for tyrosine is between 4 μM (particulate) and 15 μM (soluble) (220) whereas tyrosine levels are 50-100 μM (221). In brain tyrosine is about 80 μM. Patrick and Barchas obtained a Km of 6 μM for tyrosine in synaptosomes from brain, but they noted that the Km for tyrosine uptake is much higher (222). However Kuczenski, also working with synaptosomes reported that tyrosine concentration is not limiting (223). Application of exogeneous tyrosine does not affect tyrosine hydroxylation, indicating that tyrosine hydroxylase is normally saturated with its substrates (224).

Of the pterin cofactors, tetrahydrobiopterin (BH_4) is probably the natural one; the affinity of the

enzyme for its substate and product is higher in the presence of BH_4 than in that of others (225). The concentration of cofactor is limiting in vivo . Intraventricular injection of BH_4 accelerates DA formation in striatum (226).

An early suggestion of end-product inhibition was made on the basis of the temporary rise of biogenic amines after monoamine oxidase inhibition (227). Partially purified preparations of tyrosine hydroxylase have been used to show that catecholamines inhibit the enzyme, and do so by competing with cofactor (228,229). Patrick and Barchas found that DA inhibits its own synthesis in striatal synaptosomes, and that this inhibition is antagonized by cocaine which, in turn, inhibits dopamine uptake (222). Kuczenski found that reserpine which releases DA from vesicles into the cytoplasm, inhibited tyrosine hydroxylase in synaptosomes, whereas amphetamine, which releases DA from the synaptosomes, enhances formation (230). In striatal tissue slices (231) dopamine in the medium inhibits DA formation. The inhibitory effect is partly antagonized by benztropine, which inhibits DA uptake (232). These results indicate the importance of dopamine which has been newly taken up for end-product inhibition.

It was observed that stimulation of the vas deferens results in an increase in synthesis rate, which could be prevented by adding NE to the medium (234). It was suggested that release of transmitter by nerve firing could free tyrosine hydroxylase from end-product inhibition (234). Release of dopamine from striatal synaptosomes or slices by high K^+ concentration increases DA synthesis (222, 231). However end-product inhibition does not explain all the data, as cofactor added to stimulated vas deferens increases biosynthesis just as much as in non-stimulated vas deferens (235), and synaptosomes from amphetamine-treated animals show a decreased biosynthesis capacity (230).

b) Quantity of enzyme

The amount of tyrosine hydroxylase enzyme present in pathways of the peripheral nervous system and in chromaffin cells is increased by: brain stimulation (236), application of stress (237), injection of reserpine (238) or 6-hydroxydopamine (239). It is widely believed that the effect of these agents is correlated with their influence on neuronal firing. Agents which increase nerve firing can increase synthesis of biosynthesis enzyme. Changes

in enzyme level are not as easily obtained in brain, but an effect has been seen after some chronic treatments. These include an increase 1) of midbrain tyrosine hydroxylase after 8 days of reserpine administration (240); 2) of caudate tyrosine hydroxylase in offspring of alcoholic mother rats (241). A decrease of striatal tyrosine hydroxylase is seen after 36 hours of chronic treatment with methamphetamine (242), and after 21 days of treatment with L-dopa (243). These changes may be related to variation in the level of neuronal firing.

(ii) The Relationship of Dopamine Turnover to Neuronal Firing

Dopamine turnover appears to depend partly on the rate of neuronal firing. However, the rate of neuronal firing is itself influenced by the amount of dopamine reaching the postsynaptic receptor. Thus understanding one relationship depends on understanding the other.

a) The relationship of neuronal firing to synthesis rate

As mentioned in i.a. of this section, neuronal firing increases tyrosine hydroxylation in the vas deferens,

This was at first thought to act by a mechanism which is dependent on reduction of an end-product, cofactor-competitive inhibition. However later evidence did not agree with the predictions of this hypothesized mechanism (235). Therefore the activation of tyrosine hydroxylase must proceed by other mechanisms. Moreover these mechanisms may differ from tissue to tissue.

The activation of tyrosine hydroxylase in vas deferens involves calcium, according to evidence obtained by Morgenroth et al. (244). These workers found that the Km for substrate and cofactor is reduced and the Ki for end-product inhibitor is increased by both Ca^{++} and electrical stimulation. The effect of the latter is abolished by EGTA, which chelates calcium. Inasmuch as there is an influx of calcium into the neuron after stimulation, neuronal firing may activate tyrosine hydroxylase through Ca^{++}.

The same laboratory has found that electrical stimulation of the locus coeruleus (very rich in NE-containing cell bodies) results in a lowering of Ki of hippocampal tyrosine hydroxylase for the end-product inhibitor NE, and that this effect can be mimicked by adding Ca^{++}

or cyclic AMP (245).

However the activating role of Ca^{++} may not be applicable to striatal tyrosine hydroxylase. Removal of this ion, rather than its addition, activates striatal slice enzyme (246,247).

When dopamine receptor blockers are administered to rats, the Km of striatal tyrosine hydroxylase for pteridine cofactor is reduced. Apomorphine, which stimulates the DA receptor, antagonizes this action of the blockers (248,249). It is known that receptor blockers increase, and agonists decrease, the firing rate of the DA nigrostriatal neuron (See below). Evidence will be discussed in section iii that the activation of dopamine synthesis by neuronal firing may be through a locally acting receptor in the synaptic region.

b) Factors influencing the rate of neuronal firing

By recording the electrical activity of single DA neurons it is possible to determine the effect of drugs, applied systemically or microiontophoretically, on impulse flow in the nigrostriatal system.

Antipsychotic drugs, which are believed to block

the dopamine receptor (See sections A(iv) and D(ii)) cause an increase in DA cell firing if applied systemically (250) but not if applied microiontophoretically onto the cell body (251). As the classical antipsychotic drugs, the phenothiazines and butyrophenomes, increase DA turnover (252,253), an action prevented by sectioning the nigro-striatal pathway (254), it has been accepted that the increase in DA turnover is consequent upon increased neuronal firing caused by the activation of a feed-back loop following the blockade of DA receptors in the striatum (252).

Apomorphine, which is thought to stimulate DA receptors (140,184) depresses the firing of DA neurons when applied systemically (250) or locally by microiontophoresis, as does dopamine (251). These findings indicate that dopamine receptors are located on the dopamine neuron, and moreover, that the receptors are similar enough to the postsynaptic receptor to be activated by the same drug. As apomorphine decreases dopamine turnover, the not unreasonable conclusion has been that the decrease in turnover is related to the reduced neuronal firing. One of the criteria for new dopamine agonists is that they decrease DA turnover (See section D(i)).

Amphetamine applied systemically but not microiontophoretically also depresses firing of DA neurons. Ongoing DA synthesis is necessary for amphetamine to be effective, but not for apomorphine. A lesion just anterior to the substantia nigra results in a rapid firing of DA neurons which is not antagonized by amphetamine (251). These results provide additional evidence that amphetamine is not an agonist but acts by releasing DA, and that there is a neuronal feedback loop.

(iii) Local Receptor Control of Dopamine Metabolism

a) Inhibition of Release

It is now well established that in the peripheral noradrenergic system, there is feedback control of the release of NE from the terminal. NE inhibits its own release in two ways; by acting through prostaglandins and by activation of α-adrenoceptors. (See Langer for review and references, 255). Evidence suggests that the receptor controlling release is presynaptic (256). There also appear to be dopamine-sensitive receptors (257) and muscarinic receptors (258) that inhibit NE release, and nicotinic receptors that stimulate release (259).

Not as much work has been done with brain, but

similar influences on catecholamine release appear to be operating. If slices of cerebral cortex are preincubated with ^{3}H-NE and then electrically stimulated, the release of tritium is diminished by agents which stimulate α-adrenergic receptors and increased by agents which block α-adrenergic receptors or inhibit prostaglandin synthesis (260,261). Little work has been done with central dopamine neurons. Prostaglandin E_2 reduces slightly the release of ^{3}H-DA from striatal slices (262). Apomorphine and antipsychotic drugs have been reported by Farnebo and Hamberger to decrease and increase, respectively, ^{3}H-DA release from striatal slices (263,264). However Seeman and Lee, using the same method, obtained the opposite result with antipsychotic drugs--an inhibition of release (173). Nicotine promotes release of DA from striatal slices (265).

While the evidence obtained with neuroleptics is contradictory, the effect of noradrenergic drugs on NE release and of apomorphine on DA release indicate a precedent for the existence of receptors in the region of the synapse which can influence aspects of neurotransmitter metabolism. In section ii(a) evidence was described which indicates the presence of dopamine-sensitive receptors on the surface of the dopamine neuron. Two types of dopamine-

sensitive receptors at the synaptic site have been postulated: one that stimulates DA synthesis, and another that inhibits DA synthesis. In the next two sections, the literature on this topic will be surveyed.

b) Local Receptor Stimulation of Dopamine Synthesis

As discussed in section ii(a) nerve stimulation activates tyrosine hydroxylase. The question is how the action potential is related to the enzyme activity. For NE neurons the influx of Ca^{++} during the action potential may possibly activate tyrosine hydroxylase. For dopaminergic neurons the possibility is now emerging that DA released following the appearance of the action potential activates the enzyme through a second messenger, cyclic AMP.

Dibutyryl cyclic AMP when added to the medium bathing slices of striatum (266) or striatal synaptosomes (267) activates tyrosine hydroxylation in the tissue. Both nerve stimulation and cyclic AMP increase the affinity of tyrosine hydroxylase for substrate and cofactor, and both decrease the affinity for the end-product, which inhibits (268). High concentrations of K^+ also activate dopamine synthesis in slices (231), but these do not appear

to act through cyclic AMP (269). Dopamine agonists enhance the stimulation by cyclic AMP of tyrosine hydroxylase in synaptosomes (270). It thus appears possible that the dopamine released by the action potential activates its own synthesis through the intermediacy of an adenyl cyclase receptor. The activation of tyrosine hydroxylase by cyclic AMP may, in turn, be mediated by a protein kinase (271).

c) Receptor mediated inhibition of dopamine synthesis

This thesis attempts to answer the question whether there is a dopamine receptor which feeds back to cause inhibition of dopamine formation. The literature on this question will be reviewed up to the beginning of the project. Papers relevant to the work published during the course of the work will be discussed in Section IV.

Axotomy of the nigrostriatal tract results in a sharp increase in DA in the forebrain (272). Levels rise about 80-100%, and then reach a plateau which is constant until the axon begins to degenerate. The increased concentration suggests that tyrosine hydroxylase is released from receptor inhibition. Transsections of NE or serotonin tracts do not give rise to this effect (273). Kehr et al.

(274) found that the synthesis rate after transsection of the nigrostriatal pathway was not influenced by haloperidol, but was inhibited by apomorphine. Haloperidol given in conjunction with apomorphine was able to antagonize the inhibition by the latter. The measure used by Kehr et al. (274) was the rate of increase of dopa in rats that had received a dopa decarboxylase inhibitor. The same result was seen when the accumulation of dopamine was used as the measure (275). Moreover amphetamine also antagonized the increase in dopamine. The same results were seen if γ-hydroxybutyric acid (GOBA) was used instead of axotomy to block the flow of impulse (275). Goldstein et al. (266) found that haloperidol increased dopamine synthesis in striatal slices, while piribedil (a DA agonist) decreased synthesis. These results have been interpreted as evidence for the presence of a receptor which is activated by the agonist apomorphine with the result that synthesis is inhibited. A reputed blocker of DA receptors, haloperidol, has no positive effect by itself, but prevents stimulation by agonists.

The above hypothesis did not appear to be completely convincing or to answer all questions, so that alternative explanations for the biochemical actions of

apomorphine and haloperidol have been put forward. The approach used in the present research to deal with this problem was two-fold:

1) experimental testing of various explanations of the biochemical actions of apomorphine, and

2) the use of recently discovered dopamine agonists to determine whether they, like apomorphine, inhibit dopamine synthesis in the absence of neuronal firing.

In order to study the question of local receptor control of dopamine it is necessary to eliminate the confounding influence of neuronal firing and the feedback loop that influences it (see section ii). γ-Hydroxybutyric acid (GOBA) was employed to prevent neuronal firing. Section C reviews the actions of GOBA in brain, while section D deals with the pharmacology of the dopamine agonists and antagonists.

C. The Action of GOBA on the Dopamine System

(i) General

GOBA is a central nervous system depressant which produces sleep and has analgesic and anesthetic properties

(276). The sleep produced by GOBA is of the physiological type, but with facilitation of rapid eye movement sleep (277). It does not inhibit the reticular formation as other depressants do (278). The action of this drug is interesting because high doses produce deep anesthesia behaviourally, yet the EEG is activated.

Like other depressants, GOBA elevates brain glucose (279). Alterations of glucose metabolism have been suggested to underlie the mechanisms of sleep (278). GOBA also causes marked increases in brain acetylcholine (280) and dopamine (281).

GOBA is present naturally in small amounts in brain (282). When injected in an anesthetic dose its half-life is about one hour (283). The principal mode of metabolism is oxidation to succinic acid, which then enters the tricarboxylic acid pathway. Small amounts of Krebs-cycle-related amino acids such as aspartic acid, glutamic acid and GABA can be derived from GOBA (284).

(ii) Parallelism between GOBA Administration and Lesion of the Nigro-Striatal Tract

There are a number of parallels between the effects

of GOBA and transsection of the nigrostriatal tract. After both treatments there is a sharp increase in dopamine. After injection of GOBA, DA ascends to a peak, after which the amine level decreases as the drug is metabolized (272, 281,275,285,286). Both prevent firing of the dopaminergic neuron (287). Intrastriatal injections of KCl prevent the increase in DA after hemisection or after GOBA (288). The rise in DA is in each case inhibited by apomorphine and unaffected by haloperidol alone, although haloperidol will antagonize the effect of apomorphine (275). GOBA, as does axotomy, prevents the rise in HVA following neuroleptic treatment (289). Amphetamine prevents the rise in DA after axotomy and after GOBA (275). AMPT blocks the GOBA-induced increase in dopamine, as it does with axotomy (290,291). GOBA or transsection reduces or abolishes the decrease in DA seen after AMPT (292,288,293).

The pharmacological profile of GOBA closely resembles that seen with acute axotomy. The best explanation for this is that GOBA acts on DA metabolism the same way that axotomy does--by preventing firing of the DA neuron.

(iii) The Site and Mode of Action of GOBA

GOBA does not inhibit COMT or MAO *in vitro* or *in vivo* (281,290), thus inhibition of catabolizing enzymes is not responsible for the increase in DA. In fact, if MAO is inhibited *in vivo* so that DA is elevated, GOBA is able to produce a second elevation on top of the first (281). Whereas an enhanced diffuse fluorescence is seen in formaldehyde-treated slices of brain taken from pargyline-treated animals, GOBA increases DA in varicosities and it is thought that this reflects an increase of DA in nerve endings (294).

Addition of GOBA to the incubation medium does not produce increased conversion of ^{14}C-tyr to ^{14}C-DA by striatal tissue slices, nor does GOBA block or potentiate the K^+-induced increase in formation of ^{14}C-DA. Tyrosine uptake is unaffected (295).

The levels of γ-aminobutyric acid(GABA) and the enzymes that synthesize and degrade it,glutamic acid decarboxylase (GAD) and GABA-transaminase (GABA-T), respectively, are particularly high in substantia nigra (296). There is a GAD-containing nervous pathway which runs from striatum to substantia nigra (297). A lesion in this pathway causes

a loss of GAD in the substantia nigra (298). GABA applied microiontophoretically inhibits nigral cell firing (249). Treatment with a GABA-T inhibitor, which increases GABA in the synaptic cleft, inhibits DA turnover (300). GABA, if injected into the substantia nigra, increases brain DA, as does GOBA (301). Injection of GABA into the striatum has no effect. Given these data and the analogous structures of GOBA and GABA, the suggestion that the action of GOBA on DA neurons is through an inhibitory GABA mechanism in the substantia nigra (275) is not unreasonable.

(iv) Effect of Axotomy or GOBA on DA Synthesis

After axotomy or GOBA, the ability of AMPT to deplete DA is reduced (293,288). Moreover, the concentration of DOPAC is reduced (287). At the same time there is increased synthesis. The incorporation of label from ^{3}H-tyr into DA is increased three-fold following lesions of the nigrostriatal pathway or administration of GOBA (293,302). The decreased metabolism and increased synthesis combine to yield a sharp increase in brain dopamine.

Thus, the same treatment that prevents the dopamine cell from firing, thereby removing the influence of firing on DA metabolism, also provides the measure with

which to study receptor feedback on dopamine synthesis, namely an increase in dopamine levels. If agents known to stimulate DA receptors inhibit the rise of DA after GOBA, then evidence has been obtained that a receptor exists which acts independently of neuronal firing. At the time this project was initiated, Anden et al. (275) had shown that apomorphine inhibited the rise of DA after GOBA, and that haloperidol seemed to antagonize the inhibition by apomorphine. However, the idea that apomorphine produces its biochemical actions through receptors has recently been disputed (311,312). Therefore it was decided to study further the problem of the existence of the hypothetical receptor.

D. Drugs Acting on the Dopamine System

(i) Agonists

a) Apomorphine

The classical dopamine agonist is apomorphine. Apomorphine produces turning in rats with unilateral lesions of the nigrostriatal pathway, as does L-dopa (see Section A(iv). Injection of DA into the striatum (303) or stimulation of the substantia nigra (304) results in

asymmetric behaviour. Thus the turning action induced by apomorphine is related to the dopamine receptor.

Systemic administration of apomorphine or amphetamine has long been known to cause stereotyped gnawing behaviour (305). L-Dopa injected into the striatum does the same (184). Thus, drugs which cause stereotyped behaviour bear a relation to DA and may be implicated in dopamine receptor activity.

Apomorphine reduces the turnover of DA, as measured by the amount of HVA (306) and rate of depletion of DA in brain following administration of AMPT (140). The protection afforded by apomorphine against AMPT depletion may result from depressed firing of dopamine neurons, owing to neuronal feedback inhibition (140). Apomorphine slows firing of DA neurons, however, it is unknown whether this is due to activation of dopamine receptors on the DA cell (See Section B(ii)). As neuronal firing is slowed and as DA turnover is affected by the rate of neuronal firing, it is logical to assume that apomorphine decreases DA turnover through its actions on the dopamine receptor.

Another model for determining agonist action is that of dopamine-sensitive adenyl cyclase from homogenates

of striatum or retina (98,307). Apomorphine activates DA-sensitive adenyl cyclase (98,308).

The actions of apomorphine in inducing stereotyped behaviour, in causing turning in unilaterally lesioned animals, in decreasing DA turnover, and stimulating DA-sensitive adenyl cyclase are now regarded as the characteristic properties of DA agonists.

Recently, alternative suggestions have been made as to the mode of action of apomorphine on dopamine metabolism.

McKenzie has argued that apomorphine may decrease DA turnover through inhibition of COMT. He has reported that apomorphine is a substrate inhibitor of COMT (309) and has drawn a number of parallels between the effect of tropolone, which inhibits COMT, and the effect of apomorphine on behaviour and on DA metabolism. COMT inhibitors prolong the stereotypy produced by apomorphine (310). The incorporation of label from ^{3}H-tyr into ^{3}H-dopamine is decreased by both. Apomorphine and tropolone retard the disappearance of ^{3}H-DA. Both antagonize chlorpromazine-induced increases in DA synthesis. As tropolone does not induce stereotypy or antagonize chlorpromazine-induced

depression of rats, it was suggested that apomorphine decreases DA turnover by inhibiting COMT, and that there is, in fact, no connection between the behavioural and biochemical effects of apomorphine (311).

A second alternative interpretation of apomorphine's biochemical actions has been suggested, involving the inhibition of the other main DA metabolizing enzyme, MAO (312). Di Chiara found that apomorphine inhibits dopamine deamination by rat brain mitochondria in vitro. In favour of their hypothesis that apomorphine inhibits MAO in vivo, they found that DA levels in brain were elevated by apomorphine (as after MAO inhibitors), that HVA and DOPA were decreased, that apomorphine prevented the rise of HVA and DOPAC after reserpine and protected dopamine against the depletion produced by this drug, and that apomorphine decreased the rise of HVA and DOPAC produced by L-dopa administration. It was suggested that other workers failed to detect a rise in DA after apomorphine as methods had been used in which apomorphine interfered with the assay of dopamine. It was argued that lowering of HVA levels after apomorphine was due to inhibition of DA deamination and that the lower rate of biosynthesis of DA was due to end-product inhibition following elevated DA levels (312).

b) Other agonists

Piribedil (ET 495, Trivastal) produces contralateral turning in rats with a unilateral lesion of the nigro-striatal pathway, as does apomorphine (313). Amphetamine causes turning in the opposite direction in this model (see section A(iv)). Piribedil reduces DA turnover, as measured by the rate of depletion after AMPT (313) or by HVA concentration in rat brain (314) or cerebrospinal fluid of man (315). Stereotyped behaviour in rats is seen after piribedil (316) and the effect is enhanced if the animals have been given 6-hydroxydopamine (which causes the DA system to degenerate) as neonates. This is true if apomorphine is substituted for piribedil, but not so for amphetamine (317). These results indicate that piribedil acts postsynaptically on the DA receptor. However Costall and Naylor (316) found that abolition of DA stores by drugs or lesions prevents the stereotypy elevated by ET 495, suggesting that piribedil may also act by causing release of DA from the presynaptic site.

It appears that the metabolite of piribedil known as S584 is the active agent, because it, but not ET 495, is able to increase cyclic AMP in striatal synaptosomes (318). Also S584, but not piribedil causes asymmetry and

stereotyped behaviour when injected into the striatum (319).

Piribedil reduces tremor in monkeys with lesions of the ventral tegmental area (containing the DA nuclei) (320) and has been reported to be of some benefit in Parkinsonian patients (321).

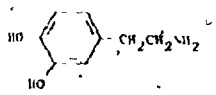
Piribedil reduces DA synthesis in striatal slices (266).

Another class of DA agonists includes the ergot alkaloids, (see section A(vi). Ergocornine and 2-bromo-α-ergocryptine, (CB 154), cause sterotypy, induce turning to the contralateral side after unilateral transsection of the nigrostriatal tract, and reduce DA turnover (322,323). CB 154 possesses long-lasting anti-tremor activity in monkeys with lesions of the ventromedial tegmental region (324).

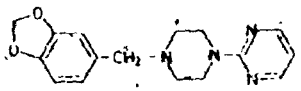
5,6-Dihydroxy-2-dimethylamino tetralin (M-7) consists of the lower portion of apomorphine. Like apomorphine it has powerful emetic actions in dogs, and causes stereotyped behaviour (325). Like dopamine, it inhibits sciatic nerve stimulation of the heart (326).

Apocodeine (10-O-methylapomorphine) induces

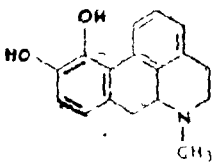
Fig. 1. Structure of dopamine and dopamine agonists .



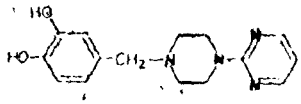
Dopamine



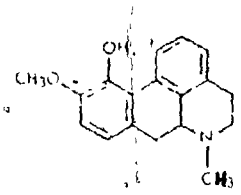
Piribedil



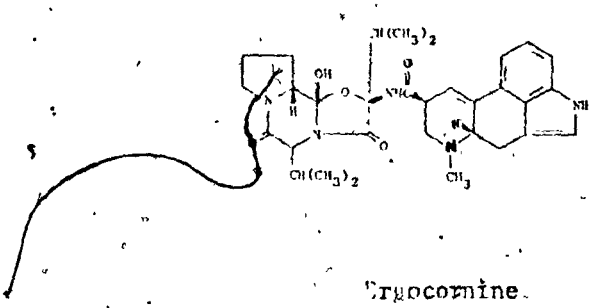
Apomorphine



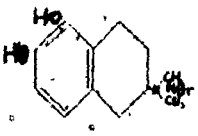
584



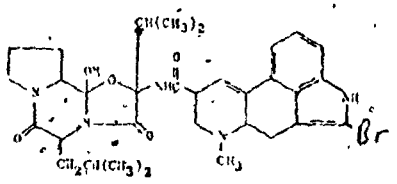
Apocodeine



Ergocornine



M7



CB 154

stereotyped behaviour, reverses reserpine-induced sedation, and reduces dopamine turnover, as measured by the concentration of HVA in the brain (327).

(ii) Antipsychotic drugs

Blockade of dopamine receptors has been suggested as the mechanism of action of antipsychotic drugs in schizophrenia (328,329). An action of these drugs on dopamine metabolism was first found by Carlsson and Lindqvist in 1963, who observed an increase in the concentration of dopamine metabolites in brain (329). It was suggested that an increase of DA turnover results from neuronal feedback. Various indicators of turnover, such as levels of acidic metabolites (330), depletion of DA after AMPT (331) and incorporation of label from labelled precursors (332) indicate an increase in dopamine synthesis and metabolism after neuroleptic treatment. As interruption of neuronal impulse flow prevents the increase in turnover (254), and as neuroleptics increase the firing of DA neurons, (section B(ii), the increase in turnover may be related to an induction of increased neuronal firing mediated by feedback from the postsynaptic receptors.

Neuroleptic drugs antagonize the turning produced

by L-dopa or apomorphine in striatectomized rats with lesions in the striatal area (333). The classical neuroleptics, such as haloperidol and chlorpromazine antagonize the turning behaviour produced by methylamphetamine in unilaterally lesioned rats, and also the stereotypy produced by amphetamine or apomorphine (334). However some antipsychotic drugs, such as clozapine and thioridazine, do not antagonize stereotypy or turning induced by agonists (335,336), leading to doubts as to whether the antipsychotic efficiency of these drugs is correlated with their action on dopamine receptors. However it has recently been shown that the atypical effect of these neuroleptics in these behavioural paradigms may be explained in terms of their antimuscarinic action (337,338). Many neuroleptics induce in animals a form of depression known as catalepsy. The tendency to induce catalepsy appears to be correlated with anti-turning potency (338).

Neuroleptics, including clozapine, are powerful blockers of stimulation of cyclic AMP by DA in striatal homogenates (for review see 339). Further evidence for blockade of dopamine receptors is indicated by the conformational similarity between dopamine and antipsychotic drugs (340), antagonism of the depressant effect of

microiontophoresed dopamine in striatal neurons by chlorpromazine (341) and the antagonism of dopamine's effect on molluscan neurons by haloperidol (342).

Another theory concerning the action of neuroleptics, proposed by Seeman and Lee, states that they interfere with the amount of dopamine reaching the receptor by interfering with impulse-coupled release. Seeman and Lee have found a good correlation between the clinical efficacy of neuroleptics and the concentration needed to inhibit stimulation-coupled release (173).

Seeman and Lee's hypothesis cannot fit all the data, such as antagonism of agonists or blockade of adenyl cyclase to dopamine. It is probable that neuroleptics interfere with dopamine transmission by both blocking the receptor and blocking impulse-coupled release. One or the other of the actions may be more important, depending on the neuroleptic.

Haloperidol was the neuroleptic selected for use in this study.

II. METHODS

A. Animals

Sprague-Dawley male rats of 130-160g body weight were used. They were kept 2 or 3 to a metal cage in a room thermostatically controlled at 22°C and relative humidity 45%. They had free access to food and water on a regular 12 hours light, 12 hours dark cycle. After being received from the supplier, animals were allowed at least 24 hours rest before use.

B. Dopamine Measurement

(i) Tissue Preparation

The extraction and purification of dopamine was based on the method of Anton and Sayre (343), with minor modifications.

a) Striatum

In an initial experiment, dopamine was measured in the striatum. The whole brain of rat was cooled in ice-cold saline (0.9% NaCl) immediately after removal from the animal. The striatum was dissected over ice: the cortex was stripped from the anterior forebrain and a cut made

through the lateral ventricle to expose the striatum, which was readily recognized because of its dark colour and striated appearance; surrounding tissue was then cut away. After weighing, each striatum was homogenized in 4ml 0.4N perchloric acid. The dopamine in each striatum from rat brain was measured separately.

b) Whole brain

In most experiments, the whole brain was used. It was cooled in ice-cold saline. After drying the organ on filter paper and removing blood vessels, the brain stem caudal to the cerebellum was excised, the brain weighed, and placed in 10ml of cold 0.4N perchloric acid, with 0.2 ml 10% EDTA added. After homogenization with a Teflon pestle, the suspension was allowed to stand for a few minutes in order to complete the process of deproteinization. Samples were then frozen. In a typical run, where drugs were administered and animals sacrificed at specific times, 10 min elapsed between sacrifice and homogenization.

(ii) Purification of Dopamine

a) Glassware and Reagents

Glass tubes, centrifuge tubes and reagent solution

bottles were soaked in 2 N HCl then rinsed and allowed to stand with deionized distilled water. The last step was repeated several times to leach out interfering fluorophores. Beakers and pipettes used during the assay were not acid-washed, but rinsed several times with deionized water. All solutions were prepared in double-distilled water.

b) Preparation of alumina columns

Woelm Al_2O_3, 300 grams, was suspended in 1.5 l. of 1 N HCl and heated for 2 hours at 60° C with constant stirring. After the alumina had settled, the supernatant was discarded. The alumina was washed and fine particles eliminated in one of two ways. The alumina was either backwashed with at least 10 l. of deionized water in a column, or washed and decanted at least 15 times in a large beaker. The second method is less time-consuming. The prepared alumina was stored in deionized water.

Before preparation of columns, the flow rate of a large number of Pasteur pipettes, with glass beads inserted, was examined. Those with a flow rate of approximately 4 ml/min were selected and alumina was added to a height of 2.5 cm above the bead. Plastic funnels, with a capacity of approximately 15 ml, were attached to the top

of the columns and filled with double-distilled water in order to wash the columns. The flow rate was again examined, and about a third of the columns rejected for speeds too fast or slow. The remaining columns were then washed with a funnel-full of 10 mM mercaptoethanol, 10 mM EDTA solution which had been adjusted to pH 8.

c) Purification of dopamine

The homogenates were thawed and centrifuged at 0°C at 12,000 g for 15 min. Care was taken to ensure that thawing did not proceed too long, as prolonged exposure of homogenates to room temperature was found to decrease recovery. Following centrifugation, the precipitate was discarded and the supernatant was adjusted at room temperature to pH 8 with 2.75 N NaOH, 0.25 M EDTA. The sample was immediately placed on the column. The column was washed, first with 1 ml of 10 mM mercapto-ethanol 10 mM EDTA pH 8.0 solution, then two funnels-full i.e. about 30 ml of double-distilled water. The sample was then eluted with 4 ml 0.1 N perchloric acid. The sample was frozen in the acidic state before or after passing through the columns.

(iii) Oxidation of Dopamine

The oxidation step is based on the procedure of Laverty and Taylor (344). From each 4 ml sample, 2 ml was withdrawn to be used as the blank. Samples were adjusted to pH 7.0 at room temperature with 1.0 N NaOH, 0.5 M KH_2PO_4. Oxidation was performed by mixing in 0.1 ml of 0.02 N I_2 in 5% NaI, followed exactly 3 min. later by 0.5 ml 2.5% Na SO_2, 1% EDTA, 2.5 N NaOH solution, again followed exactly 5 minutes later by 0.2 ml of glacial acetic acid. Reagents were added in reverse order to blanks. Samples were then heated in an oven for 40 min. at 100°.

Following cooling to room temperature in a water bath, the fluorescence of each sample was read in an Aminco-Bowman spectrofluorometer. The activation and the emission wavelengths were 315 and 375 nm, respectively. The sample compartment slits and photomultiplier slit were set at a width of 2 mm. The sensitivity was set at 100% and readings made on a ratio setting (where the intensity of excitation and the sensitivity of detection are in a constant ratio). Initially all blanks were read. In later experiments every other blank was read. Typically the fluorescence in a sample from the brain of a normal rat was 30 times that of its blank.

(iv) Calculations for dopamine content

In order to convert relative fluorescence readings into absolute values of dopamine content, and to calculate recovery, internal and external standards were used. At least 4 internal standards were used in every experiment. Typically, 4 surplus brains were homogenized in 40 ml of 0.4 N perchloric acid, 0.8 ml of 10% EDTA. The homogenate was divided into 8 equal portions of 5 ml. To one-half of 4 pairs, 1.00 µg of DA-HCl was added. Dopamine standard solutions were made by dissolving 10.0 mg of dopamine hydrochloride in 10 ml of 0.01 N HCl to form a stock solution, then diluting 0.20 ml to 10 ml to form a standard solution, from which 0.050 ml portions were taken for internal and external standards.

The internal standards were distributed in pairs at regular intervals among the samples, so that any artifactual order effect would be detected. After reading, the fluorescence of 1.0 µg DA-HCl was determined by subtracting the reading of one member of each pair from the other, then taking the average. The dopamine content of all the samples was determined by multiplying (1000 ng ÷ average fluorescence of 1.0 ng DA-HCl) and dividing by the brain weight in grams to give dopamine content in ng/g. Usually, normal rats

were found to have a dopamine content of 800 ng/g brain (as dopamine base).

To calculate recovery, 1.0 µg DA-HCl was added to 4 ml 0.1 N $HClO_4$ and adjusted to pH 7.0 just before the oxidation step. The fluorescence of this standard, after subtraction of blank, was divided into the mean internal standard fluorescence for 1.0 µg DA-HCl to give the recovery. Where whole brain was assayed, the recovery normally ranged between 60 and 70%.

C. Tyrosine Hydroxylase in vitro

(i) Purification

Purification of tyrosine hydroxylase was performed by M. Quik of this laboratory and is based on the method of Nagatsu et al. (17). The general procedure was as follows. Minced bovine adrenal medulla was homogenized in sucrose, spun down, the pellet discarded and the supernant enzyme solution partially purified by ammmonium sulfate precipitation and passage through a Sephadex G-25 column. The partially purified enzyme was diluted in Tris-acetate-sucrose buffer pH 7.6 so that the final concentration of protein was 2 mg/ml. (yielding an activity of 0.15 nmoles dopa hr^{-1}

mg^{-1}). The enzyme was stored frozen.

(ii) Assay

The assay is based on the method of Nagatsu et al. (17).

a) Reagent solutions and glassware

Tubes used in the incubation were acid-washed and scrupulously rinsed with deionized water as were tubes in the dopamine assay. Vials and beakers used in preparation of solutions were rinsed several times with deionized water.

The following reagents were purchased: 6,7-dimethyltetrahydropterin ($DMPH_4$) from Calbiochem (Los Angeles, CA); L-tyrosine -3,5-^3H (58 Ci/mmole) from New England Nuclear Corp. (Boston, MA); tropolone from Regis (Morton Grove, IL); catalase from Sigma (St. Louis, MO) and Dowex 50 W-X8 from Bio-Rad (Richmond, CA). Brocresine (m-hyroxy-p-bromobenzyloxyamine) was a gift of Lederle Laboratories (Pearl River, NY).

All solutions were prepared in doubly distilled water. A few solutions were not kept for use longer than one week: 3M sodium acetate buffer pH 6.1; 0.3 M potassium

phosphate buffer pH 6.1, 4mM tyrosine; 1mM brocresine, 10% sucrose solution. The scintillation cocktail used consisted of 700 ml. toluene, 300 ml Triton X-100 and 5 g PPO.

The radioactive tyrosine was purified before use by passage through a Dowex 50 W-X8 column, 0.5 cm. by 30 cm, and diluted by 0.01N HCl to approximately 4 x 10^6 cpm/ml. Just before the incubation an aliquot of ^{3}H-tyrosine solution was dried in a rotary evaporator at 30° and the ^{3}H-tyrosine redissolved in a volume of 4 mM tyrosine 3 or 4 times that of the original aliquot.

On the day of the incubation a buffer mixture consisting of 2 ml of acetate buffer, 1 ml of phosphate buffer, 3 ml of brocresine and 0.2 ml of catalase was prepared. Immediately before the incubation 10 mM $DMPH_4$ in 25 mM ascorbate solution was prepared. From this solution more dilute $DMPH_4$ solutions were made in ascorbate if necessary.

b) Preparation of Dowex column

Dowex 50 W-X8 H^+ (200-400 mesh) cation exchange resin was prepared for columns by M. Quik of this laboratory. The resin was prepared by backwashing in a 20 by 500 mm

column 1 l. of distilled water, washing with 2 l. of 1 NH_4Cl, 3 l. of distilled water and backwashing 1 l. distilled water.

Dowex columns (4x20mm) were prepared in Pasteur pipettes and the resin was washed with water followed by 1.5 ml of trichloroacetic acid solution (TCA) pH 1.8.

c) Incubation

To centrifuge tubes in ice, 0.1 ml of buffer, 0.2 ml of tropolone or water, 0.025 or 0.050 ml of sucrose, 0.075 or 0.050 ml. of enzyme were added. The enzyme was preincubated with shaking no longer than 15 min. in a water-bath at 30° after addition of 0.050 ml of ^3H-tyrosine solution. The reaction was started with 0.050 ml of $DMPH_4$ and allowed to proceed for 20 min at which time 0.1 ml of 25% trichloroacetic acid was added.

The reaction mixture in each tube was passed through a Dowex column, followed by 0.5 ml TCA pH 1.8 which had been used to wash the centrifuge tube. The eluate was collected in a vial containing 10 ml of scintillation cocktail. The sample was then counted for its content of tritium.

Blanks had everything added except cofactor, which was substituted by water. To internal standards tritiated water was added instead of ^{3}H-tyrosine. In order to provide a measure of recovery the same amount of tritiated water as used for internal standard was added directly to two scintillation vials, providing external standards. The ratio of internal to external standard counts provided the measure of recovery. In order to determine the amount of ^{3}H-tyrosine added to each reaction tube, 0.050 ml was added to two scintillation vials.

Activity in nmoles mg protein^{-1} hr^{-1} was calculated. The counts per minute (CPM) of each sample were a measure of tritiated water formed. As tyrosine hydroxylase hydroxylates either a normal or tritiated hydrogen, the CPM's were multiplied by two to provide a measure of number of tyrosine molecules acted upon. In fact only 90% of the theoretical maximum of tritium protons are acted upon, due to hydrogen interchanges within the tyrosine ring, so CPM was further multiplied by a correction factor of 100/90. In order to convert CPM units into n moles tyrosine acted upon, another multiplication factor was Tyr/CPM$_{tyr}$, where Tyr was the number of n moles tyrosine added to the incubation mixture, and CPM$_{tyr}$ the number of counts added,

as shown by external standards. A correction for recovery was made by dividing by the recovery, as calculated by the counts of tritiated water in the internal standard divided by those in the external standard. In order to express the results in terms of protein added, CPM was divided by mg protein added. Finally CPM was multiplied by 60/t, where t was the length of reaction in minutes, so that the result was expressed in terms of hours.

Thus the activity in n moles/mg protein/hr was determined by multiplying CPM by

$$\frac{100}{P} \cdot \frac{\text{Tyr}}{\text{CPM}_{tyr}} \cdot \frac{}{\text{mg protein}} \cdot \frac{2 \cdot 100}{90} \cdot \frac{60}{t}$$

D. Monoamine Oxidase in vitro

(i) Preparation of tissue sample

Mitochondria from brain were prepared by a modification of the method of French and Toderoff (345). Rat brains were homogenized with a Teflon pestle to form 10% homogenate in a cold solution consisting of 250 mM sucrose, 1 mM EDTA, 5 mM tris at pH 7.4. The homogenate was spun for 10 min. at 1000 g and the pellet discarded. The

supernatant was spun for 15,000 g for 5 min. The resulting supernatant was discarded and the pellet was resuspended in 25 ml of 250 mM sucrose, 5 mM tris buffer pH 7.4. The same step was repeated after spinning again at 15,000 g for 5 min.

After centrifuging 15,000 g for a third time the pellet was resuspended in 0.07 M phosphate buffer pH 7.2, so that 1.2 ml of suspension was obtained for each brain. The suspension was stored frozen.

The crude mitochemical fraction obtained likely contained cell fragments of similar density, such as synaptosomes. The protein content was determined by the method of Lowry et al. (346).

(ii) MAO assay

The assay for brain mitochondrial MAO employs DA-1-^{14}C as the substrate. Products of deamination are collected in ethyl acetate. The method was based on that of Neidle et al. (347). A total incubation volume of 1.5 ml was employed. All tubes were acid washed. To 0.9 ml of ice-cold 0.1M potassium phosphate buffer pH 7.2 was added 0.4 ml water or 0.1 ml 6 mM apomorphine solution and

or 0.3 ml haloperidol solution and/or water to make up the difference. Preincubation with shaking at 37° for no longer than 15 min. was initiated after the addition of 0.10 ml of enzyme. The reaction was started after addition of 0.100 ml of 0.1 M phosphate buffer pH 7.2 containing ^{14}C-DA and cold DA. The amount of DA varied so as to give a final concentration between 0.8 and 0.08 nM (0.6 to 0.06 uCi ^{14}C-DA).

The reaction was terminated after 15 or 20 min. with 0.1 ml of 3 N HCl. Each sample was mixed with 3.0 ml of ethyl acetate for 30 sec. After spinning in a centrifuge to effect phase separation, the ethyl acetate layer was transferred to a second tube containing 2 ml 0.3 N HCl. After mixing for 15 sec., 0.5 ml of the ethyl acetate layer was added to a vial containing 10 ml scintillation cocktail, and the sample counted.

Blanks consisted of all the reagents except enzyme. It was found that the counts in blanks depended on the amount of label added, thus a blank for each substrate concentration was employed. Internal standards consisted of a fixed amount of DA solution added to vials.

E. Drug Schedules and Doses

(i) Drugs

The following drugs were purchased: sodium γ-hydroxybutyrate and α-methyl-p-tyrosine methyl ester from Sigma (St. Louis, MO) and pargyline, pheniprazine and tropolone from Regis (Morton Grove, IL). The following drugs were gifts and are gratefully acknowledged: apomorphine HCl and apocodeine HCl from Merck-Frosst Laboratories (Kirkland, Quebec), piribedilfrom Servier (Neuilly-sur-Seine, France), DL-5,6-dihydroxy-2-dimethylamino tetralin HBr (M7) (Professor J.G. Cannon, U. of Iowa, Iowa City, Iowa),ergocornine hydrogen maleate and 2-bromo α-ergocryptine (CB 154) (Sandoz A.G., Basel, Switzerland), haloperidol (McNeil Laboratories, Don Mills, Ontario) and amphetamine (Smith, Kline and French, Philadelphia).

All drugs were injected in saline except GOBA, which was dissolved in water. CB 154 was injected as a suspension. In order to ensure stability and to facilitate dissolving, M7, apomorphine, apocodeineand ergocornine were dissolved in slightly acidic solution. Haloperidol was dissolved in a few drops of glacial acetic acid, which was then diluted with saline, and the pH adjusted to approximately 6.5. All drugs were injected intraperitoneally in a volume 1.0% of the volume of the animal except

apomorphine, which was injected in a volume 0.1% of the animal.

(ii) GOBA and haloperidol

In an initial experiment GOBA was injected in the dose of 1.5, 1.75 and 2.0 g/kg (as the sodium salt) and the brains taken for analysis at various times. In other experiments GOBA was given in a dose of about 2.2 g/kg, and the animal sacrificed 40 min. later. Whether the drug was penetrating satisfactorily was gauged by the anaesthetic effect, if an animal responded to a tail pinch it was not used in the experiment.

In all experiments haloperidol was injected in the dose of 5 mg/kg, 2 hr before sacrifice, except in one tropolone experiment, where haloperidol was injected 1 hr. 50 min. before sacrifice.

(iii) Other non-agonist drugs

Tropolone, when tested for the ability to inhibit the rise of DA after GOBA, was injected in a dose of 50 mg/kg 2 hr before sacrifice. When tested for effects on depletion of DA after AMPT, two dosage schedules were employed. In one case 100 mg/kg was injected 110 min before

sacrifice, in another case 2 doses of 40 mg/kg were administered 4 hr and 110 min before killing.

AMPT (250 mg/kg as ester) was injected 2 hr before sacrifice.

Two monoamine oxidase inhibitors were tested for their ability to inhibit the rise of DA after GOBA. Pheniprazine (4 mg/kg) and pargyline (75 mg/kg, doses refer to base) were given immediately after GOBA, 40 min before sacrifice.

In one experiment various doses of amphetamine varying from 0.3 to 10 mg/kg (as sulfate salt) were tested for their ability to inhibit the rise of DA after GOBA. Amphetamine was given 1 hr before sacrifice. When tested against GOBA and haloperidol in a 2x2x2 design in which animals received or did not receive each of the 3 drugs, 3 and 5 mg/kg of amphetamine were used in separate experiments.

(iv) Apomorphine and other agonist drugs

In one experiment apomorphine was tested for its ability to inhibit the rate of depletion of DA after AMPT. Apomorphine was injected in two doses of 5 mg/kg (as base,

in volume 1% of rat) at 110 and 60 min before sacrifice.

In all other experiments apomorphine and the other agonist drugs were tested in connection with ability to inhibit the rise of DA after GOBA. The main experiments consisted of 2x2x2 designs where every rat received either GOBA or saline, agonist or no agonist, haloperidol or no haloperidol. Thus the ability of haloperidol to antagonize the agonist in its inhibition of the rise in DA after GOBA was tested as well. In some cases various doses of agonist were tested against GOBA before the main experiment was conducted. If the agonist was effective in inhibiting the rise of DA after GOBA, a dose was selected so that the rise of DA after GOBA was inhibited approximately 50%. In this way the possibility for antagonism of the agonist by haloperidol was maximized.

Apomorphine was administered two ways when tested against GOBA and haloperidol. One group, designated apomorphine "before" received the drug (0.5 mg/kg as salt) 30 min before injection with saline or GOBA, and a second dose of apomorphine (0.5 mg/kg) 30 min before being killed. Another group, apomorphine "after"

received the first injection just after GOBA, and the second dose 20 min later.

Apomorphine was tested against GOBA in doses of 40, 20, 10 and 5 mg/kg, injected at minus 50 min (where the time of sacrifice was zero time). Subsequently, 15 mg/kg (as salt) was tested against GOBA and haloperidol.

M7 was tested against GOBA in doses of 10, 6, 3, 2, 1 mg/kg (as salt), injected at - 60 min. Subsequently 4 mg/kg of M7 was tested against GOBA and haloperidol, injected at - 50 min.

Piribedil was at first tested against GOBA and haloperidol in a dose of 10 mg/kg. In another experiment 20 mg/kg was used. Piribedil was injected immediately after GOBA, 40 min before sacrifice.

Ergocornine was first tested against GOBA in doses of 0.2, 0.5, 1.0, 3.0 and 5.0 mg/kg (as base). Subsequently 3 mg/kg was employed against GOBA and haloperidol. Ergocornine was injected immediately after GOBA, 40 min before sacrifice.

CB 154 was tested in a pilot experiment against GOBA in doses of 5 and 2 mg/kg (as base). Subsequently

CB 154 was tested against GOBA and haloperidol in a dose of 10 mg/kg. CB 154 was injected 110 min before sacrifice.

(v) Statistical tests

Difference between groups were evaluated by Student's t-test.

Dunnett's multiple range comparisons test was used to evaluate the difference between fluorescence seen in a control group and that seen in each of several experimental groups (see below) (348).

F. Quenching of dopamine fluorescence by drugs

It was necessary to determine whether catecholic drugs might be carried through the assay and quench DA fluorescence, thereby leading to artificially low values. Homogenates from control animals were divided and 1.0 ng DA·HCl added to one-half of each homogenate. In one experiment the homogenates from animals treated with 50 mg/kg tropolone 2 hr before sacrifice were treated similarly. In another experiment, in addition to the control group, 5 experimental groups were employed. These consisted of: apomorphine, 2 x 0.5 mg/kg, at -40 and -20 min; apomorphine,

10 mg/kg at -50 min; piribedil, 20 mg/kg at -40 min; M7 4 mg/kg at -50 min; and apocodeine, 15 mg/kg at -50 min. The ergot drugs were not tested, as they are not catechols, and would not bind to alumina.

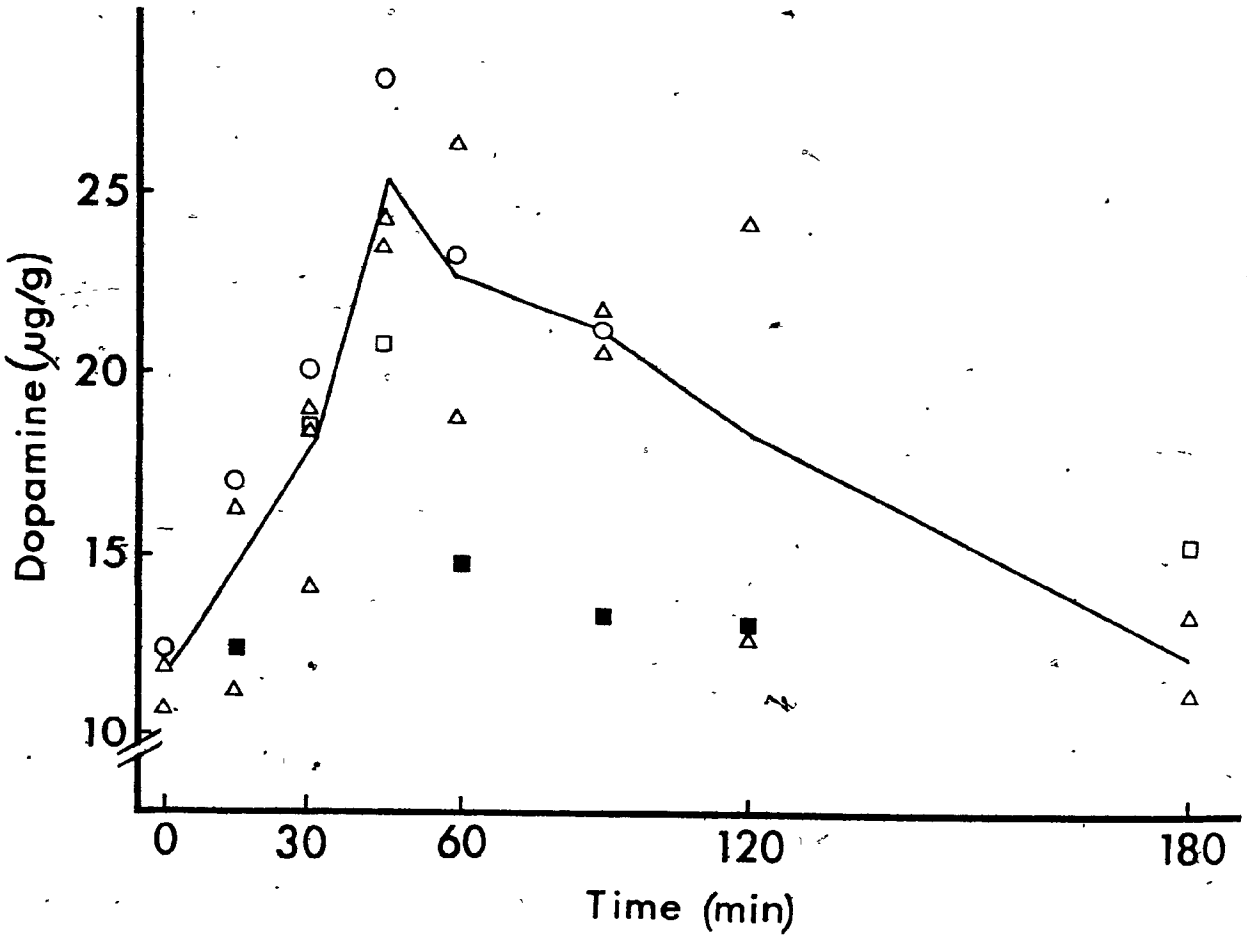
III. RESULTS

A. Effect of GOBA on dopamine Levels

The effect of different doses of GOBA, 1.5, 1.75, and 2.0 g/kg on dopamine content of rat striatum at various times was examined. See Figure 2. Each point refers to the mean of two striata from one animal. The figure indicates that dopamine in the striatum increased after the administration of GOBA to a maximum at approximately 45 min, after which the dopamine level returned to normal by 3 hr. The response depended on the dose used. When 1.5 g/kg was used, some animals did not lose the righting reflex and these animals were characterized by normal or near normal dopamine levels in the striatum. At 3 hr all the surviving animals were awake, and normal DA levels prevailed. The maximum effect was seen with 2.0 g/kg. However 2 of 8 animals given this dose died of anaesthetic-induced respiratory failure.

In subsequent experiments whole brains were taken 40 min after GOBA injection. Initially 1.75 g/kg was used, and the dose was gradually increased to 2.2 g/kg. All animals given GOBA were tail-pinched; if a rat responded at 40 min, it was rejected and not used in the experiment.

Fig. 2. Effects of various doses of GOBA on the dopamine level in striatum at various times. Doses of 1.5 (□), 1.75 (△) and 2.0 g/kg (○) were given. Closed squares indicate animals given 1.5 g/kg that did not lose the righting reflex. The line refers to the mean of 1.75 and 2.0 g/kg values.


Dopamine (µg/g)
25
20
15
10
0
30
60
120
180
Time (min)

B. The Action of Apomorphine on the Increase in DA After GOBA

(i) The Effect of Apomorphine on the Increase in DA after GOBA, with or without Haloperidol

Apomorphine was tested against GOBA in two time schedules in order to determine whether apomorphine was as effective if given after GOBA as when given before GOBA. In one, 0.5 mg/kg was injected 70 and 30 min before sacrifice ("before"), and in the other, 0.5 mg/kg was injected after GOBA, 40 and 20 min before sacrifice. Table 3 shows that apomorphine was as effective in inhibiting the rise of DA after GOBA in either schedule ($p<0.001$). Apomorphine by itself did not affect the DA level in saline controls.

Haloperidol lowered DA level significantly in both controls ($p<0.025$) and in apomorphine-injected saline controls ($p<0.001$). When all the agonist-GOBA-haloperidol experiments are considered it was generally found that haloperidol lowered DA slightly, on average, 13% ($p<0.001$). See Table 4. Haloperidol did not affect the rise in DA after GOBA. DA increased an average of 77% after GOBA.

TABLE III

Effect of apomorphine on DA levels (ng/g ± S.E.M.), in rat whole brain in saline and GOBA treated animals, with or without haloperidol. Rats received apomorphine (2 x 0.5 mg/kg) either 70 and 30 minutes before sacrifice, apomorphine "before"; or 40 and 20 minutes before sacrifice, apormophine "after." In this and subsequent experiments GOBA (2.2 g/kg) was given 40 minutes before sacrifice and haloperidol (5 mg/kg) at -2hr. Numbers in brackets refer to sample size. Comparisons are with controls.

Treatment	Control	Apomorphine	
		"before"	"after
Saline	853 ± 41 (7)	842 ± 16 (9)	
GOBA	1471 ± 35 (7)	1174 ± 35*** (7)	1140 ± 36*** (7)
Haloperidol	734 ± 25 (9)	698 ± 24 (5)	
Haloperidol + GOBA	1387 ± 49 (5)	1381 ± 51 (7)	

Student's t-tests: *** p<0.001

TABLE IV

Effect of haloperidol on DA levels (ng/g ± S.E.M.) in rat whole brain in saline and GOBA treated animals. Data are compiled from six experiments. Numbers in brackets refer to sample size. Comparisons are with controls.

Treatment	Control	Haloperidol
Saline	845 ± 13 (31)	738 ± 14*** (27)
GOBA	1493 ± 25 (32)	1446 ± 21*** (29)

Student's t-tests: *** $p<0.001$

(ii) The action of COMT inhibitors on the increase in DA after GOBA

(a) The effect of tropolone on the increase in DA after GOBA

In order to determine whether the effect of apomorphine could be mimicked by a COMT inhibitor, tropolone was tested against GOBA and haloperidol. Pyrogallol was tested but found to quench dopamine fluorescence. Table 5 indicates that tropolone, by itself, lowers the dopamine content in rat brain ($p<0.001$). Comparisons in Table 5 are made with controls in the left hand column. Tropolone completely prevented any increase in DA after GOBA ($p<0.001$). This action was not antagonized by haloperidol.

(b) Fluorescence quenching by tropolone

Small amounts of tropolone added to DA-derived fluorescence were found to quench fluorescence. In order to determine whether enough tropolone entered the brain to interfere with the assay, 3 animals were injected with 50 mg/kg and the fluorescence of a known amount of DA added to one-half of each homogenate compared with that added to each of homogenate compared with that added to each of 4 control samples. The relative fluorescence of 1.0 µg

TABLE V

Effect of tropolone on DA levels (ng/g ± S.E.M.) in rat whole brain in saline and GOBA treated animals, with or without haloperidol.
Tropolone (50 mg/kg) was given 1:50 before sacrifice.
Numbers in brackets refer to sample size.

Treatment	Control	Tropolone
Saline	854 ± 18 (9)	683 ± 28*** (6)
GOBA	1524 ± 62 (8)	848 ± 44*** (7)
Haloperidol	744 ± 33 (6)	605 ± 50* (5)
Haloperidol + GOBA	1375 ± 40 (6)	780 ± 57*** (7)

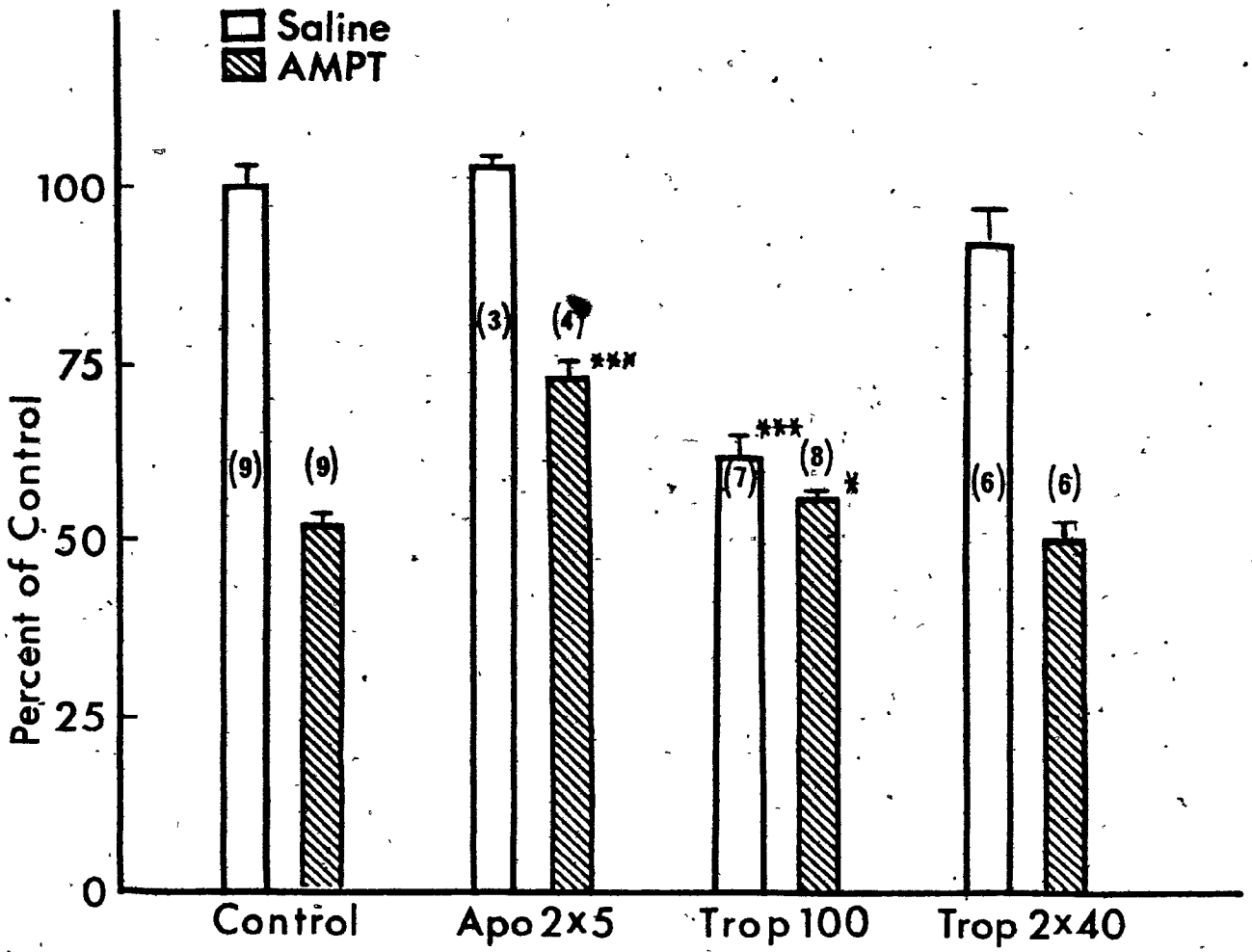
Student's t-tests: *p<0.05; ***p<0.001

DA·HCl in 4 controls was 153.2 ±4.8, while that of the tropolone sample was 158.7 ±1.8; indicating that the low values of DA seen after tropolone were not due to quenching of DA fluorescence.

(c) Inhibition of tyrosine hydroxylase in vivo by tropolone

In order to determine whether the low values after tropolone could be attributed to inhibition of tyrosine hydroxylase, the effect of tropolone on depletion of DA after AMPT was examined and compared with that of apomorphine. Fig. 3 shows that when the tyrosine hydroxylase inhibitor AMPT is given, dopamine declines about 48%. Apomorphine protected DA from depletion, so that DA dropped only 27%. Long-term exposure to tropolone, in two doses of 40 mg/kg, one dose 4 hrs before sacrifice and the other 110 min before sacrifice, failed to result in protection of DA. If a very high dose of tropolone, 100 mg/kg, was given, DA decreased 38%. If AMPT was given in conjunction. there was a very small protection of DA, so that DA decreased by 44%. It is thus apparent that the action of tropolone is not similar to that of apomorphine and may best be explained by inhibition of tyrosine hydroxylase.

Fig. 3. Effect of tropolone on the decrease in dopamine after AMPT in whole brain of rat. Tropolone was injected at the dose of 100 mg/kg 110 min before sacrifice, or in 2 doses of 40 mg/kg, 4 hr and 110 min before sacrifice. For purpose of comparison, some animals received apomorphine (2 x 5 mg/kg, as base) 110 min and 60 min before sacrifice.


Saline
AMPT
Percent of Control
100
75
50
25
0
(9)
(9)
(3)
(4)
***
***
(7)
(8)
*
(6)
(6)
Control
Apo 2x5
Trop 100
Trop 2x40

(d) Inhibition of tyrosine hydroxylase by tropolone in vitro

Fig. 4 shows that tropolone inhibits adrenal tyrosine hydroxylase in vitro. The 1/V against [Inhibitor] plot is unusual in that the lines are curved rather than straight. Similar studies of tyrosine hydroxylase in striatal homogenates were performed. While it was clear that tropolone inhibited the enzyme, the results were quite variable and are not shown here.

Fig. 5 shows a Lineweaver-Burk plot in which the amount of cofactor added to adrenal tyrosine hydroxylase was varied. Tropolone inhibited the enzyme in a cooperative fashion.

Fig. 4. Effect of various concentrations of tropolone on the activity of bovine adrenal tyrosine hydroxylase (1/V) in vitro. Assay conditions were as described in "Methods" at 0.4 mM tyrosine. Velocity is in nmoles dopa/mg protein/hr.

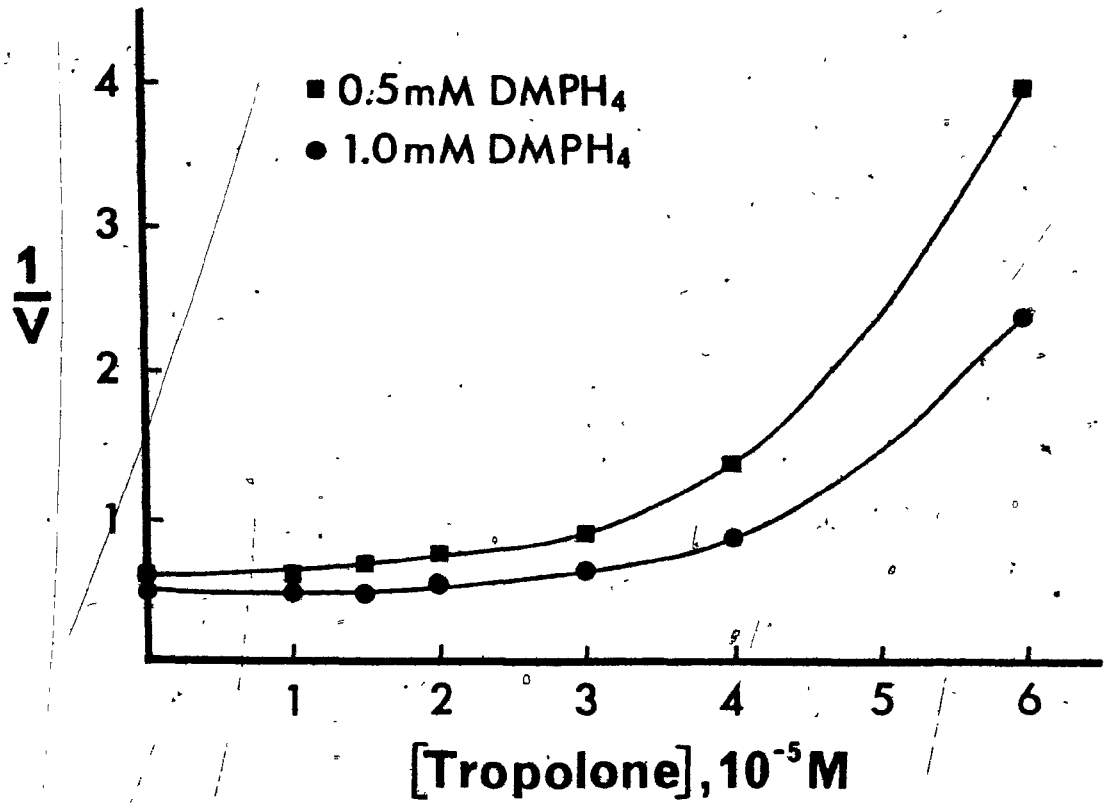
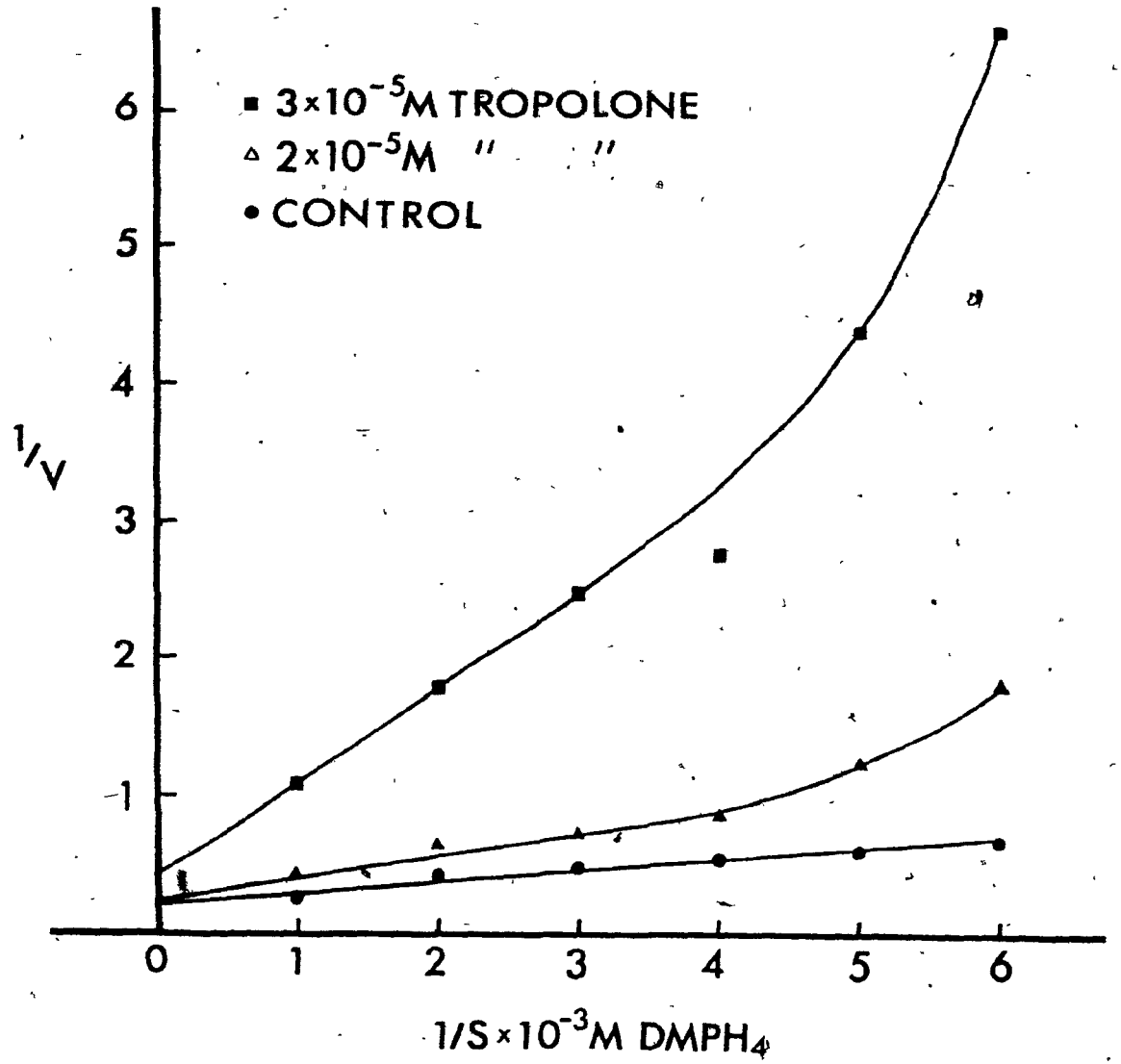

■ 0.5mM DMPH4
● 1.0mM DMPH4
1/V
4
3
2
1
1
2
3
4
5
6
[Tropolone], 10⁻⁵M

Fig. 5. Effect of tropolone on adrenal hydroxylase activity (1/V) as a function of $DMPH_4$ concentration (1/S), double reciprocal plot. Assay conditions were as described in "Methods" at 0.4 mM tyrosine. Velocity is in nmoles dopa/mg protein/hr.


■ 3×10⁻⁵M TROPOLONE
△ 2×10⁻⁵M " "
• CONTROL
1/V
6
5
4
3
2
1
0
1
2
3
4
5
6
1/S×10⁻³M DMPH₄

(iii) Apomorphine as a MAO inhibitor

(a) The effect of MAO inhibitors on the rise of DA after GOBA

In order to determine whether apomorphine would be inhibiting the rise of DA after GOBA through an inhibitory action on MAO, the effect of MAO inhibitors pheniprazine and pargyline on the rise of DA after GOBA was examined. Table 6 shows that neither pargyline nor pheniprazine, when given immediately after GOBA, inhibited the rise of DA after GOBA, in contrast to apomorphine. The MAO inhibitors were acting, as DA levels rose in saline controls.

(b) Inhibition of MAO by apomorphine in vitro

Fig. 6 shows that apomorphine in a concentration of 0.4 mM inhibits monoamine oxidase in vitro. Haloperidol, at a concentration of 5×10^{-5} M did not affect the inhibition by apomorphine of monoamine oxidase.

(iv) The effect of amphetamine on the rise in DA after GOBA, as opposed by haloperidol

(a) The effect of various doses of amphetamine on the increase in DA after GOBA

In order to determine whether the action of

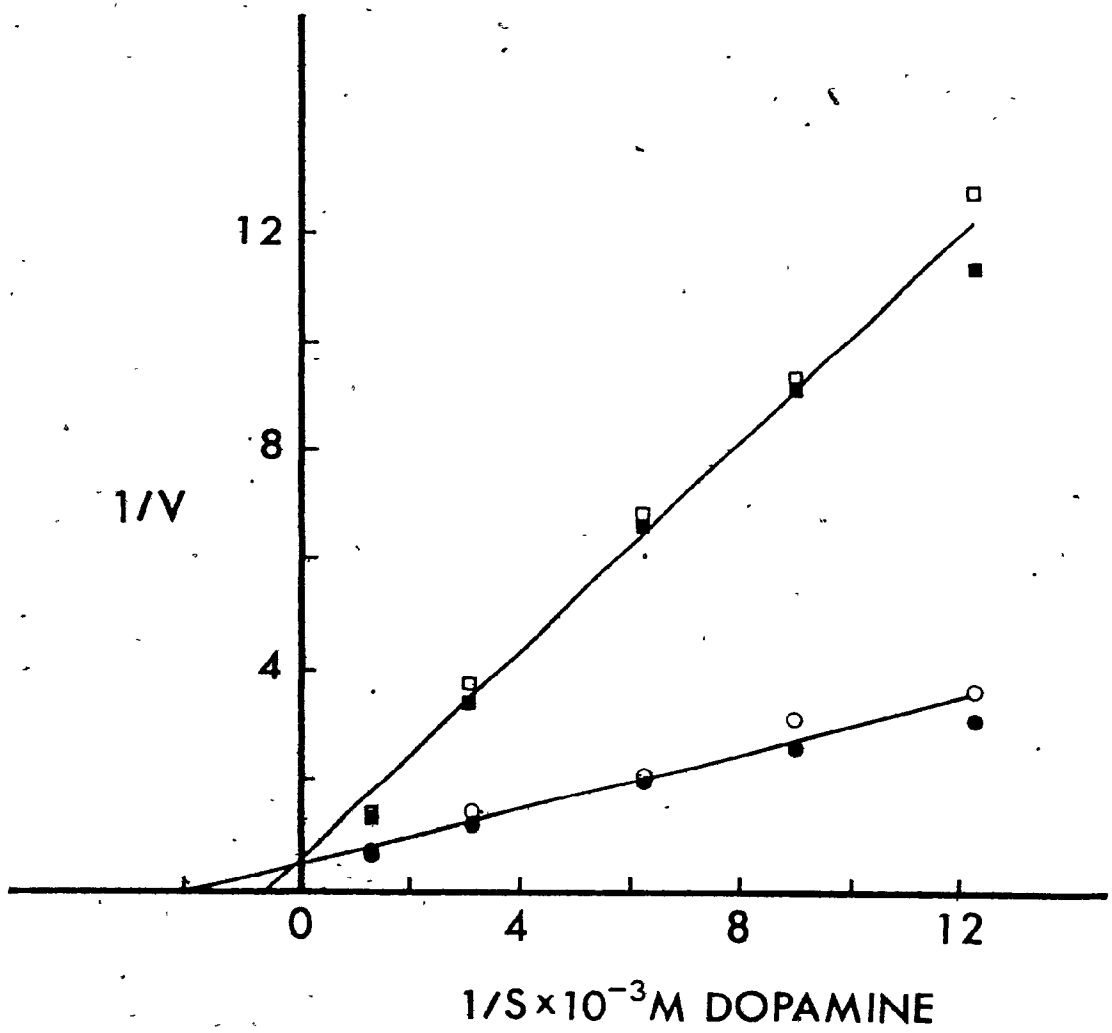
TABLE VI

Effect of monoamine oxidase inhibitors on the rise of DA (ng/g ± S.E.M.) in rat whole brain after GOBA treatment.
Pheniprazine (4 mg/kg) or pargyline (75 mg/kg) was injected immediately after GOBA, 40 minutes before sacrifice. Numbers in brackets refer to sample size.

Treatment	Saline	GOBA
Control	891 ± 43 (4)	1499 ± 72 (4)
Pargyline	1101 ± 47* (4)	1499 ± 51 (4)
Pheniprazine	1030 ± 33* (4)	1601 ± 87 (4)

Student's t-tests: * $p<0.05$

Fig. 6. Effect of apomorphine and haloperidol on monoamine oxidase activity. Double reciprocal plot of initial MAO activity (1/V) as a function of dopamine concentration (1/S) in the presence of water (●), 4 x 10^{-4}M apomorphine (■), 5 x 10^{-5}M haloperidol (○), or apomorphine + water (□). ^{14}C-Dopamine was incubated with washed mitochondria as described in "Methods". Protein concentration was 1.0 mg/ml.


12
8
4
1/V
0
4
8
12
1/S×10⁻³M DOPAMINE

apomorphine in the GOBA-haloperidol paradigm could be mimicked by a non-agonist, DA-releasing drug, the effect of amphetamine was examined. Fig. 7 shows the effect of various doses of amphetamine sulfate on the increase in DA after GOBA. Maximal inhibition was obtained with 6 and 10 mg/kg, while 0.3 mg/kg was ineffective. Fifty percent inhibition was obtained with 3 mg/kg.

(b) The effect of haloperidol on the inhibition by amphetamine of the rise in DA after GOBA

Table 7 shows that 3.0 mg/kg amphetamine, by itself raised DA levels by approximately 22% ($p<0.001$). Haloperidol, which lowered DA in saline controls ($p<0.025$) failed to antagonize the increase in DA due to amphetamine in controls. The inhibition of the increase in DA after GOBA by 3.0 mg/kg amphetamine was, however, antagonized by haloperidol. Thus DA levels rose more in animals treated with GOBA, amphetamine, haloperidol than in GOBA, amphetamine treated animals ($p<0.01$). Thus a known non-agonist, amphetamine, is able to mimic apomorphine in this respect: it inhibits the rise of DA after GOBA, and the inhibition is antagonized by haloperidol.

When the amphetamine dose was increased to 5 mg/kg

Fig. 7. Percent inhibition by various doses of amphetamine of the rise in DA after GOBA. Amphetamine sulphate was injected 1 hr before sacrifice. GOBA (2.2g /kg) was given 20 min later.

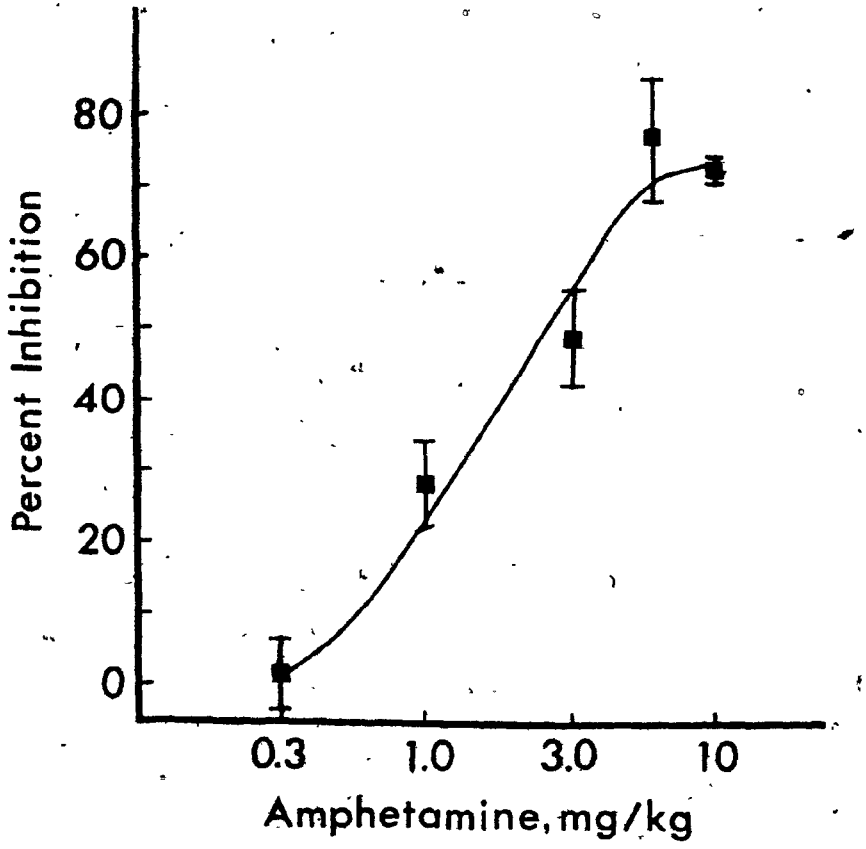

Percent Inhibition
80
60
40
20
0
0.3
1.0
3.0
10
Amphetamine, mg/kg

TABLE VII

Effect of amphetamine (3 mg/kg) on DA levels (ng/g ± S.E.M.) in rat whole brain in saline and GOBA treated animals, with or without haloperidol. Animals were given amphetamine 1 hour before sacrifice. Numbers in brackets refer to sample size. Comparisons are made with controls.

Treatment	Control	Amphetamine
Saline	884 ± 20 (9)	1080 ± 29*** (8)
GOBA	1556 ± 35 (7)	1281 ± 25*** (13)
Haloperidol	783 ± 32 (4)	1048 ± 33*** (7)
GOBA + haloperidol	1475 ± 48 (6)	1391 ± 27 (13)

Student's t-tests: *** $p<0.001$

(see Table 8), amphetamine still increased DA levels in saline controls ($p<0.05$) but only by 9%. Amphetamine (5 mg/kg) inhibited the rise of DA after GOBA by 71% ($p<0.001$) and haloperidol failed to antagonize the inhibition by amphetamine.

C. The Effect of DA Agonists on the Rise of DA after GOBA, with or without Haloperidol

(i) Apocodeine

Apocodeine (15 mg/kg as salt) did not affect DA levels in saline controls (see Table 9). Like its parent compound, apomorphine, apocodeine inhibited the rise of DA after GOBA ($p<0.001$), in this case 43%. Haloperidol lowered DA in saline controls ($p<0.025$) and in apocodeine controls ($p<0.025$). However, haloperidol did not antagonize apocodeine in its inhibitory action on the rise in DA after GOBA, so that dopamine levels in brains of GOBA-apocodeine-halpperidol rats were not significantly different from those in GOBA-apocodeine brains and significantly lower than those in GOBA-haloperidol brains (p 0.05) or in GOBA brains (p 0.025).

TABLE VIII

Effect of amphetamine (5 mg/kg) on DA levels (ng/g ± S.E.M.) in rat whole brain in saline and GOBA treated animals, with or without haloperidol. Animals were given amphetamine 1 hour before sacrifice. Numbers in brackets refer to sample size. Comparisons are made with controls.

Treatment	Control	Amphetamine
Saline	869 ± 12 (3)	946 ± 22* (4)
GOBA	1430 ± 45 (4)	1033 ± 67*** (4)
Haloperidol	732 ± 24 (3)	978 ± 35** (4)
Haloperidol + GOBA	1336 ± 6 (4)	1048 ± 27*** (5)

Student's t-tests: * $p<0.05$; ** $p<0.01$; *** $p<0.001$

TABLE IX

Effect of apocodeine on DA levels in rat whole brain (ng/g ± S.E.M.) in saline and GOBA treated animals, with or without haloperidol.

Rats received apocodeine (15 mg/kg) 50 minutes before sacrifice. Numbers in brackets refer to sample size. Comparisons are made with controls.

Treatment	Control	Apocodeine
Saline	809 ± 27 (4)	823 ± 28 (4)
GOBA	1340 ± 26 (4)	1110 ± 18*** (5)
Haloperidol	688 ± 18 (4)	681 ± 8 (3)
Haloperidol + GOBA	1324 ± 24 (4)	1138 ± 56* (6)

Student's t-tests: *$p<0.05$; ***$p<0.001$

(ii) --

An analogue of apomorphine, M7 , behaved differently from apomorphine in that it raised DA levels in saline controls ($p<0.001$) in dose used (4 mg/kg as salt) by 22%. M7 inhibited the rise of DA after GOBA by 44% ($p<0.025$). Haloperidol antagonized M7 in its tendency to raise DA in controls, but did not antagonize M7 in its inhibition of the rise of DA after GOBA, so that animals given GOBA, M7 , haloperidol had lower DA levels in the brain than animals given GOBA and haloperidol ($p<0.025$). See Table 10.

(iii) Piribedil

Piribedil (20 mg/kg) raised DA levels in saline controls slightly ($p<0.025$) (see Table 11). Like apomorphine, piribedil inhibited the rise in DA after GOBA, by 43% ($p<0.001$) and, as with apomorphine this effect was antagonized by haloperidol.

(iv) Ergocornine

Ergocornine, 3 mg/kg, like M7 and piribedil, raised DA in saline controls (19%, $p<0.001$). See Table 12. Haloperidol prevented this effect in controls. Ergocornine inhibited the increase in DA after GOBA by only 27%

TABLE X

Effect of M7 on DA levels (ng/g ± S.E.M.) in rat whole brain in saline and GOBA treated animals, with or without haloperidol.
M7 (4 mg/kg) was given 50 minutes before sacrifice. Numbers in brackets refer to sample size. Comparisons are with controls.

Treatment	Control	M7
Saline	839 ± 43 (4)	1033 ± 20** (4)
GOBA	1625 ± 87 (4)	1279 ± 62* (6)
Haloperidol	796 ± 44 (4)	763 ± 6 (4)
Haloperidol + GOBA	1475 ± 45 (4)	1248 ± 50* (5)

Student's t-tests: *$p<0.05$; **$p<0.01$

TABLE XI

Effect of piribedil on DA levels in rat whole brain (ng/g ± S.E.M.) in saline and GOBA treated animals, with or without haloperidol.
Piribedil (20 mg/kg) was administered 40 minutes before sacrifice, immediately after GOBA. Numbers in brackets refer to sample size. Comparisons are with controls.

Treatment	Control	Piribedil
Saline	845 ± 20 (4)	968 ± 35* (3)
GOBA	1475 ± 30 (4)	1207 ± 35*** (5)
Haloperidol	762 ± 43 (2)	809 ± 36 (3)
Haloperidol + GOBA	1456 ± 48 (4)	1357 ± 42 (5)

Student's t-tests: *$p<0.05$; ***$p<0.001$

TABLE XII

Effect of ergocornine on DA levels in rat whole brain (ng/g ± S.E.M.) in saline and GOBA treated animals, with or without haloperidol. Ergocornine (3 mg/kg) was given immediately after GOBA. Comparisions are made with controls.

Treatment	Control	Ergocornine
Saline	882 ± 23 (7)	1052 ± 19*** (4)
GOBA	1577 ± 43 (9)	1390 ± 30** (8)
Haloperidol	753 ± 47 (4)	809 ± 17 (4)
Haloperidol	1536 ± 35 (9)	1427 ± 29* (9)

Student's t-tests: *$p<0.05$; **$p<0.01$; ***$p<0.001$

($p<0.005$, yet haloperidol was unable to overcome the inhibition, so that DA levels in brains of rats given GOBA, ergocornine and halperidol were lower than DA levels in brains from rats given GOBA and haloperidol ($p<0.05$).

(v) CB 154

CB 154, 10 mg/kg, unlike its sister ergot alkaloid, did not alter DA levels in saline controls. See Table 13. CB 154 inhibited the rise in DA after GOBA about 36%. ($p<0.005$). Haloperidol did not antagonize the inhibition by CB 154 in GOBA-treated animals. Thus, if haloperidol was given, CB 154 still inhibited the rise of DA after GOBA by 27% ($p<0.01$).

D. Quenching of DA Fluorescence by Agonists

The effect of catechol-containing agonists on the fluorescence of assayed DA was examined. The dose and schedule of agonists were the same as in agonist GOBA experiments. In addition 4 rats were injected with 10 mg/kg of apomorphine 50 min before sacrifice. Statistical significance of the difference between controls and experimental groups was evaluated by means of Dunnett's multiple range

TABLE XIII

Effect of CB 154 on DA levels in rat whole brain (ng/g ± S.E.M.), in saline and GOBA treated animals, with or without haloperidol.
CB 154 (10 mg/kg) was given 1 hour and 50 minutes before sacrifice. Comparisons are made with controls.

Treatment	Control	CB 154
Saline	815 ± 20 (5)	850 ± 51 (4)
GOBA	1379 ± 32 (4)	1177 ± 30** (5)
Haloperidol	711 ± 29 (4)	702 ± 34 (4)
Haloperidol + GOBA	1385 ± 29 (3)	1229 ± 20** (6)

Student's t-tests: *p<0.05; **p<0.01

comparisons test (348).

As Table 14 indicates, none of the agonists altered DA fluorescence significantly, including apomorphine in a high dose of 10 mg/kg. The latter result is in contrast to report of Di Chiara et al. (312), who claimed that apomorphine quenched dopamine fluorescence.

TABLE XIV

Effect of agonists on relative fluorescence of 1.0 µg dopamine hydrochloride. Low dose of apomorphine and other agonists were administered as in GOBA-agonist experiments. High dose of apomorphine was injected 50 min before sacrifice. Numbers in brackets refer to sample size, statistical significance was evaluated by Dunnett's multiple range test.

Treatment	Dose (mg/kg)	Relative Fluorescence (units ± S.E.)
Control	-	40.5 ± 0.3 (3)
Apomorphine	10	40.3 ± 1.1 (4)
Apomorphine	2 x 0.5	37.3 ± 0.7 (3)
Piribedil	20	39.6 ± 2.1 (4)
M7	4	37.7 ± 2.7 (3)
Apocodeine	15	39.8 ± 1.7 (4)

IV. DISCUSSION

A. The Inhibition by apomorphine of the rise in DA after GOBA

The aim of this work was to examine whether dopamine receptors control DA receptors by a mechanism which does not require neuronal firing. Kehr et al. (274) showed that apomorphine inhibited dopa formation in striatum isolated by transsection, and Anden et al. (275) have shown that apomorphine inhibits dopamine formation in rat brain in which striata have been isolated by either transsection or administration of GOBA. However, interpretations of the biochemical action of apomorphine, alternative to the receptor theory have been proposed. If it can be shown that the alternative explanations are invalid, or that a number of dopamine agonists different in structure from apomorphine behave similarly, then the hypothesis of an impulse-independent receptor controlling DA synthesis remains tenable.

(i) Effect of Pre-GOBA Metabolism

Both Kehr et al. (274) and Anden et al. (275) injected apomorphine before transsection or GOBA administration. It seemed possible that the changes in DA metabolism induced by apomorphine (see Introduction, section

D(i)a) could carry over into the impulse-free period. Cheramy et al. (349) found that striatal slices from neuroleptic-treated rats exhibited increased DA synthesis, whereas the neuroleptic had no effect if added directly to the medium. Conceivably, a message or "carrier" which controls DA synthesis was involved (for example, cyclic AMP) which could alter kinetic properties of tyrosine hydroxylase. If apomorphine gave rise to such a long-lasting messenger through its inhibition of neuronal firing, then the inhibition of dopamine increase after GOBA could be interpreted as due to the continued presence of the messenger rather than due to an action on an impulse-independent receptor.

Table 3 shows that when both doses of apomorphine are given after GOBA, apomorphine is as effective in inhibiting the rise of DA after GOBA as when one dose is given before GOBA. Moreover, haloperidol, as Anden et al. found (275) has no effect on the increase in DA after GOBA, despite its well known acceleration of DA metabolism normally. It thus appears that dopamine synthesis during GOBA is independent of the synthesis rate that prevailed prior to GOBA administration. The "carryover" hypothesis cannot explain the action of apomorphine in inhibiting DA formation after

GOBA.

The finding that apomorphine inhibits DA biosynthesis after GOBA is in agreement with reports of apomorphine inhibition of tyrosine hydroxylation in vitro in striatal slices (Goldstein et al. 350) and in synaptosomes (351,270).

(ii) Apomorphine as COMT inhibitor

Recently it has been suggested that the behavioural actions of apomorphine, which are very likely mediated through a receptor (see Introduction, sections A(iv) and D(i)a), are dissociable from its biochemical actions, which according to McKenzie (311) and Di Chiara et al. (312), may not be through receptors.

(a) Tropolone tested against GOBA, haloperidol

McKenzie suggested that apomorphine is tropolone-like. If this is true, then tropolone should be apomorphine-like. Table 5 shows that, while tropolone prevented the increase in DA after GOBA, this inhibition was not antagonized by haloperidol, unlike the case of apomorphine. Moreover unlike apomorphine, tropolone decreased DA levels in controls. Thus tropolone was not found to be apomorphine-like in this paradigm .

(b) Inhibition of Tyrosine Hydroxylase by Tropolone

One way to explain tropolone's actions in the GOBA-haloperidol paradigm is to postulate a partially inhibiting action of tropolone on tyrosine hydroxylase. McKenzie rejected this possibility as he found that 40 mg/kg tropolone decreased DA in 2 cat caudates by only 23%.

If tropolone were apomorphine-like, it should protect DA from depletion after AMPT. If tropolone were an inhibitor of tyrosine hydroxylase it should have no effect. Fig. 3 shows that 40 mg/kg tropolone, given in two doses, is ineffective in protecting DA from depletion after AMPT. A high dose of tropolone depletes DA substantially, as would an inhibitor of tyrosine hydroxylase. Fig. 4 and 5 show that tropolone inhibits TH in vitro, apparently cooperatively, so that inhibition by tropolone is especially marked at high tropolone or low cofactor concentrations.

The first to show that tropolone inhibits tyrosine hydroxylase were Ozawa and Suzuki (352), who reported 93% and 49% inhibition of adrenal tyrosine hydroxylase at 5 and 1×10^{-5} M respectively. At optimal concentrations of tyrosine and $DMPH_4$ tropolone was found to inhibit tyrosine

hydroxylase at concentrations of the order of 10^{-5} M, as shown in Fig. 4. In vivo, where cofactor levels are limiting, greater inhibition could be expected. Broch (353) reported that after 100 mg tropolone, tropolone levels in striatum decreased from 21 ng/g (17×10^{-5} M) at 30 min to 3.5 ng/g (2.9×10^{-5} M) at 2.5 hr. These values are within the inhibitory range of tropolone and would explain the depleting effects of tropolone. It is therefore surprising that Broch reported an initial increase of DA in brain after 100 mg/kg tropolone, followed by normal levels. However lower brain levels of NE were found after tropolone (354) and after 5-aminotropolone (352).

Inhibition of tyrosine hydroxylase by tropolone can explain all McKenzie's data, with the exception of the decrease of disappearance of ^{3}H-DA (311). Possibly tropolone inhibits deamination as well as O-methylation and tyrosine hydroxylation.

(iii) Apomorphine as Inhibitor of MAO

Di Chiara et al. have adduced evidence that apomorphine inhibits MAO in vitro and in vivo (312). They reported that apomorphine results in protection of dopamine

and prevention of increases in metabolites after reserpine as well as prevention of dopamine metabolite increases after L-dopa administration. An increase in dopamine levels was found after apomorphine, and it was suggested that the failure of other groups to report a similar increase was due to quenching of dopamine fluorescence in the methods used. Di Chiara et al. suggested that the biochemical actions of apomorphine on dopamine metabolism may be explained as due to inhibition of deamination. If this explanation is correct, then the inhibition by apomorphine of the increase in dopamine after GOBA is due to end-product inhibition of tyrosine hydroxylase following a buildup of cytoplasmic dopamine. Moreover the antagonism of apomorphine by haloperidol should be due to antagonism of apomorphine's inhibitory action on MAO.

If apomorphine is acting as a MAO inhibitor, then other MAO inhibitors should inhibit the rise of DA after GOBA. In fact, as Table 6 shows pheniprazine and pargyline, two potent MAO inhibitors, have no effect on the rise of DA after GOBA.

Moreover the inhibition of MAO by apomorphine is not antagonized by haloperidol as Fig. 6 indicates.

Quenching of DA fluorescence by 10 mg/kg apomorphine was not found (Table 14) contrary to Di Chiara et al.'s report (312).

These data indicate that an inhibitory action of apomorphine on MAO is not a sufficient explanation of the effect of apomorphine in the GOBA-haloperidol paradigm

(iv) Apomorphine as Releaser of Dopamine

Apart from its postsynaptic agonist actions, it is conceivable that apomorphine has non-agonist presynaptic actions as well. Such suggestions have been made after findings of reduced induction of stereotyped behaviour after lesions of the substantia nigra (355,356). It seemed conceivable, that in addition to its postsynaptic actions, apomorphine possesses releasing actions. Apomorphine could thereby prevent an increase in DA after GOBA simply by releasing some of the DA. If this is the case, then the behaviour of apomorphine in the GOBA-haloperidol paradigm should be mimicked by amphetamine, which is known to release dopamine, but not act on receptors. Anden et al. (275) determined that amphetamine prevented the rise of DA after GOBA, and that haloperidol did not antagonize amphetamine. However the dose used was high (10 mg/kg) and was 100%

effective. In order to compare amphetamine with apomorphine, a dose should be employed which inhibits the rise of DA after GOBA by approximately 50%.

Table 7 shows that a low dose of amphetamine inhibited the rise of DA after GOBA 41% and this action was antagonized by haloperidol. Thus a low dose of amphetamine was similar to apomorphine in this regard. Amphetamine differed from apomorphine in that the former elevated brain DA levels of saline controls, an increase which was not overcome by haloperidol. The elevation of DA may be due to another property of amphetamine, inhibition of uptake of dopamine . Inhibitors of uptake, such as benztropine can accelerate DA synthesis (233). An acceleration of DA synthesis in saline controls by amphetamine could account for the elevated levels of DA and the lack of antagonism by haloperidol.

There are at least three ways in which amphetamine could inhibit the rise of DA after GOBA. Firstly amphetamine may simply release so much DA that the increase is attenuated. This explanation requires that the released DA be mostly metabolized or removed. Another interpretation is that amphetamine increases the pool of DA responsible for end-

product inhibition. Thirdly, the receptor theory posits that the DA released by DA acts on a dopamine-sensitive receptor to inhibit biosynthesis.

Recently Kuczenski (230) has found that amphetamine added to striatal synaptosomes increases DA synthesis. This argues against an increase in the pool of end-product inhibitor DA. Kuczenski also found that synaptosomes from amphetamine treated animals exhibited a lower rate of tyrosine hydroxylation. This finding may be interpreted as indirect evidence that DA released by amphetamine influences tyrosine hydroxylation.

Because of the uncertainty whether apomorphine was acting as a DA releasing agent, and if so, whether the released DA was acting on a receptor, it seemed fruitful to employ a second approach to the problem of impulse-independent receptor control of DA biosynthesis.

B. Agonists other than apomorphine

A number of agonists other than apomorphine were tested for their capacity to inhibit dopamine biosynthesis in the absence of neuronal flow. Two were derivatives of

apomorphine, but the other three were quite different in structure.

(i) The Effect of Various Agonists on DA Concentration

The data obtained with the DA agonists are summarized in Table 15. In contrast to the findings of some other investigators (302,322), piribedil and ergocornine elevated DA levels in saline controls, as did M7 . The other DA agonists did not affect DA levels significantly. A reduction of depletion at nerve endings by reduction in neuronal flow, seen after agonists, may tend to cause DA level to increase, as it in fact does after complete cessation of neuronal flow. Agonists may also act through a receptor to inhibit biosynthesis, tending to decrease DA levels (see below). Agonists may also activate cyclic AMP, and thereby DA synthesis through a receptor (see Introduction section B(iii)b). The final DA level would represent a balance between these factors. Other mechanisms of increasing DA may be postulated, such as inhibition of MAO or inhibition of uptake. However in view of the antagonism by haloperidol of agonist-induced increases in DA, these mechanisms are unlikely.

The view that DA levels represent a balance

TABLE XV

Effect of Dopamine Agonists in Rats on Dopamine Concentration in Brain.

	Agonist increases brain dopamine in:		Agonist inhibits GOBA-induced Decrease of DA in	
	Controls	Haloperidol-treated rats	Controls	Haloperidol-treated rats
Apomorphine	-	-	+	-
Apocodeine	-	-	+	+
M7	+	-	+	+
Piribedil	+	-	+	-
Ergocornine	+	-	+	+
CB 154	-	-	+	+

between different dopamine receptors implies that the receptors differ in structure or sensitivity, or that the agonists are distributed unevenly. There have been few studies on the distribution of agonists in brain. However it appears that agonists may be differentially distributed. Butterworth et al. (1975) found that apomorphine is low in striatum compared to other parts of brain, whereas piribedil is evenly distributed throughout the brain after injection (357).

(ii) The effect of Various Agonists on the Increase in DA after GOBA

All the DA agonists examined inhibited the rise in DA after GOBA.

The apomorphine derivatives M7 and apocodeine inhibited the rise of DA after GOBA. However the action of each was different from apomorphine, in that the inhibition by neither was antagonized by haloperidol. Moreover M7 and apomorphine differed in that one increased the level of DA in saline controls, whereas the other did not. These differences are surprising, in view of the close structural similarity among these agonists. It is not known whether M7 or apocodeine have any releasing action.

The inhibition of the rise in DA after GOBA caused by piribedil is similar to that noted by Walters and Roth (302) and is consistent with the reported reduction of tyrosine hydroxylation in slices by piribedil (320) and in synaptosomes by S 584 (270). In view of findings that the stereotypy produced by piribedil can be abolished by depleting stores of DA by treatment with AMPT and reserpine (316,358), the inhibiting action of piribedil may be regarded as secondary to release.

CB 154 and ergocornine, on the other hand, do not release DA (323). Both drugs inhibit the rise of DA seen after GOBA. Their actions on DA metabolism are not identical, as ergocornine, but not CB 154, elevates DA in saline controls.

The inhibitory action of all the agonists on dopamine formation after GOBA administration, despite their difference in structures, strongly suggests that the proposed receptor exists.

(iii) The Antagonism of Agonists by Haloperidol

Haloperidol antagonized the inhibition by apomorphine of tyrosine hydroxylation as reported by others (275)

and as in synaptosomes (351). This neuroleptic antagonizes the inhibition by piribedil of the rise in dopa in decarboxylase inhibitor—GOBA treated animals (359). Haloperidol was reported to antagonize the inhibition by piribedil of tyrosine hydroxylation in striatal slices (266).

Neuroleptics antagonize M_7 (326) in its peripheral actions and the ergot alkaloids in their ability to cause circling in rats with a unilateral 6-hydroxydopamine-induced degeneration of the nigrostriatal pathway (322). Yet it was surprising to find that haloperidol was unable to antagonize the inhibition by M7, apocodeine, ergocornine or CB 154 of the increase in DA after GOBA.

Evidently the presynaptic (assuming for the sake of argument that the receptor inhibiting DA biosynthesis is located presynaptically) receptor is not acted on in the same fashion as the postsynaptic receptor. One explanation of these results is that the presynaptic receptor is so structured that haloperidol is able to block some, but not all of the agonists. However it is difficult to visualize how apomorphine may be blocked but not apocodeine or M7, in view of their structures.

Another explanation takes into account haloperidol's

relative potency in blocking adenyl cyclase and in blocking release. Butyrophenomes, such as haloperidol, are among the most potent antipsychotics known, yet possess, compared to other antipsychotics, low potency in blocking dopamine-sensitive adenyl cyclase (360,361,362). However butyrophenomes are very potent in blocking impulse-coupled release of dopamine from presynaptic terminals (363). Therefore the latter action may be the most important aspect in haloperidol's action (359). If haloperidol does not block the presynaptic receptor but interferes with release of DA caused by apomorphine or piribedil, then the observed failure to block the other dopamine agonists may be expected. The same explanation may hold for in vitro results observed with neuroleptics and agonists in slices and synaptosomes (364,351,266,270). This hypothesis would predict that agents which are very potent in blocking receptors in the adenyl cyclase model, such as fluphenazine are most likely to antagonize agonist-induced inhibition of the rise in DA after GOBA. This hypothesis also holds that the presynaptic action of apomorphine and piribedil is primarily through release of dopamine, which then acts on the receptor. If this is true, then agents which block uptake of dopamine should potentiate the presynaptic action of apomorphine

and piribedil.

(iv) Conclusions

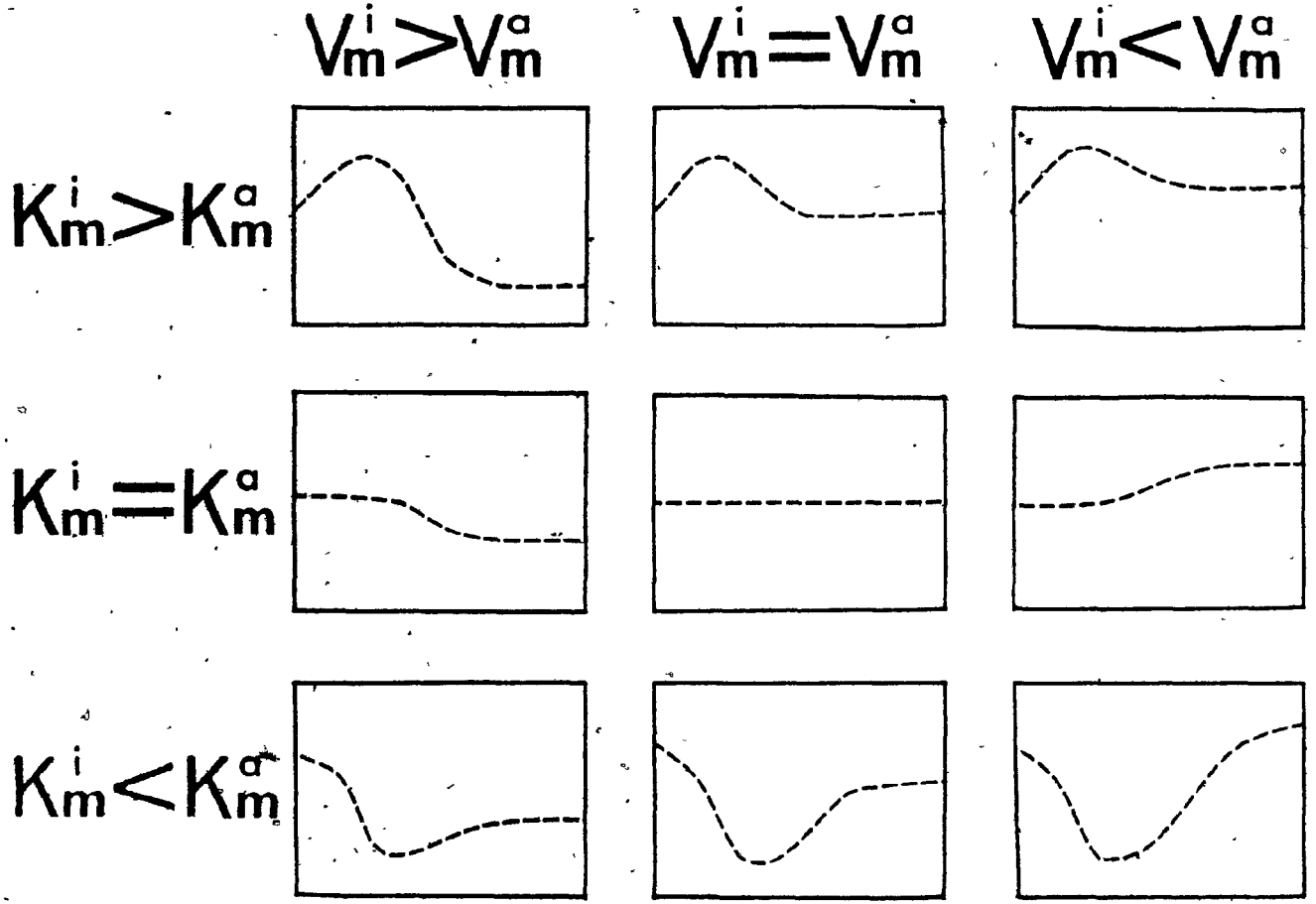
The unanimity of the agonists in inhibiting the rise of DA after GOBA strongly suggests that a receptor exists on which dopamine, most probably dopamine released by the action potential, acts to inhibit dopamine biosynthesis, by a mechanism which does not involve neuronal feedback. The action of the agonists on DA metabolism is not uniform however, as is suggested by their diverse effects on DA levels in saline controls, and the ability of haloperidol to antagonize agonist-inhibition of DA synthesis. The differing structures of the agonists indicate that it is unlikely that these agonists share another property, in addition to their agonist function, which would account for the inhibition of the rise in DA after GOBA.

There is increasing evidence that, in addition to the receptor inhibiting DA biosynthesis, there is a receptor which activates DA synthesis through adenyl cyclase (see Introduction section B(iii)b). Upon initial consideration it may seem odd that receptors exist which have opposite actions. However the receptors cancel out at all dopamine concentrations only if identical Vmax's and Km 's are shared

by the two receptors. No one has performed a study correlating rate of dopamine synthesis over all rates of neuronal firing, but fragmentary evidence suggests that the rate of DA synthesis is high when neuronal firing is zero (as after transsection), low when neuronal firing is low (as after apomorphine) and high when neuronal firing is high (as after haloperidol administration). Assuming that the two receptors exist in the synaptic region, and that the concentration of dopamine in the synaptic cleft which acts on the receptors is directly proportional to the rate of neuronal firing, then the theoretical considerations suggest that the K_m of inhibitory receptor for dopamine is lower than that for activating receptor. The Vmax's are probably about the same or V_{max} for activating receptor greater than that for inhibitory receptor. Figure 8 indicates the expected rates of DA synthesis as a function of DA concentration in the synaptic cleft, when Km 's and Vmax 's are varied. The curve in the lower right is probably the normal one.

The function of an activating receptor may be a link between the rate of synthesis and the rate of neuronal firing and utilization. If the DA concentration is high enough however this receptor will be saturated, and the

Fig. 8. Dopamine synthesis (y axis) as a function of DA concentration in the synaptic cleft (x axis), theoretical considerations. The superscripts "i" and "a" refer to maximal velocity (V_{max}) and K_m of receptors that inhibit or activate DA synthesis, respectively.


$V_m^i > V_m^a$
$V_m^i = V_m^a$
$V_m^i < V_m^a$
$K_m^i > K_m^a$
$K_m^i = K_m^a$
$K_m^i < K_m^a$

rate of DA synthesis reach a maximum. If the neuron is firing very rapidly there may be a slight depletion, as is in fact seen after haloperidol administration. Other limiting factors are end-product inhibition by newly reuptaken DA, and possibly inhibition of release of DA.

If the Km of inhibiting receptor is very low then the inhibitory receptor may serve to inhibit DA synthesis when nerve firing is low. On the other hand if the newly synthesized DA is preferentially released, then increased synthesis at very low firing rates may provide more dopamine for the receptor when the level of dopamine in the synapse is very low.

The situation is made more complex by the probable existence of other presynaptic receptors. It appears to be a general principle that neurotransmitters inhibit their own release, and dopamine appears to be no exception (See Introduction section B(iii)a). Evidence for a nicotinic receptor which causes dopamine release from slices has been described (265). Possible releasing action by other neurotransmitters has not been studied. It is possible that certain receptors fulfill multiple functions. In particular, the receptor inhibiting DA synthesis may also inhibit DA

release.

One way to examine the latter question is to compare pharmacological profiles of agonist and antagonist activity and compare the similarity of effect on release and synthesis. Another way is to examine receptor supersensitivity. Treatments such as chronic AMPT or haloperidol result in supersensitivity of the postsynaptic dopamine receptor (365,366). It remains to be determined whether the receptors controlling DA synthesis and release also become supersensitive.

While the action of the agonists in activating the impulse-independent inhibitory feedback receptor suggests that this receptor is not wholly dissimilar from he postsynaptic receptor, it is possible that agents can block or bind to the former but not the latter. One possible candidate is perlapine, a derivative of clozapine, which has no anti-psychotic activity but increases DA turnover (367).

The eventual isolation and characterization of the receptor inhibiting DA biosynthesis may be anticipated.

V. SUMMARY

Dopamine (DA) is a central nervous system neurotransmitter. Most DA terminals in brain are located in the striatum, while most DA cell bodies are located in the substantia nigra. Alterations in DA metabolism are involved in Parkinson's disease and are believed to be implicated in schizophrenia.

The regulation of DA biosynthesis and metabolism is complex. The activity of the enzyme which is rate-limiting in the synthesis of DA, tyrosine hydroxylase, is subject to end-product inhibition by DA. The amount of enzyme protein is also regulated. Evidence indicates the presence of a neuronal feedback from the striatum to the substantia nigra so that the rate of DA cell firing is inversely related to the degree of activation of the post-synaptic DA receptor. Increased DA synthesis and metabolism is related to an increase in DA cell firing. An increase in the rate of DA synthesis, but not that of metabolism has been observed after prevention of transmission of the action potential to the DA nerve terminal. It was suggested that there exists a DA-sensitive receptor which acts to inhibit DA synthesis by a mechanism which is not dependent on

neuronal firing. This study was conducted in order to examine this hypothesis.

γ-Hydroxybutyric acid (GOBA) was employed in a dose of 2.2 g/kg in order to prevent the firing of the DA cell. The increase of DA in whole brain of rat seen after 40 min exposure to GOBA was the measure. It was found, as has been reported by others, that apomorphine (2 x 0.5 mg/kg), a dopamine agonist, inhibited the rise of DA after GOBA, and that haloperidol (5 mg/kg), which blocks DA receptors, antagonized the inhibition by apomorphine.

Despite evidence that apomorphine acts on receptors to produce behavioural changes and inhibit DA cell firing, suggestions have been made that the inhibitory effect of apomorphine on DA metabolism is not due to receptor action but due to inhibition of monoamine oxidase (MAO) or of catechol-O-methyl-transferase (COMT). MAO and COMT metabolize DA. It was of interest whether the inhibitory action of apomorphine on the rise of DA after GOBA was due to inhibition of COMT or of MAO.

Tropolone (50 mg/kg), which inhibits COMT, prevented the rise of DA after GOBA. However, unlike the case with apomorphine, the inhibition was not antagonized

by haloperidol. Tropolone was found to be a potent inhibitor of adrenal tyrosine hydroxylase in vitro. In high doses (100 mg/kg) tropolone depleted DA in brain. Unlike apomorphine (2 x 5 mg/kg), tropolone (2 x 40 mg/kg) did not protect DA from depletion after administration of an inhibitor of tyrosine hydroxylase. It was concluded that apomorphine is not tropolone-like, and that the action of the latter on DA metabolism may be explained by inhibition of tyrosine hydroxylase.

The MAO inhibitors pheniprazine (4 mg/kg) and pargyline (75 mg/kg) did not inhibit the rise of DA after GOBA. Moreover the inhibition by apomorphine of brain mitochondrial MAO in vitro was not antagonized by haloperidol. It was concluded that inhibition of MAO by apomorphine was not responsible for the inhibition of the rise of DA after GOBA.

In order to determine whether the action of apomorphine could be attributed to a hypothetical releasing action on DA, the effect of a known DA releasing agent, amphetamine, was examined. It was found that a low dose of amphetamine sulphate (3 mg/kg) inhibited the rise of DA after GOBA, and the inhibition was antagonized by haloperidol.

The action of apomorphine on DA after GOBA was indistinguishable from that of a releasing agent.

Another approach to the problem of the hypothesized receptor was to test the ability of various DA agonists to inhibit the rise of DA after GOBA. The ability of haloperidol to antagonize the agonist was also examined. Each agonist was tested in a 2 x 2 x 2 experiment in which rats received either GOBA or saline, haloperidol or no haloperidol, agonist or no agonist. The agonists employed in addition to apomorphine were M7 (4 mg/kg), apocodeine (15 mg/kg), piribedil (20 mg/kg), ergocornine (3 mg/kg) and CB 154 (10 mg/kg).

Piribedil, ergocornine and M7 were found to increase slightly the DA level in saline controls, whereas the other agonists had no effect. The increase of DA seen after some agonists was interpreted as due to an imbalance between the tendency to decrease DA metabolism by inhibiting cell firing and the tendency to inhibit DA synthesis through a receptor.

All agonists tested inhibited the rise of DA after GOBA. In view of their differing structure it was unlikely that a non-receptor mechanism was responsible for the unanimous effect. This result was therefore regarded as

strong evidence for the existence of the hypothesized receptor. Haloperidol, contrary to expectations, antagonized the effect of only two of the agonists, apomorphine and piribedil. The latter result indicated that the action of the agonists was not uniform.

In conclusion, the unanimity of the agonists in inhibiting the rate of DA synthesis after the administration of an agonist known to inhibit impulse flow strongly indicates the presence of a dopamine sensitive receptor which inhibits DA synthesis by a mechanism which does not depend on cell firing. However the differing action of the agonists on DA levels in controls and in animals given GOBA and haloperidol indicates that the action of the agonists is not uniform.

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