Dopamine Receptors in the Brain

A dopamine-sensitive adenylate cyclase models synaptic receptors, illuminating antipsychotic drug action.

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During the past decade there has been an increasing realization of the importance of dopamine as a neurotransmitter in the mammalian brain, particularly in the basal ganglia and mesolimbic forebrain (*1*). In addition, there is evidence that abnormalities of dopaminergic neurotransmission in the brain may be of clinical importance. For example, degeneration of the dopaminergic pathway that normally connects the substantia nigra to the caudate nucleus and putamen is thought to be the primary feature in the etiology of Parkinson's disease (*2*). A recent article (*3*) summarized the evidence in favor of the view that the drugs used to treat schizophrenia act as dopamine antagonists in the brain. In brief, the drugs in question, such as the phenothiazines, have been shown in clinical studies to be effective in treating the fundamental symptoms of psychosis—that is, they are truly "antipsychotic"; and the results of animal experiments indicate that their principal mode of action is a blockade of dopamine receptor sites in the central nervous system (CNS). The purpose of this article is to describe recent findings which offer a new biochemical approach to testing this hypothesis. This approach depends on the discovery that activation of dopamine receptors in the brain appears to be coupled to increased formation of adenosine 3′,5′-monophosphate (cyclic AMP).

A variety of hormone-sensitive adenylate cyclases are now known to represent the primary sites of action of many peptide and catecholamine hormones (*4*). In peripheral tissues such as heart, smooth muscle, pineal gland, and fat cells, the actions of norepinephrine (NE) released from the sympathetic nerve terminals innervating these tissues and the endocrine actions of circulating epinephrine are thought to be mediated by a catecholamine-sensitive adenylate cyclase (*5*). The pharmacological properties of this system are those typical of a β-type of adrenoceptor, and indeed β-adrenoceptors in all tissues seem to be coupled to cyclic AMP production. More recently, however, attention has focused on the possible role of neurotransmitter-sensitive adenylate cyclases in mediating synaptic transmission between neurons, rather than between neurons and peripheral end organs. The adenylate cyclase activity and the concentration of cyclic AMP are higher in the brain than in any other organ. Cyclic AMP formation in slices of brain tissue can be stimulated by the addition of a variety of pharmacologically active amines or by electrical stimulation and other depolarizing stimuli (*6*). The finding that a NE-sensitive adenylate cyclase with typical β-adrenoceptor properties exists in many different regions of the brain prompted a series of investigations which suggest that the synaptic actions of NE in the CNS may be mediated by this mechanism (*7*). In the cerebellum, for example, Purkinje cells receive an inhibitory input from a diffuse system of NE-containing nerve terminals which originate from cells in the brain stem nucleus, the locus coeruleus. The iontophoretic application of cyclic AMP from microelectrodes on to the surface of Purkinje cells was found to mimic the inhibitory actions of NE on the discharge rate of these neurons. Furthermore, the inhibitory effects of both NE and cyclic AMP were potentiated by phosphodiesterase inhibitors, and the effects of NE but not those produced by cyclic AMP were blocked by β-adrenoceptor antagonist drugs. Intracellular recordings from Purkinje cells showed that both NE and cyclic AMP caused a hyperpolarization of the neurons, and that a similar hyperpolarization was produced by stimulating the NE pathway originating from the locus coeruleus. Using an immunocytochemical method for detecting cyclic AMP, the same authors showed that application of NE or stimulation of the locus coeruleus caused a large increase in the proportion of Purkinje cells that reacted positively. In other regions of the brain, such as the brain stem or pyramidal cells in the hippocampus, NE-sensitive neurons have also been found to be depressed by iontophoretic applications of cyclic AMP (*8*).

The suggestion that the inhibitory synaptic actions of NE in the CNS are mediated by an increased formation of cyclic AMP in postsynaptic neurons has been challenged and it has been proposed that the action of NE involves instead a mobilization of cellular calcium (*9*). This controversy, however, may have arisen because of technical difficulties encountered in iontophoretic experiments with cyclic AMP (*10*). It is in any case well established that changes in the availability of intracellular calcium are an essential feature of many hormone-sensitive adenylate cyclase mechanisms (*11*), so that the current views concerning the mechanism of action of NE at CNS receptors need not be mutually exclusive. Although it seems likely that a NE-sensitive adenylate cyclase with β-adrenoceptor properties does play an important role in noradrenergic synaptic transmission in the CNS, not all catecholamine-sensitive adenylate cyclase in the brain may serve such a function, since a similar β-adrenoceptor type of adenylate cyclase has been found to exist in various cultured cell lines of glial origin, in which some function other than synaptic transmission is presumably involved (*12*).

An involvement of catecholamine-sensitive adenylate cyclase in synaptic transmission is, however, strongly suggested by the results of experiments by Greengard and his colleagues on sympathetic ganglia from various species (*13*). In isolated superior cervical sympathetic ganglia of the rabbit, stimulation of the preganglionic nerve causes a rapid depolarization of the ganglionic neurons, mediated directly by activation of nicotinic cholinergic receptors on the neurons by acetylcholine released from the preganglionic nerve terminals. The excitation is followed, however, by a slow and long-lasting hyperpolarization of the ganglionic neurons which is thought to be due to a release of dopamine from small dopamine-containing interneurons in the ganglia that are also excited by acetylcholine released on stimulation of the preganglionic nerves (*14*). The inhibitory effects of dopamine appear to be mediated by a dopamine-sensitive adenylate cyclase in the ganglionic neurons. Thus stimu-

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lation of the preganglionic nerve leads to an increased concentration of cyclic AMP in isolated ganglia, and cyclic AMP can mimic the hyperpolarizing effects of dopamine when applied to ganglionic neurons. Phosphodiesterase inhibitors potentiate the hyperpolarization and the increase in ganglionic cyclic AMP induced by stimulation of the preganglionic nerve, and also potentiate the hyperpolarizing response to exogenous dopamine (*15*). Low concentrations of dopamine cause a five- to tenfold increase in the concentration of cyclic AMP in slices of bovine superior cervical ganglia, and this response is blocked by high concentrations of α-adrenoceptor antagonist drugs, but not by compounds that block β-adrenoceptors, and is potentiated by phosphodiesterase inhibition (*16*).

Greengard and his colleagues have also suggested a possible mechanism by which changes in cyclic AMP concentration could influence the membrane permeability properties of synaptic membranes, by regulating the state of phosphorylation of specific membrane proteins (*13*). The results of studies of membrane protein phosphorylation in response to cyclic AMP in nervous and nonnervous tissue are compatible with a model in which transmitter-induced changes in intracellular cyclic AMP could regulate the activity of cyclic AMP-dependent protein kinase; this would lead to changes in the level of phosphorylation of a specific protein in the synaptic membrane of the postsynaptic neuron, which would in turn modify the ion permeability characteristics of this membrane and generate a slow postsynaptic potential.

A Dopamine-Sensitive Adenylate Cyclase in the CNS

It now seems likely that the postsynaptic actions of dopamine in the CNS may also involve a cyclic AMP-dependent mechanism. Dopamine-containing neurons exist in various regions of mammalian brain, particularly in the basal ganglia and in certain regions of the mesolimbic system; they are also found in the amacrine cell population of the retina. Cyclic AMP formation in isolated retinas (*17*), and in homogenates of rat basal ganglia tissue (*18*) is stimulated by the addition of low concentrations of dopamine. This particular form of catecholamine-sensitive adenylate cyclase is found only in regions of the CNS in which dopaminergic nerve terminals are known to be present, and is absent in areas such as the cerebellum which contain only NE fibers. As will be described in detail below, the dopamine-sensitive adenylate cyclase has pharmacological properties that clearly distinguish it from either of the traditional α- or β-adrenoceptor classes. Thus the dopamine system is not activated by the potent β-adrenoceptor stimulant isoproterenol, nor is the dopamine response blocked by β-adrenoceptor antagonist drugs such as propranolol (*17, 18*).

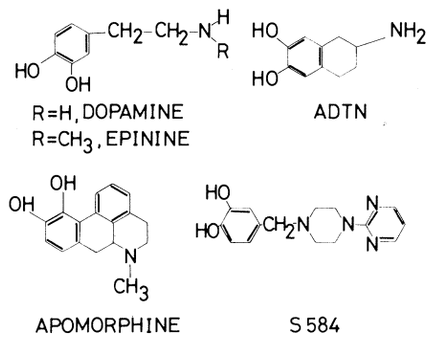

Fig. 1. Structures of dopamine and some related compounds that mimic the effects of dopamine in stimulating cyclic AMP formation in brain homogenates. The abbreviation ADTN is for 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; S584, L-(3,4-dihydroxbenzyl)-4-(2-pyrimidinyl) piperazine, is a catechol metabolite of the drug piribedil that is used against Parkinson's disease.

The dopamine-sensitive adenylate cyclase in retina can be demonstrated both in isolated whole retina incubated in vitro, and in retinal homogenates (*17*). In brain tissue, the dopamine-sensitive adenylate cyclase has been demonstrated in both striatal slices (*19*) and in membrane fragments present in cell-free homogenates of this and other dopamine-rich brain regions, although most studies have used the latter preparations (*18*). The dopamine-sensitive adenylate cyclase differs from the NE-sensitive adenylate cyclase in brain tissue in its ability to survive homogenization in hypotonic media; this procedure usually leads to a complete disappearance of the β-adrenoceptor type of cyclase, which is most readily demonstrated in isolated brain slice preparations. When isotonic sucrose homogenates of dopamine-rich rat basal ganglia are fractioned by centrifugation procedures, the dopamine-sensitive adenylate cyclase is enriched in fractions containing synaptic membranes, suggesting that it has an appropriate cellular localization for its postulated synaptic function (*19*). When the dopamine-containing nerve terminals of the basal ganglia are destroyed by surgical means, or chemically by 6-hydroxydopamine treatment, the dopamine-sensitive adenylate cyclase remains in homogenates of the denervated striatal tissue, showing that the enzyme is localized mainly postsynaptically on striatal cells rather than presynaptically in the dopaminergic nerve terminals.

Although initial reports suggested that there was no change in the activity of the dopamine-sensitive adenylate cyclase in basal ganglia tissue after such denervation (*20*), it has recently been found that the adenylate cyclase response to dopamine is considerably increased in homogenates of striatal tissue after such denervation (*21*). This adds further support to the hypothesis that the dopamine-sensitive adenylate cyclase represents the mechanism by which dopamine acts postsynaptically in the CNS, since is known that the behavioral responses elicited by dopamine-stimulating drugs are enhanced after denervation of CNS dopaminergic pathways. The behavioral results have been interpreted as a form of denervation supersensitivity in CNS dopamine receptors, and this may be reflected by the increased number of dopamine-sensitive adenylate cyclase sites present in the denervated tissues.

Stimulation by Dopamine Analogs

Most of our knowledge of the properties of α- and β-adrenoceptors and nicotinic and muscarinic cholinergic receptors derives from studies on peripheral tissues, and it is not surprising therefore that the detailed pharmacological specificity of CNS dopamine receptors has hitherto been only poorly defined, since there are few peripheral systems in which the existence of dopamine receptors can be demonstrated unequivocally. The discovery that dopamine-rich areas of CNS contain an adenylate cyclase uniquely responsive to dopamine, however, provides a simple biochemical model system to test a wide range of drugs as potential agonists or antagonists at CNS dopamine receptors.

Several studies have now been made of the actions of dopamine analogs in stimulating adenylate cyclase activity in homogenates of striatal brain tissue (*22, 23*). The results indicate a high degree of structural specificity for agonists at CNS dopamine receptors, and reveal a spectrum of activity that is different from that at either α- or β-adrenoceptors (Fig. 1). Among simple β-phenethylamine analogs of dopamine only the *N*-methyl derivative epinine is equipotent with the parent compound. Although *l*-NE is effective in stimulating adenylate cyclase, it is some 20 times less potent than dopamine; the α-methyl analog of dopamine is also considerably less potent than dopamine. Compounds that lack the catechol hydroxyl groups of dopamine, or substances in which the side chain contains one or three carbon atoms, instead of the

usual two, are without activity. Although isoproterenol is ineffective in striatal homogenates from rat brain, there may be species differences in the properties of the adenylate cyclase system, since isoproterenol was found to be weakly effective in homogenates of striatal tissue from primate brain (*23*).

A variety of compounds in which the side chain of dopamine is held in a rigid conformation in a second ring system have been tested as potential agonists (*22, 24*). The aporphine alkaloid apomorphine is thought to be a stimulant drug at CNS dopamine receptors from a variety of behavioral and biochemical evidence obtained in whole animal studies. Apomorphine is a potent stimulant of adenylate cyclase in striatal homogenates, although some reports suggest that it is only a partial agonist at such sites, that is, it does not produce a maximum response as great as that seen with dopamine. On the other hand, other studies have found this drug to behave as a simple agonist, with a potency comparable to that of dopamine. Of a variety of other apomorphine analogs tested, only *N-n*-propyl norapomorphine was found to be comparable in potency to apomorphine as an agonist. Several drugs in this series, such as bulbocapnine, have no agonist activity but behave instead as inhibitors of the stimulation produced by dopamine, that is, they are antagonists.

Among tetrahydroisoquinolines there are rigid analogs in which the side chain of dopamine is locked in a folded rather than an extended conformation; such compounds are only weakly effective as dopamine agonists. On the other hand, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (Fig. 1), which represents the fully extended conformation of the dopamine side chain, is equipotent with dopamine as an agonist. Another active compound is the catechol metabolite (S584) of the drug piribedil that is used against Parkinson's disease (Fig. 1). These findings suggest that the preferred conformation of dopamine at CNS receptor sites may be that in which the side chain is in the fully extended *trans* form (*22, 24*). It is known that this is the preferred form in the crystalline state from x-ray analysis, and in solution from nuclear magnetic resonance studies. Similar results have been obtained from theoretical calculations (see *22*).

Apart from the intrinsic value of defining more precisely the structural specificity of CNS dopamine receptors, an increased understanding in this area may prove valuable in predicting novel drugs for treating parkinsonism, in which dopaminergic agonists, or precursors such as L-dopa are known to be effective.

It is interesting that the pharmacological specificity of the dopamine-sensitive adenylate cyclase in rat brain homogenates is very similar to that described in two other animal tissues which respond to dopamine, namely the vasodilating effects of dopamine on the renal artery of the dog (*25*), and the inhibitory effects of dopamine on neuronal firing in the brain of the snail *Helix pomatia* (*26*). The former preparation in particular seems to represent a valuable peripheral model for studies of mammalian dopamine receptors.

Table 1. Inhibition of dopamine-sensitive adenylate cyclase by antipsychotic drugs. The K_i values represent dissociation constants for binding of drugs to the dopamine sites of the dopamine-sensitive adenylate cyclase; the values were calculated from the drug concentrations needed to cause 50 percent inhibition of the dopamine response (IC_{50}) determined graphically as in Figs. 3 and 4, with the assumption that all compounds acted competitively with dopamine, and use of the equation $IC_{50} = (1 + S/K_m)K_i$, where S is the concentration of dopamine used and K_m is the concentration of dopamine needed to produce half-maximal stimulation of adenylate cyclase activity. The data are from (i) Miller *et al.* (*27*), (ii) Clement-Cormier *et al.* (*29*), (iii) Brown and Makman (*30*), and Karobath and Leitich (*28*). Brown and Makman obtained their results from calf retina; the other investigators used rat striatum.

Drug*	Inhibition constant K_i (n*M*)		
	(i)	(ii)	(iii)
α-Flupenthixol	1.0		
α,β-Flupenthixol	3.5		
Fluphenazine	4.3	8.0	7.1
(+)-Butaclamol	8.8		
α-Clopenthixol	16.0		
Trifluoperazine	19.0		
α-Chlorprothixene	37.0		
Trifluoperazine		44.0	
Chlorpromazine	48.0	66.0	48.0
Spiroperidol	95.0		
Prochlorperazine	100.0	55.0	
Thioridazine	130.0	55.0	
Pimozide	140.0	122.0	
(±)-Bulbocapnine	160.0		
Clozapine	170.0		
Haloperidol		220.0	38.0
Chlorimipramine	420.0		
Ergotamine			430.0
β-Chlorprothixene	950.0		

*Compounds lacking neuroleptic activity and with K_i values > 1000 n*M*: promazine, β-clopenthixol, morphine, β-flupenthixol, (−)-butaclamol, promethazine, benztropine, imipramine, desipramine, ethopropazine, diethazine, mepazine, fenethazine, chlorpromazine sulfoxide, pyrathiazine, diphenhydramine, methdilazine, phentolamine, propranolol, prostaglandin E_1, *dl*-amphetamine, and amantadine (*27–30*).

Inhibition by Antipsychotic Drugs

The most striking pharmacological feature of the dopamine-sensitive adenylate cyclase in mammalian CNS is that it is extremely sensitive to inhibition by antipsychotic drugs (*27, 28*). The results of four independent investigations are summarized in Table 1 (*27–30*). These studies used adenylate cyclase in rat striatal homogenates or calf retina stimulated by supramaximal concentrations of added dopamine as the test system. Various antipsychotic drugs (Fig. 2) inhibited the dopamine-stimulated adenylate cyclase in a dose-dependent manner, and, in each case

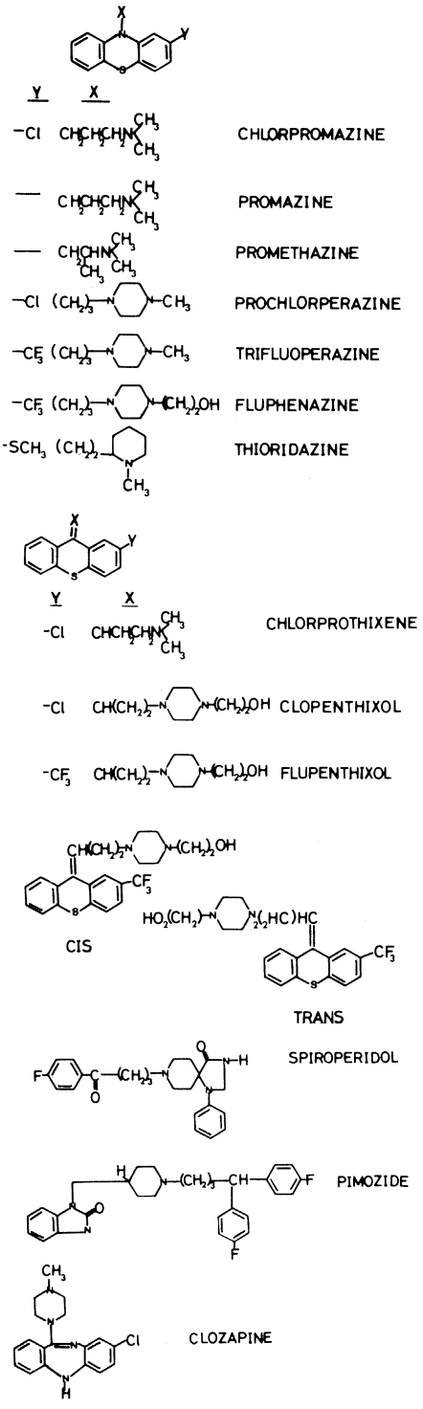

Fig. 2. Structures of some antipsychotic drugs of the phenothiazine, thioxanthene, butyrophenone, and dibenzazepine classes.

examined, kinetic analysis showed that the inhibition was competitive with dopamine. By applying a kinetic analysis similar to that used for assessing enzyme inhibition, the potency of drugs as inhibitors of the dopamine-sensitive adenylate cyclase can be expressed as K_i (inhibition constant) values, representing the dissocation constants for drug interaction with the dopamine receptive sites (Table 2). The classical antipsychotic drug chlorpromazine has a K_i value of 5 to 10 $\times$ $10^{-8}M$, and more potent antipsychotic drugs such as flupenthixol and fluphenazine have K_i values of 1 to 10 $\times$ $10^{-9}M$. This means that of all the known biological effects of such drugs, their ability to antagonize dopamine at the adenylate cyclase sites in brain is by far the most potent that has been described. Furthermore, when various phenothiazine drugs are compared (Fig. 3) the differences in their potencies as inhibitors of dopamine-stimulated cyclic AMP formation resemble the differences in their potencies in vivo as antipsychotic drugs clinically, or in their ability to act as dopamine antagonists in various animal tests, such as blockade of amphetamine- or apomorphine-induced stereotyped behavior.

This correlation also extends to other chemical classes of antipsychotic agents. The thioxanthenes present a particularly valuable test of the hypothesis that antipsychotic potency correlates with ability to inhibit the dopamine-sensitive adenylate cyclase (*27*). In this group of drugs the side chain is linked to the heterocyclic ring system by a carbon–carbon double bond. The drugs thus exhibit geometric isomerism and it is known that the *cis* isomers, in which the side chain projects toward the side of the halogen substituent on the ring system, are more potent than the *trans* isomers in various animal tests for antipsychotic activity. This is particularly marked in the compound flupenthixol (Fig. 2) in which the *cis* isomer α-flupenthixol is a potent antipsychotic drug, whereas the *trans* isomer β-flupenthixol is virtually devoid of such activity. When tested on the dopamine-sensitive adenylate cyclase, α-flupenthixol proved to be an extremely potent inhibitor, while β-flupenthixol was ineffective even at relatively high concentrations. The *cis* isomers of the other thioxanthenes also proved to be considerably more potent than the corresponding *trans* isomers.

An analogous situation was found among another group of antipsychotic drugs, the dibenzazepines. Here again those drugs corresponding to the *cis* isomers of the thioxanthenes such as loxapine and clothiapine proved to be more potent than those corresponding to the *trans* isomers such as clozapine (*31*).

Table 2. Comparison of anticholinergic and antidopaminergic properties of neuroleptic drugs.

Drug	Dissociation constant for binding to receptor sites (M)		Ratio of cholinergic to dopaminergic potency
	Muscarinic	Dopaminergic	
Atropine	5.2×10^{-10}		
Benztropine	1.3×10^{-9}	1×10^{-4}	> 75,000.0
Ethopropazine	1.0×10^{-8}		
Thioridazine	2.5×10^{-8}	1.3×10^{-7}	5.2
Clozapine	5.5×10^{-8}	1.7×10^{-7}	3.1
Chlorpromazine	3.5×10^{-7}	4.8×10^{-8}	0.14
Pimozide	1.6×10^{-7}	1.4×10^{-7}	0.87
α-Flupenthixol	2.2×10^{-6}	1.0×10^{-9}	0.0005
Trifluoperazine	4.0×10^{-6}	1.0×10^{-8}	0.005
Spiroperidol	1.2×10^{-5}	9.5×10^{-8}	0.008

Recently, another test of the predictive value of the dopamine-sensitive adenylate cyclase was provided by the compound butaclamol (Fig. 4). This is a novel neuroleptic agent which possesses pharmacological actions in animal tests that are characteristic of clinically effective antipsychotic drugs (*32*). It is unique in exhibiting stereospecificity, and only the (+)-enantiomer was active in animal tests. The (+)-enantiomer also proved to be a potent inhibitor of the dopamine-sensitive adenylate cyclase in rat brain, while the (–) form was inactive (*32*) (Fig. 4). As studies of

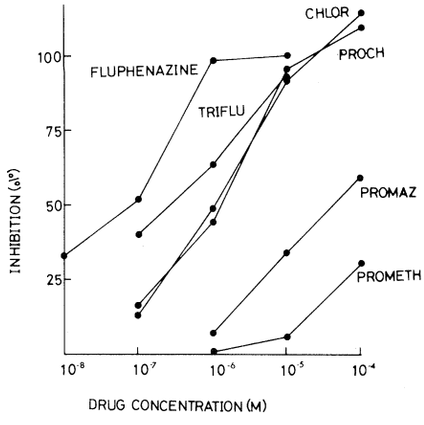

Fig. 3. Inhibition of dopamine-stimulated cyclic AMP formation in rat striatal homogenates by phenothiazines. The basal level of cyclic AMP production, without added dopamine, was 45.5 ± 3.6 picomoles per sample (equivalent to 2 milligrams wet weight of tissue) during a 2.5-minute incubation at 30°C. This was raised to 88.7 ± 9.1 pmole per sample in the presence of 100 μM dopamine (means and standard error for six experiments). Drug effects were assessed on the dopamine-stimulated component of cyclic AMP production, with a constant concentration of 100 μM dopamine. Each point is the mean of at least five separate determinations, standard errors were less than 10 percent of the means. At the highest concentrations tested some drugs caused a slight inhibition of basal activity, indicated by inhibition of greater than 100 percent. Abbreviations: *TRIFLU*, trifluoperazine; *CHLOR*, chlorpromazine; *PROCH*, prochlorperazine; *PROMAZ*, promazine; and *PROMETH*, promethazine. From (*27*).

opiate receptors in brain have been facilitated by the availability of stereochemically specific compounds such as levorphanol and dextrorphan, so the availability of a stereochemically specific antagonist of CNS dopamine receptors should also prove valuable as a research tool in further studies of this system.

Further evidence for the specificity of action of antipsychotic drugs on the dopamine-sensitive adenylate cyclase is provided by the results obtained with related compounds that lack antipsychotic activity, and which are used clinically as antihistaminic, anti-Parkinsonian, antipruritic, antiemetic, antianxiety, or antidepressant drugs. These compounds either failed to inhibit the dopamine response, or they were considerably less potent than the antipsychotic drugs (Table 1) (*27, 28*).

A molecular mechanism has been proposed that attempts to explain how chlorpromazine might block dopamine receptors (*33*). This proposal draws attention to a possible complementarity between certain portions of the x-ray structure of chlorpromazine and dopamine. At the Neurochemical Pharmacology Unit at Cambridge we are engaged in a collaborative effort to determine the x-ray structure of various active and inactive neuroleptics in an attempt to gain more insight into the structural and conformational requirements for a compound to exhibit potent neuroleptic activity. Initial results with α-chlorprothixene and α- and β-flupenthixol have added support to the above suggestion (*34*).

Although all the antipsychotic drugs that have been tested have proved to be active as inhibitors of the dopamine-sensitive adenylate cyclase, there is one chemical class of antipsychotics whose effects in this test do not correlate well with their known potencies in vivo. These are the butyrophenones and related compounds. Drugs in this class, such as haloperidol, spiroperidol, and pimozide, are among the most potent antipsychotic drugs known; they are

effective clinically and in animal tests at dose levels many times lower than those of the classical drug chlorpromazine (*35*). Haloperidol, spiroperidol, and pimozide, however, when tested on the dopamine-sensitive adenylate cyclase of rat brain or calf retina, are somewhat less potent than chlorpromazine as dopamine antagonists (Table 1) (*27, 28*). But despite their low potency, they behave, as do all other antipsychotic drugs, in a simple competitive manner. The weak effects of butyrophenones could, of course, be explained by differences in the absorption, distribution, and metabolism of such drugs in vivo when compared with other antipsychotic drugs. Potency in vivo does not necessarily correlate with drug effects observed in a system in vitro. For example, there is evidence to suggest that pimozide may be selectively accumulated in dopamine-rich areas of the brain, which might account for the apparently high potency of this compound as an antidopaminergic agent in whole animal tests (*36*). On the other hand, one must also consider the possibility that the antipsychotic action exerted by the butyrophenones depends on some pharmacological mechanisms other than inhibition at postsynaptic dopamine receptors.

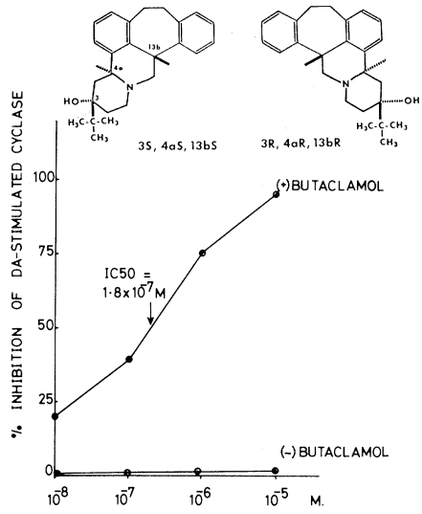

Fig. 4. Inhibition of dopamine-stimulated cyclic AMP formation in rat striatal homogenates by the (+) (3S, 4aS, 13bS) and (−) (3R, 4aR, 13bR) enantiomers of butaclamol. Other details as in Fig. 3. From Miller *et al.* (*31*).

Possible Presynaptic Actions of Antipsychotic Drugs

It is well known that antipsychotic drugs cause an acceleration of dopamine turnover in the brain (for a review, see *37*). This increase in dopamine turnover is thought to be due to an increased rate of firing of the dopamine-containing neurons that follows as a reflex consequence of the dopamine receptor blockade exerted by the antipsychotic drugs. However, at least part of the increased dopamine turnover elicited by these drugs persists after impulse traffic in the nigrostriatal neurons is abolished, either by acute lesions, or after administration of γ-hydroxybutyric acid (*38*). This suggests that the antipsychotic drugs may act in part locally at dopaminergic presynaptic nerve terminals to cause an increase in transmitter turnover. Drugs that stimulate dopamine receptors, such as apomorphine, have been found to inhibit the conversion of tyrosine to dopamine in dopaminergic nerve terminals in homogenates of striatal tissue, and this effect is partly reversed by antipsychotic drugs (*39*). It has also been reported recently that butyrophenones and other antipsychotic drugs potently inhibit the stimulation-evoked release of dopamine from nerve terminals in slices of striatal tissue (*40*). Thus, apart from the antagonistic effects of antipsychotic drugs on postsynaptic dopamine receptors, similar actions on presynaptic mechanisms should also be considered. The butyrophenones in particular appear to be very potent in blocking the stimulation-evoked release of dopamine from presynaptic terminals (*40*), and it could be that this represents the most important component in their overall actions, since this family of antipsychotic drugs proved to be relatively poor inhibitors of the postsynaptic dopamine receptors, judging by their inhibitory effects on the adenylate cyclase system.

Antipsychotic Drugs and Their Parkinson-Like Side Effects

Since Parkinson's disease is known to be associated with a deficiency of dopamine in the basal ganglia, drugs that antagonize the normal actions of dopamine at receptor sites in the brain would be expected to lead to some of the symptoms associated with parkinsonism. It is not surprising, therefore, that many antipsychotic drugs cause Parkinson-like side effects, such as muscular rigidity, a reduction in voluntary movements, and tremor. What is more puzzling is why the use of some antipsychotic drugs (*14*) is associated with only a very low incidence of such side effects, while others cause a high incidence of Parkinson-like symptoms (*41*). According to the dopamine hypothesis, antipsychotic drugs of different types would need to be given in sufficiently high doses to achieve an effective blockade of CNS dopamine receptors in order to exert an effective antipsychotic action. In that case all effective antipsychotic drugs would be expected to produce approximately the same incidence of Parkinson-like effects. The drugs that produce relatively few such side effects, such as the phenothiazine, thioridazine, and the dibenzazepine, clozapine, are approximately as potent or slightly less potent than chlorpromazine when tested as inhibitors of the dopamine-sensitive adenylate cyclase; clinically they are used in somewhat higher doses than chlorpromazine. The low incidence of extrapyramidal side effects in these compounds, however, seems likely to be associated with another pharmacological property which they possess. This is their high antagonist potency at muscarinic receptor sites in the CNS. When the antimuscarinic potency of various neuroleptic drugs was measured, clozapine and thioridazine were unique in being more potent as anticholinergic than as antidopaminergic drugs, whereas all other neuroleptic drugs tested were considerably more potent in blocking dopamine than acetylcholine (Table 2) (*42*). The drugs associated with a high incidence of Parkinson-like side effects, such as trifluoperazine, spiroperidol, and flupenthixol were only very weakly active as antimuscarinic agents. Since centrally acting anticholinergic drugs are known to be effective in counteracting the extrapyramidal side effects induced by antipsychotic drug treatment, the built-in anticholinergic properties of certain antipsychotic drugs offers a ready explanation for their lack of such side effects. This hypothesis also helps to explain the apparently anomalous behavior of such drugs as clozapine and thioridazine in several standard animal tests for antidopamine drugs, since the additional anticholinergic property tends to counterbalance the antidopamine effects of the drugs, and they may yield false negative results in such tests in vivo (*43*).

Toward an Anatomy of Psychosis

Although the nigrostriatal pathway is the largest and most thoroughly studied system of dopaminergic neurons in the mammalian CNS, it is not the only one. Dopamine-containing neurons also exist in the retina, in the basal hypothalamus, and in ascending systems from the substantia nigra region to various parts of the limbic forebrain including the olfactory tubercle, nucleus accumbens, cingulate cortex, and parts of frontal cortex (*44*). The biochemical evidence now available shows that a dopamine-sensitive adenylate cyclase can be demonstrated in homogenates of most of these areas of the CNS (*45*). Further-

more, antipsychotic drugs are as effective in inhibiting the dopamine response in such areas as they are in the basal ganglia (*29, 30, 45*). Thus, although there is strong evidence in support of the hypothesis that antipsychotic effects are mediated by a blockade of dopamine, it is not yet clear which of the various CNS dopaminergic pathways is crucial for such effects. It is attractive to imagine that the dopamine pathways in the mesolimbic system might represent a primary target for antipsychotic drugs, and this suggestion has recently been elaborated (*46*).

Conclusions and Future Directions

The antipsychotic drugs have had a very important impact on the treatment of schizophrenia, with far-reaching medical and social consequences. The therapeutic value of such drugs will increase as the search continues for compounds with a low incidence of undesirable side effects and there is a growing use of new methods of drug administration. Antipsychotic drugs are already widely used in the form of depot injections, in which a lipid-soluble derivative is injected in an oil base, providing an effective antipsychotic concentration of the drug in body fluids for several weeks after a single treatment. Such preparations are likely to allow an increasing proportion of schizophrenic patients to be treated effectively on an outpatient rather than an inpatient basis.

Scientifically, the dopamine hypothesis of the mode of action of antipsychotic drugs continues to provide a useful and stimulating area for neuropharmacological research. The dopamine-sensitive adenylate cyclase in brain tissue has proved to be a useful biochemical test preparation for systematic studies of the pharmacological specificity of CNS dopamine receptors. Much more work is needed, however, before we can be sure that this mechanism represents the predominant postsynaptic basis for dopaminergic neurotransmission in the CNS. Even if this is the case, we will need to understand how changes in the availability of cyclic AMP lead to the changes in neuronal excitability caused by dopamine in the CNS. In wider terms we also need to begin to understand the precise neurophysiological and behavioral functions served by the dopaminergic neuronal pathways in the CNS, functions which are as yet only poorly defined. The biochemical and pharmacological study of dopamine receptors, however, may prove helpful in leading to new and highly specific pharmacological tools that may assist in future studies of the functional role of dopamine in the CNS. Looking even further into the future we may hope that an improved understanding of the mode of action of antipsychotic drugs will help us to understand the biochemical and neuropharmacological basis of the psychotic state; at least this approach seems at the moment to be one of the most pragmatic available.

References and Notes

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47. I am grateful to my colleagues R. J. Miller and A. S. Horn for their help in the preparation of this article.