Dopamine-Sensitive Adenylate Cyclase in Mammalian Brain: A Possible Site of Action of Antipsychotic Drugs

(schizophrenia/extrapyramidal side effects)

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ABSTRACT Adenylate cyclase (EC 4.6.1.1), selectively stimulated by low concentrations of dopamine, has been found in the olfactory tubercle, the nucleus accumbens, and the caudate nucleus of several mammalian species. Several different classes of drugs effective in the treatment of schizophrenia (antipsychotic drugs) were potent inhibitors of the stimulation by dopamine of the enzyme from these various regions. The drugs studied included representatives of the phenothiazine, butyrophenone, and dibenzodiazepine classes. The inhibition by these antipsychotic drugs was competitive with respect to dopamine. The most potent of the antipsychotic agents tested was fluphenazine, which had a calculated inhibition constant (K_i) of about 5 × 10^{-8} M. For each of several drugs tested, the K_i for the enzyme from the olfactory tubercle was similar to that for the enzyme from the caudate nucleus. Several compounds closely related structurally to the psychoactive phenothiazines, but which have little or no antipsychotic or extrapyramidal actions clinically, had low relative potencies as inhibitors of dopamine-stimulated adenylate cyclase activity. The results, considered together with other data, raise the possibility that the therapeutic effects, as well as the extrapyramidal side effects, of these antipsychotic agents may be attributable, at least in part, to their ability to block the activation by dopamine of specific dopamine-sensitive adenylate cyclases in the human brain.

Many antipsychotic drugs produce an extrapyramidal syndrome indistinguishable from Parkinson’s disease (1, 2). There is considerable evidence that suggests that these extrapyramidal side effects may arise from the demonstrated ability of these drugs to antagonize the “dopamine receptor” of the caudate nucleus (3, 4), which many investigators have indirectly characterized in an extensive series of physiological, biochemical, and behavioral studies. Recently, a dopamine-sensitive adenylate cyclase was demonstrated in homogenates of the caudate nucleus of the rat brain (5). It was suggested (5) that an intimate association exists between this dopamine-sensitive adenylate cyclase and the “dopamine receptor” of the caudate nucleus, since the biochemical and pharmacological properties of this enzyme were similar to the reported properties of the caudate “dopamine receptor.”

A variety of evidence suggests that dopamine may serve as a neurotransmitter in several other regions of the mammalian nervous system, in addition to the caudate nucleus. Both the nucleus accumbens and the olfactory tubercle, two anatomical structures associated with the limbic system, are among the regions recently identified (6) as receiving dopaminergic innervation. Furthermore, biochemical measurements have shown the occurrence of relatively high levels of dopamine and its metabolites in these regions (refs. 7 and 8; O. Hornykiewicz, personal communication; A. Carlsson, personal communication).

The evidence that the extrapyramidal syndrome produced by the antipsychotic drugs is attributable to the ability of these drugs to block caudate dopamine receptors, together with the evidence that dopamine occurs in several other regions of the nervous system, has focused attention (e.g., refs. 9–11) on the twin hypotheses (a) that the antipsychotic drugs may achieve their therapeutic effects by virtue of blocking dopamine receptors in the brain and (b) that a hyperactivity of dopaminergic pathways in the brain may be involved in the pathophysiology of schizophrenia. The mesolimbic dopaminergic system of the brain, which projects to the olfactory tubercle and the nucleus accumbens, has figured prominently in such speculations concerning the pathophysiology of schizophrenia and the site of action of the antipsychotic drugs (11).

The results presented in this communication are consistent with a model in which the dopamine receptor of neural tissue is intimately associated with a dopamine-sensitive adenylate cyclase. The results are also compatible with the possibility that inhibition of this enzyme may provide an explanation, at the molecular level, for the therapeutic effects, as well as for the side effects, of some widely used antipsychotic agents.

MATERIALS AND METHODS

ATP, cyclic AMP, 1-norepinephrine, and EGTA were purchased from Sigma; 3-hydroxytyramine (dopamine) was from CalBiochem; inorganic salts were all reagent grade. All phenothiazines and related compounds were obtained, in high purity, from their commercial distributor.

The procedure for the dissection of the rat caudate nucleus [the nucleus caudate-putamen (12)] has been described (5). Caudate nuclei from other species were dissected in a manner similar to that used for the rat. The olfactory tubercle of the rat was removed from the ventral surface of the brain: a horizontal, medio-lateral cut was made up to the ventral surface of the anterior commissure; the lateral boundary was set by the olfactory tract. To dissect the nucleus accumbens of the guinea pig brain (13), the olfactory tubercles were removed, the brain was bisected along the midline, the lateral ventricle of each hemisphere was opened, and the nucleus accumbens was exposed and separated from the extreme rostral end of the head of the caudate nucleus.
were used in a system of activity of methane-maleate buffer with plotted enzyme as measured, min.

was per assay (c) incubation of 1114 Biochemistry:stances.

and is shown concentration. The concentrations of activity of various concentrations of dopamine, each at a maximally stimulating concentration, was no greater than with either agent alone; this nonadditivity suggests that dopamine interacts with the same receptor as does dopamine. Thus, the sensitivity of the adenylate cyclase of the olfactory tubercle to these several catecholamines was similar to that of the adenylate cyclase of the caudate nucleus, described earlier (5).

Fluphenazine, one of the most potent phenothiazine compounds both as an antipsychotic agent as well as in producing extrapyramidal side effects in patients, was investigated for its effect upon the dopamine-sensitive adenylate cyclase activity of the olfactory tubercle (Fig. 2). In the presence of a low

5–10 μM dopamine. In contrast, the β-adrenergic agonist l-isoproterenol had no significant effect on adenylate cyclase activity at concentrations as high as 1000 μM. The catecholamine, l-norepinephrine, stimulated the adenylate cyclase activity of the olfactory tubercle to the same maximal level as did dopamine (data not shown); however, considerably greater concentrations of l-norepinephrine than of dopamine were required to achieve a given increase in enzyme activity (e.g., 30 μM l-norepinephrine was required for half-maximal stimulation, and 300 μM, or greater, for maximal stimulation). The increase in enzyme activity in the presence of a combination of dopamine and l-norepinephrine, each at a maximally stimulating concentration, was no greater than with either agent alone; this nonadditivity suggests that l-norepinephrine interacts with the same receptor as does dopamine. Thus, the sensitivity of the adenylate cyclase of the olfactory tubercle to these several catecholamines was similar to that of the adenylate cyclase of the caudate nucleus, described earlier (5).

Tissues were pooled and were homogenized in 50 volumes (weight to volume) of 2 mM tris-(hydroxymethyl)aminomethane-maleate buffer (pH 7.4)–2 mM EGTA. The standard assay system (final volume 0.5 ml) for measurement of adenylate cyclase [EC 4.6.1.1; ATP pyrophosphate-lyase (cyclizing)] activity of homogenates contained (in mmol/liter): tris(hydroxymethyl)aminomethane-maleate, 80.2; ATP, 1.5; MgSO₄, 6.0; theophylline, 10; EGTA, 0.6 (including the amount introduced with the tissue homogenate); 0.05 ml of tissue homogenate; plus test substances as indicated. Incubation was for 2.5 min at 30°. The reaction was terminated, and cyclic AMP measured, as described (5). Data represent total cyclic AMP formation per assay tube. Under the experimental conditions used, enzyme activity was proportional to time and enzyme concentration. The concentrations of ATP (1.5 mM) and MgSO₄(6.0 mM) utilized were in a range such that enzyme activity was independent of the concentration of these substances.

RESULTS

The effect of various concentrations of the catecholamines, dopamine and l-isoproterenol, on the adenylate cyclase activity of a homogenate of the olfactory tubercle of the rat is shown in Fig. 1. Adenylate cyclase activity was stimulated by low concentrations of dopamine; a half-maximal increase in enzyme activity was achieved, in various experiments, with

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**Fig. 1.** Effect of catecholamines on adenylate cyclase activity in a homogenate of rat olfactory tubercle. Standard conditions were used for measurement of adenylate cyclase activity except that: (a) 0.05 ml of a 1:25 tissue homogenate was used; (b) the assay system contained 0.5 mM ATP and 2.0 mM MgSO₄; and (c) incubation was for 5 min. In the absence of added catecholamine, 24.4 ± 0.4 pmol (mean ± SEM, n = 20) of cyclic AMP was formed. The increase in cyclic AMP above this basal level is plotted as a function of catecholamine concentration. The data give mean values and ranges for duplicate determinations on each of three replicate samples.

![Graph](image-url)

**Fig. 2.** Effect of various concentrations of dopamine, alone (○) or in combination with 0.1 μM fluphenazine (●), on adenylate cyclase activity in a homogenate of rat olfactory tubercle. In the absence of added dopamine and fluphenazine, 12.0 ± 0.3 pmol (mean ± SEM, n = 9) of cyclic AMP was formed; in the presence of 0.1 μM fluphenazine (but without added dopamine), 11.0 ± 0.5 pmol (mean ± SEM, n = 6) of cyclic AMP was formed. The increase in cyclic AMP above the basal level (i.e., the level in the absence of both dopamine and fluphenazine) is plotted as a function of dopamine concentration. The data give mean values and ranges for duplicate determinations on each of three replicate samples. Inset: Double-reciprocal plot of cyclic AMP increase as a function of dopamine concentration from 3 μM to 300 μM. (A) Control; (B) 1 × 10⁻⁴ M fluphenazine.
concentration of fluphenazine (0.1 μM), higher concentrations of dopamine were required to stimulate the adenylate cyclase of the olfactory tubercle. Thus, in the absence of fluphenazine, 6 μM dopamine caused a half-maximal increase in enzyme activity, whereas in the presence of 0.1 μM fluphenazine, approximately 150 μM dopamine was required for half-maximal activation of the enzyme. The maximal stimulation of enzyme activity was the same in the presence as in the absence of fluphenazine. A double-reciprocal plot of these data is shown in the inset of Fig. 2. The data of Fig. 2 indicate that fluphenazine competitively inhibits the stimulatory action of dopamine upon the adenylate cyclase. Based on competitive kinetics, the inhibition constant, \( K_i \), of fluphenazine for the "dopamine receptor" of the adenylate cyclase was calculated to be 0.005 μM.

Since the nucleus accumbens is smaller and less well defined, anatomically, than are either the olfactory tubercle or the caudate nucleus, it was felt preferable to isolate this nucleus from the guinea pig brain, which is considerably larger than the rat brain. The effect of 40 μM dopamine was tested on the adenylate cyclase activity of a preparation of nucleus accumbens both in the presence and in the absence of 0.5 μM fluphenazine (Table 1). Low concentrations of dopamine were able to stimulate the adenylate cyclase activity of the nucleus accumbens homogenate; the stimulation by dopamine, representing approximately a 50% increase above basal activity, was less than the increase observed in either the olfactory tubercle or the caudate nucleus. This smaller stimulation of the enzyme from the nucleus accumbens may conceivably have resulted from contamination of this preparation by tissue from other (nondopaminergic) regions of the brain. Nonetheless, the increase above basal activity, seen in the presence of dopamine, was highly significant (i.e., \( P < 0.001 \)). Fluphenazine, 0.5 μM, abolished the stimulation of the nucleus accumbens enzyme by 40 μM dopamine, whereas this concentration of fluphenazine did not affect the basal enzyme activity.

With homogenates of the rat caudate nucleus, a half-maximal increase in enzyme activity was achieved, in various experiments, with 3.5–10 μM dopamine. The effect of fluphenazine was also studied on the dopamine-sensitive adenylate cyclase activity of the caudate nucleus (Fig. 3). Fluphenazine competitively antagonized the stimulatory effect of dopamine on the adenylate cyclase of the caudate nucleus. The calculated inhibition constant (\( K_i \)) of fluphenazine for the caudate enzyme was 0.008 μM. The potent and competitive nature of the action of fluphenazine as a dopamine antagonist on the caudate adenylate cyclase was similar to its action on the olfactory tubercle enzyme.

### Table 1. Effect of dopamine and fluphenazine on the adenylate cyclase activity in a homogenate of guinea pig nucleus accumbens

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cyclic AMP formed (pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.7 ± 0.12</td>
</tr>
<tr>
<td>40 μM dopamine</td>
<td>29.3 ± 0.08*</td>
</tr>
<tr>
<td>40 μM dopamine + 0.5 μM fluphenazine</td>
<td>20.9 ± 0.07</td>
</tr>
<tr>
<td>0.5 μM fluphenazine</td>
<td>19.3 ± 0.13</td>
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Data are expressed as mean value ± standard deviation for duplicate determinations of ten replicate samples.

* \( P < 0.001 \) relative to all other conditions.

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**Fig. 3.** Effect of various concentrations of dopamine, alone (○) or in combination with 0.1 μM fluphenazine (●), on adenylate cyclase activity in a homogenate of rat caudate nucleus. Standard conditions were used for measurement of adenylate cyclase activity except that 0.05 ml of a 1–25 tissue homogenate was used. In the absence of added dopamine and fluphenazine, 46.0 ± 2.0 pmol (mean ± SEM, \( n = 6 \)) of cyclic AMP was formed; in the presence of 0.1 μM fluphenazine (but without added dopamine), 45.9 ± 2.0 pmol (mean ± SEM, \( n = 6 \)) of cyclic AMP was formed. The increase in cyclic AMP above the basal level (i.e., the level in the absence of both dopamine and fluphenazine) is plotted as a function of dopamine concentration. The data give mean values and ranges for duplicate determinations on each of three replicate samples. Inset: Double-reciprocal plot of cyclic AMP increase as a function of dopamine concentration from 3 μM to 300 μM. (A) Control; (B) \( 1 \times 10^{-7} \) M fluphenazine.

In experiments similar to those illustrated for fluphenazine in Figs. 2 and 3, it was found that three other phenothiazines (triluoperazine, thioridazine, and chlorpromazine), a butyrophenone (haloperidol), and a dibenzodiazepine (clozapine), also inhibited the stimulation by dopamine of adenylate cyclase activity in homogenates from the caudate nucleus and from the olfactory tubercle of the rat. The inhibition followed competitive kinetics in all cases. The magnitude of the shift in the dose-response curve for dopamine, and the \( K_i \) calculated from this shift are tabulated in Table 2A for all six antipsychotic agents tested in this manner.

Many substances were also tested, over a range of concentrations, for their ability to inhibit the stimulation, by a constant amount of dopamine, of the adenylate cyclase activity in homogenates of the caudate nucleus and olfactory tubercle. Data obtained in this type of experiment are illustrated in Fig. 4 for fluphenazine, chlorpromazine, imipramine, and diethazine. The \( I_{50} \) for each compound tested in this manner, together with the \( K_i \) value calculated from the \( I_{50} \) on the basis of competitive kinetics, are tabulated in Table 2B.
stances where a given substance was tested in both types of kinetic study, the $K_i$ value was in fairly good agreement. Moreover, for each of several substances tested, the $K_i$ value for the enzyme from the olfactory tubercle was similar to that for the enzyme from the caudate nucleus, even though the various compounds tested varied greatly in their potency. There was greater than a 1000-fold range in the potency of the phenothiazines as antagonists of the stimulation by dopamine of caudate adenylate cyclase activity. Interestingly, fluphenazine and trifluoperazine, which are especially potent clinically both as antipsychotic agents and in causing extrapyramidal side effects, had very low $K_i$ values. Conversely, promethazine, imipramine, desmethyldiethazine, ethypromazine, and diethazine, which have little or no antipsychotic or extrapyramidal actions clinically, had relatively high $K_i$ values, even though these compounds are closely related structurally to the psychoactive phenothiazines. Not surprisingly, there are a few discrepancies between the results of various test substances observed in our enzyme system with results of clinical trials and laboratory studies in vivo. For instance, thoridazine (14), and especially clozapine (15), at therapeutically effective levels, are reputed to have a low incidence of extrapyramidal side effects. Nevertheless, these substances were approximately as potent on the enzyme from the caudate nucleus as they were on the olfactory tubercle enzyme. Our results suggest that the low incidence of extrapyramidal side effects may be attributed to some property of these compounds other than their ability to act as dopamine antagonists. In fact, Andén and Stock (8) have suggested that the ability of clozapine to block muscarinic receptors is responsible for its low incidence of extrapyramidal side effects. Another discrepancy between our enzymological results and the results of studies in vivo is the relatively low potency of haloperidol and pimozide as inhibitors of dopamine-stimulated enzyme activity, and the high potency of these compounds in vivo (16, 17). However, the low aqueous solubility of these substances where a given substance was tested in both types of kinetic study, the $K_i$ value was in fairly good agreement. Moreover, for each of several substances tested, the $K_i$ value for the enzyme from the olfactory tubercle was similar to that for the enzyme from the caudate nucleus, even though the various compounds tested varied greatly in their potency. There was greater than a 1000-fold range in the potency of the phenothiazines as antagonists of the stimulation by dopamine of caudate adenylate cyclase activity. Interestingly, fluphenazine and trifluoperazine, which are especially potent clinically both as antipsychotic agents and in causing extrapyramidal side effects, had very low $K_i$ values. Conversely, promethazine, imipramine, desmethyldiethazine, ethypromazine, and diethazine, which have little or no antipsychotic or extrapyramidal actions clinically, had relatively high $K_i$ values, even though these compounds are closely related structurally to the psychoactive phenothiazines. Not surprisingly, there are a few discrepancies between the results of various test substances observed in our enzyme system with results of clinical trials and laboratory studies in vivo. For instance, thoridazine (14), and especially clozapine (15), at therapeutically effective levels, are reputed to have a low incidence of extrapyramidal side effects. Nevertheless, these substances were approximately as potent on the enzyme from the caudate nucleus as they were on the olfactory tubercle enzyme. Our results suggest that the low incidence of extrapyramidal side effects may be attributed to some property of these compounds other than their ability to act as dopamine antagonists. In fact, Andén and Stock (8) have suggested that the ability of clozapine to block muscarinic receptors is responsible for its low incidence of extrapyramidal side effects. Another discrepancy between our enzymological results and the results of studies in vivo is the relatively low potency of haloperidol and pimozide as inhibitors of dopamine-stimulated enzyme activity, and the high potency of these compounds in vivo (16, 17). However, the low aqueous solubility of these

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two compounds may have contributed to their low potency in vitro. Moreover, problems of drug absorption, distribution, metabolism, and excretion greatly complicate the task of attempting to correlate results of studies in vivo with results obtained on putative receptors in cell-free systems.

Amantadine and d,l-amphetamine, two substances believed to achieve their clinical actions in part by affecting the release and reuptake of dopamine at presynaptic dopaminergic nerve terminals, were studied for possible effects on adenylate cyclase activity in homogenates of the rat caudate nucleus. Neither compound, in concentrations up to 1000 \( \mu \text{M} \), affected enzyme activity, either in the absence or presence of 40 \( \mu \text{M} \) dopamine.

Adenylate cyclase activity that could be stimulated by dopamine was also found in the olfactory tuberole of the guinea pig, golden hamster, and mouse (data not shown), as well as in the caudate nucleus from each of several mammalian species examined, including man (Table 3). Moreover, under experimental conditions the same as those of Table 2B, the adenylate cyclase activity of the enzyme from human caudate nucleus was inhibited, in decreasing order of potency, by fluphenazine \( (K_i = 0.033 \ \mu \text{M}) \), thioridazine \( (K_i = 0.11 \ \mu \text{M}) \), chlorpromazine \( (K_i = 0.44 \ \mu \text{M}) \), and pimozide \( (K_i = 1.11 \ \mu \text{M}) \).

**DISCUSSION**

The evidence presented here that dopamine-sensitive adenylate cyclase activity occurs in the olfactory tuberole, nucleus accumbens, and caudate nucleus of mammalian brain, that antipsychotic drugs are highly potent antagonists of this enzyme, and that clinically inactive compounds structurally related to the psychoactive phenothiazines are relatively weak antagonists of this enzyme, are compatible with several ideas: (a) that the dopamine receptor in these regions of the brain may be intimately associated with a dopamine-sensitive adenylate cyclase; (b) that the physiological effects of dopamine in these regions may be mediated through cyclic AMP; (c) that the extrapyramidal side effects of the antipsychotic drugs may be related to their ability to block the stimulation by dopamine of adenylate cyclase activity in the caudate nucleus; and (d) that the therapeutic effects of the antipsychotic drugs may be related to a similar action on a dopamine-sensitive adenylate cyclase in the limbic system (although dopamine-sensitive adenylate cyclase in another region could, conceivably, be the site of the therapeutic action of these drugs).

It has been suggested (18) that the endocrinological side effects of the antipsychotic drugs may result from a blockade of dopamine receptors in the median eminence, another region of the brain known to receive dopaminergic innervation. On the basis of our experiments, one might predict that dopamine-sensitive adenylate cyclase also occurs in those other regions of the brain receiving dopaminergic innervation, and that the antipsychotic drugs will function in those regions, as in the caudate nucleus, the olfactory tuberole, and the nucleus accumbens, by antagonizing dopamine stimulation of adenylate cyclase activity.

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