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ADENYLATE CYCLASE FROM VARIOUS DOPAMINERGIC AREAS OF THE BRAIN AND THE ACTION OF ANTIPSYCHOTIC DRUGS

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Abstract—The present report is a comparative study of adenylate cyclase activity in various areas of the brain identified as dopaminergic. Low levels of dopamine were found to stimulate adenylate cyclase from the striatum, median eminence, olfactory tubercle, nucleus accumbens and amygdala. Apomorphine, known to mimic the pharmacological and physiological effects of dopamine, stimulated adenylate cyclase from the median eminence. Several different classes of drugs effective in the treatment of schizophrenia were potent inhibitors of the stimulation by dopamine of the enzyme from these various regions. The drugs studied included representatives of the phenothiazine, butyrophenone, dibenzodiazepine and dibenzoxazepine classes. The inhibition by the dibenzoxazepine, loxapine, which is structurally very similar to the dibenzodiazepine, clozapine, was competitive with respect to dopamine. The calculated inhibition constant (K_i) for loxapine of about 15 nM was similar to that observed for some of the more potent phenothiazines. The results, considered together with previously published data, support the possibility that the therapeutic effects, as well as the extrapyramidal and endocrinological side effects, of these antipsychotic agents may be attributable to their ability to block the activation of adenylate cyclase in various select areas of the brain.

It is well known that many antipsychotic drugs produce an extrapyramidal syndrome indistinguishable from Parkinson's disease [1, 2]. These extrapyramidal side effects may arise from the ability of these drugs to block the dopamine receptor of the caudate nucleus [3, 4]. Many investigators have indirectly characterized the dopamine receptor in physiological, pharmacological and biochemical studies. Most recently, a dopamine-sensitive adenylate cyclase was demonstrated in homogenates of the caudate nucleus, olfactory tubercle and nucleus accumbens [5–9]. These studies have suggested that an intimate association exists between this dopamine-sensitive adenylate cyclase and the "dopamine receptor" of these areas, since the biochemical and pharmacological properties of this enzyme were similar to the reported properties of the dopamine receptor.

Dopamine has been implicated as a neurotransmitter in several other regions of the mammalian central nervous system in addition to those areas mentioned above. Recently, the amygdala, cerebral cortex and median eminence have been identified as regions receiving dopaminergic innervation [10–14]. Previous studies have supported the correlation between dopaminergic innervation of the limbic system and the extrapyramidal motor system and the occurrence of dopamine-sensitive adenylate cyclase in these two areas [6]. Thus, it was of interest to verify the presence of a dopamine-sensitive adenylate cyclase in the amygdala and median eminence.

It has been proposed 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 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 [6]. Furthermore, it has been suggested that the endocrinological side effects of the antipsychotic drugs may result from a blockade of dopamine receptors in the median eminence [15]. The results presented in this paper demonstrate that a dopamine-sensitive adenylate cyclase does occur in the median eminence and the amygdala and that antipsychotic drugs are capable of inhibiting dopamine stimulation of adenylate cyclase from these areas.

MATERIALS AND METHODS

ATP, cyclic AMP, and EGTA* were purchased from Sigma, and 3-hydroxytyramine (dopamine) was from CalBiochem; inorganic salts were all reagent grade; loxapine was a gift from Lederle Laboratories. 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 putament [16]), olfactory tubercle and nucleus accumbens has been described [6]. The median eminence and amygdala were dissected according to the guidelines in König and Knippel [16] for the rat brain. For the dissection of the median eminence, the ventral surface of the brain was exposed under a dissecting microscope. An iris knife was laid flat on the upturned, slightly parted

* Abbreviations used are: EGTA, ethylene glycol-bis-(β-amino ethylether)-*N*,*N*′-tetra-acetic acid; and cyclic AMP, 3′,5′-adenosine monophosphate.

basal surface of the hypothalamus and pressed down. The median eminence which protrudes the plane of the dissecting instrument was then carefully separated from the rest of the hypothalamus.

After the tissues were obtained, they were pooled and were homogenized in 50 vol. (weight to volume) of 2 mM Tris-(hydroxymethyl) aminomethane-maleate buffer (pH 7.4)-2 mM EGTA. The standard assay system (final volume 0.25 ml for the median eminence and 0.5 ml for all other areas) contained (in m-moles/liter): Tris-(hydroxymethyl) aminomethane-maleate, 80.2; ATP, 0.3; $MgSO_4$, 1.2; theophylline, 10; EGTA, 0.6 (including the amount introduced with the tissue homogenate); tissue homogenate (0.025 ml for the median eminence and 0.050 ml for other areas); plus test substances as indicated. Incubation was for 2.5 min for all areas except the median eminence which was for 5 min at 30°. The reaction was terminated by boiling, and cyclic AMP was measured as described [5]. Under the experimental conditions used, enzyme activity was proportional to time and enzyme concentration. Protein was determined essentially by the method of Lowry *et al* [17].

RESULTS

The effects of various concentrations of dopamine, norepinephrine and *l*-isoproterenol, on the adenylate cyclase of a homogenate of the median eminence is shown in Fig. 1. Adenylate cyclase activity was stimulated by low concentrations of dopamine; a half-maximal increase in enzyme activity was observed with 5 μM dopamine. In contrast, the β-adrenergic agonist *l*-isoproterenol had no significant effect on adenylate cyclase activity with concentrations as high as 1000 μM. L-Norepinephrine stimulated adenylate cyclase activity of the median eminence to the same maximal level as did dopamine. Greater concentrations of *l*-norepinephrine than of dopamine were required to achieve a given increase in enzyme activity. Half-maximal stimulation of the enzyme was obtained with 30 μM *l*-norepinephrine and maximal stimulation was obtained with 300 μM *l*-norepinephrine. Similar to results observed in the olfactory tubercle and caudate nucleus, the increase in enzyme activity in the presence of a combination of dopamine and *l*-norepinephrine, each at a concentration causing maximal enzyme stimulation, was no greater than with either agent alone. This suggests that *l*-norepinephrine interacts with the same receptor as does dopamine.

Although low concentrations of dopamine were able to stimulate the adenylate cyclase activity of the median eminence homogenate, the stimulation by dopamine represented an approximate 70 per cent increase above basal activity. This was less than the increase observed in either the olfactory tubercle or caudate nucleus but similar to that observed for the nucleus accumbens. It is conceivable that this smaller stimulation of the enzyme from the median eminence may be due to contamination of this preparation by non-dopaminergic regions surrounding the median eminence.

The effect of apomorphine, a dopamine agonist, is shown in Table 1. Low concentrations of apomorphine were found to stimulate the median eminence adenylate cyclase.

Fluphenazine, one of the most potent phenothiazine compounds, both as an antipsychotic agent as well as in producing extrapyramidal side effects in patients, has been shown to be a potent competitive antagonist of dopamine-sensitive adenylate cyclase in the olfactory tubercle and caudate nucleus [6]. It was of interest to test fluphenazine in the median

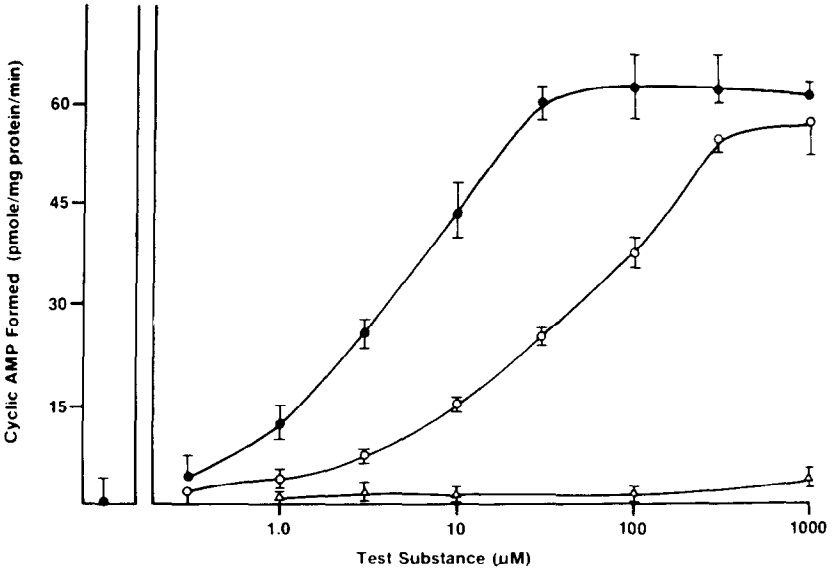

Fig. 1. Effect of catecholamines on adenylate cyclase activity in a homogenate of rat median eminence. Standard conditions were used for the measurement of adenylate cyclase activity. In the absence of added catecholamine, 78.4 ± 0.9 pmoles/mg of protein/min of cyclic AMP was formed. The increase in cyclic AMP above this basal level is plotted as a function of catecholamine concentration. The data give the mean values and ranges for duplicate determinations on each of three replicate samples. Key: (●) dopamine; (○) norepinephrine; and (△) isoproterenol.

Table 1. Effect of apomorphine on adenylate cyclase activity in a homogenate of the rat median eminence*

Addition		Enzyme activity (pmoles/mg/min)
None		89.4 ± 1.0
Dopamine	(40.0 μM)	135.7 ± 2.1†
Apomorphine	(1.0 μM)	92.4 ± 0.5
Apomorphine	(3.0 μM)	110.0 ± 1.0
Apomorphine	(10.0 μM)	125.1 ± 1.8†
Apomorphine	(30.0 μM)	100.3 ± 2.4

* Data are expressed as the mean ± S. E. M.; N = 4.
† $P < 0.001$, relative to control.

eminence in addition to the dibenzodiazepine, clozapine, a potent antipsychotic with low extrapyramidal side effects, and loxapine, a dibenzoxazepine whose chemical structure is very similar to clozapine. Table 2 shows the calculated inhibition constants (K_i) for these agents on several dopaminergic areas. In all areas studied, these agents inhibited dopamine stimulation of adenylate cyclase. Loxapine was found to be a rather potent inhibitor of adenylate cyclase in both the olfactory tubercle and the caudate nucleus. Further studies with loxapine in the olfactory tubercle (Fig. 2) and the striatum (data not shown) indicate that this compound is a competitive inhibitor of adenylate cyclase activity.

DISCUSSION

The ability of dopamine to stimulate adenylate cyclase in the median eminence may be especially important from the standpoint of neuroendocrinology. The distribution of dopamine within the median eminence correlates well with the distribution of gonadotropin releasing hormone (LHRH) [19], and dopamine has long been known to be capable of facilitating LH release through an effect that has been localized to the region of the median eminence [20]. Since most of the LHRH in this region is thought to be located in tanycytes [21], and since cyclic AMP is known to stimulate the release of hormones from other cells, such as those of the pancreas [22] and anterior pituitary gland [23], it is conceivable that dopamine facilitates the release of LHRH by stimulating adenylate cyclase in tanycytes. The implied hypothesis that tanycytes represent a significant source of the dopamine-sensitive adenylate cyclase measured in these experiments could perhaps be tested by the application

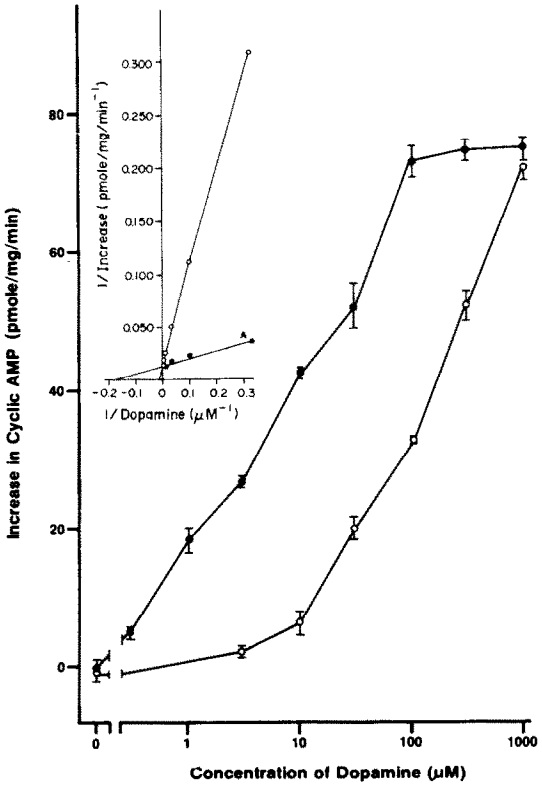

Fig. 2. Effect of various concentrations of dopamine, alone (●) or in combination with 0.25 μM loxapine (○), on adenylate cyclase activity in a homogenate of the olfactory tubercle of the rat. In the absence of added dopamine and loxapine, 70.0 ± 1.8 pmoles cyclic AMP/mg of protein/min was formed. The increase in cyclic AMP above basal level (i.e. the level in the absence of both dopamine and loxapine) is plotted as a function of dopamine concentration. Inset: Double reciprocal plot of cyclic AMP increase as a function of dopamine concentration from 3 to 300 μM. (A, ●) control; (○) 2.5×10^{-7} M loxapine.

Table 2. Calculated inhibition constants (K_i) from several dopaminergic areas of the brain for representatives of the phenothiazine, butyrophenone, dibenzodiazepine and dibenzoxazepine classes

Source of enzyme	Enzyme activity (pmoles/mg/min) Control	+ Dopamine (40 μM)	K_i* (nM) Chlorpromazine	Fluphenazine	Clozapine	Loxapine
Caudate nucleus	105.1	280.0	90	8.0	60	15
Olfactory tubercle	50.9	109.8	60	7.0	60	14
Nucleus accumbens	80.0	120.2	75†	7.5†	59	10
Median eminence	83.2	125.6	70†	7.5	61†	13
Amygdala	103.8	180.6	80			

* The K_i value was calculated from the relationship $K'_m/K_m = 1 + I/K$, where K'_m and K_m are the concentrations of dopamine required to give half-maximal activation of the enzyme, in the presence and absence of test substance, respectively, and I is the concentration of the inhibitor except where indicated.

† Where daggers appear, the K_i value was calculated from the relationship $I_{50} = K_i\ (1 + S/K_m)$, where I_{50} is the concentration of drug required to give 50 per cent inhibition of the enzyme activity, and S is the concentration (40 μM) of dopamine [5, 18].

of immunocytochemical procedures [24], and such studies are currently being planned.

The results herein also show that the dibenzoxazepine, loxapine, is a potent inhibitor of adenylate cyclase in various dopaminergic areas of the brain. The demonstration that loxapine is a potent competitive inhibitor of adenylate cyclase in the olfactory tubercle suggests that it would be a good antipsychotic agent. However, the fact that it is also a potent inhibitor of the enzyme in the caudate and median eminence suggests that its high extrapyramidal side effects may be due to its ability to antagonize the stimulation by dopamine of adenylate cyclase from these areas. 7-OH loxapine, a natural metabolite of loxapine, has recently been shown to be a potent inhibitor of the caudate enzyme [25]. Based on these experiments, we would predict that this metabolite which is thought to potentially be a good antipsychotic agent would also be a potent inhibitor of the dopamine stimulation of adenylate cyclase from the median eminence and caudate nucleus.

Finally, the evidence that a dopamine-sensitive adenylate cyclase occurs in the median eminence and that antipsychotic drugs are potent antagonists of this enzyme is compatible with the idea that the endocrinological side effects of the antipsychotic drugs may result from a blockade of dopamine receptors in this area.

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