

## Dopamine-Sensitive Adenylate Cyclase in Caudate Nucleus of Rat Brain, and Its Similarity to the "Dopamine Receptor"

(extrapyramidal disease/ganglia/neurotransmitter/dihydroxyphenylalanine/Parkinson's disease)

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**ABSTRACT** An adenylate cyclase that is activated specifically by low concentrations of dopamine has been demonstrated in homogenates of caudate nucleus of rat brain. A half-maximal increase in the activity of the enzyme occurred in the presence of 4  $\mu$ M dopamine. Concentrations of dopamine as low as 0.3  $\mu$ M stimulated the activity of the enzyme. The adenylate cyclase activity of the homogenates was also stimulated by low concentrations of apomorphine, a substance known to mimic the physiological and pharmacological effects of dopamine. The stimulatory effect of dopamine was blocked by low concentrations of either haloperidol or chlorpromazine, agents known to block the actions of dopamine in mammalian brain. The results suggest that dopamine-sensitive adenylate cyclase may be the receptor for dopamine in mammalian brain. The isolation of this enzyme from caudate nucleus should facilitate the search for new therapeutic agents useful in the treatment of extrapyramidal diseases.

Recent work has implicated dopamine as a neurotransmitter in the basal ganglia of the mammalian brain (1-4). In addition, a variety of behavioral and pharmacological evidence supports the concept of a "dopamine receptor" within the basal ganglia (5-7). Moreover, evidence has accumulated that Parkinsonism can result either from depletion of the dopamine in the basal ganglia or from blockade of the "dopamine receptor" (1, 3). It would, therefore, seem of considerable importance to identify the biochemical receptor with which dopamine interacts in the basal ganglia.

Recent studies on the role of adenosine 3':5'-cyclic monophosphate (cyclic AMP) in ganglia of the peripheral sympathetic nervous system have led to a partial clarification of the role and mechanism of action of dopamine within these peripheral ganglia. An adenylate cyclase was demonstrated in these ganglia that was specifically stimulated by very low concentrations of dopamine (8). The demonstration of this enzyme in these ganglia, together with other evidence presented elsewhere (9-12), led to the suggestion that cyclic AMP mediates dopaminergic transmission, and thereby modulates cholinergic transmission within these ganglia. More recently, a dopamine-sensitive adenylate cyclase has been reported by Brown and Makman (13) in mammalian retina, where dopaminergic amacrine cells occur. It seemed possible, particularly in view of the known balance and interaction of cholinergic and dopaminergic mechanisms in the extrapyramidal system, that a dopamine-sensitive adenylate cyclase might mediate dopaminergic transmission in the basal

ganglia, with consequent modulation of cholinergic transmission. Therefore, we undertook a search for such an enzyme in the caudate nucleus. We report the results of these studies, which demonstrate the occurrence of this enzyme, and describe some of its pharmacological properties. The similarities between the pharmacological properties of this enzyme and those of the "dopamine receptor" suggest a close relationship between these two entities.

### METHODS

Male Sprague-Dawley rats, weighing about 200 g, were killed by decapitation. The brain was rapidly excised in a cold room (4°) and placed in cold Krebs-Ringer bicarbonate buffer that contained (in mmol/liter): NaCl, 122; KCl, 3; MgSO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 1.3; KH<sub>2</sub>PO<sub>4</sub>, 0.4; NaHCO<sub>3</sub>, 25; and D-glucose, 10. This buffer had previously been equilibrated with a gas mixture of 95% O<sub>2</sub>-5% CO<sub>2</sub>, and had a pH of 7.4 at 25°. The brainstem and cerebellum were removed, and the brain was hemisected along the midline. The lateral ventricle of one cerebral hemisphere was opened, with a medial incision superior to the corpus callosum. The caudate nucleus was separated from the internal capsule and was removed. This procedure was then repeated on the contralateral hemisphere. The caudate nuclei were pooled and were homogenized in about 25 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 activity of homogenates contained (in mmol/liter): tris(hydroxymethyl)aminomethane-maleate 80; ATP, 0.5; MgSO<sub>4</sub>, 2.0; theophylline, 10; EGTA, 0.2; plus test substances as indicated. The reaction was initiated by addition of ATP. Incubation was for 2.5 min in a shaken water bath at 30°. The reaction was terminated by placing the assay tubes in a boiling-water bath for 2 min, followed by centrifugation at low speed to remove insoluble material. The amount of cyclic AMP formed in each assay tube was measured on duplicate 50- $\mu$ l aliquots by the method of Brown *et al.* (14, 15). The amount of cyclic AMP present in each aliquot was calculated from a linear standard curve, constructed with from 0.25 to 8.0 pmol of authentic cyclic AMP. Data represent total cyclic AMP formation per assay tube. Under the experimental conditions used, enzyme activity was proportional to time and enzyme concentration.

### RESULTS

The effect of various concentrations of the catecholamines, dopamine, *l*-norepinephrine, and *l*-isoproterenol on adenylate

Abbreviation: EGTA, Ethylene glycol-bis-( $\beta$ -aminoethylether)-*N,N'*-tetraacetic acid.

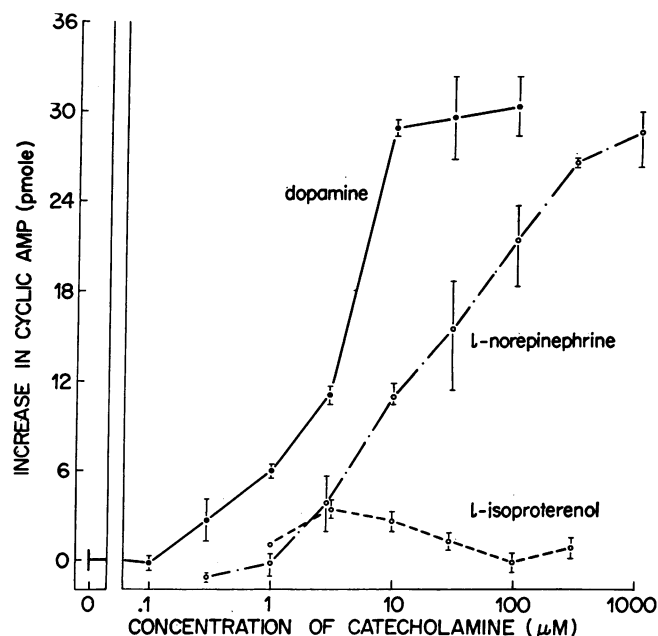


FIG. 1. Effect of catecholamines on adenylate cyclase activity in a homogenate of rat caudate nucleus. In the absence of added catecholamine,  $27.1 \pm 1.0$  pmol (mean  $\pm$  SEM,  $n = 6$ ) of cyclic AMP was formed. The increase in cyclic AMP above this basal level is plotted as a function of catecholamine concentration. The data give mean values and ranges for duplicate determinations on each of two to five replicate samples.

cyclase activity of a homogenate of the caudate nucleus is shown in Fig. 1. Adenylate cyclase activity was stimulated by very low concentrations of dopamine. A half-maximal increase in enzyme activity was achieved with about  $4 \mu\text{M}$  dopamine, and a significant increase in activity was observed with a concentration of dopamine as low as  $0.3 \mu\text{M}$ . The

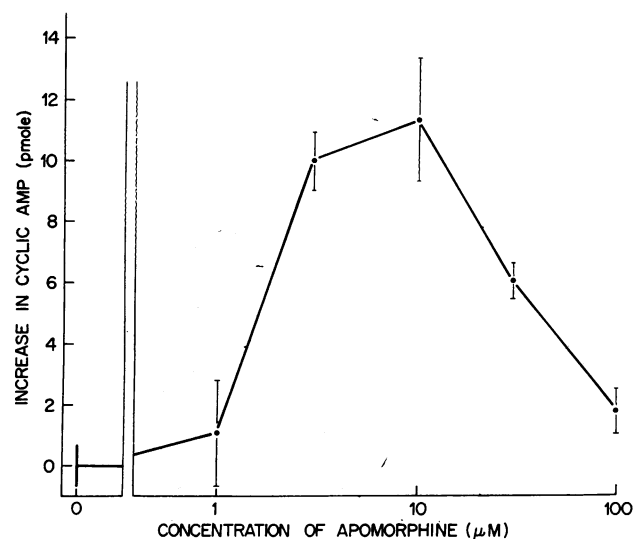


FIG. 2. Effect of apomorphine on adenylate cyclase activity in a homogenate of rat caudate nucleus. In the absence of added apomorphine,  $26.5 \pm 0.7$  pmol (mean  $\pm$  SEM,  $n = 4$ ) of cyclic AMP was formed. The increase in cyclic AMP above this basal level is plotted as a function of apomorphine concentration. The data give mean values and ranges for duplicate determinations on each of two to four replicate samples.

maximal stimulation of adenylate cyclase activity achieved by *l*-norepinephrine was equal to that observed with dopamine. However, considerably higher concentrations of norepinephrine than of dopamine were required to stimulate the enzyme. For example, a concentration of about  $28 \mu\text{M}$  *l*-norepinephrine was required to give a half-maximal stimulation of the adenylate cyclase activity. The  $\beta$ -adrenergic agonist, *l*-isoproterenol, had no significant effect on adenylate cyclase activity at concentrations as high as  $300 \mu\text{M}$ .

In control experiments, we found that the effect of dopamine on cyclic AMP accumulation in homogenates of the caudate nucleus was not due to an inhibition of phosphodiesterase activity. In those experiments, phosphodiesterase activity was assayed under the same conditions used for the adenylate cyclase assay, except that ATP was replaced by  $0.01$ – $2.0 \mu\text{M}$  cyclic AMP, and theophylline was, in some cases, omitted. We found that  $25 \mu\text{M}$  dopamine had no effect on the phosphodiesterase activity of the homogenate measured in the absence of theophylline. Moreover, in the presence of  $10 \text{ mM}$  theophylline there was less than 10% disappearance of cyclic AMP, either in the absence or presence of  $25 \mu\text{M}$  dopamine, providing further evidence against the possibility that the action of dopamine was mediated through an inhibition of phosphodiesterase activity.

Apomorphine, an agent that mimics the actions of dopamine in the caudate nucleus (6, 16), was examined for possible effects on adenylate cyclase activity. Apomorphine, in low concentrations, caused an increase in enzyme activity, with the maximal stimulatory effect occurring at concentrations between  $3$  and  $10 \mu\text{M}$  (Fig. 2). Concentrations of apomorphine higher than  $100 \mu\text{M}$  inhibited the basal activity.

The effect on adenylate cyclase activity of combinations of dopamine, *l*-norepinephrine, and apomorphine was examined. The activity of the enzyme in the presence of combinations of these stimulatory agents did not exceed that observed with optimal concentrations of the individual stimulatory agents (Table 1). These results suggest that dopamine, *l*-norepinephrine, and apomorphine activate the same adenylate cyclase molecules.

We have studied the effects of haloperidol and chlorpromazine, agents that antagonize the actions of dopamine and of norepinephrine in the caudate nucleus (5–7), on adenylate cyclase activity. Each of these antagonists was able to block completely the increase in adenylate cyclase activity caused

TABLE 1. Effect of dopamine, norepinephrine, and apomorphine, alone and in combination, on adenylate cyclase activity in a homogenate of rat caudate nucleus

Addition	Cyclic AMP formed (pmol)
None	28.0 (26.0–29.6)
$40 \mu\text{M}$ Dopamine	55.3 (53.0–57.0)
$300 \mu\text{M}$ <i>l</i> -Norepinephrine	54.0 (47.8–57.5)
$10 \mu\text{M}$ Apomorphine	42.9 (41.9–44.8)
$40 \mu\text{M}$ Dopamine + $300 \mu\text{M}$ <i>l</i> -norepinephrine	57.3 (56.6–57.6)
$40 \mu\text{M}$ Dopamine + $10 \mu\text{M}$ apomorphine	46.5 (41.3–50.1)
$300 \mu\text{M}$ <i>l</i> -Norepinephrine + $10 \mu\text{M}$ apomorphine	46.3 (39.7–50.4)

The data give the mean values and ranges for duplicate determinations on each of triplicate samples.

by the addition of dopamine. The increase in enzyme activity caused by 40  $\mu\text{M}$  dopamine was reduced 50% in the presence of either 2  $\mu\text{M}$  haloperidol (Fig. 3) or 1  $\mu\text{M}$  chlorpromazine (Fig. 4). Haloperidol had little effect on the level of enzyme activity in the absence of added dopamine; chlorpromazine inhibited the basal enzyme activity. In experiments in which 4  $\mu\text{M}$  dopamine, a concentration causing half-maximal activation of the adenylate cyclase, was used, the increase in enzyme activity due to dopamine was reduced 50% in the presence of 0.1  $\mu\text{M}$  haloperidol. We have also examined the effects of haloperidol and chlorpromazine on adenylate cyclase activity in the presence of *l*-norepinephrine. The increase in enzyme activity caused by 30  $\mu\text{M}$  *l*-norepinephrine was reduced 50% by each of these antagonists, at concentrations ranging, in various experiments, between 0.1 and 1.0  $\mu\text{M}$ .

We have examined the effect of promethazine on adenylate cyclase activity of homogenates of the caudate nucleus. This compound is closely related in structure to chlorpromazine, but does not antagonize the actions of dopamine in caudate nucleus (5). In contrast to the efficacy of chlorpromazine in blocking the increase in adenylate cyclase activity caused by addition of dopamine, promethazine, in concentrations up to 10  $\mu\text{M}$ , had no significant effect on the dopamine-mediated increase in enzyme activity.

The "dopamine receptor" of the caudate nucleus has been reported (17) to be weakly antagonized by  $\alpha$ -adrenergic blocking agents, but unaffected by  $\beta$ -adrenergic blocking agents. In addition, we have previously observed that  $\alpha$ -adrenergic blocking agents, but not  $\beta$ -adrenergic blocking agents, antagonized the actions of dopamine in stimulating cyclic AMP formation in bovine superior cervical ganglia (8). Therefore, we have investigated the effects of adrenergic blocking agents on the adenylate cyclase activity of homogenates of caudate nucleus. 80  $\mu\text{M}$  phentolamine, an  $\alpha$ -adrenergic blocking agent, reduced the stimulatory effects of dopamine and of *l*-norepinephrine by roughly 50% (Table 2). In contrast, the  $\beta$ -adrenergic antagonists propranolol (40  $\mu\text{M}$ ) and dichloroisoproterenol (40  $\mu\text{M}$ ) had no significant effect on either the resting or catecholamine-stimulated enzyme activity.

In some experiments, the cerebellum, which contains a much lower concentration of dopamine than does the caudate nucleus, was examined for dopamine-sensitive adenylate cyclase. The procedure used to study cerebellar adenylate

TABLE 2. Effect of the  $\alpha$ -adrenergic antagonist, phentolamine, on adenylate cyclase activity in a homogenate of rat caudate nucleus

Stimulatory agent	Cyclic AMP Increase	
	-Phentolamine (pmol)	+80 $\mu\text{M}$ Phentolamine (pmol)
None	25.3 (23.5-27.1)	21.9 (19.9-23.5)
4 $\mu\text{M}$ Dopamine	36.1 (33.0-38.0)	24.7 (23.6-25.3)
20 $\mu\text{M}$ Dopamine	46.8 (42.6-54.1)	31.7 (30.0-33.2)
30 $\mu\text{M}$ <i>l</i> -Norepinephrine	42.9 (39.8-47.1)	29.5 (27.1-31.8)
100 $\mu\text{M}$ <i>l</i> -Norepinephrine	45.0 (43.4-47.2)	31.8 (30.3-33.2)

The data are expressed as mean values and range for duplicate determinations on each of three to six replicate samples.

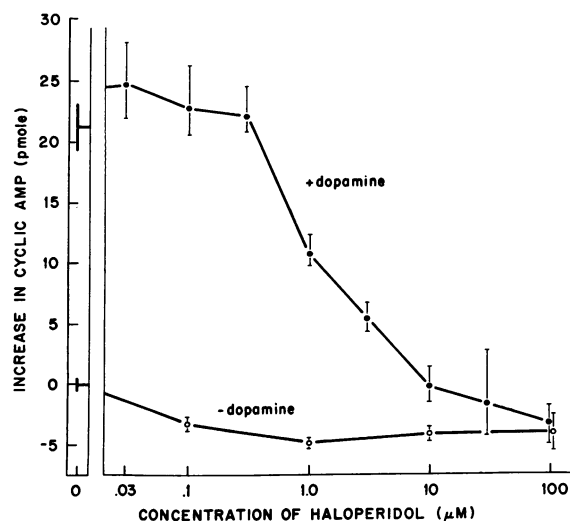


FIG. 3. Effect of haloperidol, in the absence and presence of 40  $\mu\text{M}$  dopamine, on adenylate cyclase activity in a homogenate of rat caudate nucleus. In the absence of added dopamine or haloperidol, 26.43  $\pm$  0.65 pmol (mean  $\pm$  SEM,  $n = 12$ ) of cyclic AMP was formed. Cyclic AMP accumulation is expressed as the increase above this basal level. The data give mean values and ranges for duplicate determinations on each of three samples.

cyclase activity was similar to that used to study the enzyme from caudate nucleus. Dopamine, at concentrations as high as 40  $\mu\text{M}$ , showed no significant effect on cerebellar adenylate cyclase activity. In comparison, the adenylate cyclase activity of a homogenate of caudate nuclei from the same rat was

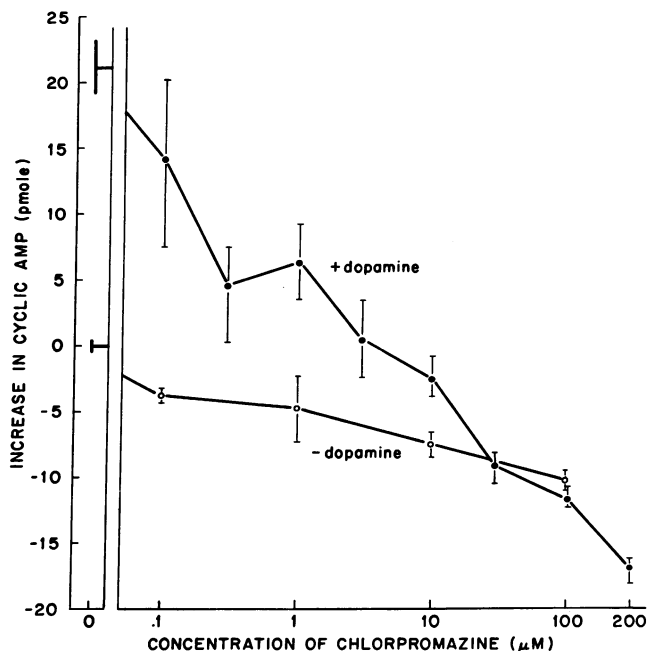


FIG. 4. Effect of chlorpromazine, in the absence and presence of 40  $\mu\text{M}$  dopamine, on adenylate cyclase activity in a homogenate of rat caudate nucleus. In the absence of added dopamine or chlorpromazine, 26.43  $\pm$  0.65 pmol (mean  $\pm$  SEM,  $n = 12$ ) of cyclic AMP was formed. Cyclic AMP accumulation is expressed as the increase in cyclic AMP above this basal level. The data give mean values and ranges for duplicate determinations on each of three samples.

stimulated 2.3-fold by 40  $\mu$ M dopamine. These observations suggest that the dopamine-mediated increase in adenylate cyclase activity of caudate nucleus was not the result of a procedural artifact.

### DISCUSSION

The present experiments have demonstrated the occurrence of an adenylate cyclase, sensitive to extremely low concentrations of dopamine, in homogenates of caudate nuclei of rats. The existence, *per se*, of this enzyme within the basal ganglia raises the possibility that the physiological effects of dopamine, which is naturally present in this region of the brain (4, 18, 19), may be mediated by cyclic AMP. Recent studies with superior cervical sympathetic ganglia of mammals may provide insight into the possible role and mechanism of action of cyclic AMP in the basal ganglia. In the superior cervical ganglion, where a dopamine-sensitive adenylate cyclase was first observed (8), a variety of experimental evidence suggests that cyclic AMP does, in fact, mediate dopaminergic synaptic transmission (8-12). Included among this evidence is the observation that dopamine can cause a hyperpolarization of the postganglionic neurons (12, 20, 21), an effect that exogenously applied cyclic AMP can mimic. In addition, the phosphodiesterase inhibitor, theophylline, can potentiate both the slow inhibitory postsynaptic potential, which is thought to be mediated by endogenous dopamine, and the hyperpolarization caused by exogenous dopamine (12). These and other observations (8-12) made on superior cervical ganglia lead one to the conclusion that the effects of dopamine on the electrophysiological parameters and on adenylate cyclase activity are causally related.

A comparison of certain characteristics of peripheral sympathetic ganglia with those of the basal ganglia reveals analogies between the two tissues that are relevant to the present discussion. Thus, the studies of DeGroat and Volle (22) on superior cervical ganglia of cats have shown that the hyperpolarizing effect of catecholamines, in this peripheral ganglion, specifically inhibits the depolarizing, excitatory, effects of muscarinic agents. Within the basal ganglia, a variety of experimental evidence (3, 23, 24) suggests that there is a similar balance between the inhibitory effects of dopamine, acting through the "dopamine receptor," and the excitatory effects of acetylcholine, acting via a muscarinic receptor. Experiments on the caudate nucleus, analogous to those done with the mammalian superior cervical ganglion (9, 12, 21, 22), should permit testing of the prediction that cyclic AMP acts as the intracellular mediator for the actions of dopamine within the caudate nucleus.

In studies of the superior cervical ganglion, dopamine not only stimulated adenylate cyclase activity in homogenates of ganglia, but also caused a 3- to 7-fold increase in the concentration of cyclic AMP in blocks of ganglionic tissue containing intact cells (8). Experiments are now in progress to determine whether it is similarly possible for dopamine, norepinephrine, and apomorphine to bring about the accumulation of cyclic AMP in preparations of caudate nucleus containing intact cells.

The caudate nucleus receives innervation from neurons located in many regions of the central nervous system. It is probable that most of these neurons utilize neurotransmitters other than dopamine. Thus, we can anticipate that some other putative neurotransmitters may also prove capable of increasing adenylate cyclase activity in homogenates of the

caudate nucleus. The observed basal activity may represent a summation of basal activities of individual types of adenylate cyclase, and this effect could account for the fact that dopamine caused only a 2-fold stimulation of activity in the present experiments.

The similarities between the "dopamine receptor," which has been characterized by others, and the dopamine-sensitive adenylate cyclase reported in this paper are consistent with the proposal that the effects of dopamine may be mediated by cyclic AMP. Low concentrations of either haloperidol or chlorpromazine antagonize the physiological and pharmacological effects of dopamine and norepinephrine within the caudate nucleus (5, 6, 7, 25), and also abolish the increased adenylate cyclase activity due to either of the catecholamines. Furthermore, the "dopamine receptor" has been reported to be weakly antagonized by  $\alpha$ -adrenergic blocking agents, but unaffected by  $\beta$ -adrenergic blocking agents (17). The dopamine-sensitive adenylate cyclase of the caudate nucleus is also weakly antagonized by  $\alpha$ -adrenergic, but not by  $\beta$ -adrenergic, blocking agents. Finally, the ability of apomorphine both to mimic the actions of dopamine upon the "dopamine receptor" of the caudate nucleus (7, 16) and to stimulate the adenylate cyclase activity of the caudate nucleus supports the idea that the dopamine-sensitive adenylate cyclase of the caudate nucleus is the "dopamine receptor" that has been characterized by others.

The existence of a dopamine-sensitive adenylate cyclase in the caudate nucleus should facilitate our understanding of the mechanism of action of dopamine within the basal ganglia. In addition, the fact that this enzyme retains its sensitivity to low concentrations of dopamine in a cell-free extract should make rapid progress possible in the search for new, clinically useful, agents that can mimic or antagonize the actions of dopamine in the central nervous system. Certainly, the results of our investigations suggest that theophylline, and other phosphodiesterase inhibitors, should be subjected to clinical trial alone, and in conjunction with L-dihydroxyphenylalanine (L-DOPA) therapy, for the treatment of Parkinsonism.

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