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Beta Adrenergic Receptor-Mediated Adenosine Cyclic 3',5'-Monophosphate Accumulation in the Rat Corpus Striatum

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SUMMARY

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After labeling with [^{14}C]adenine, the conversion of newly formed adenosine triphosphate to [^{14}C]adenosine cyclic 3',5'-monophosphate ([^{14}C]cAMP) was studied in both slices and crude mitochondrial fractions of the rat corpus striatum in the presence of a phosphodiesterase inhibitor. The observed order of potency for β-hydroxylated catecholamines in stimulating [^{14}C]cAMP accumulation in striatal slices was (−)-isoproterenol > (−)-epinephrine > (−)-norephinephrine, with EC_{50} values (concentration resulting in 50% of maximum stimulation) of 0.03, 0.7, and 3.0 μM, respectively. In comparison, non-β-hydroxylated catecholamines were much less potent, with *N*-isopropyldopamine > dopamine (EC_{50} = 60 μM); apomorphine was least active. Stereoselectivity was exhibited, with the (−) isomers of isoproterenol and norepinephrine being more potent than the racemic and (+) isomer, respectively. Over a wide range of concentrations, the response curves for (+)-norepinephrine and dopamine were similar. No "additive stimulatory effect" or significant enhancement of cAMP accumulation was demonstrated when dopamine was combined with maximum effective concentrations of norepinephrine or isoproterenol. *Beta* adrenergic antagonists, such as propranolol, MJ-1999 (sotalol), and alprenolol, were potent blockers of the dopamine-induced stimulation of cAMP formation, whereas the *alpha* adrenergic blocker phentolamine and the dopamine receptor antagonists chloropromazine and trifluoperazine were relatively inactive. Furthermore, low concentrations of propranolol displayed stereoselective inhibition, with the (−) isomer being the more potent antagonist of the stimulatory response to dopamine. A similar order of potency for catecholamines was exhibited in a crude mitochondrial preparation (P_2) of striatal homogenates: (±)-isoproterenol > (−)-norepinephrine > dopamine = (+)-norepinephrine. Dopamine did not augment the maximum response elicited by isoproterenol. Likewise, in striatal homogenates, the inhibitory potency of *beta* adrenergic antagonists contrasted markedly with the low blocking activity of trifluoperazine. Finally, in cerebral cortical slices and crude mitochondrial fractions from brain regions, including the cerebral cortex and hindbrain, which are sparsely innervated by dopaminergic terminals, a small but significant acceleration of cAMP accumulation was elicited by dopamine. Our findings in striatal slices and crude mitochondrial fractions containing synaptosomes suggest that the dopamine-induced accumulation of [^{14}C]cAMP may not be associated with a specific dopamine receptor-

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coupled adenylate cyclase but may involve a *beta* adrenergic receptor-linked cyclase which is weakly responsive to non-β-hydroxylated catecholamines, such as dopamine.

INTRODUCTION

Adenylate cyclase has been proposed to be associated with the *beta* adrenergic receptor in many tissues, including heart, liver, skeletal muscle, lung, fat, erythrocytes, pineal gland (1), and cerebellar cortex (2). Criteria utilized in identifying a *beta* adrenergic receptor-coupled adenylate cyclase system in both membrane and intact tissue preparations (1, 3), are as follows. (a) The order of potency of classical agonists in stimulating cAMP[1] synthesis should be isoproterenol > epinephrine > norepinephrine > isopropyldopamine > dopamine. (b) *Beta* adrenergic blockers, such as propranolol, should display great potency as antagonists of this activation of adenylate cyclase elicited by isoproterenol. (c) Adenylate cyclase should exhibit stereoselectivity with regard to agonists and antagonists, with the (−) isomers of these compounds being generally more active than the (+) isomers. Furthermore, it has been postulated that stimulation of cAMP synthesis may be the mechanism by which many, if not all, *beta* adrenergic effects are mediated in intact tissues (1). Accordingly, the relative potencies of agonists and antagonists in altering the activity of adenylate cyclase should correspond with their activities toward *beta* adrenoreceptor-mediated physiological responses in intact tissues, such as the rate and force of contractility of cardiac muscle, lipolysis in adipose tissues, and glycogenolysis in liver and skeletal muscle (1). Unfortunately, in the brain there is no obvious physiological response to correlate with adenylate cyclase activation, and thus evidence characterizing a specific catecholamine receptor which is associated with the production of cAMP in various brain regions has been derived by measuring relative potencies of agonists and antagonists on adenylate cyclase activity.

In the superior cervical ganglion (4), retina (5), and caudate nucleus (6), the dopamine-induced stimulation of cAMP formation has been postulated to involve an interaction of dopamine with specific dopamine receptors which are closely coupled to adenylate cyclase. The major problem in attempting to distinguish the dopamine receptor from the other *alpha* and *beta* adrenergic receptors has been the lack of specific agonists and antagonists of the dopamine receptor. Nevertheless, evidence supporting an intimate association of the dopamine receptor with the dopamine-sensitive adenylate cyclase in the caudate nucleus has been provided by observations in broken-cell preparations (6–10). Although those authors observed that maximum stimulatory concentrations of dopamine, apomorphine, or norepinephrine elicited only 2-fold rise in cAMP levels in caudate homogenates, dopamine did display greater potency than norepinephrine, while the *beta* adrenergic agonist isoproterenol was found to be inactive (6). Furthermore, the antipsychotic phenothiazines and butyrophenones, which reportedly block dopamine receptors, were the most potent agents in antagonizing the enhanced accumulation of cAMP elicited by dopamine, whereas the *beta* adrenergic blocker propranolol was without effect (6–9).

In this investigation, the hypothesis that adenylate cyclase is coupled to the dopamine receptor in the corpus striatum was further examined by employing two other types of experimental preparations. In the first series of experiments, the effects of adrenergic and dopaminergic agonists and antagonists were studied in brain slices prepared from the corpus striatum. The rationale for selecting a brain slice preparation was derived from the observations that higher concentrations of agonists were necessary to stimulate adenylate cyclase in membrane preparations than in intact tissues (11). To explain these findings, Robison *et al.* (11) suggested that a distortion of the normal receptor-cyclase coupling mechanism probably occurs in the preparation of cellular

[1] The abbreviation used is: cAMP, adenosine cyclic 3′,5′-monophosphate.

membranes. Thus, by providing a more intact tissue preparation, one might expect the adenylate cyclase to show greater sensitivity to the stimulatory effects of catecholamines in brain slices than in caudate homogenates. This study was also expanded to include a crude mitochondrial preparation containing synaptosomes, which possess high levels of neurotransmitters and the complete enzymatic machinery for synthesizing cAMP (12).

MATERIALS AND METHODS

cAMP accumulation in brain slices. Male Sprague–Dawley rats (175–200 g) were killed by decapitation, and the corpus striatum (including the caudate nucleus and putamen), cerebral cortex, or hindbrain (including the brain stem and cerebellar cortex) was dissected and sliced with a McIlwain tissue chopper into sections approximately 0.26 × 0.26 × 1.0 mm and weighed. Employing a labeling technique originally described by Shimizu *et al.* (13), the accumulation of [^{14}C]cAMP was measured according to the procedure of Perkins and Moore (14), with minor modifications. This method involved a 20-min preliminary incubation, in a Dubnoff shaker at 37° under an atmosphere of 95% O_2–5% CO_2, of striatal (12 mg/ml), cerebral cortical (120 mg/ml), or hindbrain (120 mg/ml) slices in 4 ml of low-calcium (0.76 mM) Krebs-Ringer-bicarbonate medium, buffered to pH 7.4, containing glucose (10 mM). The tissues were then washed twice by centrifugation at 600 × *g* for 30 sec, resuspended in 2 ml of fresh medium, and reincubated for 60 min with [8-^{14}C]adenine (18 μM, 1 μCi/ml; New England Nuclear), permitting maximum conversion to [^{14}C]ATP. The tissues were then washed three times with fresh medium by centrifugation and again incubated in 4 ml of Krebs medium containing ascorbic acid (0.6 mM) and a 1.0 mM concentration of the phosphodiesterase inhibitor 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724, solubilized in HCl and neutralized in Krebs medium to pH 7.4, kindly donated by Dr. W. Scott, Hoffmann-La Roche) with experimental agonists and/or antagonists. Catecholamines were made up in medium containing 0.02 mM EDTA and added in volumes of 25 μl. After 30 min, when stimulation of [^{14}C]cAMP accumulation was found to be maximal, the reaction was terminated by centrifugation at 4°, and tissues were homogenized in 5% trichloracetic acid containing carrier cAMP (Boehringer/Mannheim) and [^{3}H]cAMP (New England Nuclear) to correct for recovery. The labeled ATP and cAMP were first isolated by cation-exchange chromatography on Dowex 50W-X8 (H^+), and the cAMP fraction was further purified by passage through a column of neutral alumina oxide and subsequent barium-zinc precipitation. By paper (15) and thin-layer chromatography (16), ^{14}C-labeled ATP and cAMP were shown to be more than 95% pure, and the values of [^{14}C]cAMP were corrected for over-all recovery (approximately 60–70%) in each sample. Portions of the ATP and cAMP fractions were counted by liquid scintillation spectrometry. Results are expressed as percentage conversion of [^{14}C]ATP to [^{14}C]cAMP, i.e., (disintegrations per minute of cAMP × 100)/(disintegrations per minute of ATP + disintegrations per minute of cAMP).

To test the validity of utilizing this labeling technique for measuring cAMP formation in striatal slices, both [^{14}C]cAMP and total cAMP were determined in the same samples under standard assay conditions following incubation with various concentrations of (−)-norepinephrine. Half the sample of the Dowex column fraction of cAMP was employed to assay endogenous cAMP levels by the protein kinase stimulation procedure (17), and the other half was purified further on alumina columns to isolate [^{14}C]cAMP as described above. Over the whole concentration range of (−)-norepinephrine, the specific activity was found to be relatively constant and averaged 22.3 ± 1.7 cpm of [^{14}C]cAMP per picomole of cAMP. The percentage increases over nontreated, control levels of endogenous cAMP (6.05 ± 0.41 pmoles/mg of protein for six experiments) were 177% ± 15%, 289% ± 9%, and 387% ± 18% at 1.0 μM, 10 μM, and 30 μM (−)-norepinephrine, respectively, with a calculated

EC_{50} of 3–3.5 μM. These results are in agreement with those obtained employing the percentage conversion of newly formed [^{14}C]ATP to [^{14}C]cAMP (see Fig. 1) and thus suggest the equivalence of the two methods for measuring cAMP formation in striatal slices.

[^{14}C]cAMP accumulation in P_2, crude mitochondrial preparation. As described above for brain slices, the procedure followed for labeling the ATP pool of slices involved a 60-min incubation with [8-^{14}C]adenine. After washing three times with fresh Krebs medium, the slices were homogenized in 0.32 M sucrose (18) and centrifuged at 1000 × *g* for 15 min to sediment debris and nuclei. The supernatant was recentrifuged at 17,000 × *g*, and the P_2 or crude mitochondrial pellet (19) was resuspended in 0.5 ml of low-calcium Krebs medium containing ascorbic acid (0.6 mM) and Ro 20-1724 (1.0 mM). The partially purified synaptosomal fraction was incubated for an additional 30 min (which was shown to produce maximum accumulation of [^{14}C]cAMP by catecholamines), and the reaction was terminated with 0.5 ml of 10% trichloracetic acid containing carrier and tritiated cAMP. The labeled ATP and cAMP were isolated as described previously. In an attempt to differentiate the responsiveness of the synaptosomal fraction from other subcellular preparations, the 17,000 × *g* supernatant of the sucrose homogenate was centrifuged for 1 hr at 100,000 × *g* to prepare a microsomal pellet, and the effect of catecholamines on the accumulation of [^{14}C]cAMP in the microsomal as well as the 1000 × *g* nuclear fraction was investigated exactly as described above for the synaptosomal fraction.

Other compounds were purchased from the following sources: (−)-norepinephrine bitartrate and (±)-isoproterenol HCl, Sigma Chemical Company; (−)-epinephrine bitartrate and dopamine HCl, Calbiochem; and phentolamine HCl, Ciba Pharmaceutical Company. (+)-Norepinephrine bitartrate and (−)-isoproterenol bitartrate were donated by Sterling-Winthrop Laboratories; (−)- and (+)-propranolol HCl, by Ayerst Laboratories; *N*-isopropyldopamine, by Hoffmann-La Roche; trifluoperazine HCl and chlorpromazine HCl, by Smith Kline & French; and MJ-1999 (sotalol HCl), by Mead Johnson and Company. Alprenolol was a gift of Dr. Robert Lefkowitz, Duke University Medical School.

RESULTS

Effects of catechols on [^{14}C]cAMP accumulation in striatal slices. With respect to their ability to stimulate the accumulation of [^{14}C]cAMP in striatal slices, the β-hydroxylated catecholamines demonstrated the greatest potency, with isoproterenol > epinephrine > norepinephrine (Fig. 1). The maximum stimulation produced by (−)-norepinephrine (approximately 400%) was greater than that observed with either (−)-isoproterenol or (−)-epinephrine (about 300%). The curve generated by varying the concentration of (−)-isoproterenol to levels as low as 0.01 μM was parallel to that produced with (−)-norepinephrine. Graphical estimation of the concentration resulting in 50% of the maximum stimulation gave EC_{50} values for (−)-isoproterenol, (−)-epinephrine, and (−)-norepinephrine of 0.03, 0.7, and 3.0 μM, respectively. Catecholamines also showed

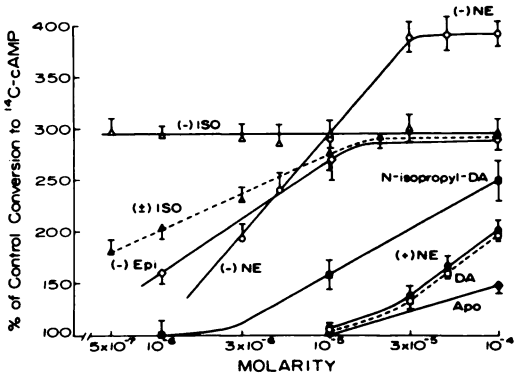

FIG. 1. *Stimulation of [^{14}C]cAMP accumulation by catecholamines and apomorphine in striatal slices*

Assays were performed according to a modified procedure of Perkins and Moore (14) as described under MATERIALS AND METHODS. Points represent percentage of control; the percentage conversion of [^{14}C]ATP to [^{14}C]cAMP for controls ranged from 0.62% to 0.69%. Each point is the mean ± standard error of determinations from three to five animals. NE, norepinephrine; ISO, isoproterenol; Epi, epinephrine; DA, dopamine; Apo, apomorphine.

stereoselectivity in their interaction with the cAMP-generating system, with the (−) isomers of isoproterenol and norepinephrine being much more potent than (±)-isoproterenol (EC_{50} approximately 0.2 μM) and (+)-norepinephrine, respectively (Fig. 1). The non-β-hydroxylated catecholamine *N*-isopropyldopamine was less potent than (±)-*N*-isopropylnorepinephrine (isoproterenol) but considerably more potent than dopamine (approximate EC_{50} of 60 μM) (Fig. 1). Furthermore, over at least a 10-fold concentration range (0.01–0.1 mM), the response curves were similar for dopamine and the (+) isomer of norepinephrine (Fig. 1). At higher concentrations (0.2 mM) of dopamine and (+)-norepinephrine, the percentage of control conversion to [^{14}C]cAMP was 235% ± 10% and 240% ± 8%, respectively, while maximum stimulation of 340% ± 12% was reached at 1.0 mM dopamine. Of all the catechols tested, apomorphine appeared least potent, producing 50% less formation of cAMP than dopamine at 0.1 mM (Fig. 1). Furthermore, in an attempt to dissociate the effects of catecholamines on cAMP synthesis from that of degradation, the phosphodiesterase inhibitor Ro 20-1724 was removed from the incubation medium. Even though the absence of a phosphodiesterase inhibitor resulted in approximately a 70% depression of the basal conversion of ATP to cAMP, the percentage stimulation in response to dopamine or norepinephrine was identical with that observed in Fig. 1, suggesting that the enhanced accumulation of [^{14}C]cAMP in response to catecholamines is due primarily to stimulation of adenylate cyclase activity rather than to inhibition of phosphodiesterase.

In view of the relative potencies of the catecholamines and stereoisomers (Fig. 1), one might postulate the presence of a *beta* adrenergic receptor-sensitive adenylate cyclase in striatal slices. However, the possible existence of a separate, specific dopamine receptor-coupled adenylate cyclase was further investigated in the next series of experiments. Simultaneous addition of dopamine to the medium produced either a very slight increase or had no significant effect upon the accumulation of [^{14}C]cAMP observed with maximum effective concentrations of norepinephrine (Table 1). Furthermore, the maximum response observed with the more specific *beta* adrenergic agonist isoproterenol was not significantly augmented by incubation with dopamine; similarly, isoproterenol did not alter the stimulatory effect of norepinephrine (Table 1).

Effects of adrenergic and dopaminergic blocking agents in striatal slices. In order to elucidate further the type of receptor-coupled adenylate cyclase in striatal slices, the effects of *beta* and *alpha* adrenergic and dopamine receptor-blocking agents were tested on the responsiveness of the cAMP-generating system to catecholamines. The augmented accumulation of [^{14}C]cAMP elicited by either (±)-isoproterenol or (−)-norepinephrine was almost completely inhibited (82% or 97%, respectively) by simultaneous addition of the *beta* adrenoreceptor antagonist (±)-propranolol (Table 2). Similarly, the *beta* adrenergic blocking agents (±)-propranolol and sotalol produced complete antagonism of the dopamine-induced increase in cAMP accumulation, whereas the *beta* blocker (−)-alprenolol reduced the stimulation elicited with dopamine by approximately 68% (Table 3). Stereoselectivity was also demonstrated for the *beta* adrenergic antagonist propranolol; at a low concentration (0.1 μM), the (−) isomer produced complete antagonism whereas the (+) isomer only partially inhibited the stimulatory response to (−)-norepinephrine (33%, Table 2) or dopamine (56%, Table 3). Further reduction of the concentration of propranolol to 0.05 μM displayed the stereoselective antagonism more markedly, with the dopamine (200 μM)-induced accumulation of cAMP now being inhibited approximately 5% and 70% by the (+) and (−) isomers of propranolol, respectively.

Upon addition to the medium of the *alpha* adrenergic blocker phentolamine, or of chlorpromazine or trifluoperazine (20 μM), no significant alteration occurred in the augmented cAMP synthesis elicited by dopamine (Table 4). Even at a higher concentration of trifluoperazine (50 μM), a potent neuroleptic agent which reportedly

TABLE 1

Effects of incubation with dopamine, norepinephrine, and isoproterenol on [^{14}C]cAMP accumulation in striatal slices

Each value represents the mean ± standard error of determinations from three to five animals (n = 3–5). All values are statistically significant in comparison with nontreated controls, at $p < 0.01$.

Additions	n	Conversion to [^{14}C]cAMP	Relative to control
		%	%
None	5	0.68 ± 0.03	100
0.1 mM dopamine	5	1.33 ± 0.04	196 ± 10
0.1 mM (−)-norepinephrine	5	2.55 ± 0.06	375 ± 18
0.1 mM dopamine + 0.1 mM (−)-norepinephrine	5	2.74 ± 0.05[a]	403 ± 19
None	4	0.68 ± 0.03	100
0.05 mM dopamine	4	1.08 ± 0.03	159 ± 8
0.05 mM (−)-norepinephrine	4	2.79 ± 0.08	410 ± 22
0.05 mM dopamine + 0.05 mM (−)-norepinephrine	4	2.80 ± 0.10[b]	412 ± 23
None	3	0.62 ± 0.01	100
0.2 mM dopamine	3	1.46 ± 0.05	235 ± 9
5.0 μM (−)-isoproterenol	3	1.76 ± 0.07	284 ± 12
0.2 mM dopamine + 5.0 μM (−)-isoproterenol	3	1.77 ± 0.10[c]	285 ± 16
None	3	0.72 ± 0.04	100
0.03 mM (−)-isoproterenol	3	2.16 ± 0.03	300 ± 17
0.03 mM (−)-norepinephrine	3	2.62 ± 0.09	364 ± 24
0.03 mM (−)-isoproterenol + 0.03 mM (−)-norepinephrine	3	2.63 ± 0.06[b]	365 ± 22

[a] Significantly different from conversion with addition of norepinephrine alone, at $p < 0.05$.
[b] Not significantly increased over conversion with addition of norepinephrine alone.
[c] Not significantly increased over conversion with addition of isoproterenol alone.

TABLE 2

Effects of propranolol on isoproterenol- or norepinephrine-induced stimulation of [^{14}C]cAMP accumulation in striatal slices

The nontreated, basal conversion rate was 0.81% ± 0.04%, and each value represents the mean ± standard error of determinations from five animals.

Additions	Conversion to [^{14}C]cAMP	Relative to control
	%	%
(±)-Propranolol (20 μM)	0.83 ± 0.02	100
(±)-Isoproterenol (20 μM)	2.41 ± 0.06	290 ± 8
(±)-Propranolol (20) μM) + (±)-isoproterenol (20 μM)	1.12 ± 0.03[a]	135 ± 4
(−)-Norepinephrine (10 μM)	2.37 ± 0.07	286 ± 11
(±)-Propranolol (20 μM) + (−)-norepinephrine (10 μM)	0.88 ± 0.04[b]	106 ± 5
(+)-Propranolol (0.1 μM) + (−)-norepinephrine (10 μM)	0.84 ± 0.09[b]	101 ± 11
(+)-Propranolol (0.1 μM) + (−)-norepinephrine (10 μM)	1.84 ± 0.05[a]	222 ± 8

[a] Significantly different from conversion with addition of propranolol alone, at $p < 0.01$.
[b] Not significantly different from conversion with addition of propranolol alone.

has specific blocking activity toward the dopamine receptor (6–9), no significant antagonism was observed of the stimulation of [^{14}C]cAMP formation in striatal slices induced by dopamine (100 μM) or norepinephrine (5.0 μM).

Effects of catecholamines and antagonists in crude mitochondrial fraction of striatal homogenates. In a crude mitochondrial fraction (P_2) of striatal homogenates, the β-hydroxylated catecholamines (±)-isoproterenol and (−)-norepinephrine

TABLE 3

Effects of beta adrenergic blockers on dopamine-induced stimulation of [^{14}C]cAMP formation in striatal slices

The nontreated, basal conversion rate ranged from 0.50% to 0.64% and each values represents the mean ± standard error of determinations from six animals.

Additions	Conversion to [^{14}C]cAMP	Relative to control
	%	%
(±)-Propranolol (20 μM)	0.57 ± 0.02	100
Dopamine (50 μM)	0.94 ± 0.05	165 ± 11
(±)-Propranolol (20 μM) + dopamine (50 μM)	0.58 ± 0.03[a]	102 ± 2
(±)-Propranolol (0.1 μM)	0.66 ± 0.04	100
Dopamine (200 μM)	1.56 ± 0.06	236 ± 17
(−)-Propranolol (0.1 μM) + dopamine (200 μM)	0.64 ± 0.04[a]	97 ± 8
(+)-Propranolol (0.1 μM) + dopamine (200 μM)	1.06 ± 0.02[b]	161 ± 10
(±)-Sotalol (20 μM)	0.63 ± 0.03	100
Dopamine (50 μM)	0.99 ± 0.02	160 ± 8
(±)-Sotalol (20 μM) + dopamine (50 μM)	0.62 ± 0.01[a]	98 ± 5
(−)-Alprenolol (50 μM)	0.49 ± 0.04	100
Dopamine (100 μM)	0.96 ± 0.06	196 ± 20
(−)-Alprenolol (50 μM) + dopamine (100 μM)	0.64 ± 0.07[c]	131 ± 17

[a] Not significantly increased over conversion with addition of propranolol or sotalol alone.
[b] Significantly different from conversion with addition of propranolol alone, at $p < 0.01$.
[c] Significantly different from conversion with addition of alprenolol alone, at $p < 0.05$.

TABLE 4

Effects of alpha adrenergic blockers on dopamine-induced increase in [^{14}C]cAMP formation in striatal slices

The nontreated, basal conversion ranged from 0.51% to 0.63%, and each value represents the mean ± standard error of determinations from six animals.

Additions	Conversion to [^{14}C]cAMP	Relative to control
	%	%
Phentolamine (20 μM)	0.63 ± 0.02	100
Dopamine (50 μM)	0.95 ± 0.04	151 ± 8
Phentolamine (20 μM) + dopamine (50 μM)	0.99 ± 0.05[a]	157 ± 6
Chlorpromazine (20 μM)	0.62 ± 0.03	100
Dopamine (50 μM)	0.97 ± 0.03	156 ± 9
Chlorpromazine (20 μM) + dopamine (50 μM)	0.99 ± 0.03[a]	160 ± 9
Trifluoperazine (20 μM)	0.53 ± 0.02	100
Dopamine (50 μM)	0.88 ± 0.03	166 ± 8
Trifluoperazine (20 μM) + dopamine (50 μM)	0.92 ± 0.01[a]	174 ± 7

[a] Not significantly different from conversion with addition of dopamine alone.

appeared to exhibit greater potency and intrinsic activity than dopamine (Table 5). The cAMP-generating system also seemed to display stereoselectivity, with the (−) isomer of norepinephrine being much more potent than the (+) isomer, which in turn appeared equipotent with dopamine (Table 5). Like the lack of "additive effect" observed in slices (Table 1), simultaneous addition of 0.2 mM dopamine did not significantly augment [^{14}C]cAMP accumulation generated by a maximum effective concentration of (−)-isoproterenol (0.01 mM). Furthermore, removal of the phosphodiesterase inhibitor Ro 20-1724 from the incubation medium produced so great a reduction of counts isolated in the cAMP fraction that basal cAMP synthesis could not be reliably determined. Although replacement of Ro 20-1724 by theophylline

TABLE 5

Effect of catecholamines on [^{14}C]cAMP accumulation in striatal homogenates

Assays were performed on crude mitochondrial fractions prepared from striatal slices incubated with [^{14}C]adenine as described under MATERIALS AND METHODS. The data represent percentage of control conversion from [^{14}C]ATP to [^{14}C]cAMP, which was 1.36 ± 0.05. Each value is the mean ± standard error of determinations from six animals.

Drug	[^{14}C]cAMP accumulation at:				
	0.01 mM	0.05 mM	0.1 mM	0.2 mM	0.5 mM
			% control		
(±)-Isoproterenol	294 ± 7	295 ± 6	310 ± 10		334 ± 20
(−)-Norepinephrine	232 ± 10	261 ± 10	288 ± 13	337 ± 8	369 ± 6
(+)-Norepinephrine		157 ± 18	182 ± 8		268 ± 15
Dopamine			174 ± 9	203 ± 7	256 ± 11

(10 mM) resulted in approximately a 40% depression of basal activity, the percentage stimulation of conversion to [^{14}C]-cAMP in response to dopamine or isoproterenol was not significantly altered. In contrast to the stimulatory effect of catecholamines in crude mitochondrial preparations, no augmented accumulation of cAMP was found when norepinephrine or dopamine was incubated with nuclear or microsomal fractions prepared from striatal homogenates.

Without altering the basal synthesis of cAMP in striatal homogenates, the *beta* blockers (±)-propranolol and (−)-alprenolol, at 50 μM, completely antagonized the stimulatory effect of dopamine; even at a lower concentration of (±)-propranolol (1.0 μM), the activation by dopamine was still abolished (Table 6). Similarly, the augmented cAMP accumulation elicited by norepinephrine was depressed approximately 80% with (±)-propranolol (Table 6). As demonstrated for agonistic potency, the blocking activity of propranolol appeared stereoselective (Table 6), since a 1.0 μM concentration of the (−) isomer was found to antagonize 82% of the (−)-isoproterenol (10 μM)-induced stimulation of cAMP formation, whereas the (+)-isomer was much less potent (23% inhibition). In contrast, the purported dopamine receptor-blocking agent trifluoperazine, at 50 μM, did not prevent the increased conversion to cAMP produced by dopamine, (−)-norepinephrine, or (−)-isoproterenol (Table 7). However, increasing the concentration of trifluoperazine in the medium to 100 μM reduced the stimulatory effectiveness of dopamine by 50% (Table 7) without altering the basal synthesis of cAMP.

Effects of catecholamines on cAMP formation in several brain regions. To determine whether the stimulation of cAMP accumulation by dopamine was unique to striatal tissues, which contain a higher concentration of dopaminergic terminals than most other brain regions, the effects of dopamine were compared with those of the β-hydroxylated catecholamines in the corpus striatum, cerebral cortex, and hindbrain (cerebellum and brain stem). In comparison with striatal slices (Fig. 1), the stimulatory activity ratio of dopamine to norepinephrine was found to be lower in cerebral cortical slices, with 0.1 mM dopamine or norepinephrine eliciting corresponding 40% ± 11% or 420% ± 22% increases in cortical cAMP formation, whereas apomorphine was inactive. In crude mitochondrial (P_2) preparations, the stimulation was about 50% greater in the striatum than in the cerebral cortex at 0.1 mM dopamine, although similar activation was reached at 0.2 mM dopamine (approximately 200%) in both brain regions (Table 8). Even in the hindbrain, where the dopamine to norepinephrine activity ratio was further diminished, dopamine was observed to generate a 46% stimulation of cAMP accumulation (Table 8).

DISCUSSION

The order of potency of β-hydroxylated catecholamines in stimulating the accumulation of cyclic AMP in striatal slices is (−)-isoproterenol > (−)-epinephrine > (−)-norepinephrine (Fig. 1). The calcu-

TABLE 6

Effects of beta adrenergic blockers on dopamine-, norepinephrine-, or isoproterenol-induced stimulation of [^{14}C]cAMP formation in striatal homogenates

The assay procedure was identical with that described in Table 5 and the text. The nontreated, basal conversion rate ranged from 1.35% to 1.42% and each value represents the mean ± standard error of determinations from five animals.

Additions	Conversion to [^{14}C]cAMP	Relative to control
	%	%
(±)-Propranolol (0.05 mM)	1.36 ± 0.05	100
Dopamine (0.1 mM)	2.37 ± 0.08	174 ± 9
(±)-Propranolol (0.05 mM) + dopamine (0.1 mM)	1.28 ± 0.02[a]	94 ± 4
(±)-Propranolol (1.0 μM) + dopamine (0.1 mM)	1.32 ± 0.04[a]	97 ± 5
(−)-Norepinephrine (0.1 mM)	3.91 ± 0.10	288 ± 13
(±)-Propranolol (0.05 mM) + (−)-norepinephrine (0.1 mM)	1.89 ± 0.13[b]	139 ± 11
(−)-Isoproterenol (10 μM)	3.47 ± 0.06	255 ± 10
(−)-Propranolol (1.0 μM) + (−)-isoproterenol (10 μM)	1.74 ± 0.03[c]	128 ± 5
(+)-Propranolol (1.0 μM) + (−)-isoproterenol (10 μM)	2.98 ± 0.01[c]	219 ± 8
(−)-Alprenolol (0.05 mM)	1.40 ± 0.04	100
Dopamine (0.1 mM)	2.41 ± 0.05	172 ± 6
(−)-Alprenolol (0.05 mM) + dopamine (0.1 mM)	1.42 ± 0.04[a]	101 ± 4

[a] Not significantly increased over conversion with addition of propranolol or alprenolol alone.

[b] Significantly different from conversion with addition of norepinephrine or propranolol alone, at $p < 0.01$.

[c] Significantly different from conversion with addition of isoproterenol or propranolol alone, at $p < 0.01$.

TABLE 7

Effect of trifluoperazine on dopamine-, norepinephrine-, or isoproterenol-induced accumulation of [^{14}C]cAMP in striatal homogenates

The assay procedure was identical with that described in Table 5. Results represent the mean ± standard error of determinations from six animals.

Additions	Conversion to [^{14}C]cAMP	Relative to control
	%	%
Control	1.31 ± 0.04	100
Trifluoperazine (0.05 mM)	1.33 ± 0.06	102 ± 6
Dopamine (0.1 mM)	2.37 ± 0.09	181 ± 9
Dopamine (0.1 mM) + trifluoperazine (0.05 mM)	2.28 ± 0.11[a]	174 ± 10
Dopamine (0.1 mM) + trifluoperazine (0.1 mM)	1.83 ± 0.03[b]	140 ± 5
(−)-Norepinephrine (0.05 mM)	3.42 ± 0.08	261 ± 10
(−)-Norepinephrine (0.05 mM) + trifluoperazine (0.05 mM)	3.32 ± 0.11[a]	253 ± 11
(−)-Isoproterenol (0.05 mM)	3.76 ± 0.08	287 ± 11
(−)-Isoproterenol (0.05 mM) + trifluoperazine (0.05 mM)	3.84 ± 0.03[a]	293 ± 9

[a] Not significantly different from conversion with addition of dopamine, norepinephrine, or isoproterenol alone.

[b] Significantly different from conversion with addition of dopamine alone, at $p < 0.01$.

TABLE 8

Effect of catecholamines on [^{14}C]cAMP formation in cortical, hindbrain, and striatal homogenates

The nontreated, control conversion to [^{14}C]cAMP was 2.79% ± 0.08% for cerebral cortex, 3.78% ± 0.10% for hindbrain (brain stem and cerebellum), and 1.36% ± 0.05% for corpus striatum (means ± standard errors).

Addition	[^{14}C]cAMP formation		
	Cerebral cortex	Hindbrain	Corpus striatum
	% control		
Dopamine (0.1 mM)	147 ± 12		174 ± 9
Dopamine (0.2 mM)	191 ± 9	146 ± 4	203 ± 7
(±)-Isoproterenol (0.1 mM)	285 ± 11		310 ± 10
(−)-Norepinephrine (0.1 mM)	284 ± 6	324 ± 25	288 ± 13

lated EC_{50} values for these catecholamines of 0.03, 0.7, and 3.0 μM, respectively, are similar to those obtained in preparations of rat erythrocyte membranes, which possess properties characteristic of a typical *beta* adrenergic receptor-sensitive adenylate cyclase (3). The finding that the non-β-hydroxylated compound *N*-isopropyldopamine appeared to be more potent than dopamine (EC_{50} 60 μM) is again similar to the results reported for the *beta* type system of the erythrocyte membrane (3). Of the catechol compounds tested, the dopamine receptor agonist apomorphine was least active in stimulating cAMP accumulation in striatal slices. By measuring the accumulation of endogenous cAMP in caudate slices, Forn and co-workers (20) reported similar EC_{50} values and order of potency, with isoproterenol > norepinephrine > dopamine > apomorphine. Although our finding that the observed maximum response (intrinsic activity) with norepinephrine was greater than that obtained with isoproterenol or epinephrine does distinguish these results in striatal slices from those reported in rat erythrocyte membranes (3), similar results were also obtained in cerebral cortical slices (21), and thus the effect does not appear unique to the striatal region, which contains a high concentration of dopamine.

Furthermore, the greater potency demonstrated by the (−) isomers of isoproterenol and norepinephrine than by (±)-isoproterenol and (+)-norepinephrine, respectively, indicates that this receptor-coupled adenylate cyclase displays stereoselectivity by favoring the (−) isomer and thereby meets another criterion identified with the *beta* adrenoreceptor (3). The observation that the dose-response curve for (+)-norepinephrine is similar to that of dopamine (Fig. 1) is in accordance with the hypothesis of Easson and Stedman (22), which predicts a two-point interaction on the *beta* adrenoreceptor for both the (+) isomer and its deoxy derivative, since the third point of interaction would involve the alcoholic hydroxyl group, which in the latter two compounds would be either in the wrong position or absent. In view of this hypothesis, Patil *et al.* (23) proposed that in the presence of a specific dopamine receptor whose configuration is distinct from that of an *alpha* or *beta* adrenoreceptor, (+)-norepinephrine should differ markedly in activity from its deoxy derivative, dopamine. However, the similarity of the stimulatory responses obtained with (+)-norepinephrine and dopamine suggests that a specific dopamine receptor-coupled adenylate cyclase is not detectable in striatal slices.

Nevertheless, in an attempt to investigate further the possible existence of a separate, specific dopamine receptor-coupled adenylate cyclase in striatal slices, dopamine was added simultaneously to the medium with maximum effective concentrations of β-hydroxylated catecholamines. The data presented in Table 1 demonstrate a lack of additivity when dopamine was incubated together with optimal concentrations of (−)-norepinephrine or (−)-isoproterenol, suggesting that dopamine and the β-hydroxylated catecholamines may interact with the same type of receptor system in striatal slices. A further delineation of the properties of the receptor-coupled adenylate cyclase was attempted by utilizing receptor-blocking agents which are relatively specific in their interactions with *alpha* or *beta* adrenergic or dopamine receptors. First, the *beta* adrenergic receptor blocker (±)-propranolol produced marked inhibition of

cAMP accumulation generated by (±)-isoproterenol (Table 2) while completely antagonizing the effects of (−)-norepinephrine (Table 2) and dopamine (Table 3). The newer *beta* adrenergic antagonists alprenolol (24) and sotalol, which reportedly lacks local anesthetic effects (25), likewise markedly reduced the dopamine-induced stimulation of cAMP synthesis without altering basal cAMP accumulation (Table 3). The greater stereoselective inhibitory potency displayed by the (−) isomer of propranolol (Tables 2 and 3) further supports the hypothesis that both norepinephrine and dopamine interact with a *beta* type of system. In contrast to the inhibitory activity of the *beta* adrenergic antagonists, incubation with the *alpha* adrenergic blocker phentolamine, as well as with the purported dopamine receptor blockers chlorpromazine and trifluoperazine, produced no significant alteration of the labeled cAMP generated by dopamine (Table 4) or (−)-norepinephrine. Thus these studies with specific receptor-blocking agents indicate that striatal slices do not exhibit properties characteristic of a dopamine receptor-coupled adenylate cyclase. Rather, it appeared that the weak stimulatory effect of dopamine was being mediated by interaction with a *beta* type of system. However, in contrast with these observations, Forn *et al.* (20) recently reported that in striatal slices the stimulatory response to dopamine is markedly inhibited by the dopamine receptor antagonist fluphenazine (at 100 μM) but is unaltered by propranolol (100) μM). Furthermore, these authors observed an additive stimulatory effect when dopamine was incubated with a maximum effective concentration of isoproterenol. Although these data were obtained by measuring the accumulation of endogenous cAMP, the difference between the two methods employed does not appear to account completely for these discrepancies in results, since in our system similar curves were generated in response to various concentrations of (−)-norepinephrine by measuring increases in either labeled or endogenous cAMP.

Further investigation of the type of receptor-mediated regulation of cAMP synthesis in a crude mitochondrial fraction containing synaptosomes (Table 5) demonstrated that the catecholamines exhibited much the same order of potency and stereoselectivity as observed in striatal slices (Fig. 1), i.e., (±)-isoproterenol > (−)-norepinephrine > (+)-norepinephrine = dopamine. The observation that (+)-norepinephrine was equipotent with dopamine is again suggestive of a possible two-point interaction of the (+) isomer and its deoxy derivative with a *beta* type of system (22, 23). Furthermore, in crude mitochrondrial fractions, the stimulation of cAMP accumulation in response to a maximum effective concentration of isoproterenol was not augmented by incubation with dopamine. Finally, the effectiveness of the *beta* adrenergic blocking agents propranolol and alprenolol in antagonizing the dopamine-induced increase in cAMP in striatal homogenates (Table 6), and the lack of inhibitory activity of the neuroleptic agent trifluoperazine (up to 50 μM) (Table 7), provide additional evidence in support of the hypothesis that dopamine acts as a weak agonist on a *beta* adrenergic receptor rather than on a specific dopamine receptor-coupled adenylate cyclase. At higher concentrations of trifluoperazine (100 μM), there was a 50% reduction in the stimulation of cAMP synthesis generated by dopamine (Table 7), which is in agreement with the inhibitory effect of 100 μM fluphenazine reported in caudate slices by Forn *et al.* (20). Although those authors interpreted their results as suggesting the existence of a specific dopamine receptor-coupled adenylate cyclase, concentrations of chlorpromazine and haloperidol in the 20 μM range have also been reported to inhibit the isoproterenol-sensitive adenylate cyclase in myocardial (26) and erythrocyte membranes (3). The inhibitory effectiveness of neuroleptic agents in tissue preparations exhibiting a *beta* adrenergic receptor-sensitive adenylate cyclase has been attributed to their ability to bind nonspecifically to membranes. (27).

Further evidence that the sensitivity of adenylate cyclase to dopamine may not necessarily involve the dopamine receptor is found in the increased formation of

cAMP elicited by dopamine in slices or crude mitochondrial fractions (Table 8) prepared from the cerebral cortex, which a recent report (28) suggested is only sparsely innervated by dopaminergic terminals. In fact, in homogenates prepared from the hindbrain (including the cerebellum and brain stem), which purportedly is devoid of dopaminergic terminals (29), dopamine is still capable of eliciting a significant increase in the accumulation of cAMP. According to the working hypothesis formulated for striatal tissues, which depicts dopamine as weakly stimulatory toward the *beta* adrenergic receptor-coupled adenylate cyclase, a similar mechanism of action might operate for dopamine in the cerebral cortex and hindbrain: this proposal is currently under investigation.

In contrast to the *beta* adrenergic receptor activity observed in striatal slices and crude mitochondrial fractions, several investigators have observed in broken-cell homogenates [prepared in hypotonic Tris buffer containing ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid] a lack of responsiveness of the adenylate cyclase system to isoproterenol (6) and *N*-isopropyldopamine (10) as well as marked reduction in the potency and efficacy of norepinephrine (6). In so far as the percentage stimulation by dopamine (up to 100 μM) is relatively similar in all striatal preparations studied, it is difficult to explain the specific loss of sensitivity to the β-hydroxylated catecholamines in tissues homogenized in Tris buffer. However, it is possible that the *beta* adrenergic receptor is partially solubilized during the homogenization procedure, which would alter or distort the normal receptor-cyclase coupling mechanism. In fact, a decreased sensitivity to norepinephrine and isoproterenol, as opposed to dopamine, occurs not only in the preparation of broken-cell homogenates of the corpus striatum but also in the cerebral cortex (30). Another possible explanation is that in more intact preparations the response elicited by the interaction of dopamine with the *beta* type system masks a weaker stimulatory effect of dopamine with a specific dopamine receptor-coupled cyclase. Accordingly, one might expect the dopamine receptor-coupled cyclase to be manifested in broken-cell preparations following the loss of responsiveness of the *beta* type system, as recently proposed to occur upon homogenization of striatal tissues under hypotonic conditions (20).

It is concluded that the pharmacological properties demonstrated for the cAMP-generating system in slices and crude mitochondrial fractions of the corpus striatum conform to many of the criteria established for the identification of a *beta* adrenoreceptor but not for a dopamine receptor-coupled adenylate cyclase. In fact, the weak stimulatory effect of dopamine on cAMP accumulation can be more readily explained by regarding dopamine as a weak agonist, possessing low affinity and efficacy for the *beta* adrenergic receptor.

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