Biochemical Pharmacology. Vol. 27. pp. 2919–2925.

0006-2952/78/1215-2919 $02.00/0

β-ADRENERGIC RECEPTOR-SENSITIVE ADENOSINE CYCLIC 3′,5′-MONOPHOSPHATE ACCUMULATION IN HOMOGENATES OF THE RAT CORPUS STRIATUM.

A COMPARISON WITH THE DOPAMINE RECEPTOR-COUPLED ADENYLATE CYCLASE*

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(*Received* 8 *December* 1977; *accepted* 23 *March* 1978)

Abstract—The conversion of newly formed [^{3}H]adenosine triphosphate (ATP) to [^{3}H]adenosine cyclic 3′,5′-monophosphate (cAMP) was studied in osmotically shocked, crude synaptosomal fractions of the rat corpus striatum. Of the β-hydroxylated catecholamines tested, the potency of isoproterenol (EC_{50} about 0.01 μM) was greater than that of norepinephrine (EC_{50} about 1.0 μM). Stereoselectivity was displayed with the (−)isomer of isoproterenol being more potent that its (+)isomer. Of the non-β-hydroxylated catecholamines studied, *N*-isopropyldopamine demonstrated greater potency than dopamine, whereas apomorphine was inactive. No "additive stimulatory effect" was observed when dopamine was combined with a maximum effective concentration of isoproterenol. The β-adrenergic antagonist propranolol, completely blocked both the dopamine- and norepinephrine-induced increases in cAMP formation. Properties characteristic of a β-type system were, likewise, exhibited by homogenates of both the cerebral cortex and hindbrain. Furthermore, the conversion to [^{3}H]cAMP was increased only slightly (about 16 per cent) by exposure to sodium fluoride, and the stimulatory response to isoproterenol was not altered significantly by high concentrations of ATP but was lost upon sonication of the tissues. In contrast, by assaying adenylate cyclase activity with a high saturating concentration of exogenous [^{3}H]ATP, striatal and cerebral cortical homogenates exhibited responses characteristic of a specific dopamine receptor-coupled adenylate cyclase. Sonication of tissues did not alter the stimulatory effect of dopamine, and sodium fluoride produced about a 2-fold stimulation of the adenylate cyclase activity. Thus, findings in osmotically shocked, crude synaptosomal fractions of the corpus striatum suggest that the particulate component of adenylate cyclase, which utilizes exogenous ATP as substrate, exhibits properties characteristic of a dopamine receptor-coupled adenylate cyclase. In contrast, the membrane-enclosed, particulate component of adenylate cyclase, which utilizes endogenously synthesized ATP as substrate, conforms to criteria identified with a β-adrenoreceptor-linked adenylate cyclase.

Previous investigations have either failed to demonstrate the presence of [1], or have shown only weak activity for [2], a β-adrenergic receptor-linked adenylate cyclase system in osmotically shocked homogenates of brain. In contrast to this lack of sensitivity of adenylate cyclase to β-adrenoreceptor agonists, such as isoproterenol [3] and *N*-isopropyldopamine [4], in osmotically lysed tissues, an active β-type system has been observed in other preparations, including slices [5, 6], crude synaptosomal fractions [6] or Krebs–Ringer homogenates [7]. In view of these observations, it has been hypothesized that the loss of activity of the β-type system may occur in the preparation of membrane fragments during the homogenization of brain tissues in a hypotonic medium [1, 5].

Our interest in these findings prompted a more extensive examination of the factors underlying this apparent loss of responsiveness of the β-type system after osmotic lysis of brain tissues. In recent years, the procedures utilized for measuring the responsiveness of adenylate cyclase to catecholamines in homogenates have involved primarily the use of exogenous ATP as substrate. However, if one were to consider the possible localization of a β-adrenergic receptor-coupled adenylate cyclase in a membrane-enclosed pool, then the failure to demonstrate a β-type system may be attributed to the poor permeability of the polar ATP molecule, resulting in diminished availability of the substrate pool serving adenylate cyclase. To test this hypothesis, the conversion of [^{3}H]ATP to [^{3}H]cAMP† was assayed by two types of experimental procedures, utilizing [^{3}H]ATP as substrate, which was either added exogenously or newly formed from [^{3}H]adenine. The rationale for selecting [^{3}H]adenine to label the ATP pool is that adenine has been shown to be readily permeable to membranes in more intact preparations [8]. Taking into consideration the high level of adenylate cyclase activity in crude synaptosomal fractions of the corpus striatum [6], as well as the high density of binding sites for both dopamine [9] and β-adrenergic receptors [10] in this region, hypo-osmotically shocked, crude

* This work was supported by Grant NS 12288 from the United States Public Health Service.

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

synaptosomal fractions from the corpus striatum were selected initially for investigating both types of receptor-cyclase systems. Moreover, to compare results obtained in the corpus striatum, which is rich in dopamine-containing nerve terminals [9, 11] with those in other brain regions, this study was expanded to include homogenates of such areas as the hindbrain and cerebral cortex, which purportedly are innervated only sparsely by dopaminergic terminals [11, 12].

MATERIALS AND METHODS

cAMP accumulation from newly formed ATP. Male Sprague–Dawley rats (150–175 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, in accordance with our previously published procedure [6]. A preliminary 45-min incubation was performed, in a Dubnoff shaker at 37° under an atmosphere of 95% O_2–5% CO_2, of striatal (96 mg/ml), cerebral cortical (240 mg/ml), or hindbrain (480 mg/ml) slices in 4 ml of a low calcium (0.75 mM) Krebs–Ringer bicarbonate medium, buffered to pH 7.4, containing glucose (10 mM) and theophylline (1.0 mM). After washing two times by centrifugation at 600 *g* for 30 sec, in most experiments, slices were homogenized in 0.32 M sucrose containing 0.6 mM EGTA and 2.0 mM Tris–HCl, buffered to pH 7.4, and centrifuged at 1000 *g* for 15 min to sediment debris and nuclei. The pellet was homogenized again in sucrose medium, centrifuged at 1000 *g*, and the combined supernatant fractions were recentrifuged at 17,000 *g*. The resultant P_2 or crude synaptosomal pellet [13] was washed with the original Krebs medium, resuspended in 2 ml of this medium and incubated at 37° under 95% O_2–5% CO_2 for 60 min with [8-^{3}H]adenine (4 μM, 25 μCi/ml; Amersham Corp., Arlington, Heights, IL), permitting maximum conversion to [^{3}H]ATP. The P_2 fraction was separated from the medium by centrifugation at 17,000 *g*, washed twice with fresh Krebs, and the crude synaptosomal fraction was rehomogenized in 1.5 ml of a hypotonic medium containing, at final concentrations: glycylglycine buffer, pH 7.4 (50 mM), EGTA (0.6 mM), $MgSO_4$ (2.0 mM), theophylline (1.0 mM), 1-methyl-3-isobutylxanthine (1.0 mM), cAMP (0.1 mM), albumin (0.1%), phosphatidylserine (5 μg/ml) [14] and an ATP-regenerating system consisting of creatine PO_4 (5 mM) and creatine phosphokinase (0.2 mg/ml). Portions of this osmotically shocked homogenate (100 μl) were incubated with experimental agonists and/or antagonists at 30° for 15 min; over this reaction period, the per cent stimulation of [^{3}H]cAMP accumulation in response to catecholamines was proportional to time and was maximal at 15 min. The reaction was terminated with 1.0 ml of 5% trichloroacetic acid containing carrier cAMP (Boehringer/Mannheim, New York, NY) and [^{14}C]cAMP (New England Nuclear, Boston, MA), to correct for overall recovery (approximately 60–70 per cent). The labeled ATP and cAMP fractions were isolated by chromatography on columns of Dowex 50W-X8 (H^+) and neutral alumina oxide and, subsequently, portions were counted by liquid scintillation spectrometry, as described in a previous publication [6]. Results are expressed as percentage conversion of [^{3}H]ATP to [^{3}H]cAMP, i.e. (dis./min cAMP × 100)/(dis./min ATP + cAMP).

In contrast to the procedure employed above, in which the crude synaptosomal fraction was incubated directly with [^{3}H]adenine, in other experiments the ATP pool was prelabeled in slices by incubating slices in 2 ml of Krebs medium for 60 min with [^{3}H]-adenine (4 μM, 12.5 μCi/ml) and, subsequently, the crude synaptosomal (P_2) fraction was prepared, broken by homogenization in hypotonic medium and assayed in a manner identical to that described already. The results obtained employing this technique were in agreement with those observed when the P_2 fraction was labeled directly (compare Tables 1 and 2 with 3) and, thus, suggest the equivalence of the two methods for measuring cAMP formation in cell-free preparations of crude synaptosomal fractions. Finally, the ATP pool was labeled directly in a striatal particulate preparation by incubating the 17,000 *g* pellet of an osmotically shocked crude synaptosomal fraction (P_2) for 60 min in 2 ml of Krebs medium containing [^{3}H]adenine (4 μM, 50 μCi/ml); after washing and resuspending the 17,000 *g* pellet in hypotonic medium, portions of this suspension were utilized to measure [^{3}H]cAMP formation in response to catecholamines.

cAMP accumulation from [^{3}H]*ATP.* After a preliminary 45-min incubation of striatal, cerebral cortical or hindbrain slices at 37°, under 95% O_2–5% CO_2 in Krebs medium containing glucose (10 mM) theophylline (1.0 mM), a crude synaptosomal fraction (P_2) was prepared in a manner identical to that described in the preceding section. After homogenization of the 17,000 *g* pellet (P_2) in 1.5 ml of hypotonic medium, the suspension was either assayed directly or recentrifuged at 17,000 *q*, and the resuspended pellet assayed for adenylate cyclase activity in a final volume of 150 μl. The reaction was initiated by the addition of [2-^{3}H]ATP (1.0 μCi, 0.5 mM; Schwarz/Mann, Orangeburg, NY) and continued for 5 min at 30°, before terminating the reaction with 1.0 ml of 5% trichloroacetic acid, as described above. In each experiment, blanks were prepared by assaying brain fractions, which were previously boiled for 5 min at 100°; radioactivity associated with this cAMP fraction was subtracted from the total activity of each sample. Adenylate cyclase activity was observed to be proportional to both reaction time for at least 5 min and protein concentration up to 0.5 mg/assay, the highest concentration studied. In the presence of the ATP-regenerating system, 90–95 per cent of the [^{3}H]ATP is recovered at the end of the 5-min incubation period. At concentrations greater than 0.2 mM ATP, cAMP formation was maximal and independent of substrate concentration. Protein was determined by the method of Lowry *et al.* [15], using bovine serum albumin as a standard.

Materials. Other compounds were purchased from the following sources: (−)norepinephrine bitartrate, Sigma Chemical Co., St. Louis, MO; (−)epinephrine bitartrate and dopamine HCl, CalBiochem, San Diego, CA; and 1-methyl-3-isobutylxanthine, Aldrich Biochemicals, Milwaukee, WI. All other chemicals were

obtained from standard sources, with the exception of the following drugs, which were gifts: (±)propranolol, from Ayerst Research Laboratories, Montreal, Canada; (−) and (+)isoproterenol, from Sterling-Winthrop Research Institute, Rensselaer, NY; *N*-isopropyldopamine, from Hoffmann-LaRoche, Nutley, NJ; and trifluoperazine HCl, from Smith, Kline & French, Philadelphia PA.

RESULTS

Effects of catechols on [^{3}H]cAMP accumulation from newly formed [^{3}H]ATP. In osmotically shocked P_2 homogenates which were prepared from striatal slices labeled with [^{3}H]adenine, the β-hydroxylated catecholamines demonstrate the greatest potency with isoproterenol being the most potent catechol studied (Table 1). Varying the concentrations of (−)isoproterenol and (−)norepinephrine, over the concentration range of 0.001 to 10 μM, generated curves which were parallel to each other, and graphical estimation of the concentration resulting in 50 per cent of maximum stimulation gave EC_{50} values for (−)isoproterenol and (−)norepinephrine of approximately 0.01 and 1.0 μM respectively. As illustrated in Table 1, the (−)isomer of isoproterenol is more potent than its (+)isomer, and *N*-isopropyldopamine is considerably more potent than dopamine, which is only stimulatory at concentrations greater than 10 μM. Incubation with apomorphine, varied over the concentration range from 1 to 100 μM, produced no significant increase in cAMP accumulation above control levels. The results in Table 2 demonstrate that the augmented accumulation of [^{3}H]cAMP elicited by either dopamine or (−)norepinephrine is completely inhibited by the simultaneous addition of (±)propranolol. In contrast, trifluoperazine has no significant effect on the dopamine-induced stimulation of cAMP formation (Table 2). Furthermore, similar findings are obtained when endogenous cAMP is measured [16] in striatal slices. In these experiments, the increase of endogenous cAMP, elicited by 100 μM dopamine (8.93 ± 0.37 above controls of 4.67 ± 0.27 pmoles/mg of protein for seven experiments), is blocked completely by 10 μM propranolol (4.52 ± 0.30 pmoles/mg of protein) but not by 10 μM trifluoperazine. After osmotic shock of a crude synaptosomal preparation, which was directly incubated with [^{3}H]adenine, the maximum response obtained with the specific β-receptor agonist, isoproterenol, is not increased significantly by coincubation with dopamine (Table 3). Moreover, dopamine as well as norepinephrine and isoproterenol, produces an approximate 25–60 per cent increase in cAMP accumulation in homogenates from both the cerebral cortex and the hindbrain (Table 4).

The next series of experiments demonstrate that the adenylate cyclase activity which is sensitive to the β-adrenergic receptor agonist, isoproterenol, is localized in the 17,000 *g* particulate fraction of an osmotically shocked crude synaptosomal preparation (Table 5, experiment I). Moreover, after osmotic shock of the prelabeled crude synaptosomal preparation, the 17,000 *g* particulate fraction is observed to retain

Table 1. Effects of catechols on the conversion of newly formed [^{3}H]ATP to [^{3}H]cAMP in striatal homogenates*

Additions	Conversion to [^{3}H]cAMP (%)	Relative to control (%)
None	1.49 ± 0.04	100
(−)Isoproterenol (0.05 μM)	2.27 ± 0.03†	152
(+)Isoproterenol (0.05 μM)	1.92 ± 0.08†‡	129
(−)Isoproterenol (0.1 μM)	2.32 ± 0.04†	156
(−)Norepinephrine (10 μM)	2.28 ± 0.03†	153
(−)Norepinephrine (1 μM)	1.85 ± 0.04†	124
(−)Epinephrine (10 μM)	2.24 ± 0.03†	150
N-isopropyldopamine (10 μM)	2.10 ± 0.04†	141
Dopamine (10 μM)	1.52 ± 0.02§	102
Dopamine (100 μM)	2.09 ± 0.03†	140
Apomorphine (100 μM)	1.44 ± 0.03§	97

* After prelabeling striatal slices with [^{3}H]adenine (25 μCi, 4 μM), the crude synaptosomal fraction was prepared, homogenized in hypotonic medium and assayed for [^{3}H]-cAMP formation, as described under Materials and Methods. Each value represents the mean ± S. E. of four determinations.

† Statistically significant in comparison with nontreated controls, at $P < 0.01$.

‡ Significantly different from conversion with (−)isomer of isoproterenol, at $P < 0.01$.

§ Not significantly increased over conversion of nontreated control.

Table 2. Effects of propranolol or trifluoperazine on catecholamine-induced stimulation of the conversion to [^{3}H]cAMP in striatal homogenates*

Additions	N	Conversion to [^{3}H]cAMP (%)	Relative to control (%)
None	5	1.52 ± 0.03	100
(±)Propranolol (50 μM)	3	1.47 ± 0.05	97
Dopamine (100 μM)	8	2.14 ± 0.05†	141
(±)Propranolol (50 μM) + dopamine (100 μM)	3	1.47 ± 0.03‡	97
(−)Norepinephrine (100 μM)	4	2.31 ± 0.04†	152
(±)Propranolol (50 μM) + (−)norepinephrine (100 μM)	4	1.60 ± 0.08‡	105
Trifluoperazine (50 μM)	3	1.50 ± 0.04	99
Trifluoperazine (50 μM) + dopamine (100 μM)	3	2.10 ± 0.03§	139

*Assays were performed in a manner identical to that described in Table 1 and Materials and Methods. Each value represents the mean ± S. E. of three to eight determinations.

† Significantly increased over conversion of nontreated control, at $P < 0.01$.

‡ Not significantly increased over conversion with addition of propranolol alone.

§ Not significantly different from conversion with addition of dopamine alone.

Table 3. Effects of catecholamines on the conversion to [^{3}H]cAMP in striatal homogenates*

Additions	N	Conversion to [^{3}H]cAMP (%)	Relative to control (%)
Control	3	1.69 ± 0.02	100
Dopamine (100 μM)	4	2.38 ± 0.10	141
(−)Isoproterenol (100 μM)	5	2.60 ± 0.08	154
Dopamine (100 μM) + (−)isoproterenol (100 μM)	6	2.64 ± 0.08†	156

* After preincubating striatal slices, a crude synaptosomal fraction (P_2) was prepared, prelabeled with [^{3}H]adenine (50 μCi, 4 μM), homogenized in hypotonic medium and assayed for [^{3}H]cAMP formation, as described under Materials and Methods. Each value represents the mean ± S. E. of three to six determinations. All values are statistically significant in comparison with nontreated controls, at $P < 0.01$.

† Not significantly increased over conversion with addition of isoproterenol alone.

about 13 and 25 per cent of the newly synthesized [^{3}H]ATP and [^{3}H]cAMP respectively. Similar increases in [^{3}H]cAMP accumulation are obtained when broken-cell particulate preparations are labeled directly with [^{3}H]adenine and subsequently stimulated with catecholamines (Table 5, experiment II). In fact, prior to labeling with [^{3}H]adenine, preincubation of the particulate preparation at 37° over a 30-min time period does not alter the basal or isoproterenol-stimulated accumulation of [^{3}H]cAMP. Furthermore, addition of ATP to the medium at a concentration as high as 0.5 mM does not alter significantly the augmented accumulation of [^{3}H]-cAMP in response to isoproterenol (Table 5, experiment I). In addition to eliciting a slight elevation of the [^{3}H]ATP pool in the particulate preparation

Table 4. Effects of catecholamines on the conversion to [^{3}H]cAMP in brain homogenates*

Additions	N	% Conversion to [^{3}H]cAMP (% of nontreated control) Cerebral cortex	Hindbrain
None	3	3.90 ± 0.09 (100)	4.92 ± 0.08 (100)
Dopamine (100 μM)	4	4.77 ± 0.02 (122)	6.20 ± 0.09 (126)
(−)Norpinephrine (100 μM)	6	5.30 ± 0.08 (136)	7.56 ± 0.14 (154)
(−)Isoproterenol (100 μM)	6	5.34 ± 0.16 (137)	7.81 ± 0.15 (159)

* After preincubating cerebral cortical or hindbrain slices, a crude synaptosomal fraction (P_2) was prepared, prelabeled with [^{3}H]adenine (50 μCi, 4 μM), homogenized in hypotonic medium and assayed for [^{3}H]cAMP formation, as described under Materials and Methods. Each value represents the mean ± S. E. of three to six determinations. All values are statistically significant in comparison with nontreated controls, at $P < 0.01$.

Table 5. Effects of catecholamines or sodium fluoride on the conversion to [^{3}H]cAMP in striatal particulate preparations*

Additions	N	Conversion to [^{3}H]cAMP (%)	Relative to control (%)
Experiment I			
None	6	1.43 ± 0.04	100
Dopamine (100 μM)	5	2.03 ± 0.09	142
(−)Norepinephrine (100 μM)	3	2.15 ± 0.09	150
(−)Isoproterenol (100 μM)	4	2.25 ± 0.10	157
(−)Isoproterenol (100 μM) + ATP (500 μM)	3	2.16 ± 0.07†	151
Sodium fluoride (10 mM)	4	1.66 ± 0.09	116
Experiment II			
None	4	1.21 ± 0.02	100
Dopamine (100 μM)	4	1.81 + 0.08	149
(−)Norepinephrine (100 μM)	4	2.01 ± 0.07	166
(−)Isoproterenol (100 μM)	4	2.00 ± 0.04	165

* In experiment I, a crude synaptosomal fraction (P_2) was prelabeled with [^{3}H]adenine (100 μCi, 4 μM), subjected to osmotic shock, and the (17,000 *g*) particulate fraction was assayed for [^{3}H]cAMP formation. In experiment II, a hypo-osmotically shocked particulate fraction was prelabeled directly with [^{3}H]adenine and subsequently assayed for [^{3}H]cAMP formation. All values are statistically significant in comparison with nontreated controls, at $P < 0.01$.

† Not significantly different from conversion with addition of isoproterenol alone.

Table 6. Effects of catechols or sodium fluoride on adenylate cyclase activity in particulate preparations*

Additions	(pmoles [^{3}H]cAMP formed/mg protein/5 min) (% of nontreated control) Cerebral Cortex	Corpus striatum
None	510 ± 22 (100)	572 ± 36 (100)
Dopamine (100 μM)	638 ± 15 (125)	852 ± 16 (149)
(−)Norepinephrine (100 μM)	620 ± 35 (121)	804 ± 27 (141)
(−)Isoproterenol (100 μM)	515 ± 21†(101)	584 + 12†(102)
Apomorphine (3 μM)	612 ± 16 (120)	840 ± 24 (147)
Sodium fluoride (10 mM)	1122 ± 37 (220)	1113 ± 42 (195)

* After preincubation of brain slices, a crude synaptosomal fraction (P_2) was prepared, subjected to osmotic shock and then centrifuged at 17,000 *g*. After resuspending the particulate fraction in hypotonic medium, the activity of adenylate cyclase in 94 or 250 μg of cortical or striatal protein, respectively, was assayed by initiating the reaction with [^{3}H]ATP (1 μCi, 0.5 mM), as described in Materials and Methods. Each value represents the mean ± S.E. of four determinations. All values except those for isoproterenol are statistically significant in comparison with nontreated controls, at $P < 0.01$.

† Not significantly increased over [^{3}H]cAMP formed in nontreated control.

(about 8 per cent), sodium fluoride at 10 mM produces a small (about 16 per cent), but statistically significant increase in the conversion to [^{3}H]cAMP (Table 5, experiment I). The finding that the removal of albumin (0.1 %) from the hypotonic medium does not alter the data presented in Table 5, experiment I, suggests that the presence of albumin does not protect the synaptosomal preparation from lysis by osmotic shock. However, by subjecting a prelabeled particulate preparation to sonication in hypotonic medium, isoproterenol, norepinephrine and dopamine no longer elicit an increase in [^{3}H]cAMP formation. In these experiments, sonication does not alter the basal conversion rate but does produce a reduction in the accumulation of both newly formed [^{3}H]ATP and [^{3}H]cAMP to about 42 per cent of the nonsonicated particulate controls.

Effects of catechols on [^{3}H]cAMP formation from exogenous [^{3}H]ATP. By incubating a saturating concentration of [^{3}H]ATP with a striatal particulate preparation of a crude synaptosomal fraction, both norepinephrine and dopamine are observed to increase the activity of adenylate cyclase approximately 40–50 per cent, whereas isoproterenol produces no stimulatory effect (Table 6). Furthermore, maximal stimulation of striatal adenylate cyclase activity is reached at 3 μM apomorphine (Table 6), with activity declining to basal levels at 100 μM apomorphine. Striatal homogenates subjected to sonication demonstrated no alteration in the basal synthesis of cAMP and similar sensitivities of the cyclase system to dopamine, norepinephrine and apomorphine. In cerebral cortical homogenates, a small but statistically significant increase of adenylate cyclase activity is observed in response to norepinephrine, dopamine or apomorphine (approximately 20–35 per cent (Table 6). However, catecholamines produced no significant alteration of cAMP formation in hindbrain homogenates. In both cerebral cortical and striatal preparations (Table 6), as well as in the hindbrain, the addition of sodium fluoride to the medium results in an approximate 2-fold stimulation of cAMP formation. Furthermore, trifluoperazine completely antagonized the dopamine-induced stimulation of adenylate cyclase activity (Table 7). The observed stimulatory response to norepinephrine was inhibited in a similar way by trifluoperazine in striatal homogenates. In contrast, propranolol does not alter significantly the augmented accumulation of cAMP elicited by dopamine (Table 7).

Table 7. Effect of propranolol or trifluoperazine on dopamine-induced stimulation of adenylate cyclase activity in striatal homogenates*

Additions	(pmoles [^{3}H]cAMP formed/mg protein/5 min) (% of nontreated control)
None	580 ± 20 (100)
(±)Propranolol (100 μM)	572 ± 36 (99)
Trifluoperazine (50 μM)	588 ± 24 (101)
Dopamine (100 μM)	864 ± 32† (149)
(±)Propranolol (100 μM) + dopamine (100 μM)	844 ± 40‡ (146)
Trifluoperazine (50 μM) + dopamine (100 μM)	592 ± 22§ (102)

* After preincubation of striatal slices, a crude synaptosomal fraction (P_2) was prepared, homogenized in hypotonic medium, and assayed for adenylate cyclase activity in about 250 μg protein by addition of [^{3}H]ATP (1 μCi, 0.5 mM) as described in Materials and Methods. Each value represents the mean ± S. E. of four determinations.

† Significantly increased over [^{3}H]cAMP formed in nontreated control, at $P < 0.01$.

‡ Not significantly different from [^{3}H]cAMP formed with addition of dopamine alone.

§ Not significantly increased over [^{3}H]cAMP formed

DISCUSSION

Employing [^{3}H]adenine as a precursor for labeling ATP, the following evidence suggests the presence of a β-adrenergic receptor-coupled adenylate cyclase in osmotically shocked, crude synaptosomal fractions of the corpus striatum. First, the β-hydroxylated catecholamines, isoproterenol, norepinephrine and epinephrine appear to exhibit greater potencies than dopamine, with the calculated EC_{50} values for (−)-isoproterenol and (−)norepinephrine being 0.01 and 1.0 μM respectively. Second, the cAMP-generating system also seems to display stereoselectivity, with the (−)isomer of isoproterenol being more potent than its (+)isomer and, thereby, meets another criterion identified with the β-adrenoreceptor. Finally, the mine and, that these stimulatory responses are in-*N*-isopropyldopamine, appears to be more potent than dopamine is again in agreement with reported findings in striatal slices [6] and for other β-type systems, such as erythrocyte membranes [4]. Although the maximum stimulatory response to catecholamines (about 154 per cent of controls) is much lower than that previously reported for striatal slices (about 300 per cent) [6], similar EC_{50} values were obtained for the stimulatory potencies of catecholamines in both slices and osmotically shocked crude synaptosomal fractions. One possible explanation for the decreased maximal stimulation in response to catecholamines may be the marked increase in the basal levels of newly formed [^{3}H]cAMP in cell-free preparations; this may be the result of the stimulatory effect of endogenous catecholamines, which are released during homogenization in hypotonic medium. Moreover, the following evidence suggests an interaction of dopamine and the β-hydroxylated catecholamines with the same β-type system. First, no additive stimulatory effect is demonstrated when dopamine is co-incubated with an optimal concentration of isoproterenol. Second, the β-adrenergic receptor blocker, propranolol, is shown to antagonize completely cAMP accumulation generated by dopamine as well as by norepinephrine. In contrast, trifluoperazine, a phenothiazine which is relatively selective in blocking the dopamine receptor [17–19], produced no significant alteration of the [^{3}H]cAMP generated by dopamine. In homogenates of another brain region, such as the cerebral cortex or hindbrain, which reportedly is sparsely innervated [12] or devoid [11] of dopaminergic terminals, respectively, dopamine is still capable of eliciting significant increases in cAMP formation. Finally, the β-type

responsiveness to catecholamines in homogenates is similar to that reported in striatal slices in which newly formed [^{14}C]cAMP or endogenous cAMP was assayed [6]. More recent observations in striatal slices show that an approximate 2-fold increase of endogenous cAMP elicited by 100 μM dopamine is antagonized completely by 10 μM propranolol but not by 10 μM trifluoperazine. In agreement with these findings, Sheppard and Burghardt [20] have presented preliminary data which demonstrate that the order of potency for stimulating endogenous cAMP production in isotonic homogenates of the rat caudate nucleus is isoproterenol $\gg$ norepinephrine > dopamine and, that these stimulatory responses are inhibited by 1 μM propranolol. These effects upon the levels of endogenous cAMP in both striatal slices and isotonic homogenates are similar to those obtained employing the prelabeling technique in which the percentage conversion of newly formed [^{14}C]ATP to [^{14}C]cAMP was calculated [6] and thus suggest the equivalence of the two methods for measuring cAMP formation. However, contrasting results have been reported by Forn *et al.* [5] in which the dopamine-induced increase of endogenous cAMP in striatal slices was inhibited markedly by fluphenazine (100 μM) but unaltered by propranolol (100 μM). At the present time. we are unable to explain the apparent discrepancies between our findings and those of Forn *et al.* [5]. On the basis of present findings and those noted in a previous report with brain slices and crude synaptosomal fractions [6], it seems likely that the stimulatory effect of dopamine on the conversion of newly formed ATP into cAMP in broken-cell preparations is mediated by an interaction with a β-type system rather than a specific dopamine receptor-coupled adenylate cyclase.

Further attempts resulted in localizing the β-type activity to particulate preparations of a crude synaptosomal fraction. This preparation of synaptosomal membranes is capable of synthesizing ATP from adenine and exhibiting responses to β-agonists, which are characteristic of a β-adrenoreceptor-linked adenylate cyclase. Furthermore, high concentrations of ATP do not alter the augmented accumulation of [^{3}H]cAMP in response to the β-adrenergic agonist, isoproterenol. This apparent inability of exogenously added ATP to decrease the specific activity of the newly formed [^{3}H]ATP suggests that the particulate fraction of [^{3}H]ATP, which serves as the substrate of adenylate cyclase, is probably synthesized in a strategic, membrane-bound pool, which is inaccessible to exogenously administered ATP. This is further supported by the finding that fluoride ions produce only minimal increases in [^{3}H]cAMP formation and in [^{3}H]ATP retained in the osmotically shocked, particulate preparation. The possibility that the fluoride ion penetrates cell membranes slowly might explain its failure to either stimulate or inhibit a membrane-enclosed component of adenylate cyclase or adenosine triphosphatase respectively. Nevertheless, the apparent lysis of intact, vesicle-filled synaptosomes during homogenization in hypotonic medium is supported by preliminary observations obtained from electron micrographs which appear to show plasma membranes of ghost-like disrupted synaptosomes which have lost their vesicular contents. In agreement with these observations is the finding that after osmotic shock of the synaptosomal fraction, the 17,000 g particulate fraction which contains the β-type system retains only 13 and 25 per cent of the newly formed [^{3}H]ATP and [^{3}H]cAMP respectively. Finally, preincubation of the particulate fraction at 37° for 30 min in hypotonic medium did not alter the β-stimulatory effect, suggesting that lysis of the membrane compartment containing the cyclase system does not appear to be a time- or temperature-dependent variable.

To test this model of the β-type system which depicts the localization of the β-adrenoreceptor-linked adenylate cyclase in a membrane-enclosed compartment, striatal homogenates first were incubated with [^{3}H]adenine and subsequently were subjected to sonication in hypotonic medium. The adenylate cyclase of a sonicated preparation appears to lose its sensitivity to the β-adrenergic agonist, isoproterenol, as well as to norepinephrine and dopamine. One possible explanation of these findings is that the loss of responsiveness of the β-type system during sonication may result from a possible disruption of the membrane-bound complex, causing the functional uncoupling of adenylate cyclase from the β-adrenergic receptor. Moreover, during sonication, newly formed [^{3}H]ATP may be released from its membrane-enclosed compartment into a free pool, which can no longer serve as substrate for the β-adrenergic receptor-coupled adenylate cyclase.

To test this hypothesis further, the properties exhibited by striatal homogenates were examined with another assay procedure, in which exogenous [^{3}H]ATP was utilized to measure adenylate cyclase activity in broken-cell preparations. The adenylate cyclase activity was assayed by employing a saturating concentration of [^{3}H]ATP (0.5 mM) to initiate the reaction. Dopamine and norepinephrine produced about a 40–50 per cent increase in [^{3}H]cAMP formation in striatal homogenates, whereas isoproterenol was without stimulatory effect. Maximal stimulation by the dopamine receptor agonist, apomorphine, was reached at 3 μM, declining to control values at 100 μM apomorphine. Furthermore, the dopamine- and norepinephrine-induced increases of adenylate cyclase activity in striatal homogenates were antagonized completely by trifluoperazine but not by propanolol. These results are in good agreement with previous findings reported by several groups of investigators [3, 4, 17–19], who utilized saturating concentrations of exogeneous ATP to measure adenylate cyclase activity. Contrasting findings have been reported [2], which demonstrate a slight stimulatory response to isoproterenol (approximately 20 per cent) in crude striatal homogenates. Unfortunately, these authors made no attempt to delineate the procedural differences in the assay of adenylate cyclase, which might explain the failure of other laboratories to observe a stimulatory effect with isoproterenol. Moreover, in the cerebral cortex, which reportedly contains dopaminergic terminals [12], and exhibits properties characteristic of α- and β-type systems [21, 22], a slight but statistically significant increase in adenylate cyclase activity is observed in response to norepinephrine, dopamine and apomorphine. These results, which are consistent with previous findings [14, 23],

suggest the possibility of a dopamine component of the adenylate cyclase response to catecholamines in cerebral cortical homogenates, when assayed with exogenous [^{3}H]ATP. In contrast, no stimulatory response is elicited by catecholamines in homogenates of the hindbrain, which is relatively devoid of dopaminergic terminals [11] but does possess binding sites for the β-adrenergic receptor [10] as well as a β-type cyclase system [6, 22]. Finally, sodium fluoride produces a marked 2-fold stimulation of [^{3}H]cAMP formation, and sonication of the tissues does not alter the stimulatory response to either dopamine or norepinephrine. Thus, it seems evident that the particulate component of a crude synaptosomal preparation of striatal adenylate cyclase, which utilizes exogenous ATP as substrate, is subject to stimulation by the fluoride ion and demonstrates properties characteristic of a dopamine receptor-coupled adenylate cyclase. In contrast, the membrane-enclosed particulate component of a crude synaptosomal preparation of adenylate cyclase, which utilizes endogenously synthesized ATP as substrate, is relatively insensitive to the fluoride ion but does conform to criteria identified with a β-adrenoreceptor-linked adenylate cyclase. Consistent with these observations, Sheppard and Burghardt [20] have similarly proposed the existence of separate compartments for the β-adrenoreceptor- and dopamine receptor-adenylate cyclase systems. Finally, it is possible that the β-type system is buried in the matrix of the synaptosomal membranes, whereas the striatal dopamine receptor-adenylate cyclase complex is localized either on postsynaptic membranes [24–26] or on glial cells [27], which contaminate synaptosomal fractions.

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