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Comparative study of the dopamine-sensitive adenylate cyclase in the striatum and hypothalamus of rat brain

B. D. ROUFOGALIS*, M. THORNTON AND D. N. WADE

Department of Clinical Pharmacology, St. Vincent's Hospital, Sydney, N.S.W. 2010, Australia

Stimulation by dopamine of adenylate cyclase in homogenates of rat brain striatum was enhanced in the presence of ATP (0·6–3 mM) and GTP (10–100 μM). The stimulation by dopamine appeared to be the result of its antagonism of inhibition of adenylate cyclase by GTP or higher concentrations of ATP. Stimulation of the enzyme by dopamine was also dependent on $MgCl_2$, and was maximal at $MgCl_2$ concentrations of at least two fold excess over ATP. While ATP did not inhibit the adenylate cyclase in homogenates of the ventral hypothalamus, GTP (10–100 μM) significantly stimulated it. Dopamine stimulated the adenylate cyclase in the hypothalamus. This action was blocked by chlorpromazine (10 μM) and phentolamine (100 μM) but not by an analogue of chlorpromazine having no neuroleptic activity or by propranolol (100 μM).

Dopamine-sensitive adenylate cyclase (ATP: pyrophosphate lyase (cyclizing) EC 4.6.1.1.) occurs in various areas of the rat brain, and its distribution appears to correlate with dopaminergic innervation of these areas (Kebabian et al 1975). In the striatum the inhibition by phenothiazine antipsychotic agents of the adenylate cyclase activity stimulated by dopamine (Kebabian & Greengard 1971; Iversen 1975) suggests a similarity between the dopamine-sensitive adenylate cyclase and dopamine receptors of the extrapyramidal system. Dopaminergic innervation has been demonstrated in the median eminence of the hypothalamus (Björklund et al 1973; Kavanagh & Weisz 1973; Ungerstedt 1971), and a dopamine-sensitive adenylate cyclase was described recently in the hypothalamus (Roufogalis et al 1975) and the median eminence (Clement-Cormier & Robison 1977). Because of the different function of dopamine in the striatum and hypothalamus (see Snyder 1972) and the apparent differences in neuronal feedback mechanisms in the two brain areas (Martin, 1973; Moore & Gudelsky 1977), we have compared the properties of dopamine stimulated adenylate cyclase in the striatum and the ventral hypothalamus. The sensitivity of the adenylate cyclases to dopamine, nucleotides, $MgCl_2$, adrenergic blocking drugs and antipyschotic phenothiazines is also described.

* Correspondence and permanent address: Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

MATERIALS AND METHODS

Materials

ATP (disodium salt), GTP, phosphoenolpyruvate (trisodium salt), pyruvate kinase (645 units mg^{-1} of protein), theophylline, bovine serum albumin, ethyleneglycol-bis-(β-aminoethylether)-*NN'*-tetra-acetate (EGTA), dopamine (3-hydroxytyramine) hydrochloride, tris (Trizma base) were purchased from Sigma Chemical Co. Chlorpromazine hydrochloride was a gift from May and Baker and the chlorpromazine analogue was kindly supplied by Dr A. R. Green (Smith, Kline and French, Australia). Phosphatidylserine (bovine) and phosphatidylinositol (plant) were obtained from Applied Science Labs. Cyclic AMP kits were obtained from the Radiochemical Centre.

Preparation of adenylate cyclase homogenates

Male Fullensdorff Albino rats, 200–300 g, after stunning and cervical dislocation, were decapitated and the brains rapidly excised at room temperature (20°C) and the brainstem and cerebellum of each were removed. The striatum (caudate and putamen, approximately 30 mg) was exposed from each hemisphere by peeling away the cerebral cortex with curved forceps. A ventral section of the hypothalamus was removed by making a medial incision at the optic chiasma and hemisecting the brain. A section approximately 1·5 mm deep and 4 mm long was removed with a fine scalpel blade (Palkovits et al 1974). The weight of hypothalamus removed from each hemisphere was approximately

15 mg (Dismukes et al 1974). Tissues were homogenized at 4 °C in 25 vol of 2 mM EGTA and 7 μg ml^{-1} phosphatidylserine in a glass homogenizer, the Teflon pestle being passed through the suspension 9–11 times in 2 min at 400 rev min^{-1}. The homogenates were kept in ice for 30–45 min because we found this to enhance the effect of added dopamine. Samples (30 μl) containing 150–300 μg of protein were used in the subsequent assays of adenylate cyclase activity. Protein contents were determined by the method of Lowry et al (1951), using bovine serum albumin as standard.

Assay of adenylate cyclase

Adenylate cyclase activity was assayed essentially as described by Clement-Cormier et al (1974) in a medium containing 80 mM tris-maleate buffer (pH 7·4 at 30° C), 6 mM $MgCl_2$ (unless otherwise stated), 0·2 mM EGTA, 10 mM theophylline, 2·5–5 μg of phosphatidylserine (and 12 μg ml^{-1} of phosphatidylinositol in the assay of homogenate of hypothalamus), 10 mM phosphoenolpyruvate, 4 μg pyruvate kinase, 15 mM ammonium sulphate (from the pyruvate kinase) and the required concentration of ATP, in a final volume 0·5 ml. Dopamine and nucleotides were added to the homogenate in this medium at 0° C for 10 min before the enzymic reaction was begun. In experiments, where the concentrations of ATP or $MgCl_2$ were varied, the reaction was initiated by the addition of the suspension of tissue homogenate. The tubes were incubated in a water bath at 30 °C for 2·5 min, and the reaction was stopped by heat at 100 °C for 2·5 min. With the experimental conditions used, enzyme activity was linear with respect to time and protein concentration. After centrifugation of the tubes for 10 min at 0–4° C, samples (50 μl) were removed for assay of cyclic (c) AMP by the method of Brown et al (1971). Neither ATP nor GTP at the concentrations used interfered with the assay. A standard curve was obtained by adding 1–6 pmol of cAMP to the enzyme homogenates in the absence of ATP. The concentration of theophylline used was sufficient to prevent any measurable hydrolysis of cAMP by endogenous phosphodiesterases (Katz & Tenenhouse 1973). Results are expressed as means ± standard error and significance was calculated using the Student's *t*-test.

RESULTS

Effect of nucleotides

The effect of ATP on basal adenylate cyclase was determined in homogenates of striatum and hypothalamus, in which the $MgCl_2$ was constant. ATP concentrations greater than 0·3 mM inhibited the basal adenylate cyclase of homogenates of striatum (Fig. 1A). In contrast, the basal activity of homogenates of hypothalamus showed a hyperbolic dependence on ATP, except at the highest ATP concentration (3 mM) where a slight inhibition occurred in every experiment (Fig. 1B). Since guanyl nucleotides regulate the activity of adenylate cyclase in some tissues, the effect of GTP on basal

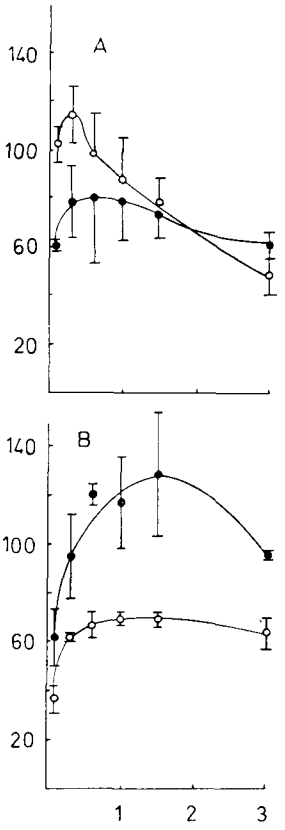

FIG. 1. Effect of ATP and GTP on adenylate cyclase activity. A: striatum; B: hypothalamus. GTP was added to the incubation mixtures at 0–4° C no longer than 10 min before starting the reaction with the enzyme. $MgCl_2$ was 6 mM. ATP was varied in the absence of GTP (○—○) and in the presence of 100 μM GTP (●—●). In A the control curve represents the mean ± s.e. of experiments from three rats and curves in the presence of GTP are the mean ± range of duplicate experiments. In B error bars represent the standard error of the mean of experiments from three rats (○—○) or the range of results from homogenates from two rats (●—●). The wide error bars resulted from the variation in absolute activities of adenylate cyclase in different rats. Ordinate: cAMP (pmol mg^{-1} min^{-1}). Abscissa: ATP (mM).

adenylate cyclase was compared in homogenates of striatum and hypothalamus. In striatal homogenates, GTP (10 and 100 μM) inhibited basal adenylate cyclase activity at low ATP concentrations, but not at high ATP concentrations (Fig. 1A; Table 1), while in hypothalamic homogenates the basal activity was significantly increased by GTP (100 μM), both at high and low ATP concentrations (Fig. 1B).

Although the different susceptibilities of the homogenates of striatum and hypothalamus to inhibition by ATP made it unlikely that the inhibition was simply due to chelation of Mg^{2+} by ATP, this possibility was tested by investigating the effect of ATP at various $MgCl_2$ concentrations. In homogenates of striatum and of hypothalamus, inhibition by ATP was observed at $MgCl_2$ concentrations from 1–9 mM (Fig. 2A, B). The differences in the effects of GTP in the two homogenates were also independent of $MgCl_2$ concentration (not shown). The inhibition of basal adenylate cyclase by ATP in homogenates of striatum which we obtained in this study appears to disagree with the results of Clement-Cormier et al (1975), who found that adenylate cyclase activity increased as the ATP concentration was increased. However, they maintained the Mg: ATP ratio at 4: 1. In these conditions we obtained a similar result, but the contribution of the progressive increase of the $MgCl_2$ to the activity of the enzyme is difficult to assess (see Fig. 3A).

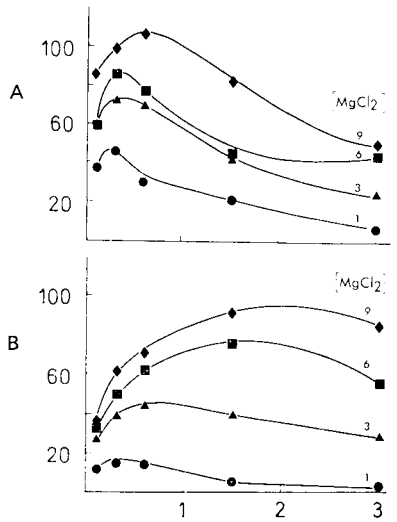

FIG. 2. Effect of $MgCl_2$ on ATP inhibition of adenylate cyclase, in homogenates of the striatum (A) and the hypothalamus (B). ATP was varied at 1 mM $MgCl_2$ (●—●); 3 mM $MgCl_2$ (▲—▲); 6 mM $MgCl_2$ (■—■); and 9 mM $MgCl_2$ (◆—◆). The curves represent a typical experiment on the same homogenate. Ordinate: cAMP (pmol mg^{-1} min^{-1}). Abscissa: ATP (mM).

Dopamine stimulation

In agreement with previous studies, the adenylate cyclase activity of striatal homogenates was stimulated by dopamine (1–50 μM). The dependence on ATP and GTP of this stimulation by dopamine was also investigated. The per cent stimulation of striatal adenylate cyclase by dopamine increased progressively with increase in ATP concentration (Table 1). The effect of GTP (10 μM) on the enyzme's stimulation by dopamine, calculated as the per cent increase over corresponding controls also containing GTP (10 μM), is also shown in Table 1. When compared with the enzyme's stimulation by dopamine in the absence of GTP, the 10 μM GTP markedly enhanced dopamine stimulation at low ATP concentrations, but only marginally at high ATP concentrations. Since inhibition by GTP is significant only at low ATP concentrations, it appears that the stimulation by dopamine under these conditions involves antagonism of GTP-induced inhibition.

As $MgCl_2$ also controls adenylate cyclase activity, the effect of its concentration on both basal and dopamine stimulated adenylate cyclase activity was investigated in homogenates of striatum. In the presence of dopamine the apparent affinity of adenylate cyclase for $MgCl_2$ was enhanced, and the concentration of $MgCl_2$ which maximally stimulated the enzyme activity was lowered, at each of the ATP concentrations examined (Fig. 3A). The increment in cAMP due to enzyme stimulation by dopamine depended on $MgCl_2$ concentration and followed a normal distribution (Fig. 3B). Maximum increase in adenylate cyclase activity by dopamine occurred at a Mg: ATP ratio of 2: 1 or greater. The results in Fig. 3B illustrate again that the absolute dopamine stimulation increased as the ATP concentration increased.

As dopaminergic pathways innervate areas of the hypothalamus (Björklund et al 1973; Kavanagh & Weisz 1973; Ungerstedt 1971), and as dopamine has been shown to stimulate cAMP formation in hypothalamic slices (Gunaga & Menon 1973), we investigated the sensitivity to dopamine of adenylate cyclase activity in homogenates of hypothalamus. Dopamine stimulated the enzyme activity in the hypothalamus, but the extent of the stimulation varied among different groups of rats. Enzyme

Table 1. Stimulation by dopamine of adenylate cyclase in homogenates of striatum at different nucleotide concentrations.

ATP (mM)	NO GTP Basal	NO GTP Dopamine (50 μM)	NO GTP % stimulation	GTP (10 μM) Basal	GTP (10 μM) Dopamine (50 μM)	GTP (10 μM) % stimulation
0·1	102·2 ± 7·7	105·2 ± 10·1 n.s.	2·9	62·3 ± 9·4**	129·8 ± 0·2**	108
0·3	114·7 ± 12·0	138·7 ± 15·0 n.s.	20·9	73·7 ± 12·3	164·3 ± 2·2	123
0·6	98·2 ± 17·6	151·5 ± 18·9*	54·3	78·7 ± 10·3	153·8 ± 9·3	95
1·0	88·0 ± 17·5	142·5 ± 19·1*	61·9	68·3 ± 2·2	168·1 ± 1·6	146
1·5	78·8 ± 10·0	141·5 ± 9·0*	79·6	50·2 ± 1·6	149·6 ± 0·7	198
3·0	48·4 ± 7·5	123·4 ± 18·7*	155·0	46·8 ± 0·6	100·3	114

$MgCl_2$ was kept constant at 6 mM and all other assay conditions are as described in Materials and Methods.
* $P<0·025$, paired t-test; n.s., not significant ($P>0·05$).
** Range of duplicate experiments.

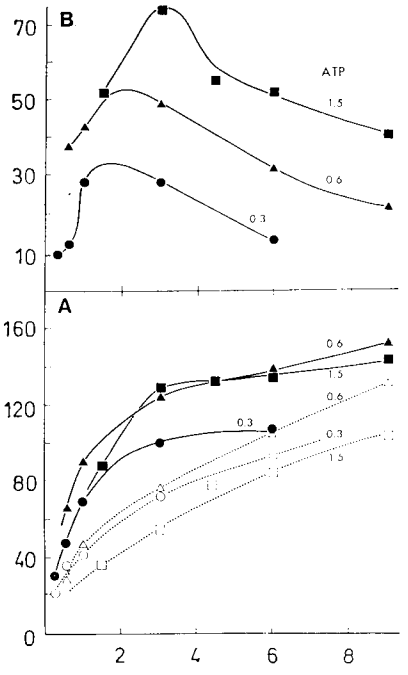

FIG. 3. Effect of $MgCl_2$ on dopamine stimulation of adenylate cyclase at various ATP concentrations in homogenates of the striatum. A: total activity in the presence (solid lines) and absence (dotted lines) of dopamine; B: increment due to dopamine stimulation. The ATP concentration was 0·3 mM in the absence (○—○) and the presence of 50 μM dopamine (●—●); 0·6 mM in the absence (△—△) and in the presence of 50 μM dopamine (▲—▲); and 1·5 mM in the absence (□—□) and in the presence of 50 μM dopamine (■—■). The curves represent the mean of experiments from 3 rats. Error bars are omitted for clarity. The s.e.'s were from 5–20% of the mean, with greater variability at the lowest $MgCl_2$ concentrations. Ordinates: (A) cAMP (pmol mg^{-1} min^{-1}) and (B) dopamine stimulation (pmol mg^{-1} min^{-1}). Abscissa: $MgCl_2$ (mM).

stimulation from 100% to as low as 5% was obtained during the study. Recently, Clement-Cormier & Robison (1977) reported maximal stimulation of around 77% in homogenates of rat median eminence. In preliminary studies it was found that when phosphatidylserine was added to homogenates of striatum or ventral hypothalamus, and phosphatidylinositol added to hypothalamus homogenates, they did not interfere with basal adenylate cyclase activity and enhanced dopamine stimulation slightly although not significantly (in contrast to phosphatidylcholine which significantly depressed both basal and dopamine sensitive adenylate cyclase activity). Although the affect of dopamine on the enzyme was not absolutely dependent on the presence of these phospholipids, they were included routinely in the assays, since they have been implicated in catecholamine sensitivity of other tissues (Sutherland et al 1962; Levey 1970; Birnbaumer et al 1971). The presence of the phospholipids did not alter the qualitative effects obtained with ATP, GTP and $MgCl_2$.

Stimulation by dopamine of the basal adenylate cyclase in hypothalamus homogenates did not require GTP or high concentrations of ATP. The dependence of the dopamine effect on $MgCl_2$ when plotted appeared to give a hyperbolic curve rather than a normal distribution as with the striatum (not shown). The specificity of the dopamine receptor responsible for the dopamine effect in homogenates of hypothalamus was examined. Dopamine's stimulatory action was blocked by chlorpromazine (10 μM) and phentolamine (100 μM), but not by the 1-chloro analogue of chlorpromazine (10 μM) which is not a neuroleptic (Roufogalis et al 1976b) or propranolol (100 μM) (Fig. 4).

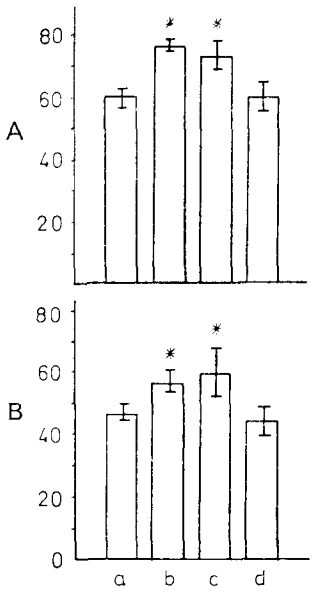

FIG. 4. Antagonism of dopamine stimulation of adenylate cyclase in homogenates of hypothalamus. ATP was 1·5 mM and $MgCl_2$ was 6 mM. A: n = 6 — 12 (6 rats). Neither propranolol nor phentolamine significantly affected the basal level, which was 57·6 ± 6·3 with propranolol (100 μM) and 65·3 ± 6·2 for phentolamine (100 μM). Abscissa: a, basal; b, dopamine (50 μM); c, dopamine (50 μM) plus propranolol (100 μM); d, dopamine (50 μM) plus phentolamine (100 μM). B: n = 3 — 6 (3 rats). a, basal, b, dopamine (50 μM); c, dopamine (50 μM) + 1-chloro analogue of chlorpromazine (10 μM); d, dopamine (50 μM) plus chlorpromazine (10 μM). *Dopamine stimulation significant at $P<0·05$, unpaired t-test. Ordinate: cAMP (p mol mg^{-1} min^{-1}).

DISCUSSION

Previous studies in a variety of tissues have demonstrated the regulation of adenylate cyclase by Mg^{2+}, ATP, nucleotides, Ca^{2+}, pH (Clement-Cormier et al 1975; Tell et al 1975; Rodbell 1975; Birnbaumer et al 1974; Londos et al 1974; Birnbaumer & Yang 1974; Lefkowitz 1974; Ebert & Schwabe 1974; Londos & Rodbell 1975; Pfeuffer & Helmreich 1975; Roufogalis et al 1976a) and Ca^{2+}-dependent protein activator (Lynch et al 1976; Brostrom et al 1975; Gnegy et al 1976). As found by earlier workers, we have found the effects of GTP and dopamine on adenylate cyclase only at Ca^{2+} concentrations maintained at the μM level or less in EGTA (unpublished observations). The results of the present study indicate that a variety of factors determine the extent of stimulation by dopamine of adenylate cyclase activity in homogenates of rat striatum. ATP and GTP as reported by others (Clement-Cormier et al 1975; Tell et al 1975) inhibited the basal adenylate cyclase activity. Although the mechanism of inhibition of the enzyme by ATP and GTP is unknown, in tissues other than those used here, the inhibition appears to result from binding of free ATP and GTP at an inhibitory allosteric site (Birnbaumer et al 1974), a mechanism that is consistent with the present results, which show more inhibition by ATP at low $MgCl_2$ concentrations compared with high concentrations (Fig. 3). Stimulation of adenylate cyclase by dopamine was most evident in conditions where the enzyme was inhibited by GTP or ATP. Optimal stimulation by dopamine of cAMP formation also depended on the $MgCl_2$ concentration and was greatest when the $MgCl_2$ was in excess of ATP. The relevance of these observations to the regulation of cAMP levels by dopamine in the striatum in vivo requires further studies on intact tissue. Clearly, the extent of dopamine's stimulatory effect will depend on the relative balance between the concentrations of ATP, GTP (or other nucleotides), Mg^{2+} and Ca^{2+} in the prevailing physiological conditions. It is also difficult to assess the extent to which dopamine's stimulatory effect in vivo results from a direct increase in adenylate cyclase activity or from antagonism of the inhibitory actions of nucleotides (De Haen 1974; Hammes & Rodbell 1976). Stimulation of striatal adenylate cyclase, as a result of apparent reversal of the inhibition caused by GTP, was also suggested by Clement-Cormier et al (1975) and Tell et al (1975). In synaptosomal preparations of striatum, dopamine's stimulation of adenylate cyclase, although absolutely dependent on the presence of GTP or related nucleotides, occurred in the absence of any apparent inhibition by nucleotide of 'basal' activity (Roufogalis et al 1976a).

Dopamine's stimulation of adenylate cyclase in homogenates of ventral segments of hypothalamus, although variable, was consistent with the activation of a characteristic dopamine receptor. The stimulation did not result from antagonism of nucleotide effects, since ATP did not inhibit the basal adenylate cyclase activity, while GTP significantly stimulated this activity. However, the possibility that dopamine stimulated different pools of adenylate cyclase in distinct hypothalamic areas cannot be eliminated. The inhibition of the dopamine stimulated adenylate cyclase in hypothalamic homogenates by neuroleptic phenothiazines but not by phenothiazines having no neuroleptic activity, parallels the ability of chlor-

promazine and other neuroleptic drugs to control the release of prolactin and growth hormone, thought to result from the blockade of dopamine receptors in the median eminence of the hypothalamus (Martin 1973; Wilson 1974).

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