# BRAIN AND CAUDATE NUCLEUS ADENYLATE CYCLASE: EFFECTS OF DOPAMINE, GTP, E PROSTAGLANDINS AND MORPHINE

G. P. TELL*, G. W. PASTERNAK and P. CUATRECASAS

*Department of Pharmacology and Experimental Therapeutics and Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 USA*

Received 19 December 1974

## 1. Introduction

Although specific membrane-bound receptors for morphine have been demonstrated in brain [1–4], the nature of the immediate biochemical events which follow the interaction of morphine with these receptors remains hypothetical. It has been suggested [5] that morphine and some morphine-like drugs can reverse the stimulation of cyclic AMP formation induced by prostaglandins in rat brain homogenates. We have tried to compare the effects in this preparation with those obtained in the dopamine-sensitive rat caudate homogenate [6]. Dopamine has been shown to stimulate modestly the adenylate cyclase activity of homogenates from basal ganglia [6], superior cervical ganglion [7], corpus striatum [8–10], cortex [11], retina [12] and nucleus accumbens and olfactory tubercle [13,14].

Using various assay conditions, neither prostaglandins ($E_1$ and $E_2$) nor morphine altered the activity of adenylate cyclase (measured with $^{32}$P-ATP) in brain or caudate homogenates. The nucleotide, GTP, which has been shown to play an important role in regulating the stimulation of adenylate cyclase by several hormones in various tissues [15–22], reproducibly inhibits the basal adenylate cyclase activity of caudate nucleus homogenates. The inhibitory effect of GTP is reversed by dopamine. This effect of dopamine is more profound and reproducible than is the stimulation of adenylate cyclase by dopamine.

* Present address: INSERM Rech. Endocr. Enfant., Hopital Debrousse, 69322 Lyon, Cedex 1, France.

## 2. Materials and methods

Brain homogenates from Sprague Dawley male rats (120–150 g) were prepared as described by Collier and Roy [5]. Brains were homogenized (seven strokes in an Elvehjam-type homogenizer with a teflon pestle) in 10 vol (v/w) of cold (4°C) buffer containing 50 mM Tris–HCl, pH 7.4, 250 mM sucrose, 1 mM $MgCl_2$, 25 mM KCl, 25 mM NaCl, 0.1 mM EDTA and 1.2 mM 2-mercaptoethanol. Ten $\mu$l of the homonate were assayed for adenylate cyclase activity in a total volume of 0.15 ml of a medium containing 50 mM Tris–HCl, pH 7.4, 2mM $MgCl_2$, 10 mM KCl, 10 mM NaCl, 0.015% BSA, 3 mM cAMP and 1 $\mu$M ATP. Both $^3$H-labelled ATP ($2.5 \times 10^6$ cpm) [5] and $\alpha$-$^{32}$P-ATP ($5 \times 10^6$ cpm) were used in separate experiments; similar data were obtained with these. After incubating the samples at 30°C for 5 to 20 min, the reaction was stopped by boiling the samples for 2 min. The labelled cAMP formed was purified by chromatography on alumina columns (23); the results were verified by isolation of cAMP by Dowex (50W × 4) chromatography followed by a double $Ba(OH)_2$ – $Zn\,SO_4$ precipitation [24]. $^3$H-Naloxone binding studies were performed as previously described [3].

For the preparation of caudate nuclei homogenates, Sprague Dawley male rats (120–150 g) were decapitated and the caudates were quickly dissected and homogenized (7 strokes in an Elvehjam-type homogenizer with a teflon pestle) in 2 mM Tris–maleate, pH 7.4, 2 mM EGTA (usually 4 caudates in 1.5 ml of the hypotonic buffer) at 4°C. Adenylate cyclase activity was assayed using 20 $\mu$l of the homogenate in a final volume of 0.1 ml containing 80 mM Tris–maleate,



*North-Holland Publishing Company – Amsterdam*

pH 7.4, 1.5 mM ATP, 6.0 mM $MgSO_4$, 10 mM theophylline, and 0.6 mM EGTA (including the EGTA added with the homogenate). We did not measure the cAMP formed by the method of Gilman [25] but used instead labelled ATP ([$\alpha$-$^{32}$P]ATP, $5 \times 10^6$ cpm per assay) which was synthesized as described by Symons [26,27] and purified (> 95% ATP) by paper chromatography (Bennett and Cuatrecasas, unpublished). After incubating the samples at 30°C for 2.5 min, the reaction was stopped in a boiling water bath for 2 min; 1 ml of [8-$^3$H] cAMP (30 000 cpm) was added to each tube to allow calculations of recovery, and the $^{32}$P-cAMP formed was purified by chromatography on alumina [23]. Over 95% of the $^{32}$P-product formed was identified as cAMP by DEAE column chromatography (Siegel and Cuatrecasas, unpublished).

## 3. Results and discussion

We were unable to demonstrate any activation of adenylate cyclase by $PGE_1$ or $PGE_2$ (0.1 to 25 $\mu$g/ml), or any effect of morphine (1.5 and 10 $\mu$g/ml), in rat brain homogenates assayed under the conditions described by Collier and Roy [5]. Since the very low basal activity (0.5–2 pmol/min/mg fresh brain) observed under these conditions could be explained by the low concentration (1 $\mu$M) of ATP used, we studied the effects of adding an ATP regenerating system (5 mM phosphoenolpyruvate and 60 $\mu$g/ml of pyruvate kinase) and of increasing the concentration of ATP. The ATP regenerating systems increased by 10-fold the activity measured with 1 $\mu$M ATP. Combining 50 $\mu$M ATP and the ATP regenerating system resulted in a 50-fold increase in enzyme activity. Even under these conditions, $PGE_1$ and/or morphine did not affect the activity of brain homogenates. The binding of $^3$H-naloxone (1 mM) was identical to that seen in brain membranes prepared by other means [1,3] and $PGE_1$ had no effect on the binding of $^3$H-naloxone in either the cyclase assay preparation or in normal binding assay homogenates. Homogenates of ileum prepared in the same way as the brain (except for homogenization, performed with a Polytron for 30 sec) were responsive to $PGE_1$. Using the basic assay conditions (50 $\mu$M ATP and regenerating system), 10 $\mu$g per ml of $PGE_1$ stimulated the adenylate cyclase activity of the ileum homogenates from 22.8 ± 0.7 to 33.7 ± 0.8 pmol cAMP/min/mg fresh tissue. However, no effect of morphine could be detected in this system.

Since under the conditions described above the brain homogenate was sensitive to 10 mM NaF (usually two-fold stimulation) but not to $10^{-3}$ to $10^{-6}$ M dopamine, we examined the dopamine sensitive caudate preparation described by Kebabian and colleagues [6,13]. Dopamine *usually* stimulated the adenylate cyclase activity of caudate homogenates with maximal effects (50–90% stimulation) occurring with $10^{-4}$ M dopamine. However, the results were extremely variable, reproducibility was poor and dopamine was less effective when the homogenate was washed (quickly) by suspension in 10 vol of buffer followed by centrifugation.*

In attempts to increase the sensitivity of the dopamine effect, GTP was examined. GTP always inhibited the basal cyclase activity in fresh as well as in washed (twice) caudate homogenates. Maximal inhibition (30–40%) was obtained with $10^{-4}$ M GTP (table 1). Dopamine very reproducibly reversed the inhibition of adenylate cyclase activity by GTP. Maximal reversal (80–100%) of the inhibition caused by $10^{-4}$ M GTP was obtained with $10^{-4}$ M dopamine (table 2). In those experiments where dopamine stimulated the basal activity (e.g., by 80%), the inhibitory effect of $10^{-4}$ M GTP was still observed and the

Table 1
Effect of GTP on adenylate cyclase activity of rat caudate homogenates

| GTP concentration | Adenylate cyclase activity* |
|---|---|
| 0 | 80.2 ± 4.6 |
| $10^{-5}$ M | 72.4 ± 3.4 |
| $5 \times 10^{-5}$ M | 63.2 ± 2.3 |
| $10^{-4}$ M | 53.6 ± 1.4 |
| $5 \times 10^{-4}$ M | 54.2 ± 1.5 |

* pmol of cAMP per min per mg of fresh caudate, ± standard error of the mean.

* The inability of certain studies [28–30] to demonstrate dopamine stimulation of adenylate cyclase activity may be related to this lack of reproducibility, which here was studied with a very sensitive and specific assay which used $^{32}$P-ATP as substrate.

Table 2
Effect of dopamine on the GTP-inhibited adenylate cyclase activity of rat caudate homogenates

| Addition | | Adenylate cyclase activity* |
|---|---|---|
| GTP | Dopamine | |
| 0 | 0 | 86.6 ± 4.3 |
| 0 | $10^{-6}$ M | 86.7 ± 3.9 |
| 0 | $10^{-5}$ M | 96.6 ± 5.2 |
| 0 | $10^{-4}$ M | 101.5 ± 4.1 |
| $10^{-4}$ M | 0 | 66.6 ± 3.2 |
| $10^{-4}$ M | $10^{-6}$ M | 65.0 ± 3.9 |
| $10^{-4}$ M | $10^{-5}$ M | 78.2 ± 4.1 |
| $10^{-4}$ M | $10^{-4}$ M | 99.6 ± 5.4 |

* pmol of cAMP per min per mg of fresh caudate, ± standard error of the mean.

activity with $10^{-4}$ M GTP plus dopamine remained slightly lower than that with $10^{-4}$ M dopamine alone.

Since GTP in the medium could be acting by lowering the concentration of free $Mg^{++}$ available for the adenylate cyclase reaction, we tested the effect of varying the concentration of $MgSO_4$. Although enzyme activity was greater at high (5:1) compared to low (1:1) $MgSO_4$ to ATP ratios, the effect of $10^{-4}$ M GTP and its reversal by $10^{-4}$ M dopamine remained the same. Varying $MgSO_4$ concentrations were also examined using various concentrations (0.5 and 2 mM) of ATP, and basically the same results were obtained.

The effect of dopamine described above appeared to be specific for brain tissue. Adenylate cyclase activity was measured in caudate homogenates and in liver membrane preparations in the same experiment, and the effects of isoproterenol and dopamine were studied in both systems in the presence and absence of GTP (table 3). Isoproterenol ($10^{-6}$ M) did not affect adenylate cyclase activity in caudate homogenates whether or not GTP ($10^{-4}$ M) was present. However, isoproterenol was effective in liver membranes when $10^{-4}$ M GTP was present. Dopamine, on the other hand, did not significantly modify liver adenylate cyclase although it was effective on the enzyme from the caudate.

The prostaglandins, $PGE_1$ and $PGE_2$, and morphine were tested in the caudate homogenate, which has very high levels of opiate binding [2]. At concentrations of 0.1 to 10 μg/ml, $PGE_1$ and $PGE_2$ did not

Table 3
Effects of GTP, dopamine and isoporterenol on adenylate cyclase activity of caudate homogenates and liver membranes

| Tissue | Addition | | | Adenylate cyclase activity |
|---|---|---|---|---|
| | GTP | Dopamine | Isoproterenol | |
| Caudate homogenate | 0 | 0 | 0 | 74.6 ± 3.2 |
| | $10^{-4}$ M | 0 | 0 | 42.3 ± 2.9 |
| | 0 | $10^{-4}$ M | 0 | 91.2 ± 4.1 |
| | $10^{-4}$ M | $10^{-4}$ M | 0 | 82.3 ± 3.6 |
| | 0 | 0 | $10^{-6}$ M | 73.0 ± 3.5 |
| | $10^{-4}$ M | 0 | $10^{-6}$ M | 42.0 ± 3.1 |
| Liver membranes | 0 | 0 | 0 | 17.6 ± 2.3 |
| | $10^{-4}$ M | 0 | 0 | 20.5 ± 2.1 |
| | 0 | $10^{-4}$ M | 0 | 16.2 ± 1.9 |
| | $10^{-4}$ M | $10^{-4}$ M | 0 | 18.2 ± 2.2 |
| | 0 | 0 | $10^{-6}$ M | 17.0 ± 1.6 |
| | $10^{-4}$ M | 0 | $10^{-6}$ M | 33.3 ± 2.7 |

* pmol of cAMP per min per mg of fresh caudate or of membrane protein, ± standard error of the mean.

alter adenylate cyclase activity in the presence or absence of $10^{-6}$ M to $10^{-4}$ M GTP and/or $10^{-4}$ M dopamine. Furthermore, morphine at 1, 5, 10, and 20 μg per ml, concentrations over 10 000 times the dissociation constant of morphine binding [1], did not modify adenylate cyclase activity in the presence or absence of $10^{-4}$ M GTP, $10^{-4}$ M dopamine, 5 μg/ml $PGE_1$ and combinations thereof.

In caudate nucleus homogenates dopamine reverses the GTP inhibition of adenylate cyclase activity more reproducibly than it stimulates enzyme activity. Although it is not yet known whether these two effects are related, or which one is physiologically more relevant, it is possible that adenylate cyclase activity in the caudate may normally be modulated by the relative concentrations of GTP and dopamine.

**Acknowledgements**

These studies were supported by grants from National Institutes of Health (AM 14956) and the

Kroc Foundation. G. Pasternak is supported by the Mutual of Omaha and United Benefit Life Insurance Companies through the Insurance Medical Scientist Scholarship Fund. P. Cuatrecasas is a recipient of United States Public Health Service Research Career Development Award AM 31464.

## References

[1] Pert, C. B. and Snyder, S. H. (1973) Proc. Natl. Acad. Sci. USA 70, 2243–2347.
[2] Kuhar, M. J., Pert, C. B. and Snyder, S. H. (1973) Nature 245, 447–450.
[3] Pert, C. B., Pasternak, G. and Snyder, S. H. (1973) Science 182, 1359–1361.
[4] Simon, E. J., Hiller, J. M. and Edelman, I. (1973) Proc. Natl. Acad. Sci. USA 70, 1947–1951.
[5] Collier, H. O. J. and Roy, A. C. (1974) Nature 248, 24–27.
[6] Kebabian, J. W., Petzold, G. I. and Greengard, P. (1972) Proc. Natl. Acad. Sci. USA 69, 2145–2149.
[7] Kebabian, J. W. and Greengard, P. (1971) Science 174, 1346–1349.
[8] Miller, R., Horn, A., Iversen, L. and Pinder, R. (1974) Nature 250, 238–241.
[9] Karobath, M. and Leitich, H. (1974) Proc. Natl. Acad. Sci. USA 71, 2915–2918.
[10] Miller, R. J. and Iversen, L. L. (1974) Trans. Biochem. Soc. 2, 256–259.
[11] von Hungen, H. and Roberts, S. (1973) Eur. J. Biochem. 36, 391–401.
[12] Brown, J. H. and Makman, M. H. (1972) Proc. Natl. Acad. Sci. USA 69, 539–543.
[13] Clement-Cornier, Y. C., Kebabian, J. W., Petzold, G. L. and Greengard, P. (1974) Proc. Natl. Acad. Sci. USA 71, 1113–1117.
[14] Horn, A. S., Cuello, A. C. and Miller, R. J. (1974) J. Neurochem. 22, 265–270.
[15] Rodbell, M., Krans, H. M. J., Pohl, S. L. and Birnbaumer, L. (1971) J. Biol. Chem. 246, 1872–1876.
[16] Rodbell, M., Birnbaumer, L., Pohl, S. L. and Krans, H. M. J. (1971) J. Biol. Chem. 246, 1877–1882.
[17] Krishna, G., Harwood, J. P., Barber, A. J. and Jamieson, G. A. (1972) J. Biol. Chem. 247, 2253–2254.
[18] Wolff, J. and Cook, G. H. (1973) J. Biol. Chem. 248, 350–355.
[19] Bockaert, S., Roy, C. and Jurd, S. (1972) J. Biol. Chem., 247, 7073–7081.
[20] Siegel, M. I. and Cuatrecasas, P. (1974) Molec. and Cell. Endocrin. 1, 89–98.
[21] Johnson, D. G., Thompson, W. J. and Williams, R. H. (1974) Biochemistry 13, 1920–1924.
[22] Deery, D. J. and Howell, S. L. (1973) Biochim. Biophys. Acta 329, 17–22.
[23] White, A. A. and Zenser, T. V. (1972) Analyt. Biochem. 41, 372–396.
[24] Krishna, G., Weiss, B. and Brodie, B. B. (1968) J. Pharmac. Exp. Ther. 163, 379–385.
[25] Gilman, A. G. (1970) Proc. Natl. Acad. Sci. USA 67, 305–312.
[26] Symons, R. H. (1968) Biochim. Biophys. Acta 155, 609–610.
[27] Flawia, M. M. and Torres, H. N. (1972) J. Biol. Chem. 247, 6880–6883.
[28] Kakiuchi, S. and Rall, T. W. (1968) Mol. Pharmacol. 4, 367–388.
[29] Shimizu, H., Creveling, C. R. and Daly, J. (1970) Proc. Natl. Acad. Sci. USA 65, 1033–1040.
[30] Weiss, B. and Costa, E. (1968) J. Pharmacol. Exp. Ther. 161, 310–319.