Biochemical Pharmacology, Vol. 27, pp. 2333–2336.

0006-2952/78/1001-2333$02.00/0

CYCLIC NUCLEOTIDE LEVELS IN THE RAT STRIATUM AND CEREBELLUM—

IN VIVO EFFECTS OF DOPAMINE AND ACETYLCHOLINE RECEPTOR AGONISTS AND ANTAGONISTS

SURENDRA K. PURI, PAUL CHOMA and LADISLAV VOLICER
Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118, U.S.A.

(*Received* 12 *July* 1977; *accepted* 25 *January* 1978)

Abstract—The role of dopamine and acetylcholine receptors in the regulation of cyclic nucleotide levels in the striatum and cerebellum was investigated after *in vivo* administration of drugs. Apomorphine increased cyclic AMP levels only in the striatum and this effect was blocked by haloperidol and dexetimide. Cyclic GMP levels were increased both in the striatum and cerebellum by apomorphine and pilocarbine. Haloperidol significantly decreased cyclic GMP levels and blocked the effect of apomorphine and pilocarpine on cyclic GMP in the cerebellum. In the striatum, haloperidol blocked only the pilocarpine-induced rise of cyclic GMP. Dexetimide increased cyclic GMP levels and failed to block the rise in cyclic GMP by either apomorphine or pilocarpine. These results suggest that the regulation of cyclic GMP levels may involve more than one mechanism.

Adenosine 3′,5′-monophosphate (cyclic AMP) and guanosine 3′,5′-monophosphate (cyclic GMP) have been implicated as second messengers in the synaptic effects of neurotransmitters, both in the central and peripheral nervous systems [1]. In the central nervous system, the concentrations of both cyclic AMP and cyclic GMP can be altered by drugs modifying catecholaminergic [2–4] or cholinergic systems [3, 5–7]. Goldberg *et al.* [8] have proposed that cyclic AMP is primarily linked to catecholaminergic neurotransmission and cyclic GMP to cholinergic neurotransmission. However, drugs like *d*-amphetamine, chlorpromazine and reserpine, which influence monoaminergic neurotransmission, also alter the concentration of cyclic GMP *in vivo* [2, 9]. In addition, other central stimulants [3, 10, 11] and stress conditions [12] increase cyclic GMP levels. Recently, Gumulka *et al.* [13] have demonstrated that dopaminergic stimulants like apomorphine, L-dopa, amantadine, nomifensine and amphetamine are capable of increasing cyclic GMP levels in the medial forebrain and cerebellum of mice. In order to investigate the mechanisms by which dopamine (DA) or acetylcholine (ACh) receptor activity participates in increasing cyclic GMP levels, we studied the effects of DA and ACh agonists and antagonists on cyclic nucleotide levels in the striatum and in the cerebellum of rat brain.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (Charles River Breeding Labs) weighing 150–200 g were used in this study. The animals were housed in colony cages in a room with thermostatically controlled constant temperature and alternate periods of light and dark. The animals received food and water *ad lib.*

Cyclic nucleotide levels. The animals were killed by microwave radiation for 6 sec focused onto the skull as described by Guidotti *et al.* [14]. This method inactivates the brain enzymes that regulate cyclic nucleotides and, therefore, allows the measurement of the steady state levels of cyclic nucleotides which are close to *in vivo* levels. The striata and cerebellum were rapidly dissected and then homogenized in 0.4 N perchloric acid containing tracer amounts of [^{14}C] cyclic AMP and [^{3}H] cyclic GMP. An aliquot of the supernatant fraction was neutralized with 4 N KOH and 3 M Tris base and the cyclic nucleotides were separated by the method of Mao and Guidotti [15]. The neutralized aliquot was passed over an alumina column and eluted with 0.6 M Tris–HCl buffer (pH 7.5). The eluate was applied to a Biorad AG 1 × 2 (Cl^-) column. Cyclic AMP was eluted with 0.05 N HCl and cyclic GMP was eluted with 0.5 N HCl. The eluates were lyophilized and reconstituted in 0.05 M sodium acetate buffer (pH 6.2). The concentrations of cyclic AMP and cyclic GMP were measured by radioimmunoassay using Schwartz–Mann antibodies. Another aliquot of cyclic AMP and cyclic GMP was counted in a liquid scintillation counter in order to determine the recovery of cyclic AMP and cyclic GMP in each sample. The concentration of radioactive cyclic AMP or cyclic GMP added to each sample was substrated in order to obtain the absolute concentrations of cyclic nucleotide levels. The results are expressed as pmoles of cyclic AMP or cyclic GMP/mg of protein. Protein concentration was measured according to the method of Lowry *et al.* [16].

Drugs. Apomorphine was dissolved in 0.1% ascorbic acid. Haloperidol was first dissolved in a small volume of 1.5% tartaric acid and then further diluted to the desired volume by saline. Pilocarpine and dexetimide were dissolved in saline. All

the drugs were administered intraperitoneally. The control animals were injected with similar volumes of saline. The statistical analysis was performed by Student's 't'-test.

RESULTS

The changes in cyclic AMP and cyclic GMP were measured 10 min after the administration of apomorphine. Apomorphine caused a dose-dependent increase in striatal cyclic AMP levels without affecting cyclic AMP levels in the cerebellum (Fig. 1). However, the administration of apomorphine produced a dose-dependent increase of cyclic GMP levels both in the striatum and in the cerebellum (Fig. 2).

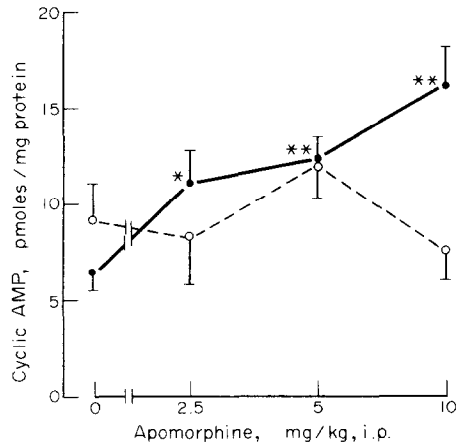

Fig. 1. Effect of apomorphine on cyclic AMP levels in the striatum and cerebellum. Solid and broken lines represent striatum and cerebellum respectively. A single asterisk (*) indicates $P < 0.05$, from saline control; a double asterisk (**) indicates $P < 0.05$, from saline control.

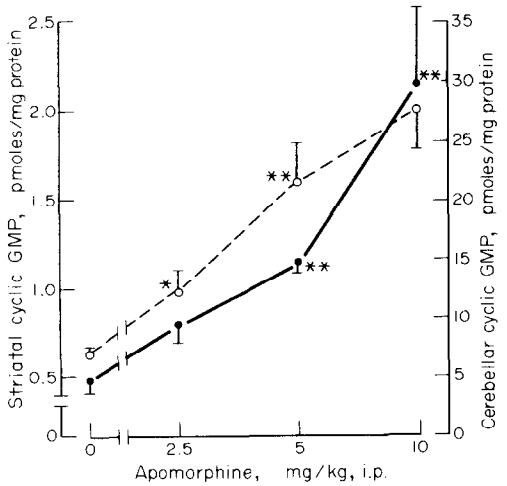

Fig. 2. Effect of apomorphine on cyclic GMP levels in the striatum and cerebellum. Solid and broken lines represent striatum and cerebellum respectively. A single asterisk (*) indicates $P < 0.05$ from saline control; a double asterisk (**) indicates $P < 0.005$ from saline control.

The administration of haloperidol did not significantly alter the levels of cyclic AMP in the striatum. However, the apomorphine-induced increase of cyclic AMP was abolished by haloperidol pretreatment (Table 1). Pilocarpine and dexetimide did not affect striatal cyclic AMP levels and, in the cerbellum, cyclic AMP levels were not altered by any drug.

Apomorphine and pilocarpine caused a significant increase of cyclic GMP levels both in the striatum and in the cerebellum (Tables 2 and 3). Haloperidol significantly decreased cerebellar cyclic GMP levels, and also blocked the effects of both apomorphine and pilocarpine. In the striatum, haloperidol did not alter cyclic GMP levels and significantly decreased only the effect of pilocarpine. A cholinergic antagonist with predominantly central effects, dexetimide [17], increased cyclic GMP levels both in the striatum and in the cerebellum. A 30-min pretreatment with dexetimide, prior to apomorphine or pilocarpine administration, did not block the rise in cyclic GMP levels (Tables 2 and 3).

Table 1. Effect of dopaminergic and cholinergic agonists and antagonists on cyclic AMP levels in striatum

Treatment	Cyclic AMP levels* (pmoles/mg protein)		
	Saline	Haloperidol†	Dexetimide†
Saline	6.38 ± 0.87	7.2 ± 0.53	8.26 ± 0.75
Apomorphine	11.08 ± 1.55‡	7.05 ± 0.6	6.86 ± 1.79
Pilocarpine	7.36 ± 0.85	8.51 ± 1.67	7.17 ± 0.83

*Expressed as mean ±S.E.; based on four animals in each group.

†Haloperidol (2 mg/kg, i.p.) was administered 60 min and dexetimide (1 mg/kg, i.p.) 30 min before apomorphine (2.5 mg/kg, i.p.) or pilocarpine (8 mg/kg, i.p.). The animals were killed 10 min after saline, apomorphine or pilocarpine.

‡$P < 0.05$, from saline controls.

Table 2. Effect of dopaminergic and cholinergic agonists and antagonists on cyclic GMP levels in cerebellum

Treatment	Cyclic GMP levels* (pmoles/mg protein)		
	Saline	Haloperidol†	Dexetimide†
Saline	6.93 ± 0.60	4.88 ± 0.53‡	9.12 ± 0.96‡
Apomorphine	12.28 ± 1.70‡	4.85 ± 0.85‡	15.49 ± 4.82
Pilocarpine	12.27 ± 1.23‡	6.52 ± 1.86	14.33 ± 0.38§

*Expressed as mean ±S.E.; based on four animals in each group.

†Haloperidol (2 mg/kg, i.p.) was injected 60 min and dexetimide (1 mg/kg, i.p.) 30 min before apomorphine (2.5 mg/kg, i.p.) and pilocarpine (8 mg/kg, i.p.). The animals were killed 10 min after saline, apomorphine or pilocarbine administration.

‡$P < 0.05$, from saline control.

§$P < 0.05$, from dexetimide–saline group.

Table 3. Effect of dopaminergic and cholinergic agonists and antagonists on cyclic GMP levels in striatum

Treatment	Cyclic GMP levels* (pmoles/mg protein)		
	Saline	Haloperidol†	Dexetimide†
Saline	0.49 ± 0.07	0.57 ± 0.02	0.89 ± 0.12‡
Apomorphine	0.79 ± 0.10‡	0.69 ± 0.09	0.93 ± 0.12‡
Pilocarpine	0.91 ± 0.16‡	0.78 ± 0.03‡§	0.84 ± 0.16

*Expressed as mean ±S.E.; based on four animals in each group.

†Haloperidol (2 mg/kg, i.p.) was administered 60 min and dexetimide (1 mg/kg, i.p.) 30 min before apomorphine (2.5 mg/kg, i.p.) or pilocarpine (8 mg/kg, i.p.). The animals were killed 10 min after saline, apomorphine or pilocarpine.

‡$P < 0.05$, compared to saline control.

§$P < 0.001$, compared to haloperidol–saline group.

DISCUSSION

The present investigation demonstrates that a DA agonist, apomorphine, increased cyclic AMP levels in the striatum without affecting the cerebellar levels. Haloperidol, DA antagonist, reversed the apomorphine-induced increase in the striatal cyclic AMP levels. This observation is consistent with the view that cyclic AMP levels in the striatum mediate the DA receptor functions [18, 19]. However, dexetimide, an ACh antagonist, also blocked the apomorphine-induced increase of cyclic AMP levels. Recent data on the inhibitory effect of atropine on DA-sensitive adenylate cyclase [20] support our results.

The increase of striatal cyclic GMP after apomorphine administration may be related, in part, to a direct stimulation of striatal dopamine receptors. However, the increase in the cerebellar cyclic GMP levels may not be a direct effect of apomorphine in the cerebellum because there is no evidence for the existence of dopaminergic innervation in that area [21, 22]. Also, the direct application of DA agonists and antagonists to the cerebellar slices does not alter the cyclic GMP content [6]. Since haloperidol prevents an apomorphine-induced cyclic GMP increase both in the striatum and in the cerebellum, it is likely that the increase is mediated by DA receptor stimulation.

The increase in cerebellar cyclic GMP concentration caused by stimulation of striatal DA receptors may be due to activation of striato-cerebellar pathways. Although there is no histological evidence, there is electro-physiological data [23, 24] to suggest that the striatum may exert an influence on cerebellar functions via polysynaptic connections between the striatum and the cerebellum. The stimulation of a striato-cerebellar pathway, therefore, may result in an enhancement of excitatory input to the cerebellum which may elevate the cyclic GMP levels. The fact that direct injection of apomorphine into the striatum of intact animals increases cyclic GMP in the cerebellum, but direct administration of apomorphine in the cerebellum does not [25], may support the existence of a striato-cerebellar pathway.

It has been suggested that cholinergic interneurons may participate in increasing the levels of cyclic GMP in the striatum as well as in the cerebellum. In order to elucidate the mechanism, we compared the effects of dopaminergic or cholinergic agonists and studied interactions with dopaminergic and cholinergic antagonists. Pilocarpine increased cyclic GMP levels in both brain areas. Haloperidol abolished not only apomorphine but also the pilocarpine-induced rise of cyclic GMP both in the striatum and in the cerebellum. Blockage of the pilocarpine-induced rise to cyclic GMP levels by haloperidol cannot be explained through a muscarinic receptor mechanism because haloperidol is devoid of anticholinergic effects [26]. Not only cholinergic agonists, but also antagonists increased the levels of cyclic GMP. Dexetimide, an anticholinergic compound which blocks pilocarpine-induced lacrimation or salivation at a submydriatic dose level [17], failed to block the pilocarpine-induced increase. These results are in agreement with the report of Opmeer *et al.* [3] who found an increase of cyclic GMP in the medial forebrain and in the cerebellum of mice after the administration of cholinergic agonists and antagonists. It is possible, therefore, that other neurotransmitter systems, sucn as adrenergic or histaminergic, are involved in the regulation of cyclic GMP levels.

Recently, a change in cyclic GMP levels has been postulated to be negatively correlated with the levels of GABA [10, 11]. The agents which are known to enhance GABAergic transmission, e.g. amino-oxyacetic acid or diazepam, were found effective in antagonizing the rise in cerebellar cyclic GMP levels due to dopaminergic stimulation [13]. Maruyama and Kawasaki [27] suggested that butyrophenones may act on cerebellar Purkinje cells by increasing GABAergic receptor activity or by possessing a GABA-like action [28]. The decrease in cyclic GMP levels induced by chlorpromazine, haloperidol and spiroperidol has also been suggested to be mediated through changes in GABA activity [13,29]. Thus, it is possible that a haloperidol-induced increase in GABAergic activity may result in the blockade of apomorphine and pilocarpine-induced increases in cyclic GMP. Recently, antipsychotic drugs, like haloperidol, have been reported to increase the turnover rate of striatal GABA [30]. The activation of the proposed striatal-cerebellar pathway by dopaminergic or cholinergic stimulation may result in the suppression of the GABAergic activity which, in turn, increases the levels of cyclic GMP.

Further experiments are needed to explain why both the cholinergic agonists and antagonists are capable of increasing cyclic GMP levels and why dexitimide blocks the effect of apomorphine on striatal cyclic AMP levels.

Acknowledgements—This work was supported by USPHS Grant DA 442. The authors also acknowledge the helpful suggestions of Charles R. Mantione and of Mrs. Marlene Lawatsch in typing the manuscript.

REFERENCES

1. J. A. Nathanson, *Physiol. Rev.* **57**(2), 157 (1977).
2. J. A. Ferrendelli, D. A. Kinscherf and D. M. Kipnis, *Biochem. biophys. Res. Commun.* **46**, 2114 (1972).
3. F. A. Opmeer, S. W. Gumulka, V. Dinnendahl and P. S. Schönhöfer, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **292**, 259 (1976).
4. T. Pasternak, *A. Rev. Pharmac.* **14**, 23 (1974).
5. V. Dinnendahl and K. Stock, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **290**, 297 (1975).
6. J. A. Ferrendelli, D. A. Kinscherf and M. M. Chang, *Brain Res.* **84**, 63 (1975).
7. J. A. Ferrendelli, A. L. Steiner, D. B. McDougal and D. M. Kipnis, *Biochem. biophys. Res. Commun.* **41**, 1061 (1970).
8. N. D. Goldberg, R. F. O'Dea and M. K. Haddon, in *Advances in Cyclic Nucleotide Research* (Eds. P. Greengard and G. A. Robinson), Vol. 3, pp. 155–223. Raven Press, New York (1973).
9. W. P. Burkard, L. Pieri and W. Haefely, *J. Neurochem.* **27**, 297 (1976).
10. C. C. Mao, A. Guidotti and E. Costa, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **289**, 369 (1975).
11. C. C. Mao, A. Guidotti and E. Costa, *Brain Res.* **83**, 516 (1975).
12. V. Dinnendahl, *Brain Res.* **100**, 716 (1975).
13. S. W. Gumulka, V. Dinnendahl, H. D. Peters and P. S. Schönhöfer, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **293**, 75 (1976).
14. A. Guidotti, D. L. Cheney, M. Trabucchi, M. Doteuchi, C. Wang and R. A. Hawkins, *Neuropharmacology* **13**, 1115 (1974).
15. C. C. Mao and A. Guidotti, *Analyt. Biochem.* **59**, 63 (1974).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. P. A. J. Janssen and C. J. E. Niemegeers, *Psychopharmacolgia* **11**, 231 (1967).
18. L. L. Iversen, *Science, N.Y.* **188**, 1084 (1975).
19. A. Carenzi, D. L. Cheney, E. Costa, A. Guidotti and G. Racagni, *Neuropharmacology* **14**, 927 (1975).
20. L. C. Tang and G. C. Cotzias, *Proc. natn. Acad. Sci. U.S.A.* **74**, 269 (1977).
21. U. Ungerstedt, *Eur. J. Pharmac.* **5**, 107 (1968).
22. U. Ungerstedt, *Acta physiol. scand.* **82** (suppl. 367), 1 (1971).
23. M. Fox and T. D. Williams, *J. Physiol., Lond.* **198**, 435 (1968).
24. M. Fox and T. D. Williams, *Brain Res.* **20**, 140 (1970).
25. G. Biggio, E. Costa and A. Guidotti, *J. Pharmac. exp. Ther.* **200**(1), 207 (1977).
26. J. Pearl, A. Spilker, W. A. Woodward and R. G. Bentley, *J. Pharm. Pharmac.* **28**, 302 (1967).
27. S. Maruyama and T. Kawasaki, *Jap. J. Pharmac.* **26**, 765 (1976).
28. G. A. Johnston, in *GABA in Nervous System Function* (Eds. E. Roberts, T. N. Chase and D. B. Towers), pp. 395–411. Raven Press, New York (1976).
29. G. Biggio and A. Guidotti, *Nature, Lond.* **265**, 240 (1977).
30. E. Marco, C. C. Mao, D. L. Cheney, A. Revuelts and E. Costa, *Nature, Lond.* **264**, 363 (1976).