THE EFFECT OF OXOTREMORINE AND ATROPINE ON cGMP AND cAMP LEVELS IN MOUSE CEREBRAL CORTEX AND CEREBELLUM

J. A. Ferrendelli, A. L. Steiner, D. B. McDougal, Jr. and D. M. Kipnis
Departments of Pharmacology and Medicine
Washington University School of Medicine
St. Louis, Missouri

Received October 19, 1970

SUMMARY

Guanosine 3',5' cyclic monophosphate (cGMP) and adenosine 3',5' cyclic monophosphate (cAMP) were measured in the cerebellum and cerebral cortex of mice after treatment with oxotremorine and atropine. Oxotremorine treatment results within 3-5 minutes in a 70% increase in cGMP in cerebral cortex and cerebellum. Pretreatment with atropine prevents the oxotremorine induced rise in cGMP. Following treatment with atropine alone, cGMP levels rise in cerebral cortex and fall in cerebellum. Atropine has no effect on cAMP levels, but oxotremorine decreases cAMP levels in both cerebral cortex and cerebellum. These results are interpreted as indicating that 1) cGMP is involved in cholinergic neurotransmission, and 2) cGMP and cAMP have independent functional roles in the central nervous system.

INTRODUCTION

Guanosine 3',5' cyclic monophosphate (cGMP) has been shown to be a natural constituent of several mammalian tissues (1,2), but a biological role for this substance has not been defined. Recent studies of George, Polson, O'Toole and Goldberg (3) demonstrating that acetycholine perfusion of the isolated rat heart results in an elevation of the myocardial cGMP level, suggest that cGMP may be involved in cholinergic neurotransmission.

The present study is an attempt to define further the metabolic role of cGMP, particularly in regard to cholinergic activity. *In vivo* levels of cGMP and adenosine 3',5' cyclic monophosphate (cAMP) were measured in the cerebral cortex and cerebellum of mice treated with oxotremorine and/or atropine, two drugs known to affect central nervous system cholinergic mechanisms.

METHODS

Six to eight week old Swiss-Webster male mice, allowed free access to food and water, were used in all experiments. Atropine sulfate (Aldrich Chemical

Company) was dissolved in distilled H_2O and injected intraperitoneally in volumes of 0.15 - 0.25 ml. Oxotremorine, obtained from Aldrich Chemical Company, was also dissolved in distilled H_2O and injected subcutaneously in volumes of 0.1 - 0.2 ml. Control animals were injected with distilled H_2O. At varying time intervals following injection, mice were rapidly frozen by immersing them head first into Freon (CCl_2F_2) maintained at its freezing point (-150°) by liquid nitrogen. The frozen brains were dissected at -20°; the entire cerebellum and the anterior-superior cerebral cortex from both cerebral hemispheres were used for nucleotide analysis. After weighing, the frozen tissues were homogenized rapidly in ice-cold 12% trichloroacetic acid, centrifuged at 15,000 RPM for 15 min. at 0° and an aliquot of the clear supernatant fluid removed and extracted three times with hydrated ethyl ether. The ether extracted aqueous phase was dried under a stream of N_2, and the sample reconstituted with 50 mM acetate buffer, pH 6.2, to give a final tissue dilution of 25 mg/ml. cAMP and cGMP were measured using the radioimmunoassay described by Steiner, Parker and Kipnis (4,5). All assays were performed in duplicate.

RESULTS

Control values - In thirteen control animals receiving no drugs, the levels of cAMP and cGMP in cerebral cortex differed significantly from those in cerebellum. The concentration (mean ± SEM) of cAMP in cerebral cortex (1.3 ± 0.1 μmoles/kg wet weight) was 62 percent higher than in cerebellum (0.8 ± 0.1 μmoles/kg wet weight). In contrast, cGMP in cerebral cortex (36 ± 2 nmoles/kg wet weight) was less than 6% of the cGMP level in cerebellum (630 ± 50 nmoles/kg wet weight). Furthermore, although the concentration of cAMP in cerebral cortex was ≈40 times greater than the concentration of cGMP, the level of cGMP approached that of cAMP in the cerebellum.

Oxotremorine - A coarse total body tremor was observed in all animals within a minute after injection of oxotremorine (5 mg/kg). The tremor reached maximum intensity by 3-5 minutes and all animals exhibited marked salivation, diarrhea and urinary frequency. These signs continued unabated for the

remainder of the 20 minute experimental period.

The changes in cGMP levels following oxotremorine treatment were similar in cerebral cortex and cerebellum (Table I). In both regions, the cGMP levels rose rapidly reaching maximum levels of 60-78% above control values 3-5 minutes after injection. The period when peak levels of cGMP were observed corresponded to the time when symptoms became maximal. Although the animals continued to remain symptomatic for 20 minutes, cGMP levels began to fall in both cerebral cortex and cerebellum 5 minutes after injection, approaching control levels in cerebral cortex and 60% of control levels in the cerebellum 20 minutes after injection.

In contrast to the increase in cGMP level, cAMP exhibited a variable but progressive fall after oxotremorine injection to 75 and 50 percent of control levels in cerebral cortex and cerebellum, respectively (Table I).

Atropine - Animals receiving from 30-120 mg/kg of atropine sulfate exhibited

TABLE I. EFFECT OF OXOTREMORINE ON cGMP AND cAMP IN MOUSE CEREBRAL CORTEX AND CEREBELLUM

	cGMP		cAMP	
Min. after Injection*	Cerebral Cortex	Cerebellum	Cerebral Cortex	Cerebellum
	Percent of Control Values**			
0	100 ± 5	100 ± 17	100 ± 10	100 ± 24
1	108 ± 11	122 ± 7	81 ± 18	79 ± 17
3	162 ± 14	178 ± 11	84 ± 9	102 ± 46
5	170 ± 11	169 ± 15	96 ± 24	107 ± 10
10	146 ± 11	117 ± 13	91 ± 6	64 ± 10
20	114 ± 16	60 ± 12	75 ± 8	53 ± 14

*Oxotremorine, 5 mg/kg injected subcutaneously.

**See text. Each value represents the mean ± SEM of 4-7 samples.

little change in behavior. Animals receiving 240 mg/kg atropine became somnolent 5-10 minutes after injection and remained depressed for the remainder of the 120 minutes experimental period.

Increasing doses of atropine (from 30-120 mg/kg), produced a progressive increase in the cGMP levels of cerebral cortex (Table II). In the animals receiving 120 mg/kg of atropine, cGMP in cerebral cortex was three times greater than that in control animals. Increasing the dose of atropine to 240 mg/kg did not result in an additional rise in cGMP. In sharp contrast to the findings in cerebral cortex, increasing doses of atropine resulted in a progressive and marked decrease in cGMP levels of the cerebellum (Table II). In animals receiving 240 mg/kg atropine, cGMP in the cerebellum was only 39% of that in control animals.

TABLE II. DOSE EFFECT OF ATROPINE ON cGMP IN CEREBRAL CORTEX AND CEREBELLUM

Dose* (mg/kg)	No. of Mice	Cerebral Cortex	Cerebellum
		Percent of Control Values**	
0	7	100 ± 6	100 ± 10
30	4	138 ± 29	74 ± 11
60	4	194 ± 28	85 ± 7
120	4	290 ± 47	51 ± 13
240	4	193 ± 24	39 ± 11

*Atropine sulfate injected I.P. and animals were frozen one hour later.

**See text.

The time course for the change in cGMP in cerebral cortex and cerebellum is shown in Table III. Cerebral cortical cGMP increased two-fold within 30 minutes after administration of 60 mg/kg atropine and then slowly returned toward control levels during the subsequent 90 minutes. In the same animals, cerebellar cGMP fell 20% during the initial 15 minutes and then decreased an

TABLE III. TEMPORAL EFFECT OF ATROPINE ON cGMP IN CEREBRAL CORTEX AND CEREBELLUM

Min. after Injection*	No. of Mice	Cerebral Cortex	Cerebellum
		Percent of Control Values**	
0	7	100 ± 6	100 ± 10
15	4	162 ± 18	80 ± 9
30	4	209 ± 19	83 ± 15
60	4	194 ± 28	85 ± 7
120	4	138 ± 25	63 ± 4

*60 mg/kg atropine sulfate injected I.P.

**See text.

additional 20% between 60 and 120 minutes.

No change in the cAMP levels in either cerebral cortex or cerebellum was observed in atropine treated mice.

Atropine and oxotremorine - Pretreatment with atropine (60 mg/kg) 30 minutes prior to the injection of oxotremorine, completely prevented the appearance of abnormal signs produced by the tremorgenic agent. Under these conditions, oxotremorine did not result in a significant increase in cGMP levels in either cerebral cortex or cerebellum (Table IV). However, 20 minutes after injection of oxotremorine, the cGMP level in cerebellum decreased 40%, a fall comparable to that observed in animals treated with oxotremorine alone. Zero time values in this experiment (i.e. - post atropine and before oxotremorine injection), reflected the effect of atropine. cGMP levels in cerebral cortex were 39% higher than in animals receiving no drugs and were not further elevated by oxotremorine.

TABLE IV. EFFECT OF OXOTREMORINE ON CEREBRAL CORTICAL AND CEREBELLAR cGMP IN ANIMALS PRETREATED WITH ATROPINE

Minutes after Injection of Oxotremorine	Cerebral Cortex	Cerebellum
	Percent of Control Values**	
0	100 ± 14	100 ± 18
1	122 ± 24	112 ± 12
3	104 ± 14	112 ± 22
5	118 ± 30	120 ± 37
10	100 ± 30	79 ± 12
20	114 ± 30	59 ± 4

*Animals were treated with 5 mg/kg oxotremorine 30 minutes after injection of 60 mg/kg atropine sulfate.

**Zero time values were 50 ± 7 nmoles/kg wet weight and 490 ± 90 nmoles/kg wet weight for cerebral cortex and cerebellum, respectively. Each value represents the mean ± SEM of 4 animals.

DISCUSSION

Oxotremorine increases the acetycholine (ACh) content of whole brain (6,7), maximum levels being observed 15 to 30 minutes after injection of the tremorgenic drug. Maximum tremor intensity, however, occurs 5 minutes after injection of oxotremorine, corresponding well with the peak rise induced in cGMP in both cerebral cortex and cerebellum. It is not clear whether the tremorgenic effect of oxotremorine is a result of increased brain ACh or is due to a direct cholinomimetic effect (7) similar to the proposed mechanism of oxotremorine action in the peripheral autonomic nervous system (8). The mechanism of action of atropine in the central nervous system is probably similar to its peripheral action; namely, blockade of cholinergic receptor sites especially those of the muscarinic type. Atropine has been shown to decrease whole brain ACh (6), but the exact mechanism by which this is accomplished has not been established.

The present inadequate understanding of the actions of oxotremorine and atropine in the CNS make any interpretation of the functional role of cGMP highly speculative. However, the present results do lead to some tentative conclusions. The temporal pattern of increase in cGMP following oxotremorine corresponds more closely to the appearance of tremor activity than to the reported increase in ACh. This observation, along with the finding that atropine prevents both the tremor and rise in cGMP level suggests that the oxotremorine induced increase in cGMP represents a post-synaptic event. The findings of George et al (3) that acetylcholine perfusion of the rat heart induces a rise in myocardial cGMP are consistent with the localization of the nucleotide at a post-synaptic site. The differential responses of the cAMP and cGMP levels observed in this study further indicate that cAMP and cGMP do not share similar functional roles in the CNS. The observation that atropine alone induces a divergent response in cGMP in cerebellum and cerebral cortex was unexpected and we have no explanation for this finding at the present time.

Although a specific role for cGMP in CNS function cannot be assigned, the fact that two drugs known to affect cholinergic processes markedly influence cGMP levels clearly indicates that this nucleotide is involved in cholinergic mechanism(s).

ACKNOWLEDGEMENTS

This work was supported, in part, by NIH Grant AM 1921, NIAMD fellowship IF3-AM 28805, NINDS fellowship 2 F11 NB 1962-02, PHS Grants NB 0600 and NINDB 5K3 NB 18005.

References

1. Goldberg, N. D., Dietz, S. B., and O'Toole, A. G., J. Biol. Chem., 244, 4458 (1969).
2. Ishikawa, E., Ishikawa, S., Davis, J. W. and Sutherland, E. W., J. Biol. Chem., 244, 6371 (1969).
3. George, W. J., Polson, J. B., O'Toole, A. G. and Goldberg, N. D., Proc. Nat. Acad. Sci., U.S.A., 66, 398 (1970).
4. Steiner, A. L., Kipnis, D. M., Utiger, R. D., and Parker, C. W., Proc. Nat. Acad. Sci., 64, 367 (1969).
5. Steiner, A. L., Kipnis, D. M., Utiger, R. D., and Parker, C. W., J. Lab. and Clin. Med. 74, 1016 (1969).
6. Holmstedt, B. and Lundgren, G., In Mechanism of Release of Biogenic Amines, (Ed. Von Euler, U. S., Rosell, S. and Uvnas, B.) Pergamon Press, Oxford, (1966).
7. Cox, B. and Potkonjak, Brit. J. Pharmacol. 35, 295 (1969).
8. Cho, A. K., Haslett, W. L., and Jenden, D. J., J. Pharmacol. exp. Ther., 138, 249 (1962).