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Regulation of Levels of Guanosine Cyclic 3′,5′-Monophosphate in the Central Nervous System: Effects of Depolarizing Agents

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SUMMARY

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Veratridine, ouabain, and K^+, agents which are known to produce depolarization of nervous tissue, increase the levels of cyclic GMP in incubated slices of mouse cerebellum. Each of the agents acts in a dose-related fashion, and each produces a maximum elevation of cyclic GMP levels which is 25–30 times control levels. The effects of all three agents on cyclic GMP accumulation are dependent on Ca^{++}. Atropine does not block and acetylcholine does not mimic the effect of the depolarizing agents. High concentrations of K^+, in addition to increasing levels of cyclic GMP, also greatly increase levels of cyclic AMP in slices of mouse cerebellum. However, the rate of accumulation of cyclic AMP is more rapid than that of cyclic GMP, induction of cyclic AMP accumulation requires higher concentrations of K^+, and theophylline markedly attenuates the accumulation of cyclic AMP produced by K^+ but not that of cyclic GMP. Furthermore, adenosine produces an accumulation of cyclic AMP but not of cyclic GMP. These data indicate that the effects of depolarization on cyclic AMP and cyclic GMP levels in nervous tissue are mediated by separate mechanisms. The effects of depolarization on cyclic nucleotide levels in cerebral cortex are different from those in cerebellum. In cerebral cortex slices, high concentrations of K^+ produce a much smaller accumulation of cyclic GMP than in cerebellum slices, whereas the K^+-induced accumulation of cyclic AMP in cerebral cortex is greater than that in cerebellum.

INTRODUCTION

There is increasing evidence that guanosine 3′,5′-monophosphate is involved in neural function and metabolism. Cholinergic agents, which were first reported to increase levels of cyclic GMP in heart (1), have been shown to produce elevation of the levels of cyclic GMP in brain, *in vivo* and *in vitro*, and atropine is capable of blocking these increases (2–4). Furthermore, cyclic GMP reproduces some of the effects of acetylcholine on neuronal membrane potentials in rabbit superior cervical ganglion (5). These findings have led to the conclusion that cyclic GMP may mediate some of the effects of acetylcholine in the central nervous system and in the peripheral autonomic nervous system. Cyclic GMP may also have a role in

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neural tissue which is not directly related to cholinergic neurotransmission. Drugs known to influence monoaminergic neurotransmission processes alter cyclic GMP levels in mouse cerebellum *in vivo*, and these effects do not appear to be linked to cholinergic neurotransmission mechanisms (6).

We have found that agents which are known to produce depolarization of nervous tissue markedly increase levels of cyclic GMP in brain. The present report describes the characteristics of cyclic GMP accumulation in slices of mouse cerebellum and cerebral cortex exposed to several depolarizing agents. The data provide additional evidence for an involvement of cyclic GMP in nervous tissue which does not appear to be directly related to cholinergic neurotransmission.

MATERIALS AND METHODS

In these experiments adult female Swiss-Webster mice (NLR strain) were decapitated, and their brains were removed and placed in cold Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 120 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 2.4 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 10 mM glucose. The cerebellum was dissected from the rest of the brain, and in some cases portions of the cerebral cortex were also obtained. The tissue samples were cut into 0.30-mm-thick slices with a Brinkman-McIlwain tissue chopper, and the slices were suspended, after several washings, in fresh Krebs buffer. In all subsequent procedures the slice suspension was maintained at 37° and gassed with a stream of 95% O_2–5% CO_2 which gently agitated the slices continuously. During this and all subsequent procedures the ratio of slice volume to incubation medium volume was 1:100–1:300.

After an incubation period of 60–75 min, the incubation medium was removed and replaced with fresh Krebs buffer or with various solutions described in the legends to the tables and figures. At various intervals thereafter aliquots of the slice suspension, containing 5–15 mg of tissue, were removed with a large-bore volumetric pipette and placed in a 15-ml test tube. The suspension was rapidly centrifuged, the medium was aspirated, and 1 ml of ice-cold 7% trichloracetic acid was added to the pelleted slices. This entire procedure for sampling the slices was completed within 45 sec. The slices were mixed vigorously and repeatedly with a Vortex mixer until the tissue was well dispersed. It appeared that maximal extraction of cyclic AMP and cyclic GMP was achieved with this procedure, since homogenization of the tissue in trichloracetic acid or homogenization and sonication of the mixture did not increase the amount of cyclic nucleotide extracted from the tissue. The mixture was then centrifuged at 20,000 × *g* for 15 min at 4°, and the clear supernatant fluid was separated from the precipitate. Trichloracetic acid was removed from the supernatant fluid by four washings with 3 volumes of hydrated ethyl ether. The neutral solution was then dried under a stream of N_2, and the resulting residue was dissolved in sodium acetate buffer, pH 6.2. This solution was assayed for cyclic GMP and, in some cases, for cyclic AMP, using the radioimmunoassay described by Steiner, Parker, and Kipnis (7). The trichloracetic acid precipitate was solubilized with 1 N NaOH and assayed for protein using the method of Lowry *et al.* (8). Exogenous cyclic AMP and cyclic GMP added to the trichloracetic acid–tissue mixture were completely recovered following centrifugation, ether washing, drying, and reconstitution with sodium acetate buffer, indicating that none of the cyclic nucleotides were lost during the extraction procedure.

In one experiment cyclic GMP was measured in the incubation medium as well as in the tissue slices. To accomplish this, the mixture of slices and incubation medium was centrifuged, the medium and slices were separated, and the slices were treated as described above. The incubation medium was placed in a test tube, which was then placed in boiling H_2O for 3 min. Precipitated protein was removed by centrifugation, and the supernatant fluid was assayed for cyclic GMP.

RESULTS

Levels of cyclic GMP in mouse cerebellum immediately after it was sliced were quite variable. Incubation of the slices in Krebs-

Ringer buffer bubbled with 95% O_2–5% CO_2 at 37° for 45–60 min reduced the concentration of cyclic GMP to a low level, 4–8 pmoles/mg of protein. This level, which is essentially the same as that observed in mouse cerebellum rapidly frozen *in situ* (9), remained constant during continued incubation in oxygenated Krebs-Ringer buffer for at least an additional 60–90 min.

Exposure of the cerebellar slices to veratridine, ouabain, or high concentrations of K^+ produced marked increases in the tissue levels of cyclic GMP (Fig. 1). Increasing the level of K^+ in the incubation medium from 6 to 64 mM or the addition of sufficient veratridine to achieve a final concentration of 50 μM in the incubation medium produced a 25–30-fold increase of cyclic GMP tissue levels within 5 min. Ouabain (1 mM) produced an equally large rise in levels of cyclic GMP, but the rate of accumulation was slower. In cerebellar slices exposed to either veratridine or ouabain, cyclic GMP levels, after increasing, subsequently fell toward control levels. In contrast, the accumulation of cyclic GMP produced by high concentrations of K^+ was sustained, essentially unchanged, for at least 30 min. However, the rise of cyclic GMP levels produced by high concentrations of K^+ was reversed by decreasing the level of K^+ in the incubation medium to 6 mM (data not shown). Measurement of cyclic GMP in both tissue and the bathing medium revealed that in the presence of 64 mM K^+ the total amount of cyclic GMP in the tissue increased from 35 to 900 pmoles in 15 min while that in the bathing medium only increased from 12 to 24 pmoles. Thus little or none of the accumulated cyclic GMP was released from the tissue depolarized with K^+.

The effect of each of the depolarizing agents was concentration-dependent (Fig. 2). On a molar basis, veratridine was the most potent of the three agents. The maximum level of cyclic GMP achieved with each of the three agents, at the time intervals tested, was 160–200 pmoles/mg of protein.

At submaximal concentrations the combined effects of any two of the three depolarizing agents were more than additive (Table 1). For example, in tissue slices treated with 0.3 mM ouabain for 3 μM veratridine, cyclic GMP levels were 25% and 35%, respectively, of the maximum. Addition of 3 μM veratridine and 0.3 mM ouabain together increased the level of cyclic GMP to 95% of the maximum. However, this is the approximate increase expected if ouabain is increased from 0.3 to 0.6 mM or if veratridine is increased from 3 to 6 μM, because on the steepest portion of each curve the slope is greater than 2 (i.e., a 10% increase in the concentration of depolarizing agent causes an increase of more than 20% in cyclic GMP concentration). At maximal concentrations, combinations of the depolarizing agents produced accumulations of cyclic GMP which were equal to or somewhat less than that observed with only one agent.

Divalent cation concentrations had an effect on the accumulation of cyclic GMP

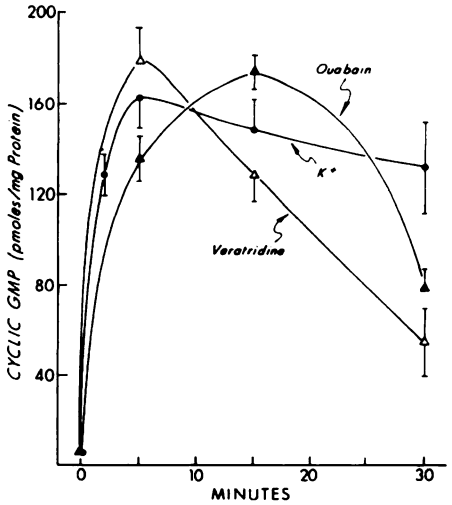

FIG. 1. *Effect of 64 mM K^+, 50 μM veratridine, and 1 mM ouabain on cyclic GMP levels in mouse cerebellum as a function of time*

Slices of mouse cerebellum were incubated for 75 min in Krebs-Ringer-bicarbonate buffer as described in the text. Then the K^+ concentration was increased from 6 to 64 mM (with a concomitant decrease in Na^+ concentration in order to maintain the total monovalent cation concentration at 151 mM), or veratridine or ouabain was added to the medium. At various intervals thereafter an aliquot of the slice suspension was removed and the slices were assayed for cyclic GMP and protein as described in the text. Each value and vertical bar represent the mean and standard error of 4–11 samples.

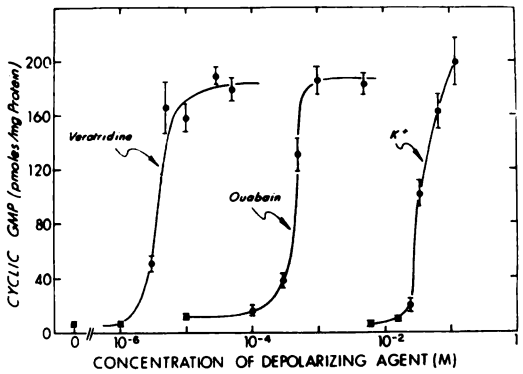

FIG. 2. *Effect of K^+, veratridine, and ouabain concentrations on cyclic GMP levels in mouse cerebellum*

Slices of mouse cerebellum were incubated for 75 min in Krebs buffer as described in the text. Then the K^+ concentration was increased by various amounts (the Na^+ concentration was simultaneously decreased an equivalent amount), or various concentrations of veratridine or ouabain were added to the incubation medium. Slices exposed to veratridine or K^+ were removed 5 min later, and those exposed to ouabain were removed 15 min later; tissue levels of cyclic GMP and protein were measured in the slices as described in the text. Each value and vertical bar represent the mean and standard error of 4–10 samples.

TABLE 1

Combined effects of K^+, veratridine, and ouabain on accumulation of cyclic GMP in mouse cerebellum

Slices of mouse cerebellum were incubated in Krebs-Ringer buffer for 75 min and then exposed to various concentrations of veratridine, ouabain, or K^+ or to combinations of these substances. At the times designated the slices were removed and assayed for cyclic GMP and protein.

Additions	Time of exposure to depolarizing Agent	Cyclic GMP	No. of samples
	min	*pmoles/mg protein*	
3 μM veratridine	5	60.4	3
0.3 mM ouabain	15	44.5	2
25 mM K^+	5	28	3
3 μM veratridine	5		
+ 0.3 mM ouabain	15	170	2
3 μM veratridine	5		
+ 25 mM K^+	5	163	2
0.3 mM ouabain	15		
+ 25 mM K^+	5	118	2

(Table 2). Under the usual assay conditions 2.5 mM Ca^{++} and 2.4 mM Mg^{++} were included in the incubation medium. When the Ca^{++} concentration was reduced to 0.5 mM or when the Mg^{++} concentration was increased to 30 mM, the K^+-induced accumulation of cyclic GMP was reduced by 30%. When Ca^{++} was omitted from the incubation media or when the Ca^{++} concentration was reduced to 0.5 mM and the Mg^{++} concentration was simultaneously raised to 30 mM, high concentrations of K^+ produced either little or no rise in cyclic GMP levels. Veratridine and ouabain also failed to increase cyclic GMP levels in the absence of Ca^{++}. Omission of Mg^{++} from the incubation medium resulted in a 60% decrease in the accumulation of cyclic GMP produced by high concentrations of K^+.

Others have demonstrated that depolarizing agents produce a marked accumulation of cyclic AMP in slices of guinea pig or rabbit cerebral cortex (10, 11). Therefore we compared the effects of K^+ on cyclic AMP and cyclic GMP accumulation in mouse cerebellum. As the K^+ concentration in the incubation medium was raised above 6 mM, tissue levels of both cyclic AMP and cyclic GMP increased (Fig. 3). Cyclic GMP was more responsive to changes in K^+ concentration than was cyclic AMP. A significant increase in the accumulation of cyclic GMP was observed with a K^+ concentration as low as 17 mM, but there was no increase in cyclic AMP levels until the K^+ concentration reached 35 mM. At K^+ concentrations of 64 mM or higher the absolute rise of both cyclic nucleotide levels was similar, but this represented a 30-fold increase for cyclic GMP and only a 15-fold increase for cyclic AMP. The time course of cyclic AMP accumulation was influenced by the K^+ concentration, but that of cyclic GMP was not. At K^+ concentrations of 35 and 64 mM cyclic AMP levels rose rapidly, reaching a maximum within 1–2 min, then fell to control levels, or nearly so, by 5 min. In the presence of 121 mM K^+ cyclic AMP rose as rapidly but remained elevated for a longer period. At all K^+ concentrations cyclic GMP

TABLE 2

Effect of Ca^{++} and Mg^{++} on cyclic GMP accumulation in slices of mouse cerebellum

Mouse cerebellum slices were incubated in Krebs-Ringer buffer for 60 min. The medium was then replaced with one containing the various concentrations of Ca^{++} and Mg^{++} designated below, and the slices were incubated for an additional 15 min. At this time the K^+ concentration was increased or veratridine or ouabain was added to the medium. At various times thereafter (indicated in parentheses) slices were removed and assayed for cyclic GMP and protein.

Additions to medium	Divalent cation concentration		Cyclic GMP	No. of samples
	Mg^{++}	Ca^{++}		
	mM	*mM*	*pmoles/mg protein*	
None	2.4	2.5	7.0 ± 0.8	6
121 mM K^+ (3 min)	2.4	2.5	185 ± 18	6
None	2.4	0	6.2 ± 0.5	4
121 mM K^+ (3 min)	2.4	0	19.8 ± 1.5	3
None	0	2.5	5.6 ± 0.6	3
121 mM K^+ (3 min)	0	2.5	66.4 ± 2.6	3
None	2.4	2.5	8.6, 6.0	2
64 mM K^+ (5 min)	2.4	2.5	177 195	2
None	2.4	0.5	8.7, 7.5	2
64 mM K^+ (5 min)	2.4	0.5	143, 144	2
None	30	2.5	6.0, 8.7	2
64 mM K^+ (5 min)	30	2.5	102, 159	2
None	30	0.5	5.6, 4.1	2
64 mM K^+ (5 min)	30	0.5	10.6, 20.0	2
50 μM veratridine (5 min)	2.4	2.5	178 ± 19	3
50 μM veratridine (5 min)	2.4	0	10.9 ± 3.1	3
1 mM ouabain (15 min)	2.4	2.5	172 ± 19	3
1 mM ouabain (15 min)	2.4	0	14.3 ± 6.1	3

levels rose slowly, reaching a maximum at 5 min, and then did not decrease significantly during the subsequent 25 min.

The addition of 1 mM theophylline to the incubation medium had no effect on control levels of cyclic AMP, but reduced the response to 64 mM K^+ almost 90% (Table 3). Others have also observed that theophylline significantly inhibits the accumulation of cyclic AMP in brain slices electrically stimulated or exposed to depolarizing agents (11, 12). In marked contrast, theophylline increased the control levels of cyclic GMP 2.5-fold and did not significantly change the response to 64 mM K^+.

Adenosine produces accumulation of cyclic AMP in guinea pig cerebral cortex (13). Shimzu and Daly (11) have suggested that some of the accumulation of cyclic AMP in brain slices produced by depolarizing agents is mediated by the release of intracellular adenosine. In support of this concept is the observation that the effects of both adenosine and depolarizing agents are inhibited by theophylline (11–13). Furthermore, extracts of brain also produce accumulation of cyclic AMP (14), presumably as a result of the adenosine or adenine nucleotides in the extracts. In mouse cerebellum adenosine (0.1 mM) produced marked increases of cyclic AMP levels, but did not influence cyclic GMP levels (Table 4). Guanosine (0.1 mM)

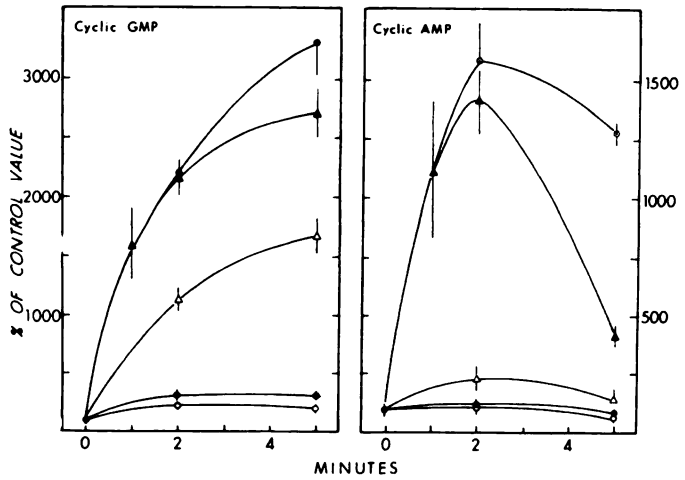

FIG. 3. *Effect of several concentrations of K^+ on cyclic AMP and cyclic GMP levels in mouse cerebellum slices as a function of time*

Tissue slices were incubated for 75 min in Krebs-Ringer-bicarbonate buffer. The K^+ concentration in the incubation medium was then raised to 17 mM (○), 24 mM (●), 35 mM (△), 64 mM (▲), or 121 mM (⊙). Tissue samples were removed at various times thereafter and extracted with trichloracetic acid as described in the text. Results are expressed as percentages of control values, which were 6 ± 0.4 and 12.8 ± 1.7 for cyclic GMP and cyclic AMP, respectively. Each value is the mean and standard error of 4–11 samples.

TABLE 3

Effect of theophylline on K^+-induced accumulation of cyclic AMP and cyclic GMP in incubated slices of mouse cerebellum

Mouse cerebellum slices were incubated in Krebs-Ringer buffer for 60 min, and then 1 mM theophylline was added as indicated to the incubation medium. After an additional 15-min incubation the K^+ concentration was increased from 6 to 64 mM and the Na^+ concentration was decreased an equivalent amount. Tissue samples removed 2 min later were assayed for cyclic AMP, and those removed 5 min later were assayed for cyclic GMP. Each value represents the mean and standard error of three to six samples.

Theophylline (1 mM)	Cyclic AMP		Cyclic GMP	
	5 mM K^+	64 mM K^+	5 mM K^+	64 mM K^+
	pmoles/mg protein		*pmoles/mg protein*	
−	11 ± 2	149 ± 31	6 ± 1	127 ± 17
+	11 ± 2	26 ± 3	15 ± 3	159 ± 37

did not influence the level of either cyclic nucleotide.

Cyclic GMP has appeared to be related to cholinergic neurotransmission in brain in several experimental models (2–4). In the present system acetylcholine (1–100 μM), either alone or in the presence of 0.3 mM eserine, a cholinesterase inhibitor, failed to produce a statistically significant increase in cyclic GMP levels. Prior treatment of the mouse cerebellum slices with 0.1 mM atropine, a concentration probably sufficient to block both muscarinic and nicotonic cholinergic receptor sites, did not significantly reduce the effect of any of the depolarizing agents on cyclic GMP levels (Table 5).

The effects of K^+ on cyclic GMP and cyclic AMP levels in cerebellum and cerebral cortex were compared (Table 6). Control levels of cyclic AMP in incubated slices from cerebral cortex were about the same as those in cerebellum. However, cyclic GMP levels were 6 times higher in cerebellum than in cortex. This difference has been observed previously in rapidly frozen mouse brain (9). Although 64 mM K^+ increased levels of cyclic AMP and cyclic GMP in cerebral cortex, as it did in cerebellum, the changes were different in the two structures. In cerebellum 64 mM K^+ increased cyclic GMP levels by approximately 150 pmoles/mg of

protein in 15 min, a 25-fold increase. In cerebral cortex 64 mM K^+ increased cyclic GMP levels only 13-fold, or 12 pmoles/mg of protein in 15 min. The K^+-induced increase in cyclic AMP levels, in contrast, were 3 times as great in cerebral cortex as in cerebellum. Moreover, in cerebellum cyclic AMP levels rapidly increased and then fell toward control levels during 15 min of exposure to 64 mM K^+, whereas in cerebral cortex cyclic AMP levels continued to rise throughout the 15-min exposure to 64 mM K^+.

TABLE 4

Effect of adenosine on levels of cyclic GMP and cyclic AMP in mouse cerebellum

Adenosine was added to a suspension of mouse cerebellum slices following an incubation for 75 min. The slices were then sampled at 5 and 20 min and assayed for cyclic AMP, cyclic GMP, and protein.

Additions to incubation medium	Cyclic AMP	Cyclic GMP
	pmoles/mg protein	*pmoles/mg protein*
None[a]	11.1 ± 3.2	4.6 ± 0.5
0.1 mM adenosine		
5 min	369, 304	4.9, 9.4
20 min	298, 280	5.7, 6.7

[a] Three control values (means ± standard errors) are represented.

DISCUSSION

Veratridine, ouabain, and extracellular concentrations of K^+ in excess of usual physiological levels produce depolarization of nervous tissue, each by a different mechanism. High extracellular concentrations of K^+ produce depolarization by decreasing the usual large difference between intracellular and extracellular K^+ concentrations (15). Veratridine increases the permeability of cellular membranes, allowing extracellular Na^+ to diffuse into cells (16). Ouabain inhibits (Na^+ + K^+)-adenosine triphosphatase, thereby preventing extrusion of Na^+ from cells (17). Thus both veratridine and

TABLE 5

Effect of atropine on accumulation of cyclic GMP in mouse cerebellum

Each value represents the mean ± standard error of four samples. Slices were incubated in Krebs-Ringer buffer for 60 min and with atropine as indicated for 15 min prior to the addition of the depolarizing agents.

Depolarizing agent	Cyclic GMP	
	No addition	Atropine (0.1 mM)
	pmoles/mg protein	
121 mM K^+, 5 min	145 ± 16	155 ± 27
50 μM veratridine, 5 min	170 ± 11	138 ± 19
1 mM ouabain, 15 min	153 ± 24	110 ± 16

TABLE 6

Effect of K^+ concentration on cyclic AMP and cyclic GMP levels in mouse cerebral cortex and cerebellum

Mouse cerebellum or cerebral cortex slices were incubated in Krebs-Ringer buffer for 75 min, and then the K^+ concentration in the incubation medium was increased from 6 to 64 mM. At various times thereafter the slices were removed and assayed for cyclic AMP, cyclic GMP, and protein.

K^+ concentration	Time	Cerebellum[a]		Cerebral cortex[b]	
		Cyclic AMP	Cyclic GMP	Cyclic AMP	Cyclic GMP
mM	*min*	*pmoles/mg protein*			
6	0	14 ± 3	6 ± 1	9 ± 1	1 ± 0.1
64	2	181 ± 18	129 ± 9	225 ± 36	7 ± 1
	5	57 ± 6	163 ± 13	327 ± 63	11 ± 1
	15	18 ± 1	149 ± 14	560 ± 41	13 ± 2

[a] Each value represents the mean and standard error of four samples.
[b] Each value represents the mean and standard error of nine samples.

ouabain probably produce depolarization by increasing the intracellular Na^+ concentration. Depolarization of nervous tissue appears to be accompanied by many changes of cellular function and metabolism (18). These include changes of cellular membrane permeability (19), increased cellular respiration (20, 21), and augmented neurotransmitter release (22). The last phenomenon may be responsible for many of the functional and metabolic alterations observed in depolarized nervous tissue.

The increases in cyclic GMP levels in mouse cerebellum produced by veratridine, ouabain, and high concentrations of K^+ are much greater than any previously reported in nervous tissue, either *in vivo* or *in vitro*. The present data reveal that all three agents have similar effects on cyclic GMP levels, although each produces depolarization of nervous tissue by separate mechanisms. Furthermore, the effects on cyclic GMP of combinations of the agents at maximal concentrations are not additive although submaximal concentrations are supra-additive. These findings suggest that the accumulation of cyclic GMP produced by the three agents is a result of a common phenomenon rather than due to independent direct effects on cyclic GMP synthesis or degradation. The only known phenomenon common to all three agents is depolarization. However, the mechanism by which depolarization may affect cyclic GMP levels is not readily apparent.

Neurotransmitter release at the neuromuscular junction is dependent on Ca^{++} (23), as is the accumulation of cyclic GMP observed here in brain slices. Furthermore, synaptic transmission is blocked, presumably by inhibition of neurotransmitter release, in rabbit superior cervical ganglia stimulated in solutions containing low (0.5 mM) Ca^{++} and high (20 mM) Mg^{++} concentrations (24). Combinations of high Mg^{++} and normal Ca^{++} concentrations or normal Mg^{++} and low Ca^{++} concentrations slightly reduce but do not prevent synaptic transmission. It is apparent from the present study that the same conditions which block neurotransmission in rabbit superior cervical ganglia also prevent the accumulation of cyclic GMP in depolarized brain slices. These parallel effects of Ca^{++} and Mg^{++} on cyclic GMP accumulation in depolarized brain slices and transmitter release at the neuromuscular junction and in autonomic ganglia suggest that the increase of cyclic GMP levels produced by depolarization is somehow related to the release of an intracellular substance, perhaps a neurotransmitter. At present we cannot ascertain whether the accumulation of cyclic GMP is the cause or the result of neurotransmitter release, or whether these are independent phenomena. Our current working hypothesis is that the accumulation of cyclic GMP is mediated by the action of a released intracellular substance in some cells in brain, and that the rise in cyclic GMP could be caused by the release of an intracellular substance at the presynaptic nerve ending or could result from the influence of the released substance on adjacent cells.

Some preliminary attempts to identify this proposed substance were made. Adenosine, which supposedly mediates the accumulation of cyclic AMP in depolarized nervous tissue, does not seem to be responsible for the accumulation of cyclic GMP. The findings that acetylcholine did not produce a significant elevation of cyclic GMP and that atropine did not block the accumulation of cyclic GMP produced by the depolarizing agents indicate that released intracellular stores of acetylcholine probably have no major role in the mechanisms underlying the effect of depolarizing agents on cyclic GMP levels.

It was surprising to find that acetylcholine did not produce an accumulation of cyclic GMP in mouse cerebellum. Previously we observed that oxotremorine, a centrally acting cholinergic agent, produces an elevation of levels of cyclic GMP in mouse cerebellum *in vivo*. Perhaps the effect of oxotremorine is not mediated by an intrinsic cerebellar cholinergic mechanism. The results of the present study also are not consistent with the observations of Lee *et al.* (4), demonstrating that acetylcholine and other choline esters produce an accumulation of cyclic GMP in slices of rabbit cerebellum. Possibly this discrepancy represents a species difference.

Many substances have been demonstrated

to produce diverse effects on the levels of cyclic GMP and cyclic AMP in several tissues (1, 2, 25). These observations have led to the proposal that the two cyclic nucleotides may have opposing effects on some cellular metabolic processes (25). High concentrations of K^+, unlike most other substances which influence cyclic nucleotide concentrations, increase levels of both cyclic AMP and cylic GMP in brain, however. It is conceivable that the increases of cyclic AMP and cylic GMP that we observed here did not occur in the same cell types. Possibly cyclic AMP levels increase in a cell population in which cyclic GMP levels fall or do not change. An alternative possibility is that the two cyclic nucleotides may increase in the same cell population, but, rather than having opposing actions, they may influence separate mechanisms.

Although high concentrations of K^+ increase levels of cyclic GMP and cyclic AMP in mouse cerebellum, the characteristics of the K^+-induced accumulation of the two cylic nucleotides are very different. We have observed the following major differences: (a) cyclic GMP accumulation is induced at a lower level of K^+ than that necessary to produce accumulation of cyclic AMP; (b) following K^+ stimulation, cyclic GMP levels increase more slowly than do cyclic AMP levels, and remain elevated for a much longer period; (c) the time course of the K^+-induced accumulation of cyclic GMP is unaffected by the K^+ concentration, while that of cyclic AMP is markedly influenced by K^+ concentration; (d) the K^+-induced accumulation of cyclic AMP is inhibited by theophylline, but that of cyclic GMP is not. These findings indicate that the accumulation of cyclic AMP and cyclic GMP produced by high concentrations of K^+ are mediated by different mechanisms, and offer additional proof that the two cyclic nucleotides probably have different roles in the central nervous system.

The present study demonstrates that the effects of high concentrations of K^+ on cyclic AMP and cyclic GMP levels in cerebellum are strikingly different from those in cerebral cortex. It appears that the effects of other agents on cyclic nucleotide levels are also not uniform throughout the central nervous system (2, 26). These findings are difficult to interpret in the light of our present incomplete understanding of the individual roles of cyclic AMP and cyclic GMP in nervous tissue. They may reflect an uneven distribution and a variable involvement of the cyclic nucleotide systems among the several morphologically and functionally distinct populations of cells in the central nervous system. Although no information is available concerning the cellular localization of the cyclic GMP systems, in support of this argument are the findings of Bloom *et al.* (27), demonstrating with the use of fluorescent antibody localization techniques that cyclic AMP can be visualized only in Purkinje neurons and granule cells in rabbit cerebellum.

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