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Heavy Metals and Adenosine Cyclic 3',5'-Monophosphate Metabolism: Possible Relevance to Heavy Metal Toxicity

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SUMMARY

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Adenylate cyclase activity in homogenates and particulate fractions of rat cerebellum, cerebral cortex, salivary gland, heart, and liver was inhibited by very low concentrations of lead ions (I_{50} < 3 μM). Both basal and hormone-stimulated activities were affected, and the inhibition was not dependent upon calcium or ATP concentration. Inhibition was reversed by 2-mercaptoethanol but not by ethylene glycol bis (β-aminoethyl ether)-*N,N'*-tetraacetic acid.) Very low concentrations (I_{50} = 1-8 μM) of zinc, copper, cadmium, mercury, uranium, silver, and gold ions also inhibited adenylate cyclase. Cyclic AMP phosphodiesterase activity was inhibited by these same heavy metals, with the exception of lead, which stimulated phosphodiesterase. The nonheavy metals aluminum, iron, and nickel had little effect on either enzyme. These effects of heavy metals *in vitro* raise the possibility that alteration of cyclic AMP metabolism, together with other membrane effects, may underlie some of the toxic signs present in certain types of heavy metal poisoning.

INTRODUCTION

Adenosine 3',5'-monophosphate plays an important role in the mechanism of action of a number of hormones, including neurotransmitters (1). Cyclic AMP is formed from adenosine 5'-triphosphate by adenylate cyclase, and hydrolyzed to adenosine 5'-monophosphate by cyclic nucleotide phosphodiesterase. For optimal activity, both these reactions require the presence of a metal cofactor, usually magnesium or manganese.

It is known that in the presence of magnesium or manganese relatively high concentrations (100 μM or more) of certain other metal ions, much as zinc or copper, will inhibit adenylate cyclase and phosphodiesterase activity (2-4). However, relatively little is known about the effects on these enzymes of heavy metal ions (e.g., Pb^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Hg^{2+}, UO_2^{2+}, Ag^{+}, Au^{3+}) at concentrations which approach those found *in vivo* (0.1-100 μM) following toxic exposure to these metals.

Recently it has been reported that micromolar concentrations of lead ion inhibit adenylate cyclase in the cerebellum (5) and in fat and pancreatic islet cells (6), and that organic mercury compounds inhibit liver adenylate cyclase (7). Both these metals are known to cause toxic manifestations *in vivo* (8, 9) at concentrations similar to those reported to inhibit cyclic AMP synthesis *in vitro*. In the present study the effects of lead on cyclic AMP metabolism are investigated in more detail. In addi-

tion, a number of other heavy metals are examined for their effects on adenylate cyclase and phosphodiesterase activity.

METHODS

Adenylate Cyclase

Male Sprague-Dawley rats weighing 150–250 g were killed by decapitation or by a blow to the head. The various tissues used (cerebral cortex, cerebellar cortex, submandibular salivary gland, heart ventricle, and liver) were removed, washed once in ice-cold NaCl, and homogenized (15 mg/ml) by hand in an all-glass homogenizer in cold 6 mM Tris-maleate buffer (pH 7.4). The rate of cyclic AMP formation was measured in an assay system containing 80 mM Tris-maleate (pH 7.4), 10 mM theophylline, 6 mM $MgSO_4$, 1.5 mM ATP, and tissue homogenate (1 mg, wet weight), plus or minus metal salt or other test substances, in a final volume of 0.3 ml. Incubation was conducted for 3 min at 30° in a shaking water bath. The reaction was initiated by the addition of ATP and terminated by boiling for 2 min, and the mixture was centrifuged at low speed to remove insoluble material. Cyclic AMP in the supernatant was measured by the method of Brown *et al.* (10) with appropriate blanks. Depending upon the experimental conditions, 3–100 pmoles of cyclic AMP were formed per tube. Aliquots containing 1–4 pmoles were assayed with a precision of ± 0.05 pmole. Under the experimental conditions used, enzyme activity was linear with respect to time and enzyme concentration.

In some cases the adenylate cyclase reaction was terminated by the addition of 0.5 ml of cold (−79°) 0.2 N HCl in ethanol. After low-speed centrifugation, the supernatant was evaporated to drynes, then redissolved in 0.3 ml of H_2O and applied to a 0.5 × 5 cm column of Bio-Rad AG 50W-X8, 200–400 mesh. The resin retained the heavy metals, and, after elution of ATP with 3 ml of 1 mM potassium phosphate buffer, pH 7.0, the cyclic AMP fraction was collected with 4 ml of H_2O, lyophilized, redissolved in 0.6 ml of H_2O, and assayed by the protein binding method (10). Passage through the ion-exchange resin removed 99.9% of the ATP and more than 99.9% of the heavy metal ions in the sample. Results obtained with this method were similar to those in which the adenylate cyclase reaction was stopped by boiling and the soluble fraction assayed directly for cyclic AMP.

When washed particulate preparations, instead of homogenates, were assayed for adenylate cyclase activity, 1.5-ml aliquots of the tissue homogenates were diluted with 2 volumes of 6 mM Tris-maleate buffer (pH 7.4) and centrifuged at 20,000 × *g* for 15 min. The resulting pellet was resuspended in 4 ml of fresh buffer, repelleted and washed twice more, and finally resuspended in 1.5 ml of buffer. Aliquots (60 μl) of this fraction were assayed for adenylate cyclase activity as described above. In some experiments aliquots of the original homogenate were incubated with a metal salt for 3 or 10 min at 30° in a shaking water bath prior to centrifugation and washing.

In the course of the studies on adenylate cyclase activity it was noted that high concentrations (0.25–4 mM) of lead, zinc, or uranyl ion caused some nonenzymatic conversion of ATP to cyclic AMP. This conversion did not interfere with the measurement of adenylate cyclase activity, however, since, at the concentrations of heavy metals used in the experiments described below (0.1–100 μM), nonenzymatic formation of cyclic AMP was neglible (less than 2%) compared with basal adenylate cyclase activity.

Phosphodiesterase

Phosphodiesterase activity was measured in an assay system containing 80 mM Tris-maleate (pH 7.4), 6 mM $MgSO_4$, tissue homogenate (1 mg, wet weight), and either 0.1 or 1 μM cyclic AMP, plus or minus metal salt or other test substances, in a final volume of 0.3 ml. Incubation was conducted for 1–5 min at 30° in a shaking water bath. The reaction was initiated by the addition of the tissue and terminated by boiling for 2 min, and the mixture was centrifuged at low speed to remove insoluble material. Cyclic AMP in the supernatant was measured as described above.

Under these conditions the rate of synthesis of cyclic AMP from endogenous ATP (in the homogenate) was neglible compared with the concentration of added cyclic AMP, and the rate of disappearance of added cyclic AMP was used as a measure of phosphodisterase activity. In some cases phosphodiesterase activity was measured as the rate of formation of [^{3}H]adenosine from tritiated cyclic AMP (11). Both procedures gave similar results.

Cyclic AMP Binding

Cyclic AMP-binding protein was prepared by the method of Brown *et al.* (10) from the cortex of frozen calf adrenal glands obtained from Pel-Freez. Binding was measured in an assay system containing 50 mM Tris-HCl (pH 7.4), 4 mM theophylline, 3 mM 2-mercaptoethanol, 4 nM cyclic [^{3}H]AMP (38.4 Ci/mmole), and a saturating concentration of binding protein, plus or minus test substances, in a final volume of 0.2 ml. Incubation was conducted for 2 hr at 0°. The reaction was initiated by the addition of binding protein and terminated with the addition of 0.1 ml of Norit SGX charcoal (100 mg/ml in 2% bovine serum albumin in the buffer described above), and the mixture was centrifuged for 15 min at 2000 × *g*. Aliquots of the supernatant were taken for the measurement of bound cyclic AMP.

Protein was determined by the method of Lowry *et al.* (12), using bovine serum albumin as the standard.

All metal salts used were of analytical grade. Cyclic AMP, ATP, theophylline, and norepinephrine were obtained from Sigma Chemical Company, and tritiated cyclic AMP, from New England Nuclear Corporation.

RESULTS

Adenylate Cyclase Activity

Lead. Lead ion was a potent inhibitor of adenylate cyclase. Concentrations of lead nitrate or lead chloride as low as 0.1 μM significantly inhibited enzyme activity. Fifty per cent inhibition occurred at about 2.5 μM, and almost complete inhibition, at 30 μM. Figure 1 shows the inhibition by lead chloride of adenylate cyclase activity in a homogenate of rat cerebellum. The extent of the inhibition by lead of adenylate cyclase activity present in washed, particulate fractions of rat cerebellum was identical with that seen in homogenates.

Under the experimental conditions used for assaying adenylate cyclase activity (in which 10 mM theophylline was present), cyclic AMP phosphodiesterase activity was nearly completely inhibited (Table 1). What phosphodiesterase activity remained was unaffected by the addition of 100 μM lead chloride, indicating that under these experimental conditions the observed decrease of cyclic AMP formation (Fig. 1) was due to inhibition of adenylate cyclase and not to stimulation of phosphodiesterase.

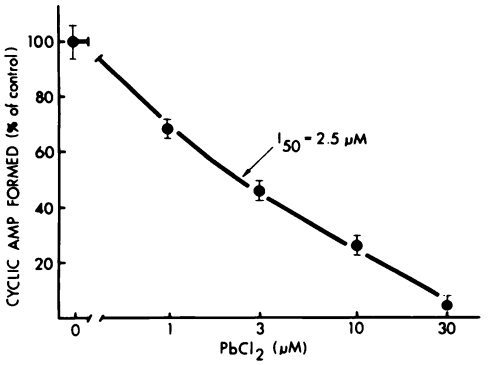

FIG. 1. *Effect of lead chloride on adenylate cyclase activity in a homogenate of rat cerebellar cortex*

Control activity in the absence of lead was 312 ± 19 pmoles/mg of protein per minute. The values shown here and in the subsequent figures and tables are the means (± mean deviations) for two to four replicate samples, each assayed in duplicate.

TABLE 1

Effect of lead on phosphodiesterase activity of rat cerebellum

Conditions	Cyclic AMP hydrolyzed at	
	0.1 μM	1 μM
	pmoles/mg protein/min	
Control	24.1 ± 1.3	167 ± 12
+Theophylline (10 mM)	3.8 ± 2.9	
+Theophylline + $PbCl_2$ (100 μM)	4.2 ± 5.0	
+$PbCl_2$ (1 μM)	29.2 ± 1.2	
+$PbCl_2$ (10 μM)	30.4 ± 1.7	
+$PbCl_2$ (100 μM)	35.4 ± 2.1	200 ± 25
+$PbCl_2$ (100 μM) + ATP (1.5 mM)	28.8 ± 3.3	

Under other conditions, in which cyclic AMP hydrolysis was not inhibited by theophylline, lead and several other heavy metals were found to alter the activity of phosphodiesterase (see below).

Other metals. In addition to lead ion, cadmium, uranyl, mercuric, cupric, zinc, gold, and silver ions also inhibited the adenylate cyclase activity of cerebellar homogenates (Table 2). Among these heavy metals, cadmium (I_{50} = 1.1 μM) was the most potent and silver (I_{50} = 8 μM) the least potent, although the range was not great. In contrast, aluminum, iron, and nickel (not usually considered heavy metals) had little effect on adenylate cyclase activity at similar concentrations. The I_{50} for these latter three metals was greater than 100 μM.

Other tissues. The adenylate cyclase activity present in homogenates of rat cerebral cortex, heart (ventricle), salivary gland, and liver was inhibited by lead in a fashion similar to that found in the cerebellum. Among these tissues, the enzyme activity of heart appeared most sensitive to inhibition by lead: 50% inhibition of basal activity (10.8 pmoles/mg of protein per minute) occurred in the presence of 1 μM lead chloride.

Hormone sensitivity. In addition to inhibiting basal adenylate cyclase activity, lead also blocked hormone activation of the enzyme. Figure 2 shows the adenylate cyclase activity in a homogenate of rat submandibular salivary gland. In the presence of 5 μM norepinephrine, a concentration causing about half-maximal activation of the enzyme, basal activity was stimulated by about 75%. Increasing concentrations of lead chloride inhibited not only basal enzyme activity (as in Fig. 1) but also the activation of the enzyme by norepinephrine. Inhibition of hormone activation by lead was proportional to the inhibition of basal activity.

In other experiments, in which the adenylate cyclase activity of cerebellar homogenates was stimulated by 10 mM sodium fluoride, 200 μM lead chloride caused partial, though not complete, inhibition of enzyme activity. [Lack of complete inhibition by lead in the presence of fluoride may have been due to the formation of insoluble lead fluoride (k_{sp} = 2.2 × 10^{-8}), which decreased the concentration of free lead ion in solution.]

TABLE 2

Effects of various metal ions on adenylate cyclase activity of rat cerebellum

Values were determined from dose-response curves consisting of six points over the range 0.1–100 μM. Each point represented the mean of two replicate samples assayed for cyclic AMP content in duplicate.

Metal salt	Adenylate cyclase inhibition (I_{50})
	μM
Cadmium chloride	1.1
Uranyl nitrate	1.5
Lead chloride	2.4
Mercuric chloride	2.5
Cupric chloride	2.6
Zinc chloride	2.6
Gold trichloride	4.0
Silver nitrate	8.0
Aluminum nitrate	>100
Ferric chloride	>100
Nickelous chloride	>100

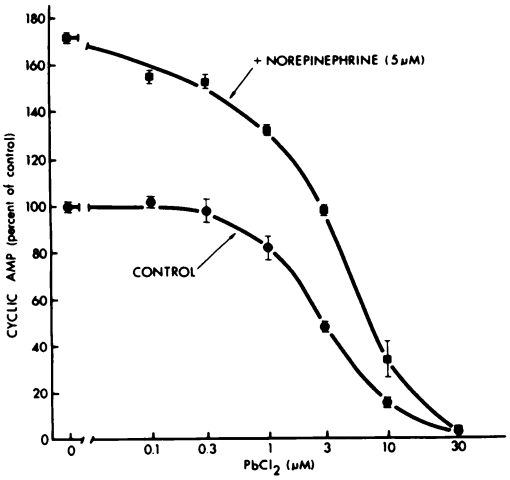

FIG. 2. *Effect of lead chloride on basal and norepinephrine-stimulated adenylate cyclase activity in a homogenate of rat submandibular salivary gland*

Control activity in the absence of lead or norepinephrine was 6.7 ± 0.1 pmoles/mg of protein per minute. In the presence of 5 μM norepinephrine, basal activity was stimulated 72%. From other data (not shown) it had been determined that 5 μM norepinephrine was the concentration necessary for about half-maximal stimulation of basal enzyme activity.

ATP, calcium, and protein concentra-

tion. Lead ion can cause nonenzymatic hydrolysis of ATP (13), which is especially detectable above concentrations of 350 μM. The inhibitory effect of lead on adenylate cyclase activity was not, however, due to a decreased concentration of enzymatic substrate (ATP). Thus, in tests at various ATP concentrations over a 25-fold range ([Mg^{2+}]:[ATP] remaining constant), the degree of enzyme inhibition by a partially inhibitory concentration of lead chloride was independent of the ATP concentration (Fig. 3). These results also suggest that the inhibition of adenylate cyclase by lead was not due to competition by lead for the ATP substrate.

In other experiments the addition of exogenous calcium chloride (10 μM–1 mM) had no effect on the concentration of lead required for constant partial inhibition of enzyme activity. This result suggests that displacement of endogenous calcium is probably not involved in the mechanism of lead-induced inhibition of adenylate cyclase activity. The degree of enzyme inhibition by 5 μM $PbCl_2$ was also not affected by varying the protein concentration of the reaction mixture over a 16-fold range. If a permanent lead-enzyme complex were required for enzyme inhibition, it might have been expected that the degree of enzyme inhibition would decrease as the amount of enzyme protein in the reaction mixture increased. However, the nonstoichiometric result actually obtained suggests that lead may cause inhibition without having to bind irreversibly to the enzyme.

Reversibility of adenylate cyclase inhibition. The addition of a concentration of EGTA[1] in excess of that of lead, prior to the addition of enzyme, prevented the inhibitory effect of lead on adenylate cyclase activity. This was presumably due to chelation of free lead ion from solution. However, if EGTA was added after the homogenate had been in contact with lead ion (Fig. 4), there was little or no reversal of enzyme inhibition, even by concentrations of EGTA in excess over lead. This latter result suggests either that the affinity of lead for the enzyme is greater than its affinity for the chelator, or, perhaps, that a permanent lead-enzyme complex is not necessary for adenylate cyclase inhibition once the enzyme has been exposed to the metal ion. This second possibility is supported by the nonstoichiometric relationship between protein concentration and activity described above.

Lead is known to bind to cysteine resi-

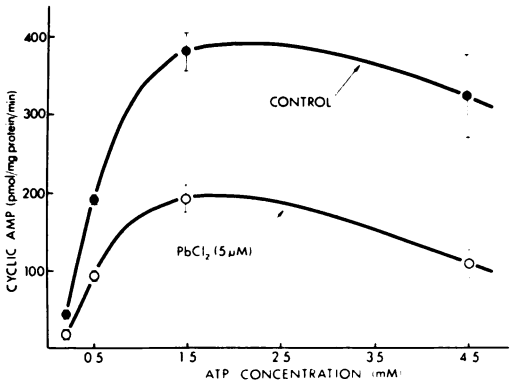

FIG. 3. *Effect of ATP concentration on adenylate cyclase activity of a rat cerebellar homogenate in the presence and absence of a partially inhibitory concentration of lead chloride*

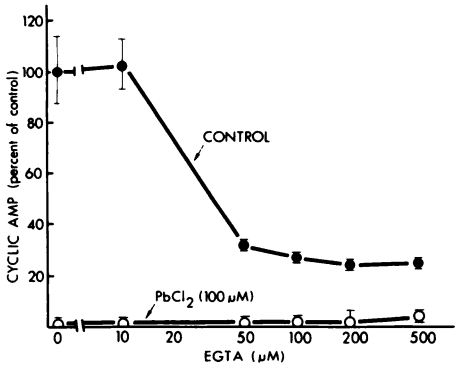

FIG. 4. *Effect of EGTA on adenylate cyclase activity of a rat cerebellar homogenate in the presence and absence of a maximally inhibitory concentration of lead chloride*

Tubes containing homogenate in buffer were incubated for 3 min at 30° in the presence or absence of 100 μM $PbCl_2$. EGTA was then added, and the tubes were incubated for an additional 3 min at 30° prior to the addition of ATP. Subsequent assay conditions were as described under METHODS. Control activity (423 ± 46 pmoles/mg of protein per minute in the absence of lead and EGTA) was depressed by concentrations of EGTA greater than about 10 μM, possibly as a result of chelation of endogenous calcium (14).

[1] The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid.

dues (15), and it has been shown that sensitivity to sulfhydryl reagents is a property of adenylate cyclases from a number of tissues (16). Therefore it was of interest to determine whether a sulfhydryl reducing agent would reverse the inhibitory effect of lead on enzyme activity. The addition of increasing concentrations of 2-mercaptoethanol to homogenates previously incubated in the presence of 5 μM lead chloride (Fig. 5) resulted in a progressive recovery of enzyme activity; 50% recovery occurred at a concentration of about 20 mM 2-mercaptoethanol. In the presence of a maximally inhibiting concentration of lead chloride (100 μM), however, much higher concentrations of 2-mercaptoethanol were required to reverse the inhibition (bottom curve, Fig. 5); less than a 50% reactivation of enzyme activity occurred in the presence of 100 mM 2-mercaptoethanol. In other experiments, in which tissue preparations were first exposed to lead, then extensively washed in lead-free buffer, and finally incubated in the presence of 2-mercaptoethanol, enzyme reactivation was similar to that shown in Fig. 5.

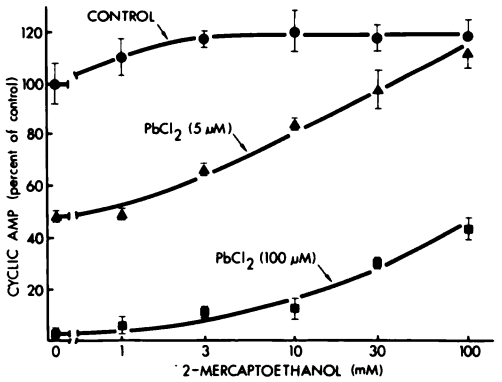

FIG. 5. *Effect of 2-mercaptoethanol on reversing inhibition by lead of adenylate cyclase activity in a homogenate of rat cerebellum*

Tubes containing homogenate in buffer were incubated for 3 min at 30° in the presence (5 μM or 100 μM) or absence of $PbCl_2$; 2-mercaptoethanol was then added, and the tubes were incubated for an additional 3 min at 30° prior to the addition of ATP. Subsequent assay conditions were as described under METHODS. Control activity in the absence of lead and 2-mercaptoethanol was 258 ± 21 pmoles/mg of protein per minute.

Phosphodiesterase

The phosphodiesterase activity of cerebellar homogenates was measured at two substrate concentrations (Table 1). In the presence of 10 mM theophylline basal enzyme activity was inhibited by 90%, and 100 μM lead chloride had no effect on the rate of cyclic AMP hydrolysis. In the absence of theophylline, however, low concentrations of several of the heavy metal ions partially inhibited phosphodiesterase activity (Table 3). At a substrate concentration of 0.1 μM cyclic AMP, inhibition ranged from 58% for 30 μM silver nitrate to 15% for 30 μM gold trichloride. Silver, cadmium, mercuric, cupric, uranyl, and zinc ions also inhibited enzyme activity at a 10-fold higher substrate concentration. The three nonheavy metals tested, nickel, iron, and aluminum, were without effect at either substrate concentration.

In contrast with the inhibition of cerebellar phosphodiesterase seen in the presence of the other heavy metal ions, 30 μM lead caused a reproducible 20–25% stimulation of enzyme activity. Table 1 shows that some activation was observed at concentrations of lead as low as 1 μM. Although retinal phosphodiesterase activation by light is dependent on the presence of ATP (17), the addition of ATP to the reaction mixture resulted in no further stimulation of cerebellar phosphodiesterase activity by lead ion.

TABLE 3

Effect of various metal ions on phosphodiesterase activity of rat cerebellum

Control activities in the presence of 0.1 and 1 μM cyclic AMP were 51.2 ± 2.7 and 278 ± 15 pmoles of cyclic AMP hydrolyzed per milligram of protein per minute, respectively.

Metal salt (30 μM)	Cyclic AMP hydrolyzed at	
	0.1 μM	1 μM
	% control	
Silver nitrate	42 ± 8	60 ± 5
Cadmium chloride	57 ± 3	75 ± 7
Mercuric chloride	62 ± 9	78 ± 17
Cupric chloride	67 ± 8	65 ± 15
Uranyl nitrate	82 ± 10	89 ± 5
Zinc chloride	82 ± 1	92 ± 5
Gold trichloride	85 ± 3	102 ± 7
Nickelous chloride	99 ± 4	110 ± 10
Ferric chloride	100 ± 17	100 ± 10
Aluminum nitrate	101 ± 7	90 ± 12
Lead chloride	122 ± 9	121 ± 5

Cyclic AMP Binding

Most of the heavy metal ions tested (Pb^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}, UO_2^{2+}, Ag^{+}, Au^{3+}) inhibited binding of cyclic AMP to the unpurified cyclic AMP-binding protein of adrenal cortex under the same conditions as, but at substantially higher concentrations than, those used in the analysis of enzyme activities. Among these, lead caused the greatest inhibition: a 50% decrease in binding occurred at a concentration of 350 μM lead chloride, and complete inhibition, at 3 mM. This inhibition of cyclic AMP binding by the heavy metal ions did not interfere with the determination of adenylate cyclase and phosphodiesterase activities, since the final concentration (0.01–2 μM) of heavy metals in the protein binding assay medium was far less than the minimum concentration (100 μM) required to affect binding. Furthermore, the inhibitory effects on cyclic AMP synthesis were unaffected when the heavy metals were largely removed from cyclic AMP samples (by retention on an ion-exchange resin) prior to the binding assay. The inhibition of cyclic AMP binding was of interest because of the possible relationship between the cyclic AMP-binding protein and the regulatory subunit of cyclic AMP-dependent protein kinase (18). In preliminary experiments we also found that lead ion stimulates the endogenous phosphorylation by ATP of particulate proteins in rat cerebellum. Possibly relevant to these observations is a report that certain organic mercury compounds bind to and induce a dissociation of muscle protein kinase (19).

DISCUSSION

These results demonstrate that very low concentrations of lead and other heavy metal ions have marked effects on cyclic AMP metabolism *in vitro*. Lead inhibited both basal and norepinephrine-stimulated adenylate cyclase activity proportionally, suggesting that the metal neither selectively affects only the receptor subunit of the enzyme (as do specific receptor blockers) nor affects just basal enzyme activity without influencing hormone sensitivity (as does EGTA). The failure of high concentrations of calcium to reverse enzyme inhibition suggests that the displacement of bound calcium is probably not involved in the mechanism of adenylate cyclase inhibition by lead.

The reactivation of lead-inhibited adenylate cyclase activity by 2-mercaptoethanol suggests that lead interacts with at least one functional sulfhydryl group on the enzyme molecule. However, from our data it is not possible to determine whether enzyme inhibition requires the formation of a permanent lead-enzyme complex. The stability constant (log K) for lead-EGTA is about 15, and for lead-cysteine, about 12 (15, 20). Therefore the ability of the divalent metal ion chelator EGTA to prevent, but not to reverse, lead inhibition may suggest that such an irreversible lead-enzyme complex is not necessary for continued inhibition after the enzyme is initially exposed to the metal.

It has previously been reported that lead nitrate (0.7–2.4 mM) inhibits the hydrolysis by brain phosphodiesterase of high concentrations (4 mM) of cyclic AMP (4). In the present experiments lead (1–100 μM) caused a small (20%) stimulation of phosphodiesterase activity at low substrate concentrations (0.1–1 μM cyclic AMP). Therefore the possibility is suggested that lead may exert differential effects on high-K_m and low-K_m phosphodiesterase activities.

The concentrations of heavy metal ions used in this study approach those found in tissues following toxic exposure to certain of these metals (15). Thus the results described here may have relevance to the metabolism of cyclic AMP *in vivo*. For example, in the mammalian caudate nucleus, a dopamine-sensitive adenylate cyclase has been identified which has properties quite similar to those of the caudate dopamine receptor (21). Blockade of this receptor, such as by the phenothiazine tranquilizers, causes extrapyramidal side effects. In Wilson's disease, a disorder of copper metabolism, copper accumulates in the basal ganglia to a concentration of 100 μM, which is 6–10 times normal (22). Interestingly, patients with Wilson's disease of-

ten display extrapyramidal neurological symptoms which improve considerably when copper is mobilized from the brain with penicillamine (23). Thus it is possible that inhibition of dopamine-sensitive adenylate cyclase by copper (which would be functionally equivalent to blockade of the dopamine receptor) might be responsible for some of the neurological manifestations of Wilson's disease. Interestingly, copper has been shown to block the increase of water permeability in toad urinary bladder by antidiuretic hormone, a compound also thought to work through the activation of adenylate cyclase (24).

Certain other heavy metals, such as mercury and lead, are also known to cause neurological abnormalities at very low concentrations (8, 9, 25). For example, in cases of experimental lead intoxication in which rats display behavioral deficits, brain lead levels range from 3 to 6 μM, a concentration which, in the present study, resulted in 50–70% inhibition of adenylate cyclase activity. This same concentration of lead also caused a stimulation of phosphodiesterase activity *in vitro,* an effect which would tend to augment the inhibition of adenylate cyclase by causing a reduction of intracellular cyclic AMP levels. Such correlations between tissue concentrations *in vivo* and effects *in vitro*, however, must be drawn with care. The availability of a metal to the target enzyme *in vivo* depends on many factors, including the cellular and subcellular distribution of the metal, the cellular and subcellular distribution of the enzyme, and the availability of various intra- and extracellular protective mechanisms. Furthermore, it should be emphasized that heavy metals are known to affect the activity of numerous enzymes and to exert many other effects on cellular metabolism, all of which may play an important role in the toxicity of these ions. Nevertheless, the potent effects on cyclic AMP metabolism of the heavy metals described in this study raise the possibility that at least some of the toxic effects of certain heavy metals may be related to their effects on cyclic AMP metabolism *in vivo.*

Finally, these results raise some questions about recently developed methods of histochemical localization of adenylate cyclase activity (26). Such procedures use millimolar concentrations of lead to precipitate the phosphate released during the adenylate cyclase reaction. Because of the potent inhibition by lead of adenylate cyclase activity described in this paper, it would seem that the interpretation of histochemical localization studies employing lead salts should be drawn with care. Similar cautions have recently been raised by Lemay and Jarett (6).

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