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STUDIES ON THE ACTION OF PORPHYRINOGENIC TRACE METALS ON THE ACTIVITY OF HEPATIC UROPORPHYRINOGEN DECARBOXYLASE

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SUMMARY

Trace metals which produce experimental uroporphyrinuria in animals during prolonged exposure inhibit uroporphyrinogen (uro) decarboxylase in rat liver extracts in vitro. Inhibitory effects are prevented by sulfhydryl reagents, suggesting metal binding to sulfhydryl groups of the enzyme as the likely mode of action. Mercury, the most potent of the metals tested with respect to sulfur binding kinetics, produces the greatest inhibition of enzyme activity. In contrast, iron, which is considered to play a role in the etiology of porphyria cutanea tarda (PCT) via inhibition of uro decarboxylase, did not inhibit the enzyme in the present test system, suggesting an indirect mode of action in vivo. These results suggest that direct inhibition of uro decarboxylase underlies uroporphyrinuria produced by prolonged trace metal exposure. Experimental inhibition of uro decarboxylase by trace metals may serve as a model for studying metal-induced uroporphyrinuria and PCT in humans.

INTRODUCTION

Numerous studies in recent years have demonstrated that trace metals may alter the regulation of heme metabolism in mammalian tissues (1-5). Specific effects have been demonstrated in this regard on several enzymes of the heme biosynthetic and degradative pathways, most notably δ-aminolevulinic acid (ALA) dehydratase (6), ferrochelatase (7), and heme oxygenase (8). Previous studies from these laboratories have demonstrated that urinary concentrations of uroporphyrin are greatly increased in animals following prolonged exposure

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to arsenic (9) and mercury (10), suggesting that uroporphyrinogen (uro) decarboxylase (E.C.4.1.1.37), the enzyme which catalyzes the stepwise decarboxylation of uroporphyrinogen to coproporphyrinogen (11), may also be susceptible to trace metal inhibition in vivo. This prospect is of particular interest in light of recent evidence suggesting that uro decarboxylase is genetically deficient in liver (12) and red cells (13) of humans suffering from porphyria cutanea tarda (PCT), the most common form of hepatic porphyria in man, and is inhibited in both animal and human tissues by a variety of environmental chemical agents (11,14,15). Thus, exposure to trace metals which also inhibit this enzyme may confound disorders of porphyrin or heme metabolism which are related to genetically or environmentally impaired uro decarboxylase activity.

The present studies were undertaken to measure the effects of several trace metals of environmental importance on uro decarboxylase activity from rat liver in vitro, and to investigate the mechanism of action of the observed effects. Metals were selected for study on the basis of observations from previous studies, suggesting an inhibitory effect on porphyrin metabolism in vivo. Inasmuch as iron has also been implicated in the regulation of uro decarboxylase (12,16), and to play a role in the etiology of PCT (17,18), the effects of iron on enzyme activity were also evaluated.

MATERIALS AND METHODS

Preparation of Tissues. Male Sprague-Dawley rats (CD strain) (150-200 gms), obtained from the Charles River Laboratories, Boston, Massachusetts, were used as the tissue source. Animals were killed by decapitation. Livers were rapidly excised, washed, perfused with buffered saline, minced, and homogenized in 9 vols. of 0.25M sucrose containing 0.05M Tris-HCl buffer, pH 7.5, using a Potter-Elvehjem homogenizer fitter with a Teflon pestle. Livers from at least 4 rats were pooled for each experiment. All assays were performed in duplicate on fresh tissues immediately following sacrifice.

Uroporphyrinogen Decarboxylase Assay. Uro decarboxylase activity in mitochondria-free liver extracts was assayed as previously described (19). The substrate, uroporphyrinogen, was derived enzymatically from exogenous porphobilinogen, using a partially purified preparation of uro I synthetase from rat spleen. The activity of the decarboxylase during a 60 minute incubation period was quantitated on the basis of coproporphyrinogen formed.

Protein determinations were made by the method of Lowry et al. (20) using bovine serum albumin as a standard.

Studies with Metals and Sulfhydryl Agents. Studies to ascertain the effects of trace metals, iron and other agents on uro decarboxylase activity were performed by adding appropriate concentrations of aqueous solutions (0.01 ml) of the test substance directly to the incubation medium at the same time the enzyme source was added. Control reaction mixtures received distilled water only. Solutions or water were added by injection into the reaction mixture through the arm of the Thunburg tube in which the assay was performed, as previously described (19). Metal salts selected for study included mercuric chloride, sodium arsenite, sodium arsenate, lead acetate, cobalt chloride, and cadmium chloride. Iron salts included ferrous ammonium sulfate and ferric ammonium sulfate. Various sulfhydryl reducing agents were also used. Final concentrations of metal salts and other substances in the reaction mixtures are given in the Tables and Figures.

Statistical Analyses. Analysis of significance of differences between groups was performed by means of Student's t test. The level of significance was chosen as $p<0.05$.

RESULTS

Previous studies from these laboratories (19) have demonstrated the dependence of uro decarboxylase in a variety of mammalian tissues on functional sulfhydryl groups. When assayed in the presence of the sulfhydryl binding reagent, N-ethyl maleimide (1 mM), for example, enzyme activity is inhibited to 19 percent of control levels. This effect is completely prevented by concomitant addition of dithiothreitol (3 mM) to the reaction mixture.

Table 1 demonstrates the inhibitory effects of trace metals *in vitro* on the activity of uro decarboxylase. Mercury is clearly the most potent inhibitor of the enzyme, followed in this respect by cadmium, cobalt and arsenic. Interestingly, lead, a potent inhibitor of ALA dehydratase and numerous other sulfhydryl enzymes (21), did not inhibit uro decarboxylase under the conditions employed.

The dose-response curves for the two most effective inhibitors, mercury and cadmium, are presented in Figures 1 and 2. Cadmium produced significant inhibition of enzyme activity at all concentrations greater than 10^{-5}M, whereas mercury significantly inhibited decarboxylase levels *in vitro* at concentrations above 10^{-6}M.

Table 1. Effects of trace metals *in vitro* on uroporphyrinogen decarboxylase activity in rat liver. The final concentration of each metal salt in the reaction mixture was 10^{-3}M. Values in this and other tables represent means ± standard errors of at least 4 experiments.

Trace Metal	Decarboxylase Activity (pmoles/hr/mg prot.)	Inhibition (Percent of Control)
Control	318 ± 20	100
Mercuric chloride	15 ± 4*	5
Sodium arsenite	263 ± 15*	83
Sodium arsenate	272 ± 14*	86
Lead acetate	300 ± 17	94
Cobalt chloride	212 ± 21*	67
Cadmium chloride	73 ± 13*	30

*Significantly ($p<0.05$) different from control value.

Results presented in Table 2 suggest that the binding of the metal to the sulfhydryl group(s) of the enzyme is probably involved in the ability of the metal to inhibit enzyme activity. As indicated, the addition of either reduced glutathione or dithiothreitol completely prevents inhibition of the enzyme by mercuric chloride, the strongest inhibitor among the metals tested.

Table 3 describes the effects of iron in both ferrous and ferric forms on uro decarboxylase activity *in vitro*. Under conditions of the assay procedure, without preincubation of iron with the reaction medium, neither ferrous nor ferric iron had a significant effect on decarboxylase levels. Moreover, the iron chelating agent, o-phenanthroline, did not alter enzyme activity when added in the absence of exogenous iron to the reaction medium.

DISCUSSION

A common property of heavy metals is their ability to bind to a number of functional groups, of which the sulfhydryl group is one of the most chemically reactive found in cells (22). The results from the present studies demonstrate inhibition of uro decarboxylase by various metal ions *in vitro* and prevention of that effect by sulfhydryl reducing agents, suggesting that metal mercaptide formation with sulfhydryl groups of uro decarboxylase underlies the

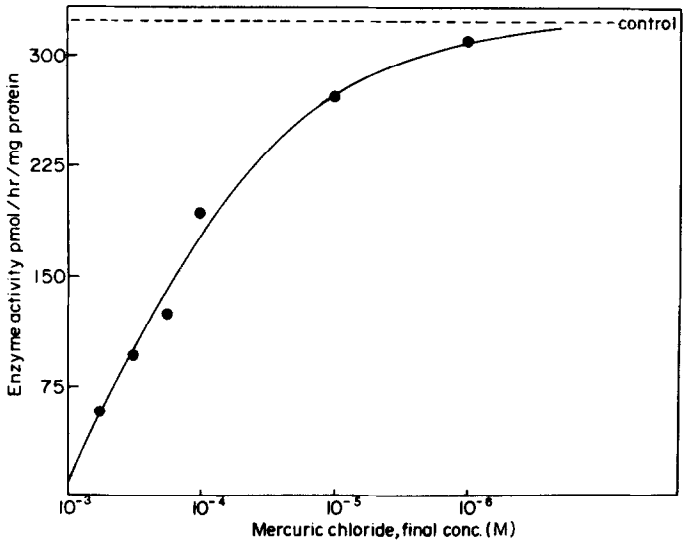

Figure 1. Effects of mercuric chloride on hepatic uroporphyrinogen decarboxylase activity in vitro.

inhibitory effects observed. The potency of trace metals with respect to inhibition of uro decarboxylase activity in vitro appears to be related to the sulfhydryl binding capacity of the metal, as demonstrated by the potent inhibition of the enzyme by mercury, the strongest of the metals tested with respect to sulfur binding kinetics (22).

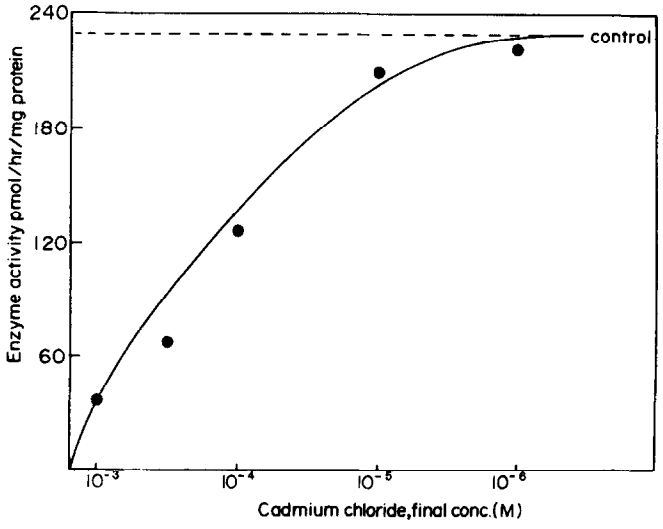

Figure 2. Effects of cadmium chloride on hepatic uroporphyrinogen decarboxylase activity in vitro.

Table 2. Effects of sulfhydryl reagents, dithiothreitol (3mM) and reduced glutathione (10mM), on the inhibition of uro decarboxylase *in vitro* by mercuric chloride (10^{-4}M).

Reagent	Decarboxylase Activity (pmoles/hr/mg prot.)	Inhibition (Percent of Control)
Control	288 ± 17	100
Reduced glutathione	296 ± 13	103
Mercuric chloride	176 ± 17*	61
Mercuric chloride and glutathione	298 ± 23	103
Dithiothreitol	309 ± 25	107
Mercuric chloride and dithiothreitol	302 ± 19	105

*Significantly ($p<0.05$) different from control value.

Lead, despite its well known affinity for sulfhydryl groups, is not an effective inhibitor of decarboxylase *in vitro*. This result is consistent with the observation that lead does not effectively inhibit enzymes possessing a single thiol group at the active site, but is a strong inhibitor of those enzymes which possess more than one thiol group, or vicinal dithiols, at the catalytic site, e.g., ALA dehydratase (23). Furthermore, concentrations of metals such as arsenate or arsenite, in the order of 10^{-3}M, required to produce a significant inhibition of the decarboxylase activity, are also in accord with the view that the enzyme possesses a monothiol rather than a dithiol configuration at the active site (24).

Cadmium, which significantly inhibits uro decarboxylase *in vitro*, does not produce uroporphyrinuria during prolonged exposure (unpublished observations), nor is known to alter the regulation of porphyrin or heme metabolism *in vivo*. These results are consistent with the known property of cadmium to induce *in vivo* and be bound in liver and possibly other organs to metallothionein (25), which effectively prevents binding of cadmium with the functional groups of other molecules such as the sulfhydryl groups of uro decarboxylase.

The present studies fail to demonstrate any significant inhibitory effects of iron on decarboxylase activity. These results differ from those of Kushner et al. (26), who reported inhibition by up to 40 percent of uro decarboxylase

Table 3. Effects of iron and o-phenanthroline (1mM) on the activity of uro decarboxylase in vitro. Glutathione (reduced form) concentration was 10mM.

Reagent	Decarboxylase Activity (pmoles/hr/mg prot.)
Control	272 ± 17
Ferrous ammonium sulfate (10^{-3}M)	248 ± 21
(10^{-4}M)	268 ± 18
(10^{-5}M)	291 ± 20
Ferrous ammonium sulfate (10^{-3}M) and glutathione	252 ± 17
Ferrous ammonium sulfate (2×10^{-3}M) and glutathione	249 ± 18
Ferric ammonium sulfate (10^{-3}M)	266 ± 16
(10^{-4}M)	319 ± 24
(10^{-5}M)	295 ± 20
o-phenanthroline	259 ± 16

activity by ferrous iron (0.7 mM) incubated with an enzyme preparation from pig liver extracts in the presence of cysteine as a reducing agent. It is possible that differences in procedures used for incubation of iron with the enzyme, or in the enzyme assay methods could account for the differences observed. In the former case, for example, it is possible that preincubation of ferrous iron with the tissue preparation produced stimulation of peroxidase activity which could have attacked sulfhydryl groups of the enzyme (27), accounting for subsequent loss of decarboxylase activity. Nevertheless, iron overload (hepatic siderosis) is considered by many to play an important etiologic role in the clinical manifestation of PCT (e.g., 28), as well as in mediating chemically-induced PCT-like symptoms in both animals and humans (29,30). Further studies on the role of iron in the regulation of uro decarboxylase are, therefore, clearly required.

Finally, although the etiology of chemically-induced PCT is not fully understood, it is clear that a variety of chemical agents and other factors may participate in the development of this condition in both humans and animal models. From previous studies conducted in these laboratories (4,9,10,31), it is apparent that various trace metals may elicit conditions resembling those of PCT during prolonged exposure. The results of the in vitro studies

conducted here suggest that inhibition of uro decarboxylase by the metals in question may play an important role in this process. Trace metal-induced uroporphyrinuria may, therefore, constitute an experimental model for studying chemically or genetically-related PCT in humans.

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