LEAD AND METHYL MERCURY: EFFECTS OF ACUTE EXPOSURE ON CYTOCHROME P-450 AND THE MIXED FUNCTION OXIDASE SYSTEM IN THE LIVER

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Lead and mercury are widespread environmental pollutants. Workers in the mining industry and in the manufacture of storage batteries, sheet-lead, paint, pipes, and ceramics are exposed to inorganic lead salts; gasoline is also a major source of environmental lead (1, 2). Acute and chronic lead poisoning from paint in impoverished urban areas is a major pediatric problem (3). Organic mercury salts have been employed for many years as fungicides in agriculture and in the paper industry (4). As a result of industrial pollution of water, it has been shown that fish accumulate mercury in the form of methyl mercury (4). In man, one of the most dangerous features of lead and methyl mercury poisoning is the insidiousness of its development. Absorption, excretion, and storage of excessive quantities of these heavy metals may occur without overt clinical manifestations of intoxication (1).

The ability to synthesize heme is a biochemical function which is common to all aerobic cells. A substantial fraction of the heme synthesized in the liver serves as a prosthetic group for the microsomal hemoprotein, cytochrome P-450. This hemoprotein plays a central role in the hepatic detoxification of drugs, hormones, and foreign chemicals. The ability of heavy metals, such as lead, to impair heme synthesis in erythroid cells through inhibition of several sulfhydryl enzymes is well known, as are the hematological consequences of this action (5, 6). The possibility that lead and other metals may similarly impair heme synthesis or the function of hemoproteins, such as cytochrome P-450, in the liver has however not been studied. In the experiments described below, the acute effects of lead chloride and methyl mercuric chloride on cytochrome P-450 and model drug metabolizing enzyme reactions coupled to the mixed function oxidase, such as the N-demethylation of ethylmorphine and the hydroxylation of aniline were examined. The acute effects of lead and methyl mercury on the duration of action of hexobarbital in the intact rat were also determined.

Male Sprague-Dawley rats each weighing 120-150 g were used. Lead chloride (PbCl₂), dissolved in 0.9% NaCl solution, was administered intravenously at a dosage of 5 mg/kg. Methyl mercuric chloride (CH₃HgCl), dissolved in corn oil, was administered intraperitoneally at a dosage of 5 mg/kg. Control rats received the vehicle only. Animals were sacri-
ficed 24 hr after administration of these compounds. Livers were removed and microsomes were prepared as described previously (7). Microsomes were suspended in 1.15% KCl solution such that each milliliter contained the equivalent of 250 mg of liver, wet weight. The composition of the incubation mixture for the determination of ethylmorphine N-demethylase activity has been described previously (7). Formaldehyde formed from the N-demethylation reaction was measured by the method of Nash (8) as modified by Anders and Mannering (9). Aniline hydroxylase activity was measured as described previously (10). Cytochrome P-450 contents were assayed by the method of Omura and Sato (11) from the carbon monoxide difference spectrum of dithionite-reduced microsomes using an extinction coefficient of 91 mM⁻¹ cm⁻¹ between 450 and 490 nm. To determine the hexobarbital sleeping time, sodium hexobarbital was administered intraperitoneally at a dosage of 100 mg/kg and the duration of loss of the righting reflex was determined. Protein contents were determined by the method of Sutherland et al. (12) using bovine serum albumin as a standard.

Ethylmorphine and aniline were chosen as substrates in these studies because they are representative of type I and type II substrates (13, 14). Type I substrates interact with the microsomal hemoprotein, cytochrome P-450, to give a difference spectrum with a λmax in the range of 385-390 μm and a λmin in the range of 418-427 μm; the λmax and λmin given by type II compounds are 425-435 and 390-405 μm, respectively (13). After administration of 5 mg/kg PbCl₂, intravenously, or 5 mg/kg CH₃HgCl, intraperitoneally, to rats, ethylmorphine N-demethylase and aniline hydroxylase activities of hepatic microsomes were decreased by 40-50% of control values in 24 hr (Table I). Concomitant with a decrease in the microsomal oxidations of ethylmorphine and aniline, there was a marked decrease in cytochrome P-450 content of liver microsomes obtained from rats administered lead or methyl mercury (Table I). Decreases in cytochrome P-450 contents and N-demethylase and hydroxylase activities of hepatic microsomes were also observable when the dosages of PbCl₂ and CH₃HgCl were decreased to 2.5 mg/kg. Hexobarbital has been widely used as a substrate for investigation of the activity of liver microsomal enzymes and hexobarbital-induced sleeping time has been used as a measure of rate of hexobarbital metabolism. As shown in

### Table I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ethylmorphine N-demethylation (μmol HCIO formed/mg protein per hr)</th>
<th>Aniline hydroxylation (μmol p-aminophenol formed/mg protein per hr)</th>
<th>Cytochrome P-450 (μmol/mg protein)</th>
<th>Hexobarbital sleeping time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.312 ± 0.034</td>
<td>41.79 ± 2.78</td>
<td>0.506 ± 0.053</td>
<td>57 ± 15</td>
</tr>
<tr>
<td>PbCl₂</td>
<td>0.151 ± 0.026*</td>
<td>25.66 ± 1.88*</td>
<td>0.331 ± 0.017*</td>
<td>106 ± 6*</td>
</tr>
<tr>
<td>Control</td>
<td>0.329 ± 0.048</td>
<td>45.54 ± 2.28</td>
<td>0.535 ± 0.014</td>
<td>40 ± 7</td>
</tr>
<tr>
<td>CH₃HgCl</td>
<td>0.182 ± 0.033*</td>
<td>25.90 ± 5.61*</td>
<td>0.321 ± 0.042*</td>
<td>65 ± 6*</td>
</tr>
</tbody>
</table>

Each value represents mean ± SE of five rats.
* Values significantly different from the respective control values (P < 0.05).
Table I, administration of lead or methyl mercury to rats resulted in a significant prolongation of hexobarbital-induced sleeping time. Thus the in vitro inhibition of microsomal oxidations is accompanied by enhanced pharmacological action of hexobarbital.

The mechanism of action of lead and methyl mercury on the hepatic mixed function oxidase system in these studies may be comparable with that of the former metal on erythroid precursors in the bone marrow. Several heme pathway enzymes, such as δ-aminolevulinic acid dehydratase, porphobilinogen deaminase, uroporphyrinogen decarboxylase, and heme synthetase possess essential sulfhydryl groups and lead and methyl mercury may exert their effects by inhibiting one or more of these enzymes, resulting in inhibition of the formation of cytochrome P-450. The short half-life of cytochrome P-450 in the liver (15) would permit such a mode of action. These heavy metals may however also exert a direct action on the hepatic mixed function oxidase since there is a suggestion of involvement of sulfhydryl groups in the integrity and electron-transfer function of cytochrome P-450 (16) as well as nicotinamide adenine dinucleotide phosphate–cytochrome c reductase activities (17), both of which are components of the microsomal drug metabolizing enzyme system.

It is not known whether the effects of acute exposure to these metals on the cytochrome P-450 system in experimental animals are comparable with those produced by acute intoxication with lead in children, for example; this question is now being investigated in clinical studies. The doses of lead employed in these experiments are however within the range of total body burden of this metal found previously lead-poisoned individuals (2).

SUMMARY

The rat liver mixed function oxidase system which is responsible for the metabolism of endogenous and exogenous compounds has been shown to be affected by lead and methyl mercury. Administration of these environmental pollutants to rats results in a decrease in cytochrome P-450 content and inhibition of in vitro N-demethylase and hydroxylase activities. The in vitro enzyme-inhibiting effects of the metals found pharmacological expression in the whole animal by prolongation of hexobarbital-induced sleeping times.

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REFERENCES