Lead Toxicity in Mice with Genetically Different Levels of δ-Aminolevulinic Acid Dehydratase

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The inhibitory effects of lead on δ-aminolevulinic acid (ALA) dehydratase, a sulfhydryl enzyme involved in porphyrin synthesis, have been extensively studied with regard to lead exposure (LITCHMAN and FELDMAN 1963; MILLAR et al. 1970; HERNBERG et al. 1970; STOPPS 1971). Lead exposure in man and experimental animals is characteristically accompanied by a decreased blood δ-ALA dehydratase activity and an increase of δ-ALA in the urine (HERNBERG et al. 1970; SELANDER and CRAMER 1970). The relationship of δ-ALA dehydratase inhibition to manifestations of lead toxicity is less clearly understood. It has been suggested that inhibition of δ-ALA dehydratase may lead to decreased cellular availability of heme. STOPPS (1971), however, demonstrated that dogs fed with lead acetate were similar to controls in their hematological functions, although blood δ-ALA dehydratase levels were severely depressed by the lead diet.

Recently, we reported that adaptation to the toxic effects of lead in mice were independent of the lead-induced inhibition of liver δ-ALA dehydratase (GARBER and WEI 1972). In this investigation, lead was administered to three strains of mice with genetically different δ-ALA dehydratase levels in order to further elucidate the relationship of δ-ALA dehydratase activity to parameters of acute and subacute lead toxicity.

METHODS

Male DBA/2, C57Bl/6 and Swiss-Webster mice, 60-70 days old, were used in these experiments. The 10 day LD_{50} for lead nitrate was calculated by the method of LITCHFIELD and WILCOXON (1949). Lead nitrate dissolved in distilled water was administered intraperitoneally at a constant volume of 0.1 ml/10 g body weight. For the LD_{50} determination of each strain, at least 10 mice were used at each of 5 different dose levels. Subacute lead toxicity was studied by feeding a 4% lead carbonate diet for 12 days. The lead diet was prepared by mixing lead carbonate with the meal form of a standard laboratory chow. Controls received the same diet without lead.

Body weight, liver and kidney weight, hematocrit, and inhibition of δ-ALA dehydratase were used as indices of subacute lead toxicity. The hematocrit was determined from samples of free-flowing blood collected in heparinized capillary tubes from the tail of the mouse. Animals were then decapitated and the liver

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and right kidney dissected and weighed. The left kidney was preserved in formaldehyde for histological assessment at a later date. Liver δ-ALA dehydratase activity was assayed according to a modification of the method described by GIBSON et al. (1955). The whole mouse liver, weighing approximately 1.5 g, was homogenized in 10 ml of ice cold 0.15M KCl and 0.5 ml of the homogenate was then mixed with 1 ml of 0.1M pH 6.8 phosphate buffer and 0.2 ml of 0.1M δ-ALA. The mixture was incubated in vacuo for 1 hr at 38°C. Under these experimental conditions, the enzyme activity, calculated as micromoles of porphobilinogen (PBG) per gram of wet tissue per hour, was a linear function of homogenate concentration.

RESULTS AND DISCUSSION

The δ-ALA dehydratase activities of the three mice strains (Table 1) used in this investigation were significantly different from each other. The observed strain differences correspond well with the values found by DOYLE and SCHIMKE (1969; DOYLE 1971), who demonstrated that genetic control of the rate of enzyme synthesis, rather than different enzyme characteristics, accounted for the strain differences. Lead nitrate, administered 20 mg/kg i.p. 24 hr before enzyme assay, significantly decreased δ-ALA dehydratase activity in all three strains (Table 1). The LD_{50} for $Pb(NO_3)_2$, however, was not correlated to the initial enzyme activity or to the percent of enzyme activity inhibited by lead (Table 1).

TABLE 1

The LD_{50} of lead nitrate for three strains of mice with different levels of δ-ALA dehydratase activity

Strain	δ-ALA dehydratase activity[a] (μmoles PBG/g liver/hr)		LD_{50}[c] (mg/kg)
	Water	Lead[b]	
DBA/2	3.92 ± .14	2.24 ± .20*	74(61-89)
Swiss-Webster	2.65 ± .10	1.41 ± .06*	148(120-182)
C57Bl/6	1.55 ± .03	1.14 ± .09*	120(102-140)

[a] Values are means ± standard errors; N = 8 for each group.

[b] Treated with lead nitrate 20 mg/kg i.p. 24 hr before assay.

[c] Ten day LD_{50} for $Pb(NO_3)_2$ i.p. determined according to LITCHFIELD and WILCOXON (1949); figures in parentheses refer to the 95% confidence limits.

* Significantly less than control group receiving water ($P < .05$).

A 4% lead carbonate diet fed over a 12-day period produced characteristic manifestations of subacute lead toxicity (Table 2). In the lead treated groups body weights decreased, the kidneys were pale and flabby and were heavier than the controls, and the hematocrit of the C57Bl/6 strain decreased slightly. Statistically significant decreases in the liver weights of the DBA/2 and C57Bl/6 strains also occurred. The lead carbonate diet diminished the liver δ-ALA dehydratase activities of the three mice strains to 54-57% of the control values. However, subacute lead toxicity was not correlated to enzyme activity present before exposure to lead or to the percent of enzyme activity inhibited by lead.

These results indicate that inhibition of δ-ALA dehydratase activity is not related to acute or subacute lead toxicity as measured by lethality, body weight loss, liver and kidney weight changes or decreases in hematocrit. Although blood δ-ALA dehydratase activity and urinary δ-ALA concentrations are sensitive indicators of lead exposure, it appears that the toxicological properties of lead are not dependent on the degree of δ-ALA dehydratase inhibition. It is possible, however, that chronic inhibition of this enzyme by exposure to low-levels of lead may lead to toxic manifestations other than those studied in this investigation.

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REFERENCES

DOYLE, D.: J. Biol. Chem. 246, 4965 (1971).

DOYLE, D., and SCHIMKE, R.: J. Biol. Chem. 244, 5449 (1969).

GARBER, B., and WEI, E.: Ind. Hyg. News Rep. 15, 3 (1972).

GIBSON, K. D., NEUBERGER, A., and SCOTT, J.: Biochem. J. 61, 618 (1955).

HERNBERG, S., NIKKANEN, J., MELLIN, G., and LILIUS, H.: Arch. Environ. Health 21, 140 (1970).

LITCHFIELD, J. T., JR., and WILCOXON, F.: J. Pharmacol. 96, 99 (1949).

LITCHMAN, H. C., and FELDMAN, F.: J. Clin. Invest. 42, 830 (1963).

MILLAR, J. A., CUMMING, R. C., BATTISTINI, V., CARSWELL, F., and GOLDBERG, A.: Lancet 2, 695 (1970).

SELANDER, S., and CRAMER, K.: Brit. J. Ind. Med. 27, 28 (1970).

STOPPS, G. J.: J. Wash. Acad. Sci. 61, 103 (1971).

TABLE 2

Subacute lead toxicity in three strains of mice fed a 4% lead carbonate diet for 12 days[a]

		δ-ALA dehydratase activity (μmoles PBG/g/hr)	Body weight (g)	Liver weight (g)	Right Kidney weight (g)	Hematocrit (%)
DBA/2	Control	3.90 ± .20	22.1 ± .6	.932 ± .031	.160 ± .005	48.2 ± .5
	Lead	1.68 ± .08*	15.9 ± .5*	.662 ± .028*	.179 ± .005*	48.0 ± .7
Swiss-Webster	Control	1.92 ± .10	32.3 ± 1.0	1.588 ± .076	.212 ± .011	47.3 ± .6
	Lead	.87 ± .04*	28.5 ± 1.2*	1.392 ± .096	.276 ± .013*	47.0 ± .6
C57Bl/6	Control	1.65 ± .08	20.3 ± .4	.870 ± .041	.119 ± .005	48.7 ± .3
	Lead	.76 ± .04*	16.3 ± .4*	.650 ± .034*	.134 ± .004*	44.9 ± .6*

[a]Values are means ± standard errors; N = 10 for each group.

*Significantly different from control group ($P < .05$)