Bull. Environ. Contam. Toxicol. (1996) 57:47-53

Bulletin of Environmental Contamination and Toxicology

δ-Aminolevulinic Acid Dehydratase Activity in Weanling and Adult Rats Exposed to Lead Acetate

A. L. S. Rodrigues,[1] J. B. T. Rocha,[2] M. E. Pereira,[2] D. O. Souza[3]

[1]Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, 88040-900, Florianópolis, SC, Brazil
[2]Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97119-900, Santa Maria, RS, Brazil
[3]Departamento de Bioquímica, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, 90046-900, Porto Alegre, RS, Brazil

Received: 11 September 1995/Accepted: 9 February 1996

Lead is an environmental pollutant with no known beneficial health effects, and environmental or occupational exposure to this metal is known to result in toxicity. It may affect numerous organ systems, including the renal, reticuloendothelial, reproductive, and nervous system (Al Saleh 1994). Children and young animals are particularly sensitive to the toxic effects of this metal, being the central nervous system the main target of lead toxicity. Adults seem to be more resistant to lead neurotoxicity, but lead nephrotoxicity is a prominent effect (Nolan and Shaikh 1992).

Lead inhibition of δ-aminolevulinic acid dehydratase (ALAD), a sulfhydryl enzyme of the heme-biosynthesis pathway, has been implicated in the pathogenesis of lead poisoning, since various critical cellular processes are affected by a reduced concentration of heme. Several studies suggest that neurotoxicity associated with lead exposure result from depletion of heme (for review, see Goering 1993). In addition, the substrate of the enzyme, δ–aminolevulinate (ALA), which accumulates in lead poisoning, has been proposed to be responsible, at least in part, for lead neurotoxicity by generating active oxygen species (Bechara et al. 1993). Several studies concerning inhibition of ALAD activity by lead have been conducted, especially in erythrocytes of various animal species (Granick et al. 1973, Rodrigues et al. 1989, Simmonds et al. 1995). ALAD reactivation index by the SH-reducing compound dithiothreitol (DTT) has also been reported to be a very sensitive parameter to evaluate ALAD inactivation (Granick et al. 1973, Sakai et al. 1980). Literature on ALAD specific activity in lead-exposed animals have mainly measured the activity of the enzyme in blood, kidney, and liver. Few studies have investigated cerebral ALAD activity in lead-exposed animals and the results reveal an insensitivity of brain ALAD to lead action (Oskarsson 1989, Rocha et al. 1995). However, in these studies lead exposure had been imposed during relatively short periods, which may not be of sufficient duration to cause an effect on the enzyme. Moreover some studies suggest that a cerebral lead-binding protein might reduce cerebral ALAD inhibition in lead-exposed animals by lowering the intracellular availability of

Correspondence to: A. L. S. Rodrigues

lead to the enzyme in a similar way that occurs in the kidney (Goering 1993). Thus, the relative insensitivity of renal and cerebral ALAD to lead would be due to this mechanism of protection. Taking this information into account, we decided to compare lead effects on forebrain, cerebellar, and renal ALAD activity and the reactivation index with DTT obtained from: a) prenatal and lactational lead-exposure of rats and b) prenatal, lactational and long-term postlactational lead-exposure of rats.

MATERIALS AND METHODS

Female Wistar rats (110-150 g) were maintained on a 12 hour light- 12 hour dark schedule in a temperature controlled room (22-26°C). The control group received deionized water (milli-Q) and lead-treated rats received 0.5 or 4.0 mM lead acetate trihydrate in drinking deionized water. All animals had free access to food (containing less than 0.5 mg lead/kg dry weight) and to drinking fluid (water or lead acetate solution). After 6 weeks of exposure, females were mated with non-lead-exposed males for 5 consecutive days and maintained on the same drinking fluid throughout this period. Pregnant rats were maintained on the same drinking solution until the end of the suckling period. Offspring were weaned at 21 days of age. Two rats from each litter were killed by decapitation and forebrain, cerebellum, and kidneys were immediately dissected on ice. From each litter, samples of forebrain or cerebellum were pooled for ALAD activity assay. Kidneys from one pup per litter were used for ALAD activity assay, whereas kidneys from the other pup of the same litter were stored at -20°C and used for lead analysis. The lead analysis was carried out by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer spectrophotometer, model 2380) using acid digestion with nitric acid (4% v/v). After decapitation, blood was collected into heparinized tubes, pooled and also stored at -20°C until lead analysis was carried out. Blood lead analysis was done using a matrix modifier which contained Triton X-100 (0.5 % v/v) and dibasic ammonium phosphate (0.2 % v/v). After weaning, the remaining rats were separated by sex and maintained on the same treatment that had been administered to the mothers until 6 months of age, when ALAD activity was measured in kidney, forebrain, and cerebellum from one animal per litter.

ALAD activity in forebrain, cerebellum, and kidney was assayed according to the method of Sassa (1982) (rate of product porphobilinogen=PBG formation), except that 70 mM sodium phosphate buffer (pH 6.8) and 2.5 mM ALA were used. Simultaneously, a set of tubes was assayed using a similar incubation medium, except that 15 mM dithiothreitol (DTT) was also added in order to obtain the reactivation index. This index indicates the extent of the reactivation of ALAD activity by DTT and is defined as follows:

$$\frac{\text{(ALAD activity with DTT - ALAD activity without DTT) x 100\%}}{\text{ALAD activity with DTT}}$$

Tissue preparation was done according to Rocha et al. (1995). The incubation was carried out for 1-3 h at 39°C. The assay was carried out under conditions of constant velocity and there was a linear relationship between protein content and PBG formation (data not shown). Enzyme activity was expressed as nmol PBG/hr/mg protein. Protein was measured by the method of Bradford (1976), using bovine serum albumin as standard.

Data were analyzed by one-way ANOVA followed by Duncan's multiple range test when appropriate. Differences between groups were considered significant if $p<0.05$. Never more than one rat from the same litter was used as an independent measure in order to avoid litter effect.

RESULTS AND DISCUSSION

The exposure of pups to lead from conception to adult age did not influence the weight gains of the animals (Table 1). The body weight values of 6-month-old rats presented in Table 1 are similar to values obtained in a study performed using a larger sample size (data not shown). The kidney-to-body weight ratio was significantly increased in 21-day-old rats exposed to the highest lead dose ($p<0.0l$), whereas both 6-month-old lead-exposed groups showed an increase in this ratio ($p<0.05$ and $p<0.01$ for 0.5 and 4.0 mM groups, respectively, Table 1). This indicates a gross anatomical alteration, that could be related to a lead-induced cell proliferation in kidney (Choie and Richter 1974) since kidney-to-body weight increase was not related to an increase in water content (data not shown). On the other hand, there were no changes in forebrain-to-body-weight ratio or cerebellum-to-body weight ratio in any lead-exposed group. This is in accordance with literature which reports no overt anatomical changes in cerebral tissues associated with low-level lead exposure in offspring (Hasan et al. 1989, Rocha et al. 1995).

Blood lead levels were measured in order to evaluate the level of intoxication attained, and to compare our study with those which determined lead levels in blood. Blood lead level of weanling rats in the 0.5 mM group fell in the range that seems to be non-neurotoxic (Davis et al. 1990) and did not differ significantly from the control group (Table 2). Conversely, the weanling rats exposed to 4.0 mM lead acetate and the 6-month-old rats exposed to 0.5 or 4.0 mM lead acetate had a significantly ($p<0.01$) higher blood lead level than the control group. The blood lead levels obtained in our lead exposure regimen are similar to the blood lead levels which cause toxicity in humans (Davis et al. 1990).

Lead deposition in kidney (Table 2) was significantly ($p<0.01$ or $p<0.05$) increased in a dose-dependent manner in lead-exposed rats. The increase was about 3- and 8-fold the control values in the 21-day-old rats exposed to 0.5 and 4.0 mM group, respectively. In 6-month-old rats, lead deposition increased 9-fold to 13l-fold above control for the 0.5 and 4.0 mM groups, respectively.

Table 1. Body weight and tissue-to-body weight ratio of 21-day-old and 6-month-old lead-exposed rats. Data are mean ± SEM (g or mg wet weight/g body weight).

	Control		0.5 mM Group		4.0 mM Group	
	21-day-old	6-month-old	21-day-old	6-month-old	21-day-old	6-month-old
	n = 7	n = 6	n = 5	n = 6	n = 8	n = 6
Forebrain-to-body-weight	19.53 ± 0.72	3.74 ± 0.38	18.18 ± 0.56	3.99 ± 0.30	20.11 ± 1.52	3.78 ± 0.29
Cerebellum-to-body-weight	3.66 ± 0.14	0.93 ± 0.11	3.51 ± 0.16	0.93 ± 0.09	3.85 ± 0.31	1.01 ± 0.08
Kidney-to-body-weight	10.56 ± 0.29	5.56 ± 0.39	10.95 ± 0.33	6.76 ± 0.31*	13.68 ± 0.40**	7.65 ± 0.34**
Body weight•	43.4 ± 1.4	M: 361 ± 6 F: 233 ± 12	42.5 ± 2.2	M: 341 ± 8 F: 236 ± 4	43.6 ± 2.8	M: 358 ± 7 F: 246 ± 3

* $p<0.05$ or ** $p<0.01$ when compared to the control group by Duncan's multiple range test. •The number of 6-month-old rats is 3 males (M) and 3 females (F) in each group.

Table 2. Blood and kidney lead levels in 21-day-old and 6-month-old lead-exposed rats. Data are mean ± SEM (μg Pb/dl whole blood or μg Pb/g kidney weight).

	Control		0.5 mM Group		4.0 mM Group	
	21-day-old	6-month-old	21-day-old	6-month-old	21-day-old	6-month-old
	n = 5	n = 6	n = 4	n = 5	n = 6	n = 6
Blood	6.53 ± 0.33	7.61 ± 1.01	9.77 ± 0.17	41.63 ± 3.66**	44.35 ± 6.23**	116.91 ± 11.89**
Kidney	0.18 ± 0.02	0.18 ± 0.01	0.54 ± 0.03*	1.68 ± 0.13*	1.38 ± 0.15**	23.66 ± 1.29**

* $p<0.05$ or ** $p<0.01$ when compared to control group by Duncan's multiple range test.

Table 3. ALAD activity and reactivation index with DTT in forebrain, cerebellum, and kidney of 21-day-old and 6-month-old lead-exposed rats. Data are mean ± SEM nmol porphobilinogen/hr/mg protein or are presented as reactivation index (%).

	Control		0.5 mM Group		4.0 mM Group	
	21-day-old	6-month-old	21-day-old	6-month-old	21-day-old	6-month-old
Forebrain	n = 7	n = 6	n = 6	n = 5	n = 8	n = 6
Specific activity	9.18 ± 0.23	8.37 ± 0.44	9.64 ± 0.40	7.07 ± 0.38	9.30 ± 0.44	6.40 ± 0.49*
Reactivation index	19.6 ± 2.0	36.7 ± 2.8	19.6 ± 0.5	34.3 ± 2.8	21.8 ± 0.9	49.9 ± 2.9*
Cerebellum	n = 6	n = 6	n = 6	n = 5	n = 8	n = 5
Specific activity	9.94 ± 0.59	8.08 ± 0.41	11.39 ± 0.87	7.17 ± 0.42	11.93 ± 0.56	7.28 ± 0.53
Reactivation index	32.1 ± 1.2	37.9 ± 5.7	30.4 ± 1.5	42.6 ± 0.7	26.6 ± 0.9	48.1 ± 6.3*
Kidney	n = 5	n = 6	n = 4	n = 5	n = 6	n = 5
Specific activity	58.80 ± 10.37	22.57 ± 1.78	61.92 ± 10.42	13.32 ± 1.37**	41.64 ± 3.68	10.33 ± 1.18**
Reactivation index	19.6 ± 2.4	26.9 ± 6.7	28.9 ± 6.0	49.0 ± 4.9*	43.8 ± 7.1*	67.0 ± 5.8**

* $p<0.05$ or ** $p<0.01$ when compared to the control group by Duncan's multiple range test.

ALAD specific activity of forebrain, cerebellum and kidney of lead-exposed rats (Table 3) can not be considered a good parameter for the evaluation of lead toxicity because there was not a consistent effect of the metal on enzyme specific activity. In contrast, reactivation index with DTT seems to give a better indication of lead intoxication, since in kidney of 21-day-old rats and in cerebellum of 6-month-old rats it revealed an effect of lead not apparent in ALAD specific activity. A similar finding describing more efficient results with DTT, was previously reported for blood ALAD (Granick et al. 1973, Sakai et al. 1980).

The reactivation index of brain (forebrain and cerebellum) from 6-month-old rats was increased by exposure to 4.0 mM lead. In contrast, cerebral enzyme activity of 21-day-old rats was not affected by lead treatment. Oskarsson (1989) reported no effect on cerebral ALAD of weanling rats (blood lead level of 147 µg/dl) raised by dams exposed to 12 mM lead acetate through drinking water during pregnancy and the suckling period. In that study the weanling rats had blood lead levels much higher than the levels obtained in our 21-day-old rats, but the blood lead levels were similar to those obtained in our 6-month-old rats exposed to the highest lead dose. It seems that a long-term lead-exposure is necessary to cause an effect on the enzyme. Our findings on cerebral ALAD suggest that this enzyme is not insensitive to lead effects, since the inhibition of ALAD activity occurred in the 6-month-old rats exposed to the highest lead dose. The regimen of lead exposure associated in the present study with cerebral ALAD inhibition caused neither undernutrition nor gross learning deficits, as previously reported by us (Rodrigues et al. 1993).

Acknowledgements. This work was supported by grants from CNPq, FAPERGS, and PROPESP-UFRGS. The authors thank the staff of Centro de Ecologia (UFRGS) for lead analyses. This study is part of A.L.S.R.'s Thesis submitted to fulfill in part the requirements for the degree of Doctor in Sciences in the Departamento de Bioquímica at Universidade Federal do Paraná.

REFERENCES

Al-Saleh IAS (1994) The biochemical and clinical consequences of lead poisoning. Med Res Rev 14:415-486.

Bechara EJH, Medeiros MHG, Monteiro HP, Hermes-Lima M, Pereira B, Demasi, M, Costa CA, Abdalla DSP, Onuki J, Wendel CMA, Mascio P (1993) A free radical hypothesis of lead poisoning and inborn porphyrias associated with 5-aminolevulinic acid overload. Química Nova 16:385-392.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254.

Choie DD, Richter GW (1974) Cell proliferation in mouse kidney induced by lead I. Synthesis of deoxyribonucleic acid. Lab Invest 30:647-651.

Davis JM, Otto DA, Weil DE, Grant LD (1990) The comparative developmental neurotoxicity of lead in humans and animals. Neurotoxicol Teratol 12:215-229.

Goering PL (1993) Lead-protein interactions as a basis for lead toxicity. Neuro Toxicology 14:45-60.

Granick JL, Sassa S, Granick S, Levere RD, Kappas A (1973) Studies in lead poisoning II. Correlation between the ratio of activated to inactivated δ–aminolevulinic acid dehydratase of whole blood and the blood lead level. Biochem Med 8: 149- 159.

Hasan F, Cookman GR, Keane GJ, Bannigan JG, King WB, Regan CM (1989) The effect of low level lead exposure on the postnatal structuring of the rat cerebellum. Neurotoxicol Teratol 11:433-440.

Nolan CV, Shaikh ZA (1992) Lead nephrotoxicity and associated disorders: biochemical mechanisms. Toxicology 73:127-146.

Oskarsson A (1989) Effects of perinatal treatment with lead and disulfiram on ALAD activity in blood, liver and kidneys and urinary ALA excretion in rats. Pharmacol Toxicol 64:344-348.

Rocha JBT, Pereira ME, Emanuelli T, Christofari RS, Souza DO (1995) Effect of treatment with mercury chloride and lead acetate during the second stage of rapid postnatal brain growth on δ-aminolevulinic acid dehydratase activity in brain, liver, kidney and blood of suckling rats. Toxicology 100:27-37.

Rodrigues AL, Bellinaso ML, Dick T (1989) Effect of some metal ions on blood and liver delta-aminolevuhnate dehydratase of *Pimelodus maculatus* (Pisces, Pimelodidae). Comp Biochem Physiol 94B:65-69.

Rodrigues ALS, Rubin MA, Souza DO, Mello CF (1993) Lead exposure and latent learning ability of adult female rats. Behav Neural Biol 60:274-279.

Sakai T, Yanagihara S, Ushio K (1980) Restoration of lead-inhibited 5-aminolevulinate dehydratase activity in whole blood by heat, zinc ion, and (or) dithiothreitol. Clin Chem 26:625-628.

Sassa S (1982) Delta-aminolevulinic acid dehydratase assay. Enzyme 28:133-145.

Simmonds PL, Luckhurst CL, Woods JS (1995) Quantitative evaluation of heme biosynthetic pathway parameters as biomarkers of low-level lead exposure in rats. J Toxic01 Environ Health 44:35l-367.