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Effects of Lead Acetate on Cerebral Glutathione Peroxidase and Catalase in the Suckling Rat

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ABSTRACT: *Exposure of immature rats to lead acetate results in hemorrhagic encephalopathy of variable evolution. As the maintenance of adequate protection against peroxides may be critical in this condition, the activities of selenium-glutathione peroxidase and catalase in cerebrum and cerebellum of suckling rats poisoned with lead acetate were studied from day six to day sixteen post-exposure. Age-related decreases of glutathione peroxidase and catalase activities in both controls and lead poisoned animals were observed. An increase in catalase activity was observed in cerebrum and cerebellum of lead-treated rats compared to controls. Glutathione peroxidase activity did not change significantly in cerebrum over the period studied. By contrast, glutathione peroxidase activity in cerebellum of lead-treated rats remained at about twice the control level over most of the study period. This apparent increase in glutathione peroxidase activity may be due either to a slower ontogenic decrease of its specific activity or to enzyme induction in response to oxidant stress in cerebellum.*

KEY WORDS: Lead Poisoning, Glutathione Peroxidase, Selenium, Catalase, Development

INTRODUCTION

Lead has been recognized as a major toxicological factor due to its wide distribution in the human environment. Lead acetate exposure at high doses in humans, especially children, can cause encephalopathy with convulsions, stupor or coma, sometimes followed by death (Popoff *et al.*, 1963; Pentschew, 1965). Since Pentschew and Garro (1966) described hemorrhagic lead encephalopathy in immature rats, severals reports (Krigman *et al.*, 1974; Toews *et al.*, 1978; Holtzman *et al.*, 1980 and 1982) have added to our understanding of this model and its relationship to human lead encephalopathy. Spontaneous clinical improvement and regression of the morphological abnormalities in the capillaries have been observed by several authors (Pentschew and Garro, 1966; Toews *et al.*, 1978; Lefauconnier *et al.*, 1983) but the mechanism or mechanisms whereby lead acetate produces encephalopathic changes have not yet been established.

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It has been shown that after continuous lead acetate poisoning, the concentration of lead is non-homogeneous throughout the brain, highest levels of lead being observed in the cerebellum (Shafiq-ur-Rehman *et al.*, 1982; Lefauconnier *et al.*, 1983). The percentage change in the rate of lipid peroxidation and the intensity of hemorrhages followed a pattern similar to that of lead concentration in these cerebral areas. There is some evidence that lipid peroxidation increases following lead administration (Levander *et al.*, 1975; Gerber *et al.*, 1978; Sifri and Hoekstra, 1978; Shafiq-ur-Rehman, 1984). On the other hand, one study has appeared reporting that lead toxicity is not associated with an increase in lipid peroxidation in the brain (Gelman *et al.*, 1979). In vivo, lipid peroxidation is a process in which some activated form of oxygen reacts with polyunsaturated fatty acids to yield semistable peroxides, presumably via a free-radical mechanism (Girotti, 1985). It has been found that several defense mechanisms protect the central nervous system against reactive oxygen species, glutathione peroxidase and catalase being among the main detoxifying enzymes in this protective system (Prohaska and Ganther, 1976; Brannan *et al.*, 1980; Carmagnol *et al.*, 1983). The present study was undertaken to elucidate whether or not lead poisoning in immature rats could alter glutathione peroxidase (EC 1.11.1.9) and catalase (EC 1.11.1.6) activities in cerebrum and cerebellum, as such alterations might play a critical role in the development of lead encephalopathy.

MATERIALS AND METHODS

Pregnant female Sprague-Dawley rats were obtained on the 14th day of pregnancy. On arrival, they were individually housed in plastic cages and given a commercial solid diet plus tap water ad libitum. The day following parturition, when the offspring were distributed at random among the nursing mothers (eight rats per nursing mother), was designated as day one. Animals were maintained in a temperature-controlled room (23° C) with an alternating 12:12 hour light:dark cycle.

From the fifth day after birth, the animals received a daily i.p. injection of lead acetate (60 µg/g body weight) according to the procedure described by Lefauconnier *et al.* (1983). Lead acetate was prepared every day in freshly boiled distilled water in order to prevent precipitation of lead carbonate. Control litters were injected with the same volume of a solution containing the same concentration of sodium acetate. Lead acetate was administered daily from day 5 to day 21 after birth. Animals were killed six, twelve or sixteen days after beginning lead administration. The number of pups used for each experiment is shown in Tables 1 and 2.

Animals were anesthetized with ethyl ether and perfused intracardially with 10 ml 0.9% (w/v) NaCl before decapitation to reduce blood contamination of cerebral tissues. Each cerebral region (cerebellum and cerebrum) was homogenized in 7 vol. of 50 mM Tris-HCl, 0.1 mM EDTA, adjusted to pH 7.4. This homogenate was centrifuged at 3000 g for ten minutes. Aliquots of the supernatant solution were used for enzymatic analyses and protein determinations.

Glutathione peroxidase activity was measured according to Paglia and Valentine (1967) using cumene hydroperoxide as substrate. 20 mM sodiumazide was added to the reaction mixture in order to inhibit catalase activity. Blank values obtained without addition of samples were subtracted from assay values. The mixture components, with the exception of hydroperoxide, were preincubated at 37° C for at least 2 min before initiation of the reaction. Linear rates of NADPH oxidation were recorded spectrophotometrically (340 nm) at 25° C. Brain catalase activity was measured according to the method of Aebi (1974). The decomposition of H_2O_2 was recorded as the decrease of absorbance at 240 nm at 25° C. Since the results of enzymatic analyses were expressed as specific activity, proteins were quantified in all samples according to Lowry *et al.* (1951). The differences in activity between controls and poisoned rats were tested for significance with Student's t-test.

Since brain homogenates were contaminated with blood from the cerebellar hemorrhages, it was necessary to correct for possible erythro-

cyte contribution to brain glutathione peroxidase and catalase activities. The erythrocytes of three experimental animals from each group (6, 12 and 16 days after lead acetate administration) were individually isolated by centrifugation at 800 g for 10 min. The cells were washed twice with 0.9% NaCl and lysed with distilled water. The percentage of hemoglobin in each lysed erythrocyte preparation and both cerebral and cerebellar supernatant solutions was measured using a standard hemoglobin curve. Glutathione peroxidase and catalase activities and proteins in these preparations were measured by the methods described above. Enzyme activities in each brain sample were corrected for erythrocyte contribution (averages of enzyme activities of the 3 erythrocytes samples from each group) by the following formula: Δ E/min (brain corrected)= [Δ E/min (brain)] - [Δ E/min/mg Hb (RBC) x mg Hg (brain)], as described by Kaplan and Ansari (1984).

RESULTS AND DISCUSSION

Tables 1 and 2 show catalase and glutathione peroxidase activities in controls and lead poisoned animals after lead administration. As expected, blood contamination in cerebrum (from control and lead poisoned animals) and in cerebellum of control rats was null. By contrast, cerebellum in lead-treated rats was contaminated by blood due to cerebellar hemorrhages. Both uncorrected and corrected data for blood contamination are presented (Tables 1 and 2).

Our results demonstrate significant ontogenic changes in glutathione peroxidase and catalase activities in both controls and lead poisoned animals, in agreement with previous reports (Prohaska and Ganther, 1976; Gelman *et al.*, 1979). An increase in catalase activity was seen in the cerebrum (12 and 16 days after intoxication) and in the cerebellum on day 12 after lead administration in poisoned animals compared to controls (Table 1). Such findings could be related to an enhancement of the detoxication process in both regions. Nevertheless, it is difficult to establish a direct relationship between the increase of catalase activity and the participation of this enzyme in cerebral detoxication processes following lead poisoning.

Moreover, lead acetate exposure in suckling rats significantly increased cerebellum glutathione peroxidase activity on days 12 and 16 respectively after intoxication (Table 2). Glutathione peroxidase activity in the developing cerebellum of lead-treated rats remained at about twice the control level over most of the study period. Our corrections for contamination by erythrocyte enzymes should insure that we are measuring only cerebral activities. Even though one cannot exclude the possibility that hemoglobin may be degraded faster than erythrocyte glutathione peroxidase in the brain, this would hardly explain why the cerebellum glutathione peroxidase activity ratio remains constant over a period of ten days. Overall, our results provide evidence for a much higher glutathione peroxidase activity in the cerebellum of suckling rats intoxicated with lead acetate than in controls. Nevertheless, the origin of this increased activity is uncertain, as it was observed at a time when glutathione peroxidase activity steadily decreases as a result of anticipated ontogenic factors. Bull *et al.* (1983) reported that perinatal lead administration delays the development of the cerebral cortex of rat pups, while Ahtee *et al.* (1985) suggested that such a delay also occurs in the cerebellum as a consequence of perinatal lead exposure. In this sense, it is interesting to note that lead exerts a detrimental effect upon the morphologic and biochemical features of cerebral ontogenesis (Michaelson, 1973). The mass of both gray and white cerebral matter is reduced due to the retardation in neuronal growth and maturation (Krigman *et al.*, 1974). A slower ontogenic decrease in its specific activity could explain the data. If a delay in age-related decreases of enzyme activity were related to the observed changes, the different kinetics of cerebellar maturation between control and treated rats would have to be cited. For example, inhibition of developmental growth of the mitochondrial pool could conceivably maintain higher levels of glutathione peroxidase activity if the cytosolic specific activity were higher

Table 1. Effects of Lead Acetate on Cerebral and Cerebellar Catalase Activity in the Suckling Rat.

			Days After Lead Administration		
Sample	Pretreatment		6	12	16
Cerebrum	Control	(+)	10.77 ± 1.10[a]	0.19 ± 0.04	0.19 ± 0.04
		(++)	10.77 ± 1.10[a]	0.19 ± 0.04	0.19 ± 0.04
	Lead poisoned	(+)	12.44 ± 1.62[b]	0.95 ± 0.07	0.51 ± 0.03
		(++)	12.44 ± 1.62[b]	0.95 ± 0.07*	0.51 ± 0.03*
Cerebellum	Control	(+)	27.62 ± 5.21[a]	0.68 ± 0.16	0.68 ± 0.16
		(++)	27.62 ± 5.21[a]	0.68 ± 0.16	0.68 ± 0.16
	Lead poisoned	(+)	37.90 ± 5.22[b]	2.96 ± 0.32	2.54 ± 0.84
		(++)	35.80 ± 5.46[b]	2.46 ± 0.28*	2.14 ± 0.71

Catalase activity is expressed as substrate nmol x mg protein $^{-1}$ x min $^{-1}$. Values represent mean ± S.E. of four animals except (a) = 3 animals, and (b) = 7 animals. (+) Uncorrected data, (++) Corrected data for blood contamination. (*) $P < 0.001$, different from controls.

Table 2. Effects of Lead Acetate on Cerebral and Cerebellar Glutathione Peroxidase Activity in the Suckling Rat.

Sample	Pretreatment		Days After Lead Administration 6	12	16
Cerebrum	Control	(+)	26.51 ± 3.88[a]	5.60 ± 1.04	5.60 ± 1.04
		(++)	26.51 ± 3.88[a]	5.60 ± 1.04	5.60 ± 1.04
	Lead poisoned	(+)	22.16 ± 0.43[b]	8.25 ± 1.46	7.62 ± 1.40
		(++)	22.16 ± 0.43[b]	8.25 ± 1.46	7.62 ± 1.40
Cerebellum	Control	(+)	57.90 ± 0.90[a]	8.03 ± 0.55	8.03 ± 0.55
		(++)	57.90 ± 0.90[a]	8.03 ± 0.55	8.03 ± 0.55
	Lead poisoned	(+)	94.71 ± 8.58[b]	23.26 ± 0.72	22.29 ± 3.05
		(++)	73.18 ± 8.19[b]	18.30 ± 0.54**	18.06 ± 2.88*

Glutathione peroxidase activity is expressed as substrate nmol x mg protein^{-1} x min^{-1}. Values represent mean ± S. E. of four animals except (a) = 3 animals, and (b) = 7 aniimals. (+) Uncorrected data, (++) Corrected data for blood contamination. (*) $P < 0.02$, (**) $P < 0.001$, different from controls.

than that of the mitochondria.

Hence, delays in age-related decreases of enzyme activity are not the only possible explanation, as enzyme induction could be involved as well. If lead intoxication of the cerebellum were related to enzyme induction, this would certainly support the initial hypothesis of a free radical injury. We can speculate that an increase in the formation of lipid hydroperoxides in the lead-intoxicated cerebellum may have served as a signal to maintain higher levels of glutathione peroxidase activity in order to enhance the detoxication process. In this sense, it is interesting to note that glutathione peroxidase is a selenoenzyme (Rotruck *et al.*, 1973). Selenium at the active site is especially sensitive to reversible inactivation by heavy metals (Splittgerber and Tappel, 1979; Chaudiére and Tappel, 1984) and therefore, higher levels of glutathione peroxidase protein would be necessary to maintain a constant level of catalytic activity in the presence of lead. The endogenous inhibition of glutathione peroxidase would be expected to undergo reversal in our assay procedure, in which reduced glutathione and EDTA would still greatly exceed residual lead. This type of reversible inhibition could therefore be a major cause of both oxidant stress and enzyme induction. Further investigations will be needed to provide definitive interpretations of these interesting observations.

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