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Effect of acute and chronic lead exposure on the level of sulfhydryl groups in rat brain

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Short communication

Abstract. Lead is known to be a potent neurotoxic agent. The interactions of lead with proteins are considered to be at least one of the mechanisms due to which lead exerts its toxicity. In the present work we demonstrate that acute and chronic models of exposure to lead affected the level of total and protein-bound SH groups in homogenates and synaptosomes obtained from rat brains. The concentrations of SH groups were lowered significantly ($P<0.05$) in both types of poisoning. Different classes of cellular proteins were considered to play a role in high affinity binding of lead to these ligands.

Key words: lead, SH groups, lead-protein interactions

It is already established that lead exposure produces neurotoxicity including behavioural, morphological, biochemical and electrophysiological effects (Silbergeld and Hruska 1980). Current public awareness of the dangers of lead neurotoxicity, especially to young organisms, is now probably higher than at any other time in history. Lead has no known biological function in man i.e. it is not a trace element, but it can be found in every human in some concentration. It is therefore in the interest of public health to learn precisely the mechanism of action of that dangerous neurotoxin.

The mechanisms by which metals cause toxicity to specific tissue are multifactorial and highly individualistic and as yet poorly understood. It is believed that the metal toxicity occurs primarily through its interaction with sulfhydryl groups on critical target protein but it is risky to generate that hypothesis basing only on *in vitro* studies and on chemical properties of the metal. It is therefore interesting to search for evidence of lead toxicity in brain using *in vivo* models due to the lack of that sort of studies.

With the aim of determining the mechanism of toxic lead effect we examined the level of sulfhydryl groups in homogenates and synaptosomal fractions obtained from brains of lead-poisoned rats.

The study was performed on male Wistar rats. Two models of poisoning were used. In the model of chronic toxicity lead acetate in concentration of 200 mg/L distilled water was given to 3-week-old rats to drink *ad libitum* for 3 months. Control animals received distilled water without lead added. In the model of acute toxicity 15 mg $Pb(CH_3COO)_2$/kg b.w. was injected intraperitoneally for 7 days into rats weighting about 200 mg. Control animals were injected with distilled water. Animals were decapitated, brains were removed and homogenised in sucrose medium to 10% of final concentration. Synaptosomal fraction was isolated from the hemispheres of rats using a discontinuous Ficoll gradient as described by Booth and Clark (1978). The level of SH groups was determined by the method of Sedlak and Lindsay (1968). For determination of total SH groups, samples (homogenates or synaptosomes) were mixed with 0.2M TRIS buffer pH 8.2 and 0.1M dithionitrobenzoic acid (DTNB). Non protein SH groups were estimated after addition of 50% TCA to each sample. The tubes were shaken and centrifuged at approx. 3,000 g. The absorbances of supernatant were read within 5 min at 412 nm after addition of 0.4 TRIS buffer pH 8.9 and DTNB, against a reagent blank.

The protein-bound SH groups contents were calculated from total and non protein SH groups. The content of lead in brain homogenates and synaptosomes was measured using atomic absorption spectrometer (Perkin Elmer 1100B).

Protein content was determined by the method of Lowry (Lowry et al. 1951) using bovine albumin as a standard.

Lead level significantly increased in brains of toxicated rats and accumulated preferentially in nerve endings particles (Table I).

Levels of non protein sulfhydryl groups were practically the same in control and lead-treated samples (Table II), but there was statistically significant ($P<0.05$) decrease in the content of total as well as of protein-bound SH groups in both models of toxicity.

In the case of chronic exposure the content of SH groups in synaptosomes was diminished to 0.56±0.05 (mmol/ml sample) while in the control it was 0.65±0.04 . In acute treated animals the levels were 0.56±0.08 and 0.62±0.04 respectively (Fig.1A).

In the homogenates a decrease in protein-bound SH groups levels was also observed. The respective values were: 0.91±0.04 for control and 0.82±0.07 for chronically treated animals and 0.97±0.11 for

TABLE I

The lead level (μg/g w.w.).The values represent the means ± SD for 3 independent preparations for each sample. ([a] under sensitivity of method)

Model of toxicity	Sample	Control	Lead-treated
chronic	homogenates	<0.019a	0.03±0.01
	synaptosomes	<0.019a	0.74±0.16
acute	homogenates	<0.019a	
	synaptosomes	<0.019a	1.3±0.32

TABLE II

The content of -SH groups in synaptosomes and homogenates obtained from acute and chronic models of toxicity. The values represent mean ±SD for three independent preparation for each tissues

model	materials	Level of non protein -SH groups (mmole/ml sample) control	Pb^{2+}-toxicated
acute	homogenates	0.06±0.01	0.07±0.01
	synaptosomes	0.02±0.005	0.02±0.006
chronic	homogenates	0.07±0.01	0.06±0.01
	synaptosomes	0.03±0.004	0.03±0.005

control and 0.82±0.02 for acute treated animals (Fig. 1B). Data presented in Fig. 1A and B are expressed as percent of control.

The results taken together show reduction in number of total and protein-bound sulfhydryls in brains of rats treated acutely or chronically with lead. The rate of this decrease was similar independently of the type of exposure.

It might be suggested that lead entering cells, including neurones, is tightly bound to subcellular constituents. The binding to target proteins may influence the activity of some enzymes and conformation of protein receptors which may be the primary reason of disturbances in neurotransmitters transport, energy metabolism and subsequently the morphologicall (mainly mitochondrial) damage. (Goering 1993, Jabłońska et al. 1994, Strużyńska and Rafałowska 1994).

Chemical properties of lead allow interactions with diverse bio-ligands. A hierarchy of binding is based on availability of chemical groups Pb can interact with. Primarily the presence of sulfhydryls, but in the form of vicinal ditiol, makes the proteins susceptible to lead binding. Amine, phosphate and carboxyl are other groups of high importance for this process (Goering 1993).

Diversity of ligands in cellular compartments may dictate both the site and direction of lead bind-

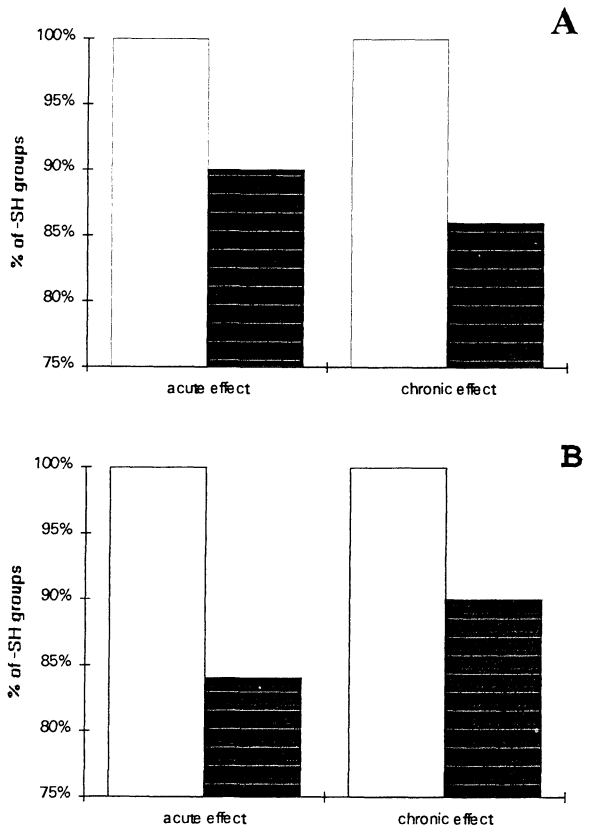

Fig. 1. Effect of lead acetate on the level of protein bound SH groups in synaptosomes (A) and homogenates (B) obtained from acute and chronic lead-poisoned rat brains. Open bar, control; filled bar, Pb poisoned

ing and hence determine its toxicity. The present studies did not show significant quantitative differences in the decrease of SH groups concentration between the two models of lead toxicity. However it does not mean that lead-protein interactions are the same. Several high-affinity cytosolic lead-binding proteins (PbBPs) have been isolated from rat kidney and brain (Oskarson et al. 1982, Mistry et al. 1985, Duval et al. 1989). The reaction of lead with those protein may influence the bioavailability, intracellular distribution and transport of Pb. Although the concentration of PbBPs in brain is low (Ebadi et al. 1987, Duval et al. 1989) may be enough to affect Pb activity in brain reducing or preventing Pb interactions with molecules of great importance for the cell. It was established that lead-binding pro-

teins as well as glutathione (GSH) appear soon after metal exposure and might be a protective factor diminishing the susceptibility to lead toxicity (Ebadi et al. 1987, Goering 1993, Legare et al. 1993). Thus, while the decrease of SH concentration in the acute model may be detrimental, it may be neuroprotective in the chronic model.

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