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Acute lead toxicity and energy metabolism in rat brain synaptosomes

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Abstract. Although the neurotoxic effects of lead (Pb) are well documented, the subcellular mechanisms of its action in the central nervous system are not fully understood. The present work examined some parameters of energy metabolism in nerve endings of the brains of adult rats exposed to Pb. We applied the model of acute Pb toxicity *in vivo*, imitating the acute action of lead observed in occupationally exposed workers or in occasional incidents of poisoning. The measurement of Pb levels in the synaptosomal fraction exhibited its significant accumulation under applied conditions. Oxygen consumption increased in synaptosomes from Pb-treated rats whereas the activity of cytochrome c oxidase did not change. The intrasynaptosomal levels of ATP and CrP were significantly elevated, as was the activity of creatine kinase, suggesting the activation of the CrP/CK system. On the other hand, the activity of the synaptosomal Na^+,K^+-ATP-ase decreased. We suggest that under acute Pb toxicity conditions the mobiliaztion of CrP/CK system may take place to protect the cell against the effects of decreased Na^+,K^+-ATP-ase activity.

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INTRODUCTION

Lead (Pb) exposure is still a major medical problem both in the environment and in occupational settings. The clinical effects of chronic and acute lead toxicity are well documented (Davis et al. 1993, Grandjean 1993). The main target of Pb toxicity is the central nervous system (CNS) (Davis et al. 1993) and the major neurological manifestations are acute Pb encephalopathy and neuropathy (Goldstein et al. 1974, Holtzman et al. 1984). Although the neurotoxic effect of Pb is well documented, the subcellular mechanisms of its neurotoxicity are not yet clear.

Our previous investigations showed that chronic administration of low doses of Pb to rats (imitating environmental exposure), affected some parameters of energy metabolism in nerve endings of adult rat brains (Rafałowska et al. 1996). In the present work we have examined changes in levels of some energetic metabolites and related enzymes, as well as alterations in oxygen consumption rates in synaptosomes isolated from brains of rats subjected to acute Pb intoxication. Obtained results were compared to those observed in the model of chronic Pb toxicity.

METHODS

Experimental procedure

The animal model of acute Pb toxicity *in vivo* was applied. The study was performed on male Wistar rats weighing about 200 g, arranged into two groups, each consisting of 50 animals. In the experimental group, 15 mg of $Pb(COOH)_2$ per kg of body weight was injected intraperitoneally into each animal for 7 days. Distilled water was injected into the animals from the control group.

Preparation of fractions

The study was performed on a synaptosomal fraction obtained from rats from both groups and on a mitochondrial fraction isolated from synaptosomes.

Synaptosomes were isolated from the forebrain of the rats using a discontinuous Ficoll gradient as described by Booth and Clark (1978). The synaptosomal pellet was washed once in a Krebs-Ringer buffer (140 mM NaCl, 5 mM KCl, 10 mM Tris-HCl, 1.4 mM $MgSO_4$, 1 mM Na_2HPO_4, pH 7.4) and the final synaptosomal pellet was suspended in Krebs-Ringer buffer to obtain a protein concentration of approx. 5 mg/ml. As was demonstrated in the previous experiments, synaptosomes obtained by this procedure exhibit a high purity and well - maintained energy metabolism (Rafałowska et al. 1980, Deutsch et al. 1981). Synaptic mitochondria were isolated according to Lai et al. (1977) using a Ficoll-sucrose gradient.

Lead determination

The content of Pb in synaptosomes from control and Pb-poisoned rats was determined using a Perkin Elmer 1100B atomic absorption spectrophotometer. Levels of Pb in blood samples were also estimated.

Respiratory studies

The rate of oxygen consumption by synaptosomes was measured with Clark-type electrode at 22°C in a Krebs-Ringer buffer (pH 7.6) containing 10 mM respiratory substrate (pyruvate or succinate) and 2 mM $CaCl_2$, as described previously by Rafałowska et al. (1980a).

Metabolite levels

The perchloric acid extracts of synaptosomes were neutralized with 2.5 M $KHCO_3$ and centrifuged. Supernatants were used for the determination of metabolites by standard enzymatic techniques.

The level of ATP was measured according to the method of Lamprecht and Trautschold (1974). Creatine phosphate (CrP) was assayed in the same reaction mixture after the addition of 1mM ADP (Lamprecht et al. 1974).

The levels of ADP and AMP were measured according to the method of Jaworek et al. (1974). Creatine (Cr) was assayed in the reaction mixture used for the measurement of ADP according to the procedure of Bernt et al. (1974).

Enzyme assays

The cytochrome c oxidase activity in synaptic mitochondria isolated from the control and Pb-intoxicated rat brain was measured spectrophotometrically according to the method of Wharton and Tzagoloff (1955).

The ATP-ase activity of synaptosomes isolated from control and Pb-poisoned rats was measured spectrophotometrically according to the method of Fox et al.

(1991) by determining the inorganic phosphate liberated from ATP. For measurement of the enzyme activity synaptosomes were suspended to the final concentration (f.c.) of protein of approx. 1mg/ml, in the reaction mixture containing 30 mM Tris-HCl (pH 7.4), 130 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM ATP, 2 mM EGTA with or without 2 mM ouabain. The reaction was stopped after 45 min by the addition of ice-cold TCA (f.c. 5%). The samples were centrifuged for 10 min at 1,500 x g and the inorganic phosphate concentration was determined in a protein-free supernatant. The difference between the activity recorded in the absence (total ATP-ase) and the presence (Mg^{2+}-ATP-ase) of ouabain was the Na^+,K^+-ATP-ase activity.

The creatine kinase (CK) activity in synaptosomes and in synaptic mitochondria obtained from the control and Pb-treated rat brains was measured spectrophotometrically according to the enzymatic method of Booth and Clark (1978a). Triton X-100 (f.c. 0.5%) was added to the synaptosomal fraction which was sonicated for three minutes before enzymatic assay.

Protein determination

The protein concentration was measured according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Statistical analysis

The data were analysed using a Student *t*-test. Differences between groups in all measurements were regarded as significant if $P<0.05$.

RESULTS

This study revealed high levels of Pb in the blood of Pb-treated rats. Acute Pb administration to the rats caused an increase in blood lead levels from 1.9 g/dl in control rats to 112.5 µg/dl in Pb-treated rats. (Table I). However, during the daily observations within the time of exposure, the animals did not exhibit signs of seizures and there were no deaths within the experimental group of rats. The measurement of Pb levels in the synaptosomal fractions exhibited a trend towards the significant penetration of lead from blood to the brains of the exposed rats. In the synaptosomes obtained from the control and Pb-treated rats, Pb levels were <0.019 and 1.8 µg/g w.w., respectively (Table I).

TABLE I

The content of lead in the synaptosomes and blood in the control and lead- treated rats

Model of toxicity	Sample	Pb level	
		Control	Pb-treated
Acute	Synaptosomes µg/g w.w.	<0.019*	1.8 ± 0.4
	Blood µg/dl	1.9 ± 0.2	112.5 ± 1.0

The values represent the mean ± SD of measurements from six independent preparations. *Non-detectable (under sensitivity of the method).

The rates of oxygen uptake by synaptosomes isolated from control and Pb-treated rats are shown in Fig.1. It can be seen that oxygen consumption increased significantly in synaptosomes from Pb-treated rats as compared to the control, independent of the substrate (pyruvate and succinate).

In rats receiving Pb the activity of cytochrome c oxidase measured in synaptic mitochondria did not change (Table II).

The levels of intrasynaptosomal adenine nucleotides (ATP, ADP, AMP), creatine (Cr) and phosphocreatine (CrP) in Pb-treated rats were changed as compared to the control values. The results are summarized in Table III.

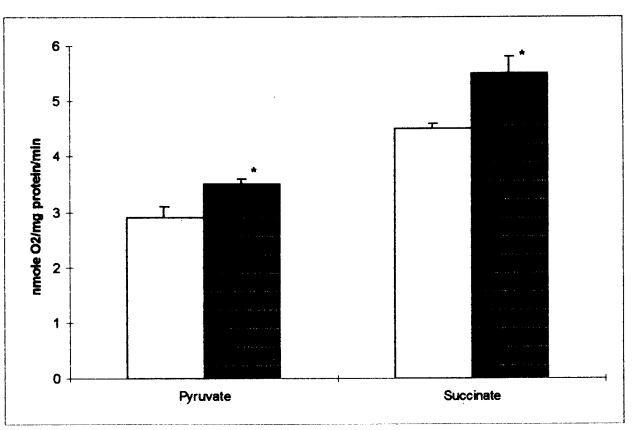

Fig. 1 The effect of lead on the oxygen consumption rates in synaptosomes isolated from the brain of control and acutely Pb-treated rats. Results represent mean values ± SD of five experiments, *$P<0.05$. Open bars, control rats; filled bars, Pb-treated rats.

TABLE II

Activity of cytochrome oxidase in synaptosomal mitochondria obtained from control and Pb-treated rats

Sample	Activity of cytochrome oxidase μmoles/mg protein/min		
	Control	Pb-treated	% of control
Synaptosomal mitochondria	39.1 ± 4.3	38.8 ± 2.8	99

The values represent the means ± SD of five experiments.

Intracellular concentration of ATP increased significantly in synaptosomes obtained from the brains of Pb-treated rats as compared to the control ones (about 75%), whereas the levels of ADP and AMP were only slightly elevated. Consequently, the [ATP]/[ADP] ratio was higher by about 58%. Since the concentration of CrP in synaptosomes from Pb-treated rats was elevated (by 124% of control) and the concentration of Cr decreased by about 30%, the [CrP]/[Cr] ratio was three times higher than control ratio (over 300% of control).

The activity of creatine kinase increased drastically under Pb-toxicity conditions. The measurement of the synaptosomal activity of this enzyme (corresponding to the sum of "cytosolic" and mitochondrial activities) exhibited an 80% increase. On the other hand the mitochondrial activity of the enzyme rose by about 30% (Table IV).

TABLE III

Metabolite levels in synaptosomes isolated from control and acutely Pb- treated rats

Metabolite μmol/mg protein	Synaptosomes	
	Control	Pb-treated
ATP	3.66 ± 0.35 *(9)*	6.42 ± 0.39 *(8)**
ADP	1.11 ± 0.05 *(17)*	1.22 ± 0.07 *(17)*
AMP	0.51 ± 0.04 *(7)*	0.61 ± 0.05 *(7)*
CrP	2.20 ± 0.15 *(6)*	4.93 ± 0.68 *(7)**
Cr	4.53 ± 0.31 *(8)*	3.28 ± 0.26 *(7)**
ATP/ADP	3.33	5.26
CrP/Cr	0.48	1.50

Results represent mean values ± SD for the number of experiments shown in parantheses. Values are significantly different from their respective controls at the level of $P<0.05$.

TABLE IV

Activity of creatine kinase in synaptosomes and synaptosomal mitochondria obtained from control and acutely Pb-treated rats

Sample	Activity of creatine kinase μmoles/mg protein/min		
	Control	Pb-treated	% of control
Synaptosomes	0.022 ± 0.002 *(5)*	0.040 ± 0.007* *(5)*	180
Synaptosomal mitochondria	0.041 ± 0.016 *(9)*	0.053 ± 0.016* *(10)*	130

The values represent the means ± SD for the number of experiments shown in parantheses, *$P<0.05$.

The synaptosomal activity of Na^+-K^+-ATPase, the enzyme that maintains cellular fluid balance and provides the electrochemical gradients essential for synaptic potentials, decreased by about 30% in the applied model of acute lead toxicity (Table V).

TABLE V

Activity of Na^+-K^+-ATPase in synaptosomes obtained from control and Pb-treated rats

Sample	Activity of Na^+-K^+-ATPase μmol/protein/h	
	Control	Pb-treated
Synaptosomes	7.00 ± 0.20	5.00 ± 0.20

The values represent the means ± SD of six experiments, $P<0.05$.

DISCUSSION

Our earlier data showed disturbances in the mitochondrial structure (Jabłońska et al. 1994) and in neurotransmission (Strużyńska et al. 1994a) in brain synaptosomes isolated from rats poisoned chronically with low doses of Pb. Those results suggested that the observed disturbances might be connected with changes in energy metabolism of synaptosomes and synaptic mitochondria

under chronic lead toxicity conditions (Rafałowska et al. 1996).

The aim of the present study was to assess the sensitivity of energy metabolism in brain synaptosomes and synaptic mitochondria to acute Pb toxicity in an animal model imitating the acute action of Pb observed in occupationally exposed workers, or in occasional incidents of poisoning.

The magnitude of exposure to this metal is well reflected by the Pb concentration in blood. As the biological half-life of Pb in blood is a few weeks only, the level of blood Pb (PbB) reflects recent exposure and the measurement of this parameter is useful for monitoring current exposure levels or acute poisoning.

High blood Pb levels in the treated rats presented in Table I were about three times higher than in the chronic model of Pb toxicity described by Rafałowska et al. (1996). We also observed a significant penetration of Pb from blood to the brain, expressed by its accumulation in nerve endings (synaptosomal fraction) (Table I). The tendency of Pb to accumulate in the brain has been demonstrated earlier (Collins et al. 1982), although its concentration in the brain is among the lowest found in various organs of the body. It is known that Pb can penetrate through the blood-brain barrier of not only immature organisms (Thomas et al. 1973) but also of adults (Strużyńska et al. 1997). Synaptosomes isolated from acutely poisoned rats contained much more Pb than those in the chronic model of toxicity (Rafałowska et al. 1996). These higher levels of Pb did not affect the activity of cytochrome c oxidase in synaptic mitochondria. In contrast to what is seen in chronic toxicity, we observed an increase of synaptosomal respiration rates and in levels of ATP. Oxidative phosphorylation is stoichiometrically coupled to oxygen reduction and the rate of respiration corresponds to the rate of ATP synthesis which depends on the intramitochondrial $[NAD^+]/[NADH]$ and the cytosolic [ATP]/[ADP] ratio and the flux through cytochrome c oxidase (Wilson et al. 1979). Our results suggest that the substrate oxidation in synaptosomal mitochondria is potentiated, causing an increase in the availability of reducing equivalents for the respiratory chain. Creatine kinase (CK) is the enzyme that transfers energy between phosphagens in the reversible reaction: phosphocreatine + MgADP+H^+ $\Leftrightarrow$ creatine + MgATP. CrP acts as a source of energy, especially for ATP-ases (Wallimann et al. 1992). It has been shown that the drop in CrP concentration occurs together with the activation of the Na^+,K^+-ATP-ase, which is the major energy - utilizing enzyme in stimulated nerve cells (Blum et al. 1991). In our experiments we observed a reverse situation i.e. an increased level of CrP and high CK activity was accompanied by a decrease in Na^+,K^+-ATP-ase activity. The CrP shuttle in neurones is probably involved in maintaining a high cellular ATP/ADP ratio (Brown 1992). The more rapid CK-catalyzed phosphate fluxes are thought to function to provide energy under pathological conditions with high energy demand (Holtzman et al. 1991). In brain synaptosomes, mitochondrial ATP production has a significant effect on Na^+,K^+-ATP-ase activity (Erecińska and Dagani 1990) and the CrP/CK system, transferring ATP from mitochondria to cytosol, serves as a source of ATP for this enzyme. Our results indicate that under acute Pb toxicity condition, the energy mobilization of the cell may take place. Both respiration and the CrP/CK system are activated to maintain a high ATP concentration. What is a direct stimulus to that kind of cell defence is not known. We also confirmed that Pb may exert an inhibitory effect directly on Na^+,K^+-ATP-ase. It is known that Na^+,K^+-ATP-ase, especially brain-derived Na^+,K^+-ATP-ase, is among the enzymes particularly affected by lead (Siegel et al. 1977, Fox et al. 1991, Strużyńska et al. 1994). This fact may relate to the different α subunit composition of Na^+,K^+-ATP-ase of different tissues. Enzyme extracted from brain has mostly $\alpha 2$ and $\alpha 3$ isozymes present in significant amounts (Brodsky and Guidotti 1990, Gerbi et al. 1990). Substantial levels of $\alpha 3$ isoform have been reported, besides in the nervous system, only for heart and pineal gland (Mc Grail et al. 1991). All the subunits of the enzyme ($\alpha 1$, $\alpha 2$, $\alpha 3$) differ with the number of reactive sulfhydryl groups in the catalytic site (Brodsky and Guidotti 1990) and $\alpha 2$ and $\alpha 3$ forms of enzyme are much more sensitive to the inhibitory effect of Pb that the $\alpha 1$ form is (Fox et al. 1991). Thus it can be suggested that one of the possible mechanisms of the inhibition may be the interaction of Pb with -SH groups in the catalytic site of the enzyme. It was demonstrated that in the model of acute lead poisoning levels of both total and protein-bound SH groups in the brain homogenates and synaptosomes were lowered significantly (Dąbrowska-Bouta et al. 1996). Not all the enzymes containing sulfhydryl groups are equally sensitive to the inhibition by Pb. Marcovac and Goldstein observed that protein kinase C is, on the contrary, stimulated by Pb at concentrations as low as 10^{-15}M (Marcovac and Goldstein 1988a) although its catalytic and regulatory domains contain cysteine-rich sequences (Goering 1993). Among the

multiplicity of factors which dictate whether the function of protein will be altered or not are localiaztion of protein in the cell and the nature of the catalytic site of the enzyme, i.e., the presence of sulfhydryls in the form of vicinal dithiol (Goering 1993).

It seems from this study that under conditions of the acute Pb toxicity activation of the energy metabolism in the nerve ending represents a protection mechanizm against the decrease in the essential enzyme (Na^+,K^+-ATP-ase) activity. The role of that "energetic mobilization" is probably the diminution of the acute toxic effect of Pb on CNS.

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