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Is lead toxicosis a reflection of altered energy metabolism in brain synaptosomes?

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Abstract. The aim of the experiments presented here was to discern whether prolonged consumption of leaden water, that imitates environmental exposure, affects some energetic parameters in nerve endings of adult rat brains. Our results indicated that during chronic lead intoxication the oxidation chain of synaptic mitochondria remains intact. The oxygen consumption by synaptosomes and activity of cytochrom oxidase in synaptic and pericarionic mitochondria obtained from intoxicated rats did not change in comparison to those from the control samples. Compared with the control samples, the concentration of ATP decreased and the concentration of creatine phosphate (CrP) increased drastically in fractions obtained from Pb^{2+} intoxicated animals with simultaneously increased activity of creatine kinase (CK). It seems likely that, the CrP/Cr/CK system constitutes a satisfactory regulatory mechanism for chronic Pb^{2+} toxicity effects on energy metabolism in nerve endings of the adult rats.

Review

Key words: lead, synaptosomes, energy metabolism

INTRODUCTION

A broad range of physiological, biochemical and behavioural dysfunctions has been ascribed to lead (Pb) toxicosis (Davis et al. 1993, Grandjean 1993). The main target for 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. 1994). Several mechanisms by which Pb^{2+} can produce toxicity in the CNS have already been suggested. The toxic effect usually involves an interaction between Pb^{2+} and specific targets such as enzymes or cell membrane structure or cation mimicry (Vallee and Ulmer 1972, Petering 1974, Pounds 1984, Knowles and Donaldson 1990). One of the acknowledged targets of Pb^{2+} is synaptic transmission (Silbergeld et al. 1974, Jabłońska et al. 1994, Strużyńska and Rafałowska 1994a).

Even though the neurotoxic effect of Pb is well documented, the subcellular mechanism underlying the neurotoxicity of Pb^{2+} is not yet clear. Up to now, information on the effects of lead on energy processes in synaptic mitochondria of the brain is lacking.

Our earlier investigations (Jabłońska et al. 1994) have shown that chronic Pb administration to rats changed the morphology of the synaptosomes and disturbed the structure of synaptic mitochondria. Such results suggested that inner mitochondrial disturbances may be responsible for changes in the energy state of synaptosomes, thus influencing mitochondrial metabolic and functional energy-dependent processes.

In the present work we have examined changes in levels of some metabolites and related enzymes, as well as alterations in oxygen consumption rates by mitochondria present in synaptosomes isolated from the brains of rats subjected to chronic Pb intoxication. A preliminary account of this work has appeared elsewhere (Strużyńska and Rafałowska 1994b).

METHODS

The study was performed on synaptosomal fractions obtained from male Wistar rats (approx. 300 g weight) and on mitochondrial fractions isolated from synaptosomes.

In the *in vivo* experiments lead acetate in 200 mg/l concentration was given to three-week-old rats in drinking water for three months. Control rats received distilled water. Eighty one Pb-intoxicated rats and the same number of control rats were used in the experiments.

Synaptosomes were isolated from the forebrain of the rats using a discontinuous Ficoll gradient as described by Booth and Clark (1978b). In one experiment, brains from four rats were pooled for obtained of synaptosomal fraction. The synaptosomal pellet was washed once in a Krebs-Ringer buffer (140 mM NaCl; 5 mM KCl; 10 mM Tris-HCl; 1.4 mM $MgSO_4$ and 1 mM Na_2HPO_4, pH 7.4) and the final synaptosomal pellet from the rats was suspended in a Krebs-Ringer buffer in a protein concentration of approx. 5 mg/ml.

Previous investigations demonstrated that synaptosomes have a high purity and exhibit a well maintained energy metabolism (Rafałowska et al. 1980b, Deutsch et al. 1981).

Synaptic and pericarionic mitochondria were isolated according to Lai et al. (1977) using a Ficoll-sucrose gradient.

The content of Pb^{2+} in synaptosomes from control and lead-poisoned rats was determined in a Varian atomic absorption spectrophotometer aquipped with a graphite furnace. The levels of Pb^{2+} in blood samples was also recorded.

Oxygen consumption was measured with a Clark-type oxygen electrode at 22°C in a Krebs-Ringer buffer (pH 7.6) containing 10 mM respiratory substrate and 2 mM $CaCl_2$ according to Rafałowska et al. (1980a).

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

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

Levels of ADP and AMP were measured according to the method of Jaworek et al. (1974).

Creatine (Cr) was assayed in the incubation mixture used for the measurement of ADP according to the procedure of Bernt et al. (1974).

The volume of synaptosomal water was measured according to Deutch and Rafałowska (1979). Tritiated water and [^{14}C] polyethylene glycol (M.W. 4,000) (New England Nuclear, Boston, Massachusetts, USA) were added to the suspension of synaptosomes. Following centrifugation of the synaptosomes through silicone oil (specific gravity 1.03) using a Beckman centrifuge, the total water volume of the pellet was determined from the content of [^{3}H], whereas the extrasynaptosomal water was determined from the content of [^{14}C].

The ATPase activity of synaptosomes isolated from the control and Pb-poisoned rats was measured spectrophotometrically according to Fox et al. (1991) by determining the inorganic phosphate liberated from ATP.

The incubation mixture contained: synaptosomes (1 mg protein/ml 30 mM Tris-HCl (pH 7.4), 130 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM ATP, 2 mM EGTA and the presence or absence of 2 mM ouabain.

The reaction was stopped after 45 min by the addition of ice-cold TCA (5% final concentration). Samples were centrifuged for 10 min at 1,500 x g and the inorganic phosphate was determined in a protein free supernatant.

The creatine kinase (CK) activity in synaptosomes and in synaptic mitochondria obtained from the control and Pb-intoxicated rats brain was measured enzymate-spectrophotometrically according to the method of Booth and Clark (1978 a). Triton X-100 (final concentration 0.5%) was added to the synaptosomal fraction that was sonicated for three minutes before enzymatic assay.

Protein concentration was measured according to the method of Lowry et al. (1951).

The data were analysed using a Student *t*-test. Probability (*P*) values >0.05 were regarded as insignificant.

RESULTS

Chronic lead administration to the rats caused increase in the Pb^{2+} level from 1.9 μg/dl in the blood of control rats to 37.8 μg/dl in the blood of lead treated rats. In the synaptosomes obtained from control and Pb^{2+}-treated rats, lead level were <0.019 and 0.73 μg/g respectively (Table I).

The rates of oxygen uptake by synaptosomes isolated from control and chronic Pb-treated rats is shown in Fig. 1. It can be seen that the oxygen con-

TABLE I

The content of lead in the synaptosomes and blood in the control and Pb^{2+} -treated rats

Model of toxicity	Sample	Lead level	
		Control	Pb^{2+}-treated
Chronic	Synaptosomes (μg/gdw)	<0.019*	0.73±0.2
	Blood (μg/dl)	1.9±0.4	37.8±9.0

The values represent the mean ± SD of measurements from six independent preparations, $P<0.05$. *Non-detectable (under sensitivity of method).

TABLE II

Activity of cytochrome oxidase in synaptosomal and pericarial mitochondria obtained from control and chronic Pb^{2+} -treated rats

	Activity of cytochrome oxidase μmole/mg protein/min	
	Control	Pb^{2+}-treated
Synaptosomal mitochondria	32.14±6.43	39.13±5.83
Pericarial mitochondria	40.61±7.08	43.16±6.36

The values represent the means ± SD of seven experiments. Observed differences are statistically insignificant, $P>0.05$.

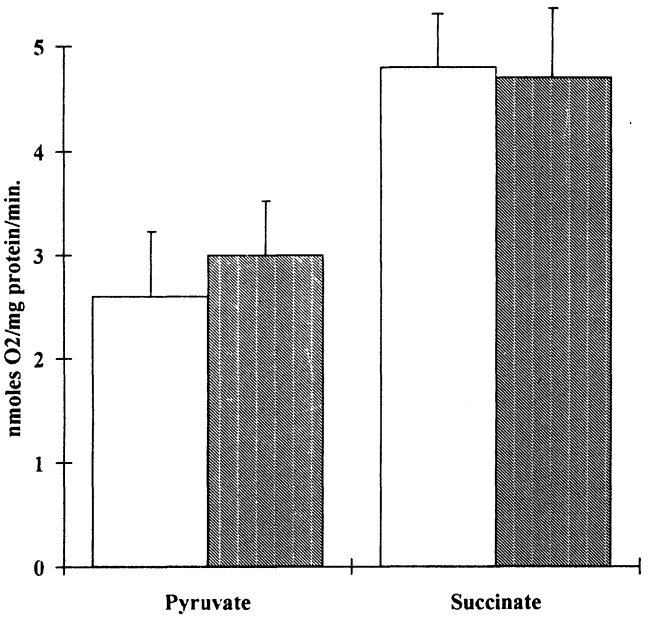

Fig. 1. The effect of lead on oxygen consumption rates in synaptosomes isolated from control and chronic Pb^{2+}-treated rat brains. The experimental conditions are described under Methods. Results represent mean values ± SD for four experiments. Differences are statistically insignificant, $P>0.05$. open bars, control; filled bars, Pb^{2+} toxic *in vivo*.

sumption was essentially the same in the control and Pb-intoxicated synaptosomes independently from the substrate. Small differences were statistically insignificant.

The rates of respiration were unaffected by the addition of ADP and calcium (data not shown): the latter were added routinely to the incubation mixture. Fig. 1 also shows that the rates of respiration with the succinate seems to be oxidised about 50% faster when compared to the pyruvate.

The activity of cytochrome oxidase measured in Pb^{2+}-intoxicated synaptic mitochondria and in perikaryonal mitochondria did not change in comparison to those from the control sample (Table II).

The intracellular volume of synaptosomes did not change in a chronic Pb^{2+} toxicity condition and was found to be 4.0 µl/mg protein (Table III).

The concentrations of intrasynaptosomal adenine nucleotides and phosphocreatine in synaptosomes obtained from control and Pb-treated rats are summarized in Table IV.

TABLE III

Intrasynaptosomal water volume in control and Pb^{2+}-treated synaptosomes

	Intrasynaptosomal water volume µl/mg protein
Synaptosomes of control rats	4.01±0.06
Synaptosomes of Pb^{2+}--treated rats	4.00±0.09

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

Compared to the control sample, it can be seen that concentration of ATP, [ATP] decreased significantly and the [CrP] increased drastically in the fractions obrained from Pb^{2+}-intoxicated animals. [ADP],[AMP] and [Cr] did not change. Small differences in AMP concentration are statistically insignificant. The [ATP]/[ADP] ratio decreased by about 35% and [CrP]/[Cr] ratio increased by about 100%.

TABLE IV

Metabolite concentrations in synaptosomes isolated from control and Pb^{2+}-treated brains

	Synaptosomes		
	Control	Pb^{2+}-treated	
µmol/ml intracellular H_2O			
ATP	2.99±0.19 (12)	2.17±0.12 (15)*	
ADP	0.50±0.10 (21)	0.57±0.09 (17)	
AMP	1.24±0.21 (6)	0.98±0.20 (9)*	
CrP	0.98±0.21 (7)	1.78±0.60 (7)*	
Cr	1.52±0.13 (6)	1.52±0.18 (6)	
ATP/ADP	5.86	3.81	35%
CrP/Cr	0.65	1.16	80%

Results represent mean values ± SD for the number of experiments in parentheses. *Values significantly different from their respective controls at the levels of $P<0.05$.

TABLE V

Activity of creatine kinase in synaptosomes and synaptosomal mitochondria and Na^+-K^+-ATPase in synaptosomes obtained from control and chronic Pb^{2+}-treated rats

	Activity of creatine kinase μmoles/mg/min		Activity of Na^+-K^+-ATPase μmoles/mg/h	
	Control	Pb^{2+}-treated	Control	Pb^{2+}-treated
Synaptosomes	0.050±0.019	0.090±0.027	7.10±0.22	5.00±0.20
Synaptosomal mitochondria	0.044±0.029	0.070±0.019		

The values represent the means ± SD of six experiments. All values are significantly different from their respective controls at the levels of $P<0.05$.

Na^+-K^+-ATPase plays a key role in the conduction of nervous impulses and in synaptic transmission. Therefore, because the activity of this enzyme decreased significantly in our model of toxicity, it cannot be responsible for the decrease in the concentration of ATP (Table V).

The increased phosphocreatine concentration in Pb-intotoxicated synaptosomes is in close correlation to the increased activity of creatine kinase.

DISCUSSION

Lead is one of the most hazardous agents to living matter and is a focal point of concern in contemporary toxicology. Earlier investigations by Goyer et al. (1968) have demonstrated that mitochondria from kidneys of Pb-intoxicated rats became extremely swollen and displayed ruptured outer membranes and vacuolated inner compartments. Our work (Jabłońska et al. 1994) has also shown the disturbances of mitochondrial structure in brain synaptosomes isolated from Pb-poisoned rats. Data from this research have already demonstrated the effects of lead on the metabolism and on the function of mitochondria. Therefore, the aim of the experiments presented here was to discern whether prolonged consumption of leaden water, that imitates environmental exposure, affects some energetic parameters in nerve endings responsible for neurotransmission functions in the brain.

The type of lead salt, time and method of administration were chosen on the basis of available literature (Grant et al. 1980, Cory-Slechta and Wichowski 1991).

Nerve endings were isolated as a synaptosomal fraction. This fraction was metabolically intact and could be considered as a good simple nerve-ending model (Rafałowska et al. 1980, Deutsch et al. 1981). Nerve endings have the ability to accumulate lead. The level of Pb in the synaptosomal fraction obtained from Pb^{2+}-treated rats was many times higher than that in the synaptosomal fraction obtained from the control animals. These results are in agreement with earlier data concerning Pb^{2+} penetration from blood into nerve endings (Jabłońska et al. 1994, Strużyńska and Rafałowska 1994a).

The results described here show that synaptosomes isolated from chronic Pb^{2+}-poisoned rats, did not show changes in oxygen uptake compared to control synaptosomes. These data, supported by lack of differences in the activity of cytochrome oxidase in synaptic mitochondria obtained from control and chronic Pb^{2+}-toxic synaptosomes, suggest that damage to synaptosomal or mitochondrial membranes observed in Pb-toxic conditions (Goyer et al. 1968, Jabłońska et al. 1994) is rather mild and cannot modify substrate oxidation in mitochondria, and influences the availability of reducing equivalents for the respiratory chain.

The lack of damage to the mitochondrial membrane by lead is supported by the fact that the oxi-

dation of the succinate, which is catalysed by a tightly membrane-bound succinate dehydrogenase, is not inhibited more than the oxidation of the pyruvate. Furthermore, the presence of lead did not change the intrasynaptosomal water volume.

The results presented in this work demonstrate that during chronic Pb intoxication the oxidation chain of synaptic mitochondria remains intact. On the other hand, the data have shown that the concentration of ATP decreased significantly and the [ATP]/[ADP] ratio dropped by about 35%.

The two most likely explanations for this discovery are: metabolic changes (i.e., changes in the rate of synthesis and/or degradation), and changes in the morphology of the synaptosomes (e.g., synaptosomes containing more or less mitochondria). The main degradation factor of ATP in synaptosomes is Na^+-K^+-ATPase, which plays a key role in the nervous system in the conduction of nervous impulses and in synaptic transmission by regulating the ionic gradient involved in the maintenance of membrane potentials. However, in the chronic, Pb-poisoned conditions, the activity of this enzyme decreases (Strużyńska et al. 1994). Therefore, the reason for the diminished ATP concentration results from degraded synthesis and/or changes in the number of synaptic mitochondria observed in electron microscopy after Pb-intoxication (Jabłońska et al. 1994).

It is also interesting to note that, simultaneously to the decrease of the ATP concentration, we observed an increased concentration of creatine phosphate in synaptosomes and enhanced activity of creatine kinase in synaptosomes and in synaptic mitochodnria. The increase in the synthesis of creatine phosphate is the componsatory factor that replaces ATP in the energy metabolism of synaptosomes.

It seems likely that, the CrP/Cr/CK system constitutes a satisfactory regulatory mechanism for chronic Pb^{2+}-toxicity effects on energetic process in nerve endings.

However, the possibility remains that the effects may be different in the cases when level of Pb accumulated by synaptosomes is higher, for example: in acute Pb^{2+} poisoning and/or in very young animals with immaturity of blood-brain barrier.

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