ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1994, 54: 201-207

The effect of lead on dopamine, GABA and histidine spontaneous and KCl-dependent releases from rat brain synaptosomes

Lidia Strużyńska and **Urszula Rafałowska**[1]

Department of Neurochemistry, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa St., 00-784 Warsaw, Poland

Abstract. The effect of lead on the release of [^{14}C]GABA, [^{3}H]dopamine and [^{14}C]histidine (as a precursor of histamine) was studied in synaptosomes obtained from chronically lead-treated rats and in synaptosomes with *in vitro* lead added. *In vivo* treatment of rats with lead acetate results in a decrease in the K^+-depolarization-dependent release of GABA and dopamine and histidine. Lead given *in vitro* itself (independently of depolarizing condition) stimulated the release of previously accumulated neurotransmitters in synaptosomes (GABA and dopamine). This effect depends on lead acetate concentration. On the other hand lead, in different concentrations, did not cause changes in the histidine release. The results show that lead can attack the synaptic neurotransmission in two ways: by depressing the Ca-KCl-evoked release of GABA, dopamine and histidine and by a selective stimulation of a spontaneous release (independent of depolarization conditions) of GABA and dopamine but not histidine.

[1]To whom correspondence should be addressed

Key words: lead, GABA, dopamine, histidine release, synaptosomes

INTRODUCTION

Lead ions are known to be toxic to the nervous system of man and animal (Davis et al. 1993). Both acute and chronic exposures to lead result in behavioral and neurological symptoms: vertigo and headache, insommia, restlessness and irritability and major manifestations such as convulsions and coma. Lead can induce encephalopathy with accompanying cerebral edema and haemorrhages. At the peripheral level, lead poisoning gives rise to a neuromuscular syndrome known as lead palsy, which includes muscle weakness, easy fatigability and eventually paralysis (Silbergeld 1974). In lead neurotoxicology an important issue relates to how lead interferes with chemical neurotransmission. It was reported that lead interfered with neuromuscular (Kalton and Yaari 1982) and neuromuscular and neuronal transmission (Silbergeld and Goldberg 1975, Silbergeld 1977) and induced changes in dopaminergic sensitivity (Cory-Slechta et al. 1993). The effect of lead on the release mechanism of neurotransmitters was investigated on brain homogenates by Bondy et al. (1979).

It was postulated that Pb^{2+}/Ca^{2+} interactions might play an important role in the toxicity of lead in a neurotransmission process (Silbergeld 1977, Kalton and Yaari 1982, Simons 1993). Our investigations suggest the existence of several mechanisms of lead toxicity (especially for uptake process), related to the individual neurotransmitters which are not necessarilly connected with Pb^{2+}/Ca^{2+} interaction (Jabłońska and Rafałowska 1993a, Jabłońska et al. 1994).

In this study we examined and compared the effects of lead acetate on KCl dependent and spontaneous releases of GABA, dopamine and histidine (as precursor of histamine) from rat-brain synaptosomes.

Preliminary results concerning this problem were presented in abstract form (Jabłońska and Rafałowska 1993b).

EXPERIMENTAL PROCEDURE

Animal material

The study was performed on synaptosome fractions obtained from male Wistar rats (approx. 300 g weight).

In experiments *in vivo* lead acetate in 100 mg/l concentration was given to 3-week-old rats in drinking water for 3 months. Control rats received drinking water without Pb^{2+} added. The model of acute intoxication was used only to check the Pb^{2+} level in the blood. In this model 15 mg $Pb(CH_3COO)_2$/kg b.w. was injected intraperitoneally for 7 days into rats weighing approx. 200 g. In experiments *in vitro*, lead (as lead acetate) was added to the incubating medium containing isolated synaptosomes. In experiments *in vivo* we used 63 Pb-intoxicated rats and the same number of control rats.

In the investigation *in vitro* 36 rats were used to obtain synaptosomes for experiments with lead and the same number for control. Each synaptosomal preparation was obtained from 4 rats. Numbers of experiments are marked under the tables and in the legend of the figure.

Preparation of rat brain synaptosomes

Synaptosomes were isolated from the forebrain of rats using a discontinuous Ficoll gradient as described by Booth and Clark (1978). As demonstrated earlier the synaptosomes had a high purity and exhibited a well maintained energy metabolism (Rafałowska et al. 1980, Deutsch et al. 1981).

Rats were killed by decapitation and the forebrain of the animal was rapidly removed. The forebrains were dropped into ice-cold isolation medium (0.32 M sucrose, 1 mM potassium EDTA, 10 mM Tris-HCl, pH 7.4) and chopped into small pieces. All the procedure was performed at 0^{o}-4^{o}C. The blood was washed out by adding more isolation medium and decanting the supernatant from the top of the minced tissue. The chopped tissue was then homogenized in a Dounce-type glass homogenizer by 7 up-and-down strokes with a glass pestle (total

clearance 0.1 mm). This homogenate was centrifuged at 1,300 g for 3 min. The supernatant from this spin was centrifuged at 17,000 g for 10 min and obtained pellet was resuspended in 5 ml of isolation medium, diluted with 12% Ficoll/sucrose medium. The suspension was introduced into a centrifuge tube and above this 5 ml of 7% Ficoll/sucrose medium was carefully layered. Finally, on top of this 5 ml of isolation medium was layered. The tubes were centrifuged at 99,000 for 30 min in a swing out rotor. The free mitochondria and myelin layer was removed. The synaptosomes were gently sucked off from the interphase, diluted to 60 ml and homogenized as above in a Potter homogenizer and spun at 5,500 g for 10 min.

The synaptosomal pellet was washed once in Krebs-Ringer buffer (140 mM NaCl; 5 mM KCl; 10 mM Tris-HCl; 1.3 mM $MgSO_4$ and 1 mM Na_2HPO_4, pH 7.4) and the final synaptosomal pellet from rats was taken up in Krebs-Ringer buffer in a protein concentration of approx. 5 mg/ml.

Measurement of Pb^{2+} concentrations

The contents of Pb^{2+} in synaptosomes from control and lead-poisoned rats were measured using the X-ray fluorescence with an energy dispersion method. This very sensitive method is applied in biochemical investigations with the same good results as the better known atomic absorption spectrophotometer method (Beitz 1975, Kaufman et al. 1976, Pawłowski et al. 1992).

Additionally the contents of Pb^{2+} in blood samples were recorded.

Measurement of GABA, dopamine and histidine release

The measurement of release was preceded by an uptake procedure. Synaptosomes from control or Pb-poisoned rats (final concentration about 5 mg protein/ml) were suspended in the Krebs-Ringer medium containing 10 mM glucose, 2.5 mM $CaCl_2$, 2 mM aminooxyacetic acid or 0.1 mM pargyline and preincubated 5 min at 30°C.

The uptake of GABA, dopamine and histidine was measured by the method of Troeger et al. (1984). The measurements started with the addition of radioactive reagents obtained from Amersham: [^{14}C]GABA (2 µM; 22.6 mCi/mmol), [^{3}H]dopamine (0.4 µM; 675 mCi/mmol) and [U-^{14}C]histidine (1 µM; 24.0 mCi/mmol) in the incubation medium.

In the eighth minute of the uptake (time of optimal neurotransmitters uptake to the synaptosomes) (Troeger et al. 1984, and unpublished data from our laboratory) an aliquot of synaptosomal suspension was withdrawn and diluted 10-fold with the same medium or with the same medium enriched with KCl or $Pb(CH_3COO)_2$. This procedure prevented interference of reuptake. The ionic strength was retained by an equivalent reduction of NaCl concentration. Samples of the suspension (200 µl) were taken out at 7 min after KCl or lead addition and rapidly centrifuged for 1 min through the layer of silicone oil (specific gravity 1.03) on Beckman microfuge. Pellets were solubilized with NCS tissue solubilizer and the radioactivity was determined with a Beckman LS 9000 scintillation counter using Bray scintillation fluid.

Protein assay

Protein concentrations were measured by the method of Lowry et al. (1951).

RESULTS

The Pb^{2+} levels in the blood of control and chronically poisoned rats were practically equal; the slight differences observed were statistically insignificant. On the contrary, acute intoxication (intraperitoneal injection of $Pb(CH_3COO)_2$ 15 mg/kg b.w. of rat) increased Pb^{2+} level in blood significantly (Table I).

The content of lead in the synaptosomes fraction obtained from the lead-treated rats was more than two times higher than in synaptosomes from the control rats (Table I).

The depolarization of control synaptosomes by 75 mM KCl caused a drastic release of [^{14}C]GABA

TABLE I

Lead level in blood and synaptosomes obtained from control and lead-treated rats *in vivo*

	Blood			Synaptosomes	
	Control	Chronically Pb-treated rats	Acute Pb-treated rats	Control	Chronically Pb-treated rats
Lead level μg/g d.w.	11.0±0.2	11.4±0.3	12.54±0.2**	1.6±0.2	3.5±0.2*

Values are means from 3 different experiments measured in triplicate (±SD), *$P<0.05$, **$P<0.01$. Student's t test.

leading to a decrease of [^{14}C]GABA content in synaptosomes from 510 to 50 pmoles/mg prot. This release was decreased in the case of synaptosomes obtained from the Pb-treated rats, as corresponding values for the synaptosomal content were 400 and 110 pmoles of [^{14}C]GABA per mg prot. Similarly, KCl released [^{3}H]dopamine from the control synaptosomes (from 47 to 25 pmoles) and only (from 55 to 47 pmoles) from the Pb-intoxicated nerve endings. Conversely, in the case of [^{14}H]histidine, which was released by KCl from the control synaptosomal fraction (from 65 to 45 pmoles) and practically not released from the Pb intoxicated *in vivo* synaptosomes (Fig. 1). These results obtained in the presence of 75 mM KCl correspond to values obtained in the presence of veratridine as the depolarization agent (unpublished data).

The addition of lead acetate to the synaptosomal fraction (independently from depolarizing conditions in the absence of high concentration of KCl) stimulated the release of previously accumulated radioactive neurotransmitters. This effect depended on the concentration of lead acetate. Already 10 μM concentration, which is close to *in vivo* Pb^{2+} intoxication conditions, significantly increased GABA and dopamine release, but did not change the histidine release. The lack of this effect was independent of Pb^{2+} concentration (Table II).

DISCUSSION

The aim of this work was to assess the sensitivity of brain synapses to lead toxicity, especially its influence on the release of neurotransmitters or their precursors.

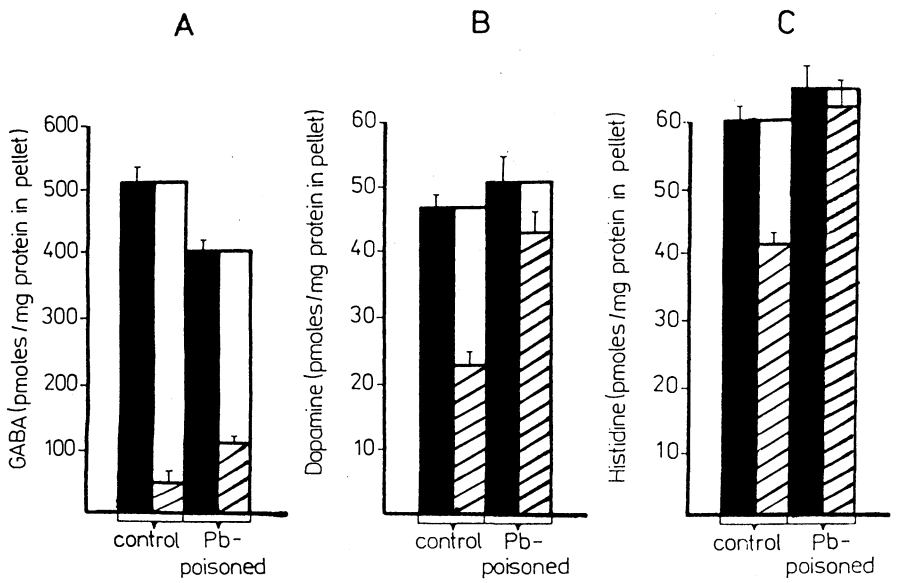

Fig. 1. Release of [^{14}C]GABA (A), [^{3}H]dopamine (B) and [^{14}C] histidine (C) by 75 mM KCl from synaptosomes of control and lead poisoned rats. Black bars, radioactivity in pellet (KCl no added); hatched bars, radioactivity remaining in pellet after depolarization by KCl; open bars, radioactivity released from pellet after depolarization by KCl. Values are means (SD) from 4 different experiments. Release of GABA, dopamine and histidine in control and lead poisoned synaptosomes differs, significantly $P<0.05$ (Student's t test).

TABLE II

Stimulation of the release of previously accumulated [^{14}C]GABA, [^{3}H]dopamine and [^{14}C]histidine from synaptosomes by lead acetate

Concentration of lead acetate	% Release		
	GABA	Dopamine	Histidine
1 μM	22±1	16±0.5	0
10 μM	31±2	20±2.0	0
100 μM	35±1	25±1.0	0
1 mM	60±5	50±3.1	0
2.5 mM	79±4	64±6.2	0

100% = the amount of neurotransmitter accumulated by synaptosomal preparation. Values are mean from 3 different experiments (±SD).

Nerve endings were isolated as a synaptosomal fraction. This synaptosomal fraction was metabolically intact and could be considered as a good, simple nerve-ending model (Rafałowska et al. 1980, Deutsch et al. 1981). These synaptosomes had the ability to accumulate lead. The Pb^{2+} level in the synaptosomal fraction obtained from the Pb-treated rats was more than two times higher than in the control fraction.

Pb^{2+} level in the blood from the control rats was a little higher than the results obtained in other countries. This effect can be connected with a greater contamination of the environment in Poland. The lack of significant differences between Pb^{2+} level in blood of control and poisoned adult (4 months old) rats could be caused by the quick penetration of lead from blood to body in the chronic toxic model. Our results confirm the data of Nakagawa et al. (1980), who have shown that after administration of lead to rats, its level was elevated significantly only in prenatal animals, while in the same conditions, Pb^{2+} level in blood of adult rats was unchanged.

The mechanisms through which inorganic lead affects behaviour and neurochemistry are unknown. It has been hypothesized that Pb^{2+} and Ca^{2+} may competitively interact at the sites involved in the release of neurotransmitters, resulting in the inhibition of Ca-dependent stimulus-coupled release. Some evidence for this hypothesis has been found in studies on lead on cholinergic and dopaminergic neurotransmission (Silbergeld 1977).

Our investigation on synaptosomal fractions obtained from chronic Pb-treatment rats confirm this hypothesis. Chronic treatment of rats with lead acetate was associated with the inhibition of KCl dependent release of GABA, dopamine and histidine (as precursor histamine). Histamine could not be used, because this neurotransmitter does not penetrate through the membrane into synaptosomes (Rafałowska et al. 1987).

The inhibition of KCl dependent release by lead acetate was different, related to the individual neurotransmitters. Less effect of Pb^{2+} on KCl depolarization dependent release was found in the case of GABA. Higher inhibition was observed in the case of dopamine release and practically 100% inhibition was found in the case of histidine. Our results are in agreement with Kalton and Yaari (1982), Silbergeld et al. (1974), Manalis and Cooper (1973) data concerning the release of acetylocholine from nerve-muscle preparations.

We have shown that lead blocks synaptosomal transmission by depressing the neurally evoked release of GABA, dopamine and histidine. These results presumably are the consequence of blocking the influx of calcium ions into the presynaptic terminals during their depolarization by the nerve action potential. It has been proposed that ionic calcium and lead interact in a competitive manner at the synapse (Silbergeld 1977, Silbergeld 1978). Probably, the effect of Pb^{2+} on Ca^{2+} influx at the different endings is selective, so we have observed different effects of lead acetate on GABA, dopamine and histidine.

In the non-depolarized synaptosomes incubated in the absence of high concentration of KCl, or in the case of electrically- induced depolarization, the presence of lead acetate activated GABA and dopamine but not histidine release. The lead-induced increase in a spontaneous release may be caused by inhibition of mitochondrial calcium uptake (Silbergeld

et al. 1977c). Lead ions may also produce an increase in spontaneous release more indirectly by inhibition the Na^+-K^+-ATPase from the presynaptic axolemma. Incubating effect of Pb on Na^+-K^+-ATPase was found in the erythrocyte membrane (Selhi and White 1975), brain homogenates (Nechay and Saunders 1978) and in synaptosomes (Jabłońska and Rafałowska 1993b).

Elevation of intracellular sodium has been shown to augment spontaneous neurotransmitter release (Baker and Crawford 1976). Organometallic compounds like tributyl lead acetate or lead tetraethyl are more active than lead acetate (used in our experiments) in the disturbances of neurotransmitters translocation. This may be partially due to the fact that lead in organometallic compounds is able to dissolve and diffuse through lipid-rich membranes easier (Wood et al. 1978). We used lead acetate but organometallic compounds have been studied as well as ionic salts since they can be found in plenty in the environment. Such chemicals may be man-made (lead tetraethyl) or can be converted from inorganic industrial waste by bacteria commonly found in lake and river sediments (Jensen and Jernelov 1969).

Used in our experiments, the small concentrations of lead acetate (1-10 μM) that we found could change neurotransmitter release are comparable with the level of lead (ranges from 0.3 to 1.0 x 10^{-6} M) found in brains of people living in North America (ranges from 0.3 to 1.0 x 10^{-6} M (Schroeder and Tipton 1968, Massaro et al. 1974).

So, this report indicates that lead acetate at such concentrations can modify the translocation of neurotransmitters to a considerable extent, and this may account in part for the neurotoxicity of lead.

REFERENCES

Baker P.F., Crawford A.C. (1976) A note on the mechanism by which inhibitors of the sodium pump accelerate spontaneous release of transmitter from motor nerve terminals. J. Physiol. 247: 209-226.

Beitz L. (1975) X-ray fluorescence and X-ray diffraction analysis in the model laboratory. Electromedica 1: 13-19.

Bondy S.C., Anderson C.L., Harrington M.E., Nasad P. (1979) The effects of organic and inorganic lead and mercury on neurotransmitter high-affinity transport and release mechanisms. Environmental Res. 19: 102-111.

Booth R.F., Clark J.B. (1978) A rapid method for the preparation of relatively pure, metabolically competent synaptosomes from rat brain. Biochem. J. 176: 365-370.

Cory-Slechta D.A., Widzowski D.V., Pokora M.J. (1993) Functional alterations in dopamine systems assessed using drug discrimination procedures. NeuroToxicology 14: 105-114.

Davis J.M., Elias R.W., Grant L.D. (1993) Current issues in human lead exposure and regulation of lead. NeuroToxicology 14: 15-28.

Deutsch C., Drawn C., Rafałowska U., Silver I.A. (1981) Synaptosomes from rat brain: morphology, compartmentation and transmembrane pH and eletrical gradients. J. Neurochem. 36: 2063-2072.

Jabłońska L., Rafałowska U. (1993a) Lead as an inductor of morphological and functional changes in synaptosomes from rat brain. Symposium on heavy metal induced alterations in excitable membranes. Tihany, Hungary, Abstr. No 10.

Jabłońska L., Rafałowska U. (1993b) The basis of lead action on synaptosomal release of neurotransmitters after its chronic administration. 14th Meeting of the International Society for Neurochem, Montpellier, France, Abstract S33D.

Jabłońska L., Walski M., Rafałowska U. (1994) Lead as inductor of morphological and functional changes in synaptosomes from rat brain; are one or more mechanisms of lead toxicity in chemical neurotransmission? NeuroToxcicology (in press).

Jensen S., Jernelöv A. (1969) Biological methylation of mercury in aquatic organism. Nature (London) 223: 753-754.

Kaufman L., Deconinck F., Price D.C., Gnesvy P., Wilson C.J., Hruska B., Swann S.J., Camp D.C., Voegele A.L., Friesen R.D., Nelson J.A. (1976) An automated fluorescent excitation analysis system for medical applications. Invest. Radial. 11: 210-215.

Kolton L., Yaari Y. (1982) States of action of lead on spontaneous transmitter release from motor nerve terminals. Israel J. Med. Sci. 18: 165-170.

Lowry O.H., Rosebrough A.L., Farr A.L. Randal R.J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Manalis R.S., Cooper Y.P. (1973) Presynaptic and postsynaptic effects of lead at the frog neuromuscular function. Nature 243: 354-356.

Massero E.J., Yatte S.J., Thomas C.C. (1974) Mercury levels in human brain skeletal muscle and body fluids. Life Sci. 14: 1939-1946.

Nakagawa K., Asami M., Kuriyama K. (1980) Inhibition of release of lysosomal enzymes in young rat brain by lead acetate. Toxical Appl. Pharmacol. 56: 86-92.

Nechay B.R., Sanders J. (1978) Inhibitory characteristic of lead chloride in sodium and potassium dependent adenosine triphosphate preparations derived from Kidnay, brain and haart of several species. J. Toxociol. Environ. Health 4: 147-159.

Pawłowski Z., Cudny W., Marzec J., Walentek J., Zaremba K., Konarzewski B. (1992) X-ray fluorescence analysis in determination of trace elements in the body. Probl. Techn. Med. 23: 115-124.

Rafałowska U., Erecińska M., Wilson D. (1980) Energy metabolism in rat brain synaptosomes from nembutal-anesthetized and nonanesthetized animals. J. Neurochem. 34: 1380-1386.

Rafałowska U., Waśkiewicz J., Albrecht J. (1987) Is neurotransmitter histamine predominantly inactivated in astrocytes? Neurosci. Lett. 80: 106-110.

Schroeder H.A., Tipton I.H. (1968) The human body burden of lead. Arch. Environ. Health 17: 965-978.

Selhi H.S., White I.M. (1975) The effect of lead on the red cell membrane. Postgrad. Med. J. 51: 765-769.

Silbergeld E.K. (1977) Interaction of lead and Ca on the synaptosomal uptake of dopamine and choline. Life Science 20: 309-318.

Silbergeld E.K., Adler H.S. (1978) Subcellular mechanisms of lead neurotoxicity. Brain Res. 148: 451-467.

Silbergeld E.K., Adler H.S., Costa I.L. (1977) Subcellular localization of lead in synaptosomes. Res. Commun. Chem. Pathol. Pharmacol. 17: 715-725.

Silbergeld E.K., Goldberg A.M. (1975) Pharmacological and neurochemical investigations of lead-induced hyperactivity. Neuropharmacology 14: 431-444.

Silbergeld E.K., Fales J.T., Goldberg A.M. (1974) The effects of inorganic lead on the neuromuscular junction. Neuropharmacology 13: 795-801.

Simons T.J.B. (1993) Lead-calcium interaction in cellular lead toxicity. NeuroToxicology 14: 77-86.

Troeger M.B., Rafałowska U., Erecińska M. (1984) Effect oleate on neurotransmitter transport and other plasma membrane functions in rat brain synaptosomes. J. Neurochem. 42: 1735-1742.

Wood J.M., Cheh A., Dizikes L.J., Ridley W.P., Rokow S., Lakonier J.R. (1978) Mechanisms for the biomethylation of metals and metalloids. Fed. Proc. 16: 21.

Received 11 January 1994, accepted 17 May 1994