Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 27 (3-4)
Sofia ● 2020

# Influence of Lead on the Secretion of Amyloid Precursor Protein in Mouse Brain

*Ludmil Kirazov[1]*, Evgeni Kirazov[1], Emilia Petrova[1], Yordanka Gluhcheva[1], Juliana Ivanova[2]*

*[1] Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
*[2] Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria*

* Corresponding author e-mail: lkirazov@yahoo.com

It is known that heavy metals and especially lead (Pb) are toxic to the organism and particularly to the brain. Lead is assumed to participate in the etiology of neurodegenerative diseases including Alzheimer's disease (AD). Little is known about the relationship between Pb and the metabolism of the amyloid precursor protein (APP) which is considered to have a key role in this type of dementia.

In our study we examined the effect of Pb on the secretion of the amyloid precursor protein and found that it reduces the secretion in the cerebral hemispheres and cerebellum and had no significant effect in the other mice brain regions studied.

*Key words:* lead, amyloid precursor protein secretion, mice brain regions

## Introduction

Changes in the biological levels of essential metal ions, which regulate the function of a number of enzymes, can influence numerous biochemical processes in the body [11]. In the brains of persons who suffered from AD a disruption of metal cation levels has been reported [10], which is associated with subsequent cognitive loss and neurodegeneration. These observations were used by Bush [2] to formulate the "Metals hypothesis of AD", stating that the preservation of metal homeostasis is a critical point for neuronal function.

Wu et al. [14] point out that exposure to heavy metals (such as Pb) during brain development affects the metabolism of APP at later stages, and conceivably the processes of amyloidogenesis.

Lead has long been known as a toxic metal. It can replace divalent cations and affect the concentration of other ions, which are important cofactors of a number of enzymes and are also involved in various metabolic processes.

Lead induces oxidative stress by disruption of the defense mechanisms against reactive oxygen species (ROS). Furthermore Pb can even at picomolar concentrations

replace calcium and affect the sodium ion concentration, thereby affecting vital biological activities in the excitatory tissues like cell to cell interactions, thus disrupting neuronal communication [4].

It has been reported that Pb inhibits APP translation [8] through replacement of iron (Fe)-ions in Fe-regulated pathways. On the other side APP is a protein participating in the maintenance of Fe homeostasis, regulating its efflux [1, 3, 8]. McCarthy et al. [7] proposed that this function is furnished specifically by the secreted APP forms.

The Pb-mediated lowering of APP concentration, and thus the APP-regulated Fe-efflux, results in raised cytosolic Fe levels which can become toxic due to their catalyzing the generation of ROS. Enhanced APP expression counteracts this process [9].

Bringing about elevation of the intracellular Fe concentration, and thus the generation of ROS, Pb has been shown to impair mitochondrial membrane function and to influence the calcium balance in this organelle which results in cell death [13]. This process has also been termed "ferroptosis" [12].

Data on the direct influence of Pb on APP secretion are scarce in the literature which prompted us to address this problem. We found that exposure to Pb decreases the secretion of APP in mice cerebral hemispheres and cerebellum, while in the other studied areas (forebrain, hindbrain) there was no significant effect detectable.

## Materials and Methods

The experimental design for inducing subacute Pb intoxication was developed and implemented by Ivanova et al. [5]. The cerebral hemispheres, cerebellum, forebrain and hindbrain of experimental animals were dissected and processed as follows.

The tissues were homogenized and membrane-containing and soluble protein fractions were prepared by centrifugation for 1 h at 100 000 g at 4ºC. The fractions were subjected to dodecylsulfate-polyacrylamide gel electrophoresis on 7% gels. APP was detected through immunoblotting with the monoclonal antibody 22C11 (Boehringer Mannheim) and visualized with the diaminobenzidine-$H_2O_2$ technique. Quantification of the grey values was performed by densitometric image analysis using the software package TINA 2.0 (Raytest). Results were normalized using actin as internal loading control.

## Results and Discussion

Using the experimental protocol for intoxication with Pb, described by Ivanova et al. [5], we studied the effect of Pb on the secretion of APP in cerebral hemispheres, cerebellum, forebrain, and hindbrain of the treated mice.

The monoclonal antibody 22C11 employed in this study binds to an epitope in the N-terminal portion of the APP molecule, i.e. it recognizes both the intact APP molecules as well as the metabolites, obtained as a result of the activity of the proteases acting at the C-terminal. These comprise the secreted forms of APP which are found in the fraction containing the soluble proteins.

To verify the comparison between the studied fractions we used actin for normalizing the results. Under ideal circumstances normalization would not be necessary, but factors as transfer efficiency and sample loading make this step essential. The amount of actin detected allows the correction of deviations in protein content.

Representative immunoblot showing the effect of Pb on the secretion of APP in the cerebral hemispheres is shown on **Fig. 1**, and respectively for the effect of Pb on the secretion of APP in cerebellum – on **Fig. 2**.

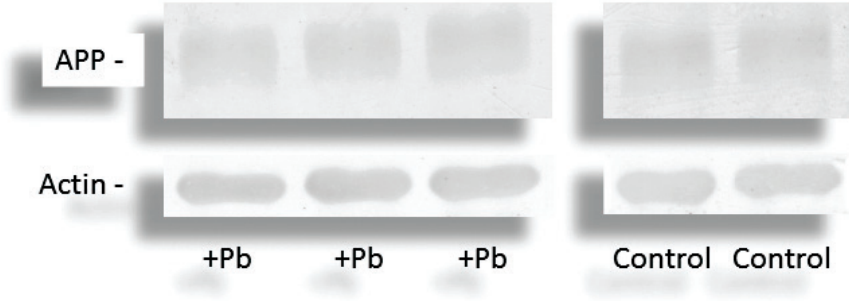


**Fig. 1.** Representative immunoblot showing the effect of lead (Pb) on the APP secretion in cerebral hemispheres.

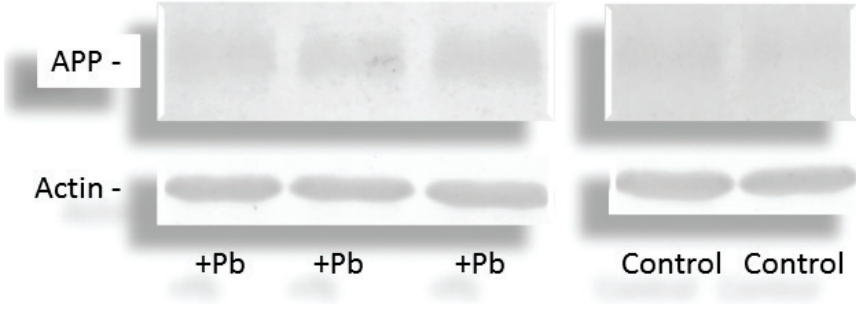


**Fig. 2.** Representative immunoblot showing the effect of lead (Pb) on the APP secretion in cerebellum.

The numerical quantification of these results is shown on **Fig. 3**. It can be seen that Pb decreases the secretion of APP in the cerebral hemispheres by 10% and in cerebellum by 19%.

We also studied the effect of Pb on the secretion of APP in front brain and hindbrain, where we did not find any significant effects of Pb intoxication (data not shown).

In a previous study we have shown that the content of APP in the cortex containing structures cerebral hemispheres and cerebellum is much higher as compared to that in other brain structures [6] (**Fig. 4**). It could explain the significant influence of Pb in the cerebral hemispheres and cerebellum.

We also performed immunoblotting with the membrane containing fractions of the studied brain regions and found no significant changes in the APP content after ~~the~~ Pb treatment (data not shown).

The results of our study show that exposure to Pb leads to decreased secretion of APP in the cerebral hemispheres and cerebellum. In accordance with the proposal of McCarthy et al. [7] that secreted APP participates in the maintenance of Fe homeostasis, regulating its efflux, we can suggest a mechanism of Pb toxicity – Pb decreases APP

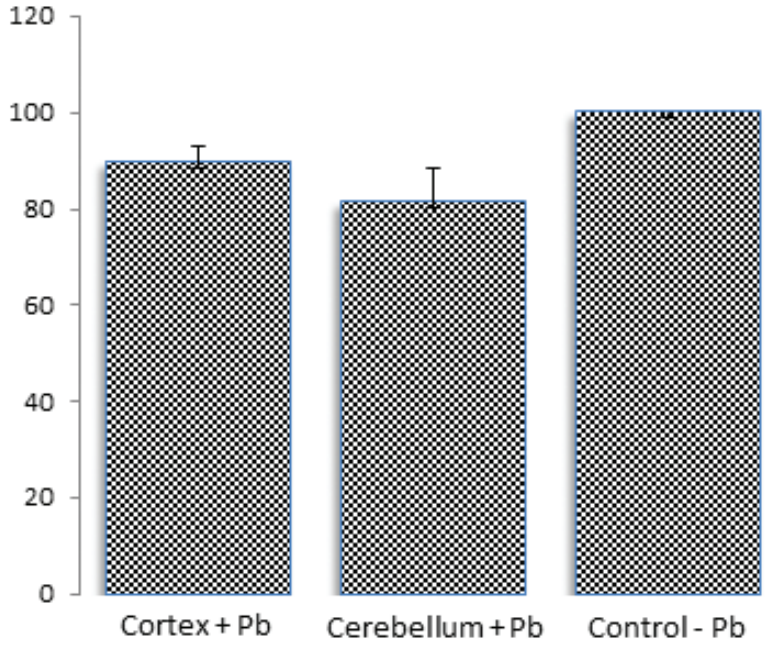


**Fig. 3.** Numerical quantification of the effect of lead (Pb) on the APP secretion in cerebral hemispheres and cerebellum. The data are calculated as grey values/µg protein and the value of control is taken as 100%. The data are the means of three experiments, each performed in duplicate.

**Fig. 4.** Expression pattern of APP695 mRNA. Representative autoradiogram from sagittal section through 90-day-old rat brain. The figure is part of the data presented by Kirazov et al. 2001 [6]. For details please refer this publication.

secretion which results in increased Fe concentration and thus causing a number of disturbances in cellular metabolism leading to cell- respectively neurotoxicity.

The mechanism of the effect of Pb on the secretion of APP evidently needs further investigation.

*Acknowledgements:* This study was financially supported by Sofia University "St. Kliment Ohridski" Science Fund (grant 162/2014).

## References

1. **Ayton, S., P. Lei, D. J. Hare, J. A. Duce, J. L. George, P. A. Adlard, C. McLean, J. T. Rogers, R. A. Cherny, D. I. Finkelstein, Al. Bush.** Parkinson's Disease Iron Deposition Caused by Nitric Oxide-Induced Loss of beta-Amyloid Precursor Protein. – *J. Neurosci.*, **35**(8), 2015, 3591-3597.
2. **Bush, Al.** The metal theory of Alzheimer's disease. – *J. Alzheimers Dis.,* **33**(Suppl. 1), 2013, 277-281.
3. **Duce, J. A., A. Tsatsanis, M. A. Cater, S. A. James, E. Robb, K. Wikhe, S. L. Leong, K. Perez, T. Johanssen, M. A. Greenough, H. H. Cho, D. Galatis, R. D. Moir, C. L. Masters, C. McLean, R. E. Tanzi, R. Cappai, K. J. Barnham, G. D. Ciccotosto, J. T. Rogers, Al. Bush.** Iron-export ferroxidase activity of beta-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. – *Cell,* **142**, 2010, 857-867.
4. **Flora, G., D. Gupta, A. Tiwari.** Toxicity of lead: A review with recent updates. – *Interdiscip. Toxicol.,* **5**(2), 2012, 47-58.
5. **Ivanova, J., Y. Gluhcheva, D. Dimova, E. Pavlova, S. Arpadjan.** Comparative assessment of the effects of salinomycin and monensin on the biodistribution of lead and some essential metal ions I mice, subjected to subacute lead intoxication. – *J. Trace Elem. Med. Biol.,* **33**, 2016, 31-36.
6. **Kirazov, E., L. Kirazov, V. Bigl, R. Schliebs**. Ontogenetic changes in protein level of amyloid precursor protein (APP) in growth cones and synaptosomes from rat brain and prenatal expression pattern of APP mRNA isoforms in developing rat embryo. – *Int. J. Devl. Neurosci.,* **19**, 2001, 287-296.
7. **McCarthy, R. C., Y. H. Park, D. J. Kosman.** sAPP modulatesiron efflux from brain microvascular endothelial cells by stabilizingthe ferrous iron exporter ferroportin. – *EMBO Rep.,* **15**, 2014, 809-815.
8. **Rogers, J. T., V. Venkataramani, C. Washburn, Y. Liu, V. Tummala, H. Jiang, A. Smith, C. M. Cahill.** A role for amyloid precursor protein translation to restore iron homeostasis and ameliorate lead (Pb) neurotoxicity. – *J. Neurochem.,* **138**, 2016, 479-494.
9. **Rogers, J. T., N. Xia, A. Wong, R. Bakshi, C. M. Cahill.** Targeting the iron-response elements of the mRNAs for the Alzheimer's amyloid precursor protein and ferritin to treat acute lead and manganese neurotoxicity. – *Int. J. Mol. Sci.,* **20**(4), 2019, 994.
10. **Salvador, G. A., R. M. Uranga, N. M. Giusto.** Iron and mechanisms of neurotoxicity. – *Int. J. Alzheimers Dis.,* **2011,** 720658, 2010.
11. **Sastre, M., C. W. Ritchie, N. Hajji.** Metal ions in Alzheimer's disease brain**.** – *JSM Alzheimer's Dis. Related Dementia,* **2**(1), 2015, 1014.
12. **Stockwell, B. R., J. P. FriedmannAngeli, H. Bayir, Al. Bush, M. Conrad, S. J. Dixon, S. Fulda, S. Gascon, S. K. Hatzios, V. E. Kagan, K. Noel, X. Jiang, A. Linkermann, M. E. Murphy, M. Overholtzer, A. Oyagi, G. C. Pagnussat, J. Park, Q. Ran, C. S. Rosenfeld, K. Salnikow, D. Tang, F. M. Torti, S. V. Torti, S. Toyokuni, K. A. Woerpel, D. D. Zhang.** Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. – *Cell.,* **171**, 2017, 273-285.
13. **Verstraeten, S. V., L. Aimo, P. I. Oteiza.** Aluminium and lead:molecular mechanisms of brain toxicity. – *Arch.Toxicol.,* **82**, 2008, 789-802.
14. **Wu, J., M. R. Basha, B. Brock, D. P. Cox, F. Cardozo-Pelaez, C. A. McPherson, J. Harry, D. C. Rice, B. Maloney, D. Chen, D. K. Lahiri, N. H. Zawia.** Alzheimer'sdisease (AD)-likepathology inagedmonkeysafterinfantileexposure to environmentalmetallead (Pb): evidencefor a developmen taloriginandenvironmentallinkfor AD. – *J. Neurosci.,* **28**(1), 2008, 3-9.