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Effects of Pb^{2+} and Cd^{2+} Heavy Metal Ions on the Rat Liver Mitochondrial ATP-Dependent Potassium Channel

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ABSTRACT:
In the article, rat liver of Pb^{2+} and Cd^{2+} ionsthe effect on mitoK_{ATP}-channel activity in mitochondria was studied.Inhibition of mitoKATF-channel activity was observed experimentally in the presence of 200 µM concentration of ATP in the incubation medium of mitochondria isolated from rat liver.In the absence of ATP in the incubation medium, 8.0 µM concentration of Pb^{2+} and Cd^{2+} salts was noted to reduce the permeability of liver mitoK_{ATP}-channel activity in both energized and de-energized states compared to the control.

Keywords:
Cd^{2+}, Pb^{2+}, Heavy Metals, ATP-Sensitive Potassium Channel, Rat Liver Mitochondria, ATP.

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INTRODUCTION

Environmental pollution of heavy metals is increasingly becoming a problem and has become of great concern due to the adverse effects it is causing around the world. These inorganic pollutants are being discarded in our waters, soils and into the atmosphere due to the rapidly growing agriculture and metal industries, improper waste disposal, fertilizers and pesticides. This review shows how pollutants enter the environment together with their fate. Some metals affect biological functions and growth, while other metals accumulate in one or more different organs causing many serious diseases such as cancer (Jessica Briffa, Emmanuel Sinagra, Renald Blundell, 2020).

Heavy metal pollution not only poses a serious threat to both animal growth and public health, but also to aquatic life. Mitochondria are the first target sites for a variety of heavy metals, and recently great attention has been made on the mechanisms of toxicity of heavy metals on mitochondria. (Qiuyu Sun, Ying Li, Lijun Shi, Riaz Hussain, Khalid Mehmood, Zhaoxin Tang, Hui Zhang, 2022).

Among the chemical substances classified as global environmental pollutants, heavy metals form a special group of anthropogenic toxicants, which largely determine the unfavorable environmental situation and the increase in morbidity in industrialized areas (Ponomareva L.A., 2003; Skachkov M.V., Skachkova M.A., Vereshchagin N.N., 2002; Gutnikova A.R., Makhmudov K. O., Saidkhanov B. A., Tadzhikulova O.D., Ergashev N.A., Asrarov M. I., Kosnikova I. V., 2009). Over the years, anthropogenic factors have led to cadmium (Cd) accumulation in the environment causing various health problems in humans. Cadmium (Cd) is considered to be of major concern for

public health by the World Health Organization (Satarug, S., 2012; Ambily Ravindran Nair., Olivier DeGheselle., Karen Smeets., Emmy Van Kerkhove and Ann Cuypers., 2013). Agricultural and industrial activities have led to the entry of Cd into the soil and subsequently into ground and drinking water. Due to the highly soluble nature of Cd compounds as compared to other metals, they are readily taken up by plants resulting in storage in crops for food and feed production (Sarwar, N.; Malhi, S.S.; Zia, M.H.; Naeem, A.; Bibi, S.; Farid, G., 2010; Ambily Ravindran Nair., Olivier DeGheselle., Karen Smeets., Emmy Van Kerkhove and Ann Cuypers., 2013). Environmental and occupational lead contamination is harmful to human health. Many countries and regions have made a series of measures to control Pb contamination (Levin, R.; Zilli, V.C.; Rosenbaum, M.H.; Bischoff, K.; Mordarski, D.C.; Brown, M.J. 2021; Charkiewicz, A.E.; Backstrand, J.R., 2020; Jianbin Zhang., Peng Su., Chong Xue., Diya Wang, Fang Zhao., Xuefeng Shen and Wenjing Luo., 2022), but there are still many people are suffering from Pb contamination, especially in developing countries (Obeng-Gyasi, E., 2019). Pb exposure exerts irreversible damage effects on nervous system cognition, learning, and memory capabilities (Mason, L.H.; Harp, J.P.; Han, D.Y., 2014; Cassleman, K.L.; Dorrance, K.A.; Todd, A.C., 2020; Jianbin Zhang., Peng Su., ChongXue., Diya Wang, Fang Zhao., Xuefeng Shen and Wenjing Luo., 2022).

Mitochondrial dysfunction, as known today, plays a critical role in aging, cell death and different diseases, including hereditary ones. It was found also that mitochondria are target organelles for dangerous environmental pollutants such as heavy metals (Belyaeva et al., E.A. Belyaeva, S.M. Korotkov, N.E. Saris., 2011). Mitochondrial membrane performs the task of ensuring metabolic exchanges between the cell cytoplasm, and through the function of ion transport systems located in the membrane, the exchange of ions required for the process of oxidative phosphorylation is carried out in the inner part of the mitochondria. (Cardoso A.R., Queliconi B.B., Kowaltowski A.J., 2010; Nicholls D.G., S. Fergusson., 2002; Garlid K.D and Paucek P., 2003). Mironova and a number of other authors revealed the existence of a uniport transport system with a selective effect on K^+ ions in the inner membrane of mitochondria. (Mironova G., Fedotcheva N., Makarov P., Pronevich L., Mironov G.P.,1981). The mitoK^+/ATP-channel located in the mitochondrial membrane has been reported to act as a protector in tissue cells (Facundo H.T., R.S. Carreira, J.G. de Paula, C.C. Santos, R. Ferranti, F.R. Laurindo, A.J. Kowaltowski., 2006; Fornazari M., J.G. de Paula, R.F. Castilho, A.J. Kowaltowski.,2008).

MATERIALS AND METHODS

Rat liver mitochondria were isolated using the Schneider method of differential centrifugation (Schneider W.C., Hogeboom G.H., 1951). Composition of separation medium: 250 mM sucrose, 10 mM tris-chloride, 1 mM EDTA, pH 7.4.For this purpose, after the rat was decapitated, its liver was thawed and placed in the isolation medium, and its weight was weighed on a scale. For grinding, it was passed through a micropress with holes of 1 mm. Minced liver tissue was placed in a molybdenum glass homogenizer, 1:6 separation medium was added to it, and homogenized using a Teflon pestle. Homogenization was carried out using a pestle mounted on an electric motor at a speed of 600-800 revolutions per minute. The resulting homogenate was poured into a centrifuge tube.Centrifugation was carried out at a temperature of 0-2°C. The centrifugation process was carried out in 2 stages. In the first stage, centrifugation at a speed of 1500 rpm (relative centrifugal acceleration of 600 g) lasted 7-8 minutes. In this case, the large cellular components of the non-disintegrated parts of the tissue were precipitated. The supernatant was transferred to another clean tube and used for a second centrifugation. In the second step, centrifugation was carried out for 15 minutes at a speed of 6000 revolutions per minute.After the end of centrifugation, liquid residues and oil particles on the test tube walls were cleaned using filter paper.Mitochondria purified from the isolation medium were taken into a beaker using an autopipette. For experiments, mitochondria were diluted 1:1 in EDTA-free

isolation medium and stored in an ice-cold beaker in the freezer.

ATP-dependent potassium channel conductance of mitochondria (0.3-0.4 mg/ml) was determined by changes in optical density at 540 nm wavelength in 3 ml cells. IM as follows: 125 mM KCl, 10 mM Hepes, 5 mM succinate, 1 mM $MgCl_2$, 2.5 mM K_2HPO_4, 2.5 mM KH_2PO_4, 0.005 mM rotenone and 0.001 mM oligomycin (pH-7,4)

RESULTS

We determined the inhibitory effect of Pb^{2+} and Cd^{2+} ions on mitoK_{ATP}-channel activity in rat liver mitochondria.The inhibition of mitoK_{ATP}-channel activity was determined experimentally in the presence of 200 µM concentration of ATP in the incubation medium of mitochondria isolated from rat liver.In the absence of ATF in the incubation medium, a concentration of 8.0 µM of Cd^{2+} salts was noted to reduce the conductance of liver mitoK_{ATP}-channel activity in both energized and deenergized states compared to control (Figure 1 a and b). Therefore, it was found that heavy metal Cd^{2+} salts act as a blocker on mitoK_{ATP}-channel activity, which plays a key role in controlling membrane potential and matrix size in mitochondria. In the above experiment, mitoK_{ATP}-channel inhibition was observed under the influence of Cd^{2+}salts even in the absence of ATP in the incubation medium.In our next experiments, inhibition of mitoK_{ATP}-channel activity was noted under the influence of 8.0 µM concentration of Pb^{2+} salts in the absence of ATP in the incubation medium (Fig. 2 a and b).

Heavy metals slow down the entry of K^+ ions into the mitochondrial matrix and inhibit channel activity. As a result, under the influence of heavy metals, the mitoK_{ATP} channel became dysfunctional (Figures 1 and 2).

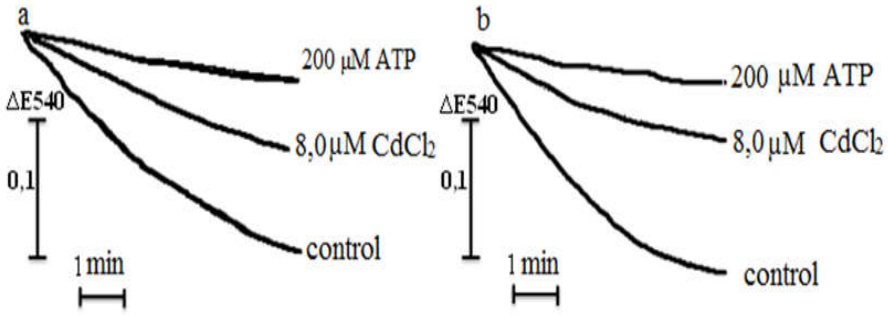

Figure 1: Effect of Cd^{2+} ions on mito$K^+/_{ATP}$ - channel activity. a) energized mitochondria; b) deenergized mitochondria; 200 µM ATP; 8.0 µM Cd^{2+}, n=5.

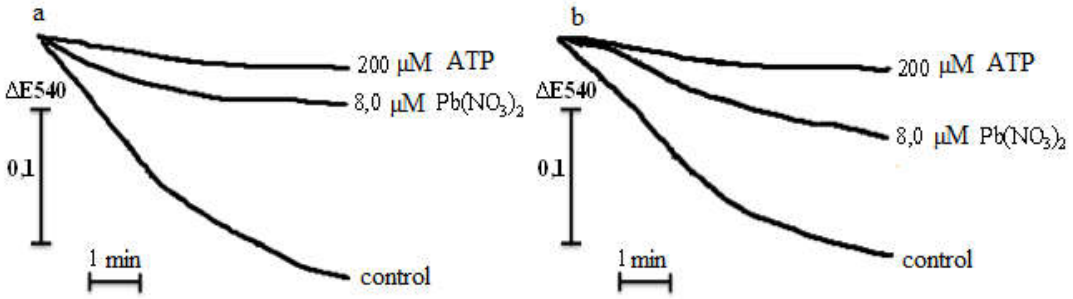

Figure 2. Effects of Pb^{2+} ions on mito K+/ATP -channel activity. a) energized mitochondria; b) deenergized mitochondria; n=5.

DISCUSSION

MitoK_{ATP}-channel plays a key role in cell volume control, and channel activity is derailed by various toxic effects. As a result of the increase in the concentration of K^+ ions in the matrix of mitochondria, under the influence of osmotic pressure, the entry of water molecules into the mitochondria increases and, in turn, the mitochondria collapse. Changes in the size of mitochondria have a direct effect on its optical density. According to the obtained results, the rate of transport of K^+ ions in the matrix of mitochondria depends on the oxidation of substrates, in which the rate of transport of K^+ ions in NAD-dependent substrate oxidation was lower than in the case of succinate oxidation. It was also shown that in the presence of ATP in the incubation environment, the entry of K^+ ions into the matrix is inhibited, and K^+ ions enter the matrix through the mitoK_{ATP}-channel. Inhibition of mitoK_{ATP}-channel by adenine nucleotide may be due to its binding to a specific domain, but not to phosphorylation of the protein molecule. Under these conditions, diazoxide increases mitoK_{ATP}-channel activity.

CONCLUSION

In conclusion, it can be said that the effect of Pb^{2+} and Cd^{2+} ions on ATP-dependent potassium channel in rat liver mitochondria isolated by differential centrifugation was studied in this study. The obtained results showed that Pb^{2+} ions have an inhibitory effect on mitochondrial ATP potassium channel. Also, the effect of Cd^{2+} ions as a blocker was determined.

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