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Short communication

Influence of toxic metal mixture on acetylcholinesterase activity in subchronic exposure in rats

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Introduction

The biological function of acetylcholinesterase (AchE) is to degrade acetylcholine and some other cholines. The importance of the optimal activity of this enzyme is reflected in the fact that acetylcholine is one of the most important neurotransmitters in the human body. Thus, the normal activity of AchE is of great importance for the optimal functioning of the organism. It is known that many compounds can affect the activity of this enzyme, from organophosphates, through drugs for Alzheimer's disease and myasthenia gravis, to metals such as mercury (Hg), chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni) and arsenic (As*)* (Frasco et al., 2005; Whittaker, 1990). In everyday life, the general population is more often exposed to a combination of different metals, even those with toxic effects, than individual metals. That is why more relevant results are obtained if the effects of metal combinations, i.e. the effects of mixtures, are examined, because such results better describe the actual exposure of the general population (Hernandez et al., 2019). The aim of this study was to examine the effect of a mixture of these metals on the activity ofAchE, namely the AchE present in the brain of experimental animals in a subchronic exposure experiment.

Materials and methods

Experiment design

Groups of experimental rats (Wistar rats, males, 5 per group) received a mixture of metal dissolved in water via an oral tube for 90 days. The doses to which the animals were exposed corresponded to the results of a human biomonitoring study obtained in male subjects. The control group received water. The M1 group received doses corresponding to human doses calculated to give rise to blood metal concentrations corresponding to the medians obtained in the human biomonitoring study. The M2 group received doses corresponding to the doses estimated to be required to achieve 95th percentile exposure concentrations. Group M3 received doses corresponding to BMD (Benchmark dose, BMD). BMD was calculated in relation to the effects of metals on certain hormones, with BMDs having the best confidence interval selected. Water and food were available *ad libitum* to the animals during the experiment. The metals were given as aqueous salt solutions of the corresponding metals. On the 90^{th} day of the experiment, the animals were sacrificed in accordance with the ethical principles (permission of the Ethics Committee for Work with Experimental Animals of the Faculty of Pharmacy, number 323-07-11822/2018-05) and the brains of the animals were collected.

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Determination of AchE activity

The collected material was used to determine AchE activity by the Elman method. Acetylthiocholine iodide (ASChJ) was used as a substrate, and Elman's reagent (5,5-dithio-bis-(2-nitrobenzoic acid), DTNB) was used as an indicator. Collected brain samples were homogenized in phosphate buffer pH 7.4, in the mass ratio brain(g):phosphate buffer(mL)=1:3. After homogenization, 2 0µL of tissue homogenate was transferred to a spectrophotometer cuvette, along with 2500 µL ofDTNB indicator solution. The mixture was then incubated for 5 minutes at 25°C. After incubation, 20µL of substrate was added to the mixture. In parallel with the analysis of the sample, a blank test was performed (instead of 20 µL of the homogenate, 20 µL of the physiological solution was added to the mixture). The change in absorbance (Abs/min) was monitored for 3min on a CARY60 UV-VIS spectrophotometer.

Results and discussion

The results obtained by measuring the absorbance on the spectrophotometer were used to calculate the enzyme activity. Enzyme activity was calculated by the formula: ΔA/min x (ξ x l) -1 x Vuk x Vuz -1 x dilution =activity (µmol hydrolyzed acetylthiocholine iodide/min per gram of tissue), where ξ is the moral absorption coefficient (1.36 $ml\mu mol^{-1}$ cm^{-1}), l is the optical path length (1cm), Vuk is the total volume of the reaction mixture and Vuz is the volume of the sample.

Using the Kolmogorov-Smirnov test, the normal distribution of the data was determined, and then using the ANOVA test followed by the Tukey test, no statistically significant difference was found betweenAchE activity measured in brain tissue of control rats and test groups. F = 0.404; p = 0.752; mean ± standard deviation for groups was: 2.74 ± 0.84 µmol/min/g; 3.23 ± 1.33 µmol/min/g; 3.24 ± 0.55 µmol/min/g; 3.37 ± 1.04 µmol/min/g; 3.14 ± 0.93 µmol/min/g. There is a slight trend of increasing values of enzyme activity, however, this increase was not statistically significant.

A group of scientists studied the effect of a mixture of metals on AchE in the brains of zebrafish. The metals used in this case were zinc, cadmium, lead and mercury, and acute, subchronic and chronic exposure was investigated, which lasted for 24 hours, 96 hours and 30 days, respectively. The results obtained indicate that zinc and cadmium did not lead to statistically significant changes in enzyme activity, while this was not the case for lead and mercury. In the first 24 hours, lead and mercury led to a slight decrease in AchE activity, after 96 hours there was a slight increase in activity, and after 30 days AchE activity returned to normal. These results are in agreement with the results obtained in our experiment (Richetti et al., 2011). Another significant study from the perspective of mercury studies consisted of applying different doses of mercury to crayfish and measuring acetylcholinesterase activity after 72 hours. The conclusion within that study was that mercury decreased enzyme activity at higher concentrations, while lower mercury concentrations did not lead to statistically significant changes in enzyme activity (Gunderson et al., 2018).

Conclusion

Low doses of six metals, which correspond to a real-life scenario of environmental exposure, do not lead to statistically significant changes in acetylcholinesterase brain activity.

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