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ACETYLCHOLINE CONTENT AND CHOLINESTERASE ACTIVITY IN THE PONTO-MEDULLARY REGION OF BRAIN IN RATS TREATED WITH ARMIN AND OBIDOXIME

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Abstract—The lethal dose of armin (0.4 mg/kg, s.c.) produced an increase in the total acetylcholine content and a marked degree of cholinesterase inhibition in the region of pons and medulla oblongata of rats. The intraperitoneal injection of obidoxime (25 mg/kg), given 5 min after armin administration, significantly prevented the rise of acetylcholine content (25%) and produced a satisfactory reactivation of cholinesterase in the same tissue (33%). The decrease of accumulated acetylcholine by obidoxime seems to be in good correlation with degree of cholinesterase reactivation in the investigated tissue.

The reactivating effect of pyridinium aldoximes on the activity of brain cholinesterase in animals poisoned with cholinesterase inhibitors and the resulting changes in brain acetylcholine have been the subject of relatively few reports, dealing generally with the whole brain [1, 2].

However, alterations in the cholinesterase activity and concentration of acetylcholine in the whole brain do not necessarily reflect the corresponding changes in the vital sites, such as those in the medulla oblongata, the depression of which immediately contributes to the fatal outcome of the intoxicated organism.

Because of this, in the present study the ponto-medullary part of the brain was used for the estimation of cholinesterase activity and acetylcholine content in rats treated with armin, as a cholinesterase inhibitor, and obidoxime, as a powerful reactivator of phosphorylated cholinesterase.

MATERIALS AND METHODS

Albino rats of both sexes, weighing 150–200 g were used in all experiments. The rats were randomly divided into groups and treated with armin (Ethyl-4-nitrophenyl ethylphosphonate), obidoxime dichloride (Toxogonin "Merck") or sodium chloride (see Tables 1 and 2). A volume of 0.2 ml/100 g body wt was used for all injections.

Table 2. Cholinesterase activity in the ponto-medullary region of brain in rats treated with armin and obidoxime (LüH-6)

Armin mg/kg	LüH-6 mg/kg	n	ChE activity $\mu l\ CO_2$/mg/30 min	Change (%)
—	—	6	0.525 ± 0.04	
0.4	—	6	0.098 ± 0.02	81*
0.4	25	6	0.238 ± 0.03	33†

* Inhibition.
† Reactivation.

The tissue was removed as quickly as possible and immediately immersed into the frog Ringer-eserine (10 μg/ml) solution, without $NaHCO_3$, and adjusted to pH 4 with 0.1 N HCl. The frog Ringer-eserine solution of the same composition was used for the extraction of acetylcholine from the tissue.

The acetylcholine was extracted by the method described by Rothschuh [3] and partly modified by Lewartowski and Bielecki [4]. The acetylcholine was estimated biologically on the isolated frog rectus abdominis [3]. All results were expressed as acetylcholine chloride in μg/g of fresh tissue.

Cholinesterase activity was determined in a Warburg apparatus at 37° with the following reaction mixture: 0.15 M sodium bicarbonate, 0.164 M sodium

Table 1. Acetylcholine (ACh) content in the ponto-medullary region of brain in rats treated with armin and obidoxime (LüH-6)

Armin mg/kg, s.c.	LüH-6 mg/kg, i.p. 5 min. after armin	n	ACh μg/g	Change [%] in comparison with control	P
—	—	12*	2.29 ± 0.17		
0.4	—	20†	3.42 ± 0.14	+50	<0.01
0.4	25	20‡	2.86 ± 0.11	+25	
—	25	6‡	2.25 ± 0.15		

* Controls, killed by decapitation 55 min after subcutaneous injection of saline.
† Animals decapitated immediately after cessation of respiration.
‡ Animals killed by decapitation 55 min after injection of obidoxime.

chloride and acetylcholine chloride as substrate (0.4 ml 0.1% solution in distilled water). The total vol. 2.6 ml; pH 7.4; gas phase nitrogen + carbon dioxide (95:5). Enzyme activity was expressed in μl of carbon dioxide liberated per mg of fresh tissue in the course of the first 30 min. All results were corrected for non-enzymatic hydrolysis.

The inhibition and reactivation of cholinesterase (ChE) activity was calculated as follows:

$$\text{Inhibition } (\%) = \frac{\text{ChE (control)} - \text{ChE (armin)}}{\text{ChE (control)}} \times 100$$

$$\text{Reactivation } (\%) = \frac{\text{ChE (armin + obidoxime)} - \text{ChE (armin)}}{\text{ChE (control)} - \text{ChE (armin)}} \times 100$$

RESULTS

The results of these experiments are shown in Tables 1 and 2. As can be seen from the tables, the subcutaneous administration of armin (0.4 mg/kg) produces a significant rise of total acetylcholine (50%) in the tissue of pons and medulla oblongata of rats, and a high degree of cholinesterase inhibition (81%).

The intraperitoneal injection of obidoxime (25 mg/kg), given 5 min after armin administration, partly prevents the increase (25%) of total acetylcholine content in the same region with a satisfactory enzyme reactivation (33%).

DISCUSSION

In the tissue of pons and medulla oblongata of rats treated with armin a high degree of cholinesterase inactivation (81%) and an increase in the total acetylcholine content (50%) were found. Similar findings concerning other cholinesterase inhibitors have been published [5].

Our results also show that the intraperitoneal injection of obidoxime produced a significant decrease in the amount of acetylcholine together with a satisfactory enzyme reactivation in the ponto-medullary part of the brain in poisoned rats. This finding indicates that obidoxime, in spite of its quaternary structure, penetrates the blood-brain barrier in a concentration sufficient to reactivate the phosphorylated cholinesterase in the ponto-medullary region. According to Bajgar [6], the therapeutic effect of oximes may be based on the reactivation of acetylcholinesterase in the respiratory centre in medulla oblongata to a "minimum level" that is necessary for the life of the organism. This agrees with the findings of Erdmann [7], who has shown that in decerebrate rats on artificial respiration the central respiratory paralysis following paraoxon poisoning is overcome by obidoxime, but not by pralidoxime, which penetrates the blood-brain barrier to a lesser degree than obidoxime. The reactivation of phosphorylated cholinesterase in the ponto-medullary part of the brain of poisoned rats is probably the main factor in the observed reduction of accumulated acetylcholine. However, our results do not exclude other possible mechanisms, either, especially the diffusion of accumulated acetylcholine from the brain into the periphery which contains active (oxime reactivated) cholinesterase [2, 8].

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