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Influence of trimedoxime and atropine on acetylcholinesterase activity in some parts of the brain of mice poisoned by isopropylmethyl phosphonofluoridate

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THE COMMONLY accepted principles of treatment of acute organophosphate poisoning comprise the use of parasympaticolytic drugs and cholinesterase-reactivating substances, atropine[1,2] being the preferred parasympaticolytic. There is less agreement on the choice of cholinesterase-reactivating substances for the treatment of organophosphate poisoning. From many investigated reactivators pralidoxime (1-methyl-2-hydroxyiminomethylpyridinium iodide),[3–5] obidoxime [1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxapropane dichloride][6,7] and trimedoxime [1,1′trimethylenebis(4-hydroxyiminomethylpyridinium) dibromide][7–10] are considered as the most effective ones.

However, there exist some doubts about the use of cholinesterase reactivators in organophosphate poisoning: According to Oettel[11] reactivation of inhibited blood cholinesterase by obidoxime is very doubtful. Prinz[12] has established that through the action of oximes it is possible to obtain reactivation of cholinesterases *in vitro* but not *in vivo*. Nevertheless, the therapeutic effect of a combination of

parasympaticolytics and cholinesterase reactivators is indisputable. In this paper the therapeutic effect of the combination of atropine (in a constant dose) and trimedoxime (in different doses) against intoxication by isopropylmethyl phosphonofluoridate (IMPF) in mice is correlated with acetylcholinesterase (AChE, EC 3.1.1.7) activity in four parts of the mouse brain.

Material and methods

Preparation of homogenates of the brain parts. White male mice (Mezno), weighing 15–17 g, were killed by bleeding the carotid artery. The brains were removed and four parts of the brain were dissected out—pons with medulla oblongata, mesencephalon, diencephalon and basal ganglia. Each part of the brain was homogenized (Ultra Turrax, Janke and Kunkel, Germany) to make a 10% homogenate (0·2 M tris–HCl buffer pH 7·6).

Enzyme assay. AChE activity was measured by a modification[13] of the method of Ellman *et al.*,[14] using acetylthiocholine iodide (Lachema Brno, Czechoslovakia) as substrate and 5,5′-dithio-bis-(2-nitrobenzoic) acid as chromogen (Serva, Heidelberg, Germany). AChE activity was expressed as ΔE/min, corresponding to 1 mg of wet tissue (412 nm, Vitatron, Sci. Instr., Holland).

The influence of the treatment on AChE activity of the brain parts. The mice were randomly divided into 11 groups, with six animals in each group. The control group was injected i.m. with saline. The groups of intoxicated mice were poisoned by IMPF, in doses of 0·2 and 0·4 mg/kg (i.e. 1 × and 2× LD_{50}). The groups of treated mice were poisoned by IMPF in the dose 0·4 mg/kg, and 30 sec after intoxication they were treated i.m. by atropine sulphate (Spofa, Praha, Czechoslovakia) in a constant dose (21·0 mg/kg) and by trimedoxime bromide (Léčiva, Měcholupy, Czechoslovakia) in the doses of 2·06–4·63–10·4–15·6–23·3–35·0–52·5 and 79·0 mg/kg. After 2 hr the animals were killed, and homogenates of the brain parts were prepared. Activity of AChE of the control group was given as 100 per cent, AChE activities in experimental groups were expressed as a percentage of normal activity.

Statistical evaluation. The homogeneity of experimental groups was tested by Bartlett's test. The differences between groups were evaluated by regression analysis, using MINSK 22 computer programmes.[15]

Results

Among the brain parts under study normal AChE activity was highest in the basal ganglia, followed by the pontomedullar part. Lowest activity was observed in mesencephalon and diencephalon.

AChE activity in the brain parts of the mice, poisoned by IMPF in two doses, decreased depending on the injected dose. The remaining activity was lowest in the ponto-medullar part, and highest in the basal ganglia (Fig. 1). The higher dose of IMPF (0·4 mg/kg) killed all of the experimental animals. This dose of IMPF was used for testing the therapeutic effect. Trimedoxime had no effect on the AChE activity in mesencephalon, diencephalon and basal ganglia. The increase of AChE activity (from 3 to 15 per cent of control) was observed only in the ponto-medullar area. Figure 2 shows the dependence of AChE activity in the ponto-medullar part on the toxic effect, expressed as mortality.

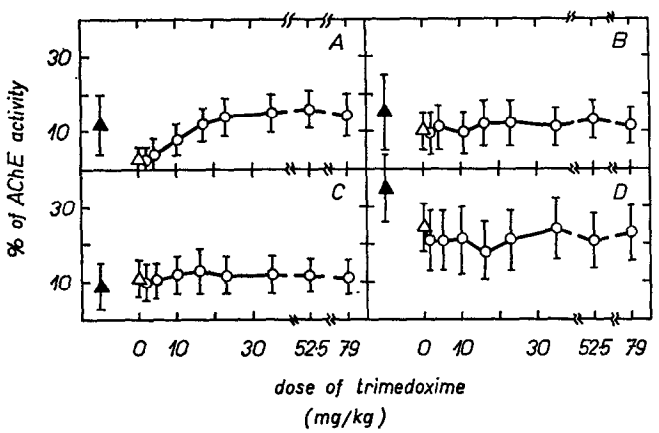

FIG. 1. AChE activity in brain parts of mice after IMPF poisoning and its treatment. ▲, IMPF in the dose 0·2 mg/kg. △, IMPF in the dose 0·4 mg/kg. ○, IMPF in the dose 0·4 mg/kg, and 30 sec after intoxication atropine (21·0 mg/kg) and trimedoxime (in different doses) were administered.

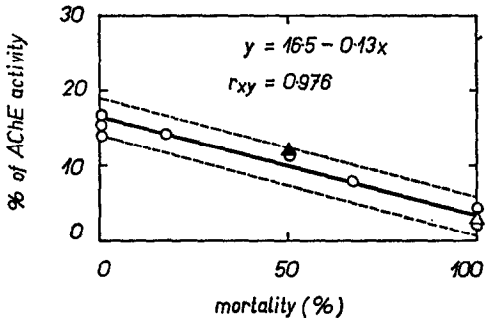

FIG. 2. Correlation between AChE activity in the pontomedullar part of mouse brain and mortality after IMPF poisoning. IMPF without treatment in the doses 0·2 mg/kg (▲) and 0·4 mg/kg (Δ), with treatment by atropine and trimedoxime (○).

The results are given for treated and untreated mice, poisoned by IMPF. A correlation between AChE activity of the ponto-medullar part and mortality was demonstrated. In other parts no correlation was observed.

The normal AChE activity of the mouse basal ganglia was about 2·3 times higher than that of rats,[16] with acetylthiocholine as substrate. The decrease of AChE activity in IMPF poisoning was similar to the decrease of AChE in the brain of IMPF poisoned rats.[17] For another organophosphate, pinacolylmethyl phosphonofluoridate, the highest remaining AChE activity has also been observed in basal ganglia of the rat.[18] The total AChE inhibition was observed in other studied brain parts (medulla oblongata, pons and frontal cortex).[18]

The non-uniform inhibition of AChE in the different parts of the brain after IMPF poisoning and its treatment suggests that AChE activity in the ponto-medullar area has a great importance in the toxidynamics of this compound. A relatively low increase of activity in this part was sufficient for the survival of the organism. In this connection, some authors suggested that a central reactivation effect of oximes is very important for prognosis of organophosphate poisoning.[6,18] The penetration of trimedoxime into the brain is probably very low, and selective for the ponto-medullar area. If the AChE activity were measured in the whole brain homogenate, no reactivation effect could be observed.

From our results it appears that trimedoxime caused a reactivation of inhibited AChE to a "minimum level", that is necessary for the life of organism. The immediate cause of death in IMPF intoxication was described as respiration failure.[19,20] The therapeutic effect of trimedoxime may be based on the reactivation of AChE in the part of respiratory centre in medulla oblongata to a "minimum level". For verification of this hypothesis it would be necessary to perform topical histo- and biochemical studies of AChE activity in this part of the brain.

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Effect of diethylnitrosamine on the respiratory and enzymic response of rat liver to corticosterone

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CHANGES in the respiratory and enzymic activity of rat liver preparations induced *in vitro* by incubation with corticosterone have been shown[1] to be associated with the saturation of two types of hormone receptor sites.[2] Hormonal action on tissue respiration has been associated with increased activity of enzymes of the tricarboxylic acid cycle and an increased transaminase activity of the tissue.[3] The regulatory response of gluconeogenic enzyme activity and glycogen level in rat liver to glucocorticoid injection has been described by Webber *et al.*[4] and it has been shown that this hormonal response is blocked by inhibitors of protein synthesis. The steroid response was found to be limited in slow growing hepatomas and absent in fast growing hepatomas.[5]

In the present work we have studied the change in corticosterone response of rat liver preparations taken from rats fed diethylnitrosamine[6] with a normal diet (Oxoid 86), in order to investigate effects of carcinogens leading to a loss of hormone response.

A group of 20 male rats (8 week old) were fed diethylnitrosamine (50 mg/l.) in the drinking water over a period of 6 months. A group of 20 litter mates were kept over the same period as control animals. At the start of the experiment six male rats were killed by a blow on the head and the livers exsanguinated by infusion of 0·9% heparinized saline via the hepatic portal vein. Animals for each experiment were killed at mid-morning,[7] the livers were excised, pooled and minced using a stainless steel press through 1·2-mm dia. holes. The oxygen consumption, succinate dehydrogenase activity and tyrosine transaminase activity were measured in this liver preparation using procedures described previously by Dalton and Snart[1] in the presence and absence of 10^{-9}M and 10^{-7}M corticosterone. These studies were repeated at the 3 and 6 month stage of treatment using six treated and control animals at each stage. The livers taken from animals at the 6 month stage of treatment with diethylnitrosamine showed tumor development as evidenced by an observed increase in size and apparent surface hepatomas with some necrosis. However, no detailed examination of the tissue was undertaken. All the tissue was used in preparation of the experimental sample. A 100,000 *g* supernatant protein was obtained from a 50 per cent homogenate of livers taken from 6 month treated and control animals. Each supernatant fraction was incubated with low specific activity ^{3}H corticosterone for 1 hr at 20°, after which 1 ml of each sample was applied to a 2 × 50 cm Sephadex G50 column and the corticosterone binding capacity of the liver protein measured in terms of the amount of radioactivity eluting with the protein peak.

The enzymic response of the control liver, Fig. 1, confirmed our previous observation that the succinate hydrogenase activity is maximally stimulated by 10^{-9}M corticosterone, whereas the tyrosine transaminase activity was maximally stimulated by 10^{-7}M corticosterone. The level of succinate