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Acetylcholine content in the brain of rats treated with paraoxon and obidoxime

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Summary

1. The effect of obidoxime on the rise in brain acetylcholine caused by the anticholinesterase paraoxon was studied in the rat.

2. In animals poisoned with a sublethal dose of paraoxon and thereafter treated with obidoxime the levels of both " free " and total brain acetylcholine were practically the same as those in rats injected with paraoxon only.

3. After poisoning with doses of paraoxon which are lethal unless an oxime is also given, the total acetylcholine in the brain of obidoxime-protected rats continued to accumulate, reaching a peak 2 h after injection of paraoxon. At this time no signs of central effects such as convulsions or tremor were seen.

4. Atropine, given 30 min before paraoxon, markedly reduced the rise in total brain acetylcholine seen when the anticholinesterase is given alone.

5. In rats pretreated with atropine and obidoxime excessive doses of paraoxon which are lethal in the absence of the antidotes produced a rise in total brain acetylcholine which was directly proportional to the dose of paraoxon administered.

Introduction

The penetration of quaternary pyridinium aldoximes into the brain, and their ability to reactivate phosphorylated brain acetylcholinesterase (AChE) have been the subject of discussion for several years (see Hobbiger, 1963). Previous studies (Milošević & Andjelković, 1966 ; Andjelković & Milošević, 1966) have shown that relatively large doses of pralidoxime (P-2AM) and obidoxime (Toxogonin ; Lü H6) can effectively reactivate the phosphorylated functional AChE in the brain cortex of rats. Hobbiger & Vojvodić (1967) confirmed and extended these observations but concluded that reactivation of brain AChE, at least in rats and mice, does not appear to be of primary importance for the antidotal action of oximes. According to Schaumann (1960) oxime-induced reactivation of brain AChE is not essential at all for survival, because even a minimum amount of enzyme activity (1% of normal or less) may be sufficient to prevent the accumulation of toxic concentrations of acetylcholine in the central nervous system. To gain additional information on the significance of the reactivation of phosphorylated AChE in the brain, the effect of obidoxime on the rise in brain acetylcholine induced by the anticholinesterase paraoxon was investigated.

Methods

Albino rats of both sex, weighing 120 to 150 g, were injected intravenously with various doses of diethyl-4-nitrophenyl phosphate (paraoxon, E 600). The injections were given at a controlled rate of 0·1 ml/20 s. Obidoxime dichloride and atropine sulphate were used in doses of 0·035 and 0·14 mmol/kg, respectively. Both drugs were given intraperitoneally at the time stated in each experiment. The injection volume for all drugs was 1 ml/kg.

At an appropriate time after the treatment, the animals were killed by decapitation, the brain (excluding the cerebellum) was quickly removed and immediately placed in ice-cold frog-Ringer solution containing physostigmine (eserine) sulphate (0·03 mM). An MSE homogenizer was used for the preparation of a 10% homogenate which then was divided into two equal portions. One part was centrifuged for 10 min at 3,000 rev/min and the supernatant immediately thereafter assayed for " free " acetylcholine. The other part of the homogenate was acidified with 0·5 M HCl to pH 3–4 and placed for 2 min in boiling water. After neutralization with 0·5 M NaOH, the supernatant was assayed for the total acetylcholine content.

The bioassay was carried out on the eserinized rectus abdominis muscle of the frog. The results are expressed in nmol of acetylcholine per gram of fresh tissue. In control experiments it was shown that the extracts did not contain the drugs used or substances other than acetylcholine in concentrations which affected the sensitivity of the assay preparation to acetylcholine.

Drugs used were acetylcholine chloride (Pliva, Zagreb), physostigmine (eserine) sulphate (Merck), atropine sulphate (B.D.H.), N,N'-oxydimethylene-bis(pyridinium-4-aldoxime) dichloride (obidoxime dichloride, Toxogonin " Merck "). The organophosphate diethyl 4-nitrophenyl phosphate (paraoxon, E 600) was kindly provided by Dr. G. Kroneberg, Farbenfabriken Bayer AG, Wuppertal-Elberfeld.

Results

Sublethal dose of paraoxon

One hour after intravenous injection of a sublethal dose of 0·0007 mmol/kg of paraoxon there was a marked increase in the amount of acetylcholine in rat brain. The results, summarized in Table 1, show that on a percentage basis " free " acetylcholine was increased to a greater extent than was total acetylcholine.

Obidoxime alone, 0·035 mmol/kg intraperitoneally, produced no change in the acetylcholine content of rat brain. This dose was previously found to reactivate

TABLE 1. *Effect of a sublethal dose* (0·0007 *mmol/kg*) *of paraoxon, obidoxime* (0·035 *mmol/kg*) *and a combination of the two on the "free" and total acetylcholine* (*ACh*) *content of rat brain*

Drug	No. of rats	nmol ACh g tissue	
		Free ACh	Total ACh
None	10	4·43±0·26	14·57±0·65
Obidoxime	7	4·39±0·21	14·77±0·70
Paraoxon	7	8·83±0·37	22·48±1·12
Paraoxon + Obidoxime	7	9·69±0·24	22·65±1·07

Animals were killed 60 min after the injection of paroxon intravenously or obidoxime intraperitoneally. Animals injected with paraoxon and obidoxime received the oxime intraperitoneally 10 min after the organophosphate and were killed 60 min from the time of injection of paraoxon. The ACh content of the brain is expresssed by the mean±S.E.

about one third of the " functional " AChE in the brain cortex of rats poisoned with paraoxon (Andjelković & Milošević, 1966). The intraperitoneal administration of the same dose of obidoxime 10 min after paraoxon did not affect the rise in brain acetylcholine. One hour after the administration of paraoxon the levels of both " free " and total brain acetylcholine were practically the same as those in rats injected with paraoxon only.

Lethal dose of paraoxon

Immediately after injection of a lethal dose of 0·0014 mmol/kg of paraoxon there were severe signs of intoxication, and all animals died in convulsions within 3 to 8 min. The mean value for the total acetylcholine content in the brains of these animals at death was increased over that of controls by 117% ($P<0·001$).

The intraperitoneal injection of 0·035 mmol/kg of obidoxime 2 to 5 min after the administration of paraoxon, at the time when the animals exhibited signs of severe intoxication, saved all animals. One hour after the injection of the oxime, the animals were in a relatively good condition, showing only minor signs of intoxication. However, at the same time the mean total acetylcholine content in their brain was even higher than that found at the time of death in rats injected only with paraoxon.

Figure 1 summarizes these experiments and shows the time-course of changes in total acetylcholine content of the brain of rats treated with paraoxon and obidoxime. The same figure also shows that atropine, given 30 min before paraoxon, markedly reduced the acetylcholine rise induced by paraoxon.

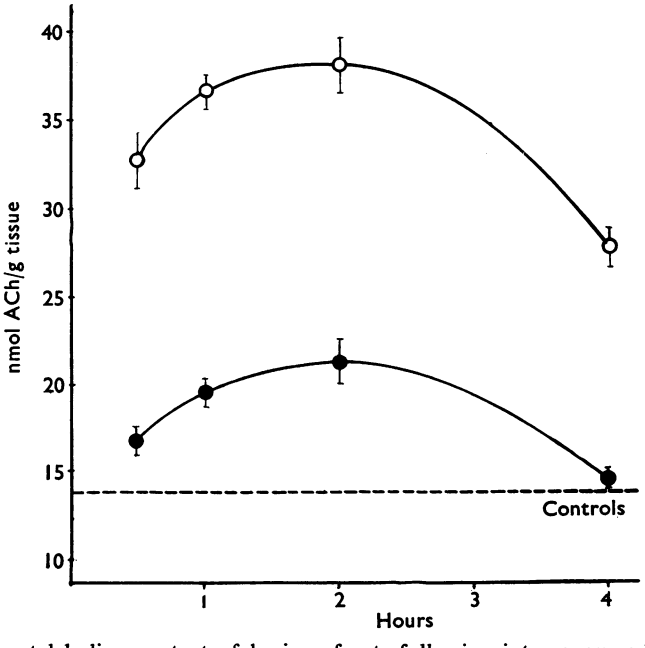

FIG. 1. Total acetylcholine content of brains of rats following intravenous administration of paraoxon, 0·0014 mmol/kg. ○——○. Results in rats which received 0·035 mmol/kg of obidoxime intraperitoneally 2–5 min after paraoxon; ●——● results obtained in rats which received 0·14 mmol/kg of atropine sulphate intraperitoneally 30 min before paraoxon. Abscissa: time in hours after injection of paraoxon. Means ± S.E. are shown.

Excessive doses of paraoxon

Pretreatment with obidoxime and atropine protects rats against several LD50s of paraoxon. It was interesting, therefore, to investigate the brain acetylcholine content in rats surviving excessive doses of paraoxon since it may be assumed that AChE in the brains of these animals is completely inhibited (Schaumann, 1960).

Groups of six or more rats were injected intraperitoneally with atropine and obidoxime, and then with increasing doses of paraoxon which in the absence of the antidotes were lethal. The results are shown in Fig. 2. As can be seen, the rise in total brain acetylcholine content in these conditions was directly proportional to the dose of paraoxon administered. After 0·14 mmol/kg of paraoxon (a dose corresponding to about 25 LD95–100), some rats died and in the survivors the total brain acetylcholine content was increased by 200% over that of controls.

Discussion

The experiments described show that obidoxime-induced reactivation of phosphorylated functional AChE in the brain (Andjelković & Milošević, 1966) cannot prevent the increase of the so-called " free " form of acetylcholine which is, for the most part, responsible for the rise in the amount of total brain acetylcholine during poisoning with organophosphorus anticholinesterases. In this respect obidoxime is similar to pralidoxime (Milošević, 1969).

Schaumann (1960) has expressed the opinion that acetylcholine accumulating in the brain after the inhibition of AChE can be removed by diffusion from the brain

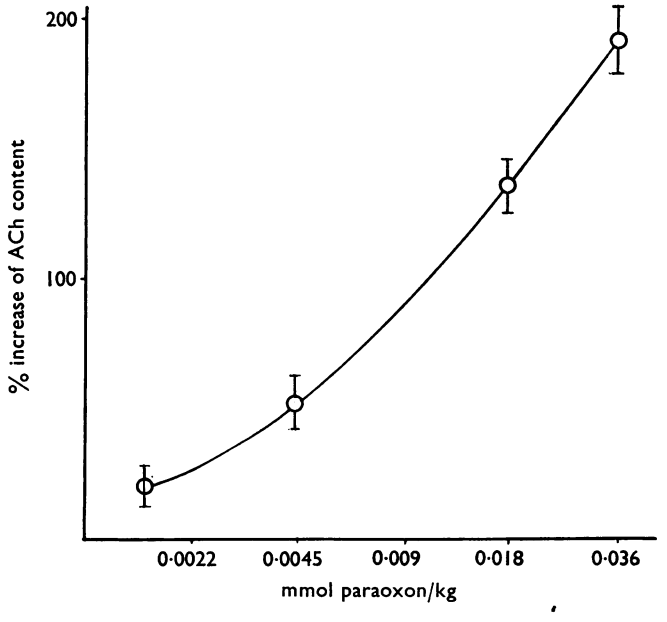

FIG. 2. Total acetylcholine content of brains of rats pretreated with 0·14 mmol/kg of atropine sulphate intraperitoneally and 0·035 mmol/kg of obidoxime intraperitoneally and then injected intravenously with large doses of paraoxon (atropine and oxime were injected together 10 min before paraoxon). Animals (at least six in each group) were killed 60 min after the administration of paraoxon. Means ± S.E. are shown.

into the periphery which contains an active (oxime-reactivated) enzyme. It seems, however, that the diffusion, if operative at all, cannot effectively remove from the brain the accumulated acetylcholine, at least during the initial period of poisoning.

Holmstedt, Härkönen, Lundgren & Sundwall (1967) have shown that the rise in brain acetylcholine induced by organophosphorus anticholinesterases is closely related to central nervous symptoms, such as convulsions and tremor, which appear when the level of total brain acetylcholine has risen by 60% or more over its control value. It is also well established that the onset of tremor induced by tremorine and other tremorgenic drugs coincides with an increase in the brain acetylcholine content of 20 to 40% above the normal value (Pepeu, 1963 ; Holmstedt, Lundgren & Sundwall, 1963 ; Crossland & Slater, 1968). It is interesting, therefore, that in the experiments reported here animals protected by obidoxime against an otherwise lethal dose of paraoxon exhibited no signs of central origin in spite of the abnormally high concentrations of acetylcholine in their brain.

It seems, therefore, reasonable to suppose that in the presence of abnormally high concentrations of acetylcholine the cholinergic receptors in the brain rapidly become less sensitive to acetylcholine. Such an adjustment to constant high levels of acetylcholine has been previously reported by DuBois (1963) and Brodeur & DuBois (1964).

The antidotal action of atropine in anticholinesterase poisoning is attributed to its ability to block the cholinergic receptors to an increased amount of acetylcholine. The results presented make it clear that, in addition, atropine may affect the metabolism of brain acetylcholine. A decrease in total brain acetylcholine content by atropine was first described by Giarman & Pepeu (1962) and has since been confirmed by Holmstedt & Lundgren (1965) in animals treated with organophosphorus anticholinesterases and atropine.

After treatment with atropine and obidoxime the animals survive very large doses of paraoxon, which should be more than sufficient to inhibit all the acetylcholinesterase present in the brain. The fact that even under such conditions the amount of total acetylcholine in the brain progressively rises with increasing doses of inhibitor suggests that the acetylcholinesterase in the brain is not completely inactivated.

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