Acetylcholine content in the brain of rats treated with paraoxon and pyridinium-2-aldoxime methylchloride

Evidence indicating that pyridinium-2-aldoxime methylchloride (pralidoxime, P-2-AM) reactivates functional acetylcholinesterase in the brain of rats *in vivo* has been obtained by Milošević & Andjelković (1966) and Hobbiger & Vojvodić (1967). I have now investigated whether P-2-AM prevents the accumulation of acetylcholine in the brain of animals given a lethal dose of paraoxon.

Male albino rats, 150 to 200 g, were injected subcutaneously with 0·5 mg/kg of (*O*,*O*-diethyl-*O*-*p*-nitrophenyl) phosphate (paraoxon, E 600). Ten min later, 20 mg/kg of P-2-AM (chloride) was given intravenously and, 1 h after, the animals were decapitated. The brain, excluding the cerebellum was removed without delay and the total acetylcholine extracted with hydrochloric acid without the addition of physostigmine, by the method of Elliott, Swank & Henderson (1950).

The acetylcholine content of the extracts was estimated on the ileum of the guinea-pig in oxygenated Tyrode solution containing neostigmine, 5 μg/ml, and morphine, 10 mg/litre (Paton, 1957). Amounts of acetylcholine were assessed as chloride equivalents. In additional 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.

A lethal dose of 0·5 mg/kg of paraoxon increased the total amount of acetylcholine in the brain by approximately 80% (Table 1). Rats injected intravenously with P-2-AM survived, but the acetylcholine content in the brain of these animals was even greater than that in animals treated with paraoxon only.

Table 1. *Total acetylcholine content (mean ± s.e. μg/g tissue) in the brain of rats treated with paraoxon and P-2-AM*

Substance (mg/kg)	No of rats	Time (h)	Acetylcholine	Change (%)
None	15	—	2·9 ± 0·3	—
Paraoxon (0·5)	10	0·5*	5·1 ± 0·4	+75
Paraoxon (0·5) + P-2-AM (20) ..	10	1	5·8 ± 0·5	+100
	10	2	7·5 ± 0·8	+158
	8	4	4·3 ± 0·3	+48
	8	6	3·9 ± 0·3	+34
P-2-AM (20)	10	1	2·6 ± 0·3	—

* All animals died within 30 min of injecting paraoxon.

These findings indicate that neither the extent of P-2-AM-induced reactivation of phosphorylated functional acetylcholinesterase in the brain (Hobbiger & Vojvodić, 1967), nor redistribution of accumulating acetylcholine from the brain to the periphery containing active enzyme (Schaumann, 1960) can prevent the increase of brain acetylcholine induced by paraoxon. However, in spite of an abnormally high concentration of acetylcholine in the brain, the rats treated with P-2-AM exhibited no symptoms of central origin such as convulsions or tremor. The results are reminiscent of those of Brodeur & Dubois (1964) who found that in the presence of high concentrations of acetylcholine after inhibition of acetylcholinesterase, the cholinergic receptors may become less sensitive to acetylcholine.

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Determination of dihydroxypropyltheophylline in plasma

There are numerous methods in the literature for the detection of dihydroxypropyltheophylline (diprophylline) in various solutions, but none are sensitive enough for its determination in plasma after therapeutic doses (Bukowska & Gierlowska, 1960, Ott & Wittman-Zinke, 1958; Raber, 1964).

The procedure of Schack & Waxler (1949) for theophylline has now been modified for the determination of diprophylline in plasma.

The procedure involves an extraction of the drug from plasma into chloroform–isopropanol (10:1) from which the drug is re-extracted with 20% v/v sulphuric acid. The absorbance of this solution is then read in a spectrophotometer. The absorbance of a standard solution of the drug in 20% v/v sulphuric acid is also measured. The peak absorbance is at 268 nm. The absorbance peak for theophylline in 0·1 N sodium hydroxide is at 277 nm.

Method. Plasma (2·0 ml) is extracted with chloroform (spectrograde)—isopropanol (nanograde) (10:1) (50 ml) by shaking vigorously for 10 min in a 120 ml separatory funnel. The solvent layer is then filtered through anhydrous sodium sulphate, the filter washed with fresh solvent (1 ml) and the filtrates combined. The plasma (aqueous) layer is then extracted a second time with another portion of chloroform-isopropanol (50 ml) for 10 min and the organic layer filtered to remove water as above and added to the previous 50 ml portion. The combined solvent layers (102 ml) are evaporated on a water bath to a final volume of 10 ml. This is placed in a 30 ml vial and extracted with 20% v/v sulphuric acid (reagent grade; 4·0 ml) by shaking vigorously for 10 min. The vial is then centrifuged to break the slight emulsion that forms. The sulphuric acid layer is pipetted into a cuvette and the absorbance read at 268 nm against a reagent blank consisting of plasma samples without diprophylline treated in the same way as the samples.

The percent recovery of diprophylline is 85–90% in the range of 10·0 to 50·0 μg/ml of plasma. The method is equally effective for human and rat plasma, is simple and fairly rapid and inexpensive.

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