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Time course of acetylcholinesterase inhibition in the medulla oblongata of the rat by *O*-ethyl *S*-(2-dimethylaminoethyl) methylphosphonothioate *in vivo*

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Summary

1. The time course of acetylcholinesterase inhibition in three parts of the medulla oblongata of the rat following intramuscular injection of *O*-ethyl *S*-(2-dimethylaminoethyl) methylphosphonothioate was studied.

2. The inhibition in the three parts of the medulla oblongata proceeded at different rates and this indicates that penetration of the inhibitor through various parts of the blood-brain barrier is not uniform.

Introduction

O-Ethyl *S*-(2-dimethylaminoethyl) methylphosphonothioate (EDMM) belongs to the group of organophosphates which are potent inhibitors of acetylcholinesterase (AChE, EC 3.1.1.7) and cholinesterase (EC 3.1.1.8) (Tammelin, 1957; Patočka & Tulach, 1969) and have a high toxicity (Aquilonius, Fredriksson & Sundwall, 1964). Inhibition of AChE, determined on homogenates of the whole brain of rats, poisoned by EDMM, at the time of death amounts to only 50% (Bajgar, Tulach, Jakl & Patočka, 1971). Histochemical results indicate a lack of uniformity of AChE inhibition in different parts of the brain, with inhibition being highest in the reticular formation of the pons-medullary area and lowest in the basal ganglia (Urban, 1969). In this paper, AChE inhibition in three parts of the medulla oblongata of rats injected with EDMM has been studied. Simultaneously the inhibition of cholinesterases in the blood was determined.

Methods

Female Wistar rats (Mezno), weighing 150–170 g, were randomly divided into 8 groups of 6–10 animals. Each animal in the control group received an intramuscular injection of saline, and the animals of experimental groups each received an intramuscular injection of 0·035 mg EDMM/kg. The animals were killed by bleeding from a carotid artery 1, 2, 4·5, 10 and 19 min after the injection, or immediately after respiration had stopped (about 30 min after injection), and blood and homogenates of parts of the medulla oblongata were used for measurements of cholinesterase activity.

The medulla oblongata was subdivided into parts A, B, and C. Part A, the middle part of the medulla, contained largely reticular nuclei, the nuclei of cranial

nerves X and XII, and the nucleus ambiguus. Part B, the lateral part of the medulla, contained mainly the nucleus cuneatus lateralis and the nuclei of cranial nerves V and VIII. Part C consisted mainly of the oliva inferior. The weights of these parts in all experimental groups were: for the whole medulla oblongata $33{\cdot}1 \pm 2{\cdot}6$ mg; for part A, $11{\cdot}3 \pm 1{\cdot}3$ mg; for part B, $11{\cdot}0 \pm 1{\cdot}5$ mg; and for part C, $10{\cdot}7 \pm 1{\cdot}6$ mg.

The three parts of the medulla were homogenized with an Ultra-Turrax homogenizer (Janke & Kunkel, Germany), in 0·2 M tris-HCl buffer (99 ml/g tissue), pH 7·6, and the blood was haemolyzed in distilled water (98·8 ml/ml blood).

The cholinesterase activity was measured at 25° C, by the method of Ellman, Courtney, Anders & Featherstone (1961), with 1 mM acetylthiocholine iodide (Lachema, Brno, Czechoslovakia) as substrate, and 1 mM 5,5′-dithiobis-2-nitrobenzoic acid (Serva, Heidelberg, Germany) as chromogen, 0·2 M tris-HCl buffer, pH 7·6. Absorption was determined with a Vitatron scanner (Sci. Instr., Holland) at 412 nm (glass cuvettes, reaction volume 2·0 ml), and the activity was expressed as (μmol of acetylcholine hydrolyzed/min)/g wet weight of tissue, or as a percentage of controls.

It has been shown by Hobbiger & Lancaster (1971) that hydrolysis of 0·5 mM acetylthiocholine by cholinesterase contributes less than 1% to the enzymic hydrolysis of the ester by rat brain homogenate, and thus the activity measured with 1 mM acetylthiocholine as substrate gives relevant information on AChE in brain homogenates.

The hydrolysis of acetylthiocholine by cholinesterases in the blood is due to the combined action of both enzymes, for the most part by AChE which contributes 71% to the hydrolysis, and then by cholinesterase which contributes 29% to the hydrolysis.

The final concentrations of tissue and blood used for assay were 2·5 mg/2 ml and 3 μl/2 ml, respectively. All estimations of enzyme activity were made in duplicate. The content of SH-groups (tissue blank) was measured in the brain and blood of the rats 30 min after the injection of EDMM and in the brain and blood of control rats, and was found to be negligible.

Homogeneity of experimental groups was tested by Bartletts' test and differences between groups were calculated by regression analysis with a MINSK 22 computer programme (Hraška & Tulach, 1966).

Results

EDMM, 0·035 mg/kg i.m., first produced symptoms of poisoning, i.e., alteration of respiration and fasciculation, approximately 10 min after administration. Convulsions were observed 13 min after injection and 30 min after EDMM administration all experimental animals were dead.

The intramuscular administration of 0·035 mg EDMM/kg decreased cholinesterase activity in blood (Fig. 1). Inhibition of AChE in different parts of the medulla oblongata was not uniform (Fig. 1). It was faster in part A than in part B and slowest in part C. At the time of death, the AChE activity in part A was approximately 10% of controls and in the other two parts 30–60% of controls. From these results the half-times ($t_{0\cdot5}$) for inhibition *in vivo* were calculated (Table 1).

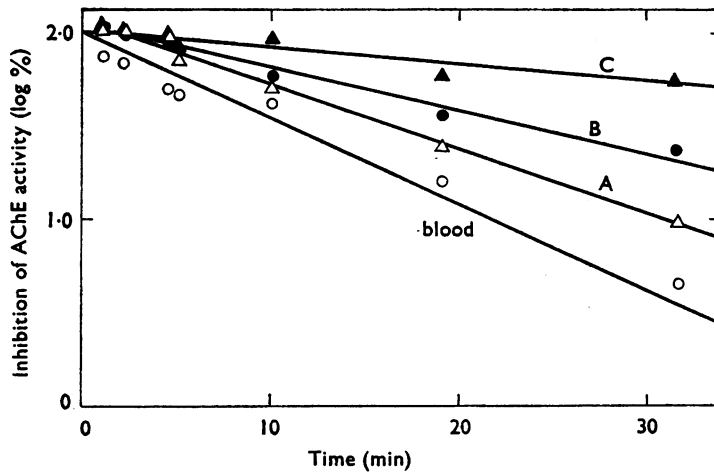

FIG. 1. Time-course of inhibition of cholinesterases in the blood and of acetylcholinesterase in the medulla oblongata of rats injected with 0·035 mg EDMM/kg, i.m. ○—Blood, △—part A, ●—part B, ▲—part C of the medulla oblongata.

TABLE 1. *Values of the half-time* ($t_{0\cdot5}$) *of inhibition of cholinesterases in blood and of acetylcholinesterase in three parts of the medulla oblongata in rats injected with* 0·035 *mg EDMM/kg, i.m.*

	$t_{0\cdot5}$ (min)*	Correlation coefficient
Blood	6·7 (6·3–7·2)	0·9787
Part A	12·0 (11·0–13·4)	0·9676
Part B	17·0 (15·0–19·7)	0·9102
Part C	44·0 (39·6–49·6)	0·8607

* Results are means with their 95% confidence limits.

Discussion

The inhibition of AChE in the medulla oblongata was delayed. This delay is probably caused by slow absorption of EDMM from the muscle and by slow penetration through the blood-brain barrier. A major part of the injected EDMM will be protonated at physiological pH, and it is known that quaternary anticholinesterases relative to their tertiary analogues penetrate more slowly through the blood-brain barrier (Koelle & Steiner, 1956; Schaumann & Job, 1958).

The results obtained suggest that EDMM penetrates into individual parts of the brain at different rates. Measurements of AChE inhibition of whole brain homogenate can thus be misleading as to the inhibition at vital sites such as those in the medulla oblongata.

During homogenization the 'free' EDMM was 1,000 times diluted. It is thus unlikely that the level of AChE activity observed in these experiments was affected by 'free' inhibitor during homogenization.

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REFERENCES

AQUILONIUS, S. M., FREDRIKSSON, T. & SUNDWALL, A. (1964). Studies on phosphorylated thiocholine and choline derivatives. I. General toxicology and pharmacology. *Toxicol. Appl. Pharmac.*, **6**, 269–279.

BAJGAR, J., TULACH, J., JAKL, A. & PATOČKA, J. (1971). Differences in anticholinesterase action of some organophosphorus compounds *in vivo*. *Acta biol. med. germ.*, **27**, 171–178.

ELLMAN, G. L., COURTNEY, D. K., ANDERS, V. & FEATHERSTONE, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.*, **7**, 88–95.

HOBBIGER, F. & LANCASTER, R. (1971). The determination of acetylcholinesterase activity of brain slices and its significance in studies of extracellular acetylcholinesterase. *J. Neurochem.*, **18**, 1741–1749.

HRAŠKA, M. & TULACH, J. (1966). Contribution to automation of evaluation of experimental data in experimental medicine. Programmes for MINSK 22 computer (in Czech), VLVDÚ Hradec Králové.

KOELLE, G. B. & STEINER, E. C. (1956). The cerebral distributions of a tertiary and a quaternary anticholinesterase agent following intravenous and intraventricular injection. *J. Pharmac. exp. Therap.*, **118**, 420–434.

PATOČKA, J. & TULACH, J. (1969). Interaction of *O*-ethyl-*S*-(2-dimethylaminoethyl) methylthiophosphonate with acetylcholinesterase. *Coll. Czechoslov. Chem. Commun.*, **34**, 3191–3195.

SCHAUMANN, W. & JOB, C. (1958). Differential effect of a quaternary cholinesterase inhibitor, phospholine, and its tertiary analogue, compound 217–AO, on cerebral control of respiration and on neuromuscular transmission. The arrest caused by morphine. *J. Pharmac. exp. Ther.*, **123**, 114–120.

TAMMELIN, L. E. (1957). Dialkoxy-phosphorylthiocholines, alkoxymethyl-phosphorylthocholines and analogues choline esters. Synthesis, pK_a of tertiary homologues and cholinesterase inhibition. *Acta chem. scand.*, **11**, 1340–1349.

URBAN, R. (1969). Changes of acetylcholinesterase activity in the central and peripheral nervous system following organophosphate intoxications and their treatment (in Czech.) *Sborník věd. prací VLVDÚ Hradec Králové*, **45**, 89–104.

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