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Relationship between acetylcholine and cholinesterase activity in the brain following an organophosphorus cholinesterase inhibitor

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THERE are relatively few reports on the effect of cholinesterase inhibitors on the acetylcholine content in tissues and blood. The injection of DFP (di-isopropylphosphorofluoridate), TEPP (tetraethyl pyrophosphate) or eserine in large doses has been shown to produce an increase in the total acetylcholine (ACh) content in the brain of the rat[1,2] and the rabbit.[3] After Parathion poisoning, free ACh in the brain is also increased.[4] The appearance of ACh-like substances in the blood of some mammals after very large doses of cholinesterase inhibitors has also been reported.[2,5,6]

The present investigation was carried out to obtain information on the relationship between cholinesterase activity and increase in ACh in the brain following an organophosphorus cholinesterase inhibitor. The compound used is 2(diethoxyphospinylthio)-ethyldimethylammoniumhydrogen oxalate.[7]

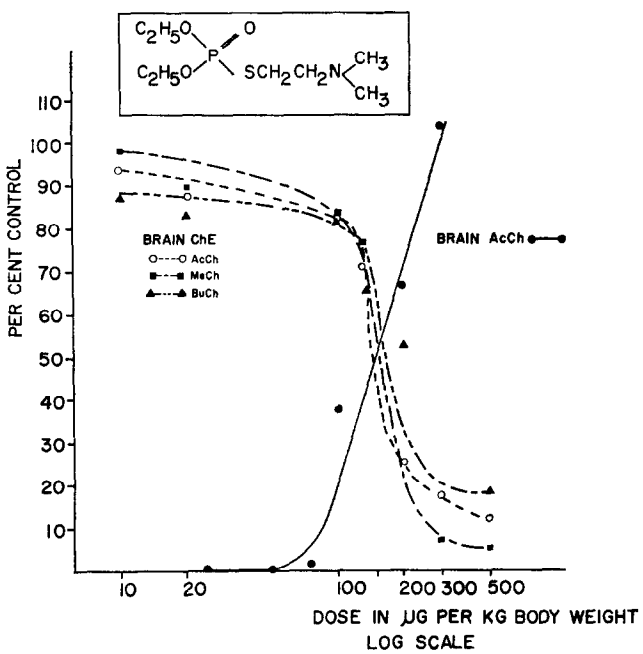

FIG. 1. Relationship between inhibition of brain cholinesterase and increase in brain acetylcholine. Per cent control refers to—for ACh, per cent increase above control value, for brain cholinesterase activity decrease in per cent of control activity.
ACh = acetylcholine iodide $7{\cdot}4 \times 10^{-3}$M
MeCh = acetyl-β-methylcholine iodide 6×10^{-3}M
BuCh = butyrylcholine iodide $6{\cdot}47 \times 10^{-3}$M
All molarities in final concentrations.

Male albino rats, descendants of the Sprague–Dawley strain, were used as experimental animals. The inhibitor was injected intraperitoneally in the doses 10–500 µg/kg (LD_{50} in mice 530 µg/kg). After 30 min the animals were killed by a blow on the head. The brains were immediately removed and

homogenized in 0·4 N cold perchloric acid. The ACh level was determined using the frog rectus muscle. For the measurement of the ChE activity, the brains were homogenized in 0·1 M CO_2-free KCl at pH 8 (40 mg wet weight/ml) and assayed by a titrigraphic method.[8] To measure all types of cholinesterases, the following substrate and final concentrations were used: acetyl-β-methylcholine iodide (MeCh) 6×10^{-3} M, acetylcholine iodide (ACh) $7{\cdot}4 \times 10^{-3}$ M and butyrylcholine iodide (BuCh) $6{\cdot}47 \times 10^{-3}$ M. In Fig. 1 the ACh content and ChE activities have been plotted. Per cent control refers to, for ACh, per cent increase above control value for brain cholinesterase activity decrease in per cent of control activity.

As seen, the lowest dose which caused an increase in the ACh content was 100 μg/kg. This dose produced about 15 per cent inhibition of brain AChE. No toxic symptoms were seen. At 200 μg/kg when ACh had increased to about 65 per cent above normal and ChE activity decreased to about 25 per cent of normal the animals developed fasiculations. Following 500 μg/kg severe dyspnoe, tremor and convulsions occurred and some of the animals died. The activity of ChE was below 20 per cent of normal.

In order to study ChE inhibition it was also tested at a suboptimal substrate concentration (ACh $7{\cdot}4 \times 10^{-5}$ M). The substrate concentration was kept constant by means of a continuous addition through a second syringe of a solution of acetylcholine equimolar to that of the NaOH used for titration. The results were the same as when $7{\cdot}4 \times 10^{-3}$ M ACh was used.

In the present work the increase in the ACh content was achieved without giving lethal doses of the compounds as was reported earlier concerning some other cholinesterase inhibitors.[1,2] Because a correlation was found between the dose-response curves of acetylcholine and cholinesterase activity it is quite clear that the rise in the brain ACh bears a close relationship to the inhibition of cholinesterases regardless of the substrate used.

When the total amount of ACh in the brain rose to 60 per cent above normal, corresponding to an enzyme inhibition of 75% symptoms did appear and then only of a peripheral nature (fasciculations and diarrhoea). Tremor and convulsions appeared at still higher level of brain ACh. Tremor and convulsions with intoxication by cholinesterase inhibitors have a complex pharmacodynamics and can partly be due to asphyxia.[9] The symptoms of peripheral and central origin as compared to the increase in the brain amine thus differ from that of cholinergic drugs such as oxotremorine and arecoline which latter drugs have been proven to give rise to tremor, salivation and lacrimation at a rise of brain ACh of 20 per cent above normal.[10,11]

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Effect of a nitrogen mustard on amino acid incorporation by thymus cell fractions*

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NITROGEN mustards have been shown to alkylate nucleic acids and proteins and to inhibit protein synthesis. Studies of the mechanism of action of alkylating agents have been reviewed by Wheeler[1] and Brown.[2] Brookes and Lawley have studied the alkylation and subsequent changes in structure of DNA.[3] Rutman *et al.*[4] have discussed the alkylation of protein, DNA, RNA, and lipid constituents of the cell. However, the mechanism of inhibition of protein synthesis is not known, nor whether the inhibition is a primary or secondary effect.

In a previous report the effect *in vitro* of whole-body X-irradiation on amino acid incorporation by isolated thymus nuclei and cytoplasmic fractions was described.[5] Incorporation by the intact nuclei was inhibited at 4 hr, but the isolated cytoplasmic ribosomal fractions showed no inhibition of amino acid incorporation until 6 hr after irradiation. It was also demonstrated that an early manifestation of nuclear damage was the presence of DNA material in the isolated cytoplasmic fractions.[5, 6] The presence of DNA material in the cytoplasmic fractions after treatment with a nitrogen mustard was also reported.[5] The effects of a nitrogen mustard on the incorporation of amino acid by the thymus nuclei and cytoplasmic fractions have been studied. A comparison of the effects of X-irradiation and a radiomimetic agent on these systems was made in order to attempt to distinguish a similar or dissimilar mode of action.

In all experiments, male rabbits 10 to 12 weeks old were used. Treated animals received an intravenous injection of the nitrogen mustard, Mustargen [methyl-bis(β-chloroethyl)amine hydrochloride; Merck Sharp and Dohme]. Mustargen was dissolved in 0·04 M sodium phosphate buffer, pH 7·4, at a concentration of 4 mg/ml. Injections were given within 1 min of dissolving the mustard. Final pH of the solution was 6·6. Animals were given 4 mg Mustargen/kg body weight. Control animals were injected with buffer containing 40 mg NaCl/ml to make it comparable to the concentration of NaCl in the dissolved Mustargen preparation.

At 4, 6, 8, 12, and 18 hr after treatment, control and treated animals were decapitated and the thymuses removed and chilled. All further operations were carried out at 4°. The thymuses from four to six animals were pooled in the respective groups. All experiments have been repeated a minimum of three times with essentially the same results.

The nuclei, cytoplasmic microsomal and ribosomal fractions, and 5·1P fraction were prepared as described previously.[5, 7] Conditions for incorporation *in vitro* of leucine-1-^{14}C by the various fractions were those used in previous studies.[5, 7]

Incubations were terminated by addition of an equal volume of 10% perchloric acid. The precipitates were washed with cold and hot perchloric acid, ethanol, and ether. Protein residues were plated on tared planchets and counted in a thin-window, low-background counter, for a sufficient time to obtain 5 per cent statistical accuracy. All calculations are corrected for both self-absorption and counting efficiency.

Fractions for nucleic acid analysis were precipitated and washed with cold 4% perchloric acid, 95% ethanol, absolute ethanol, and ethyl ether. The RNA and DNA content of the dried protein–nucleic acid residues was determined by using the methods of Webb and Levy.[8, 9]

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