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Inhibition of Cholinesterases by Tetrahydroaminacrin

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The influence of tetrahydroaminacrin upon acetylcholinesterase and butyrylcholinesterase is described. The compound acts as a reversible inhibitor, partly competitive with the substrate. It is a more potent inhibitor of BuChE than of AChE.

Tetrahydroaminacrin, 1,2,3,4-tetrahydro-5-aminoacridine, also called Tacrin or THA, has been described in 1953 by Shaw and Bentley [1]. They mentioned that the substance inhibits cholinesterase [2]. A short time ago the compound gained new interest as a substance able to prolong *in vivo* the muscle relaxing effect of succinylcholine [3,4]. This prolongation is supposed to be connected with the ability of tetrahydroaminacrin to inhibit cholinesterase. Cholinesterase, especially butyrylcholinesterase, is considered to be responsible for the *in vivo* breakdown of succinylcholine. However, it has not yet been settled, whether the synergism of cholinesterase inhibitors with succinylcholine depends upon an acetylcholine action or on inhibition of the breakdown of succinylcholine.

The present paper describes the result of a study of the effect of Tacrin upon acetylcholinesterase and of a more detailed investigation of the butyrylcholinesterase inhibition.

EXPERIMENTAL

An automatic recording titrator [5] was employed in all experiments except in the determination of the pI_{50}-values and the *in vivo* experiments, where an electrometric method [6] was used. Both methods were used at pH 8.0, $t = 25°C$. pI_{50}-Determinations were made with human erythrocytes and human plasma. Studies on the type of inhibition were made with a butanol-washed preparation of stroma from bovine blood and on a horse serum fraction prepared according to Strehlitz [7]. Substrates were acetylcholine iodide, butyrylcholine iodide and acetyl-β-methylcholine iodide. In one experiment sarin was used as inhibitor. Values obtained in *in vitro* experiments and used for calculations were corrected for spontaneous hydrolysis of the substrate. The compound was tested in a dog.

RESULTS

In vitro experiments. The pI_{50}-value (incubation time 30 min) for acetylcholinesterase (AChE) was found to be 6.2 and for butyrylcholinesterase (BuChE) 7.6. The inhibition was controlled during 0—30 min and found to be constant during this interval.

Table 1. Incubation of butyrylcholinesterase with 1.5 × 10^{-8} M Tacrin in the presence or absence of 10^{-2} M acetylcholine iodide.

	% inhibition
Acetylcholine present	66, 65, 67
Acetylcholine absent	77, 77, 76
Acetylcholine and Tacrin added together	66, 66, 67

Incubation of the enzyme with the inhibitor in the presence or absence of the substrate [10^{-2} M] showed only a small difference in the degree of inhibition as is seen in Table 1. It was therefore expected that Tacrin might not act as a competitive inhibitor. This was studied in the usual way [8], *i.e.* the concentration quotient substrate/inhibitor was varied and the activity determined. The results are seen in Figs. 1 and 2 and in Table 2. They indicate that the inhibition is of the "mixed" type [8], with a tendency towards competitive inhibition at higher substrate concentration and towards noncompetitive inhibition at lower substrate concentration. The curves seen in Fig. 1 could be reproduced in other experiments. K_I calculated from the competitive part of the curve seen in Fig. 1 is 1.5 × 10^{-9}, which fits in Table 2.

Experiments with 1.25 × 10^{-7} M (final solution) sarin in the presence or absence of Tacrin (7.5 × 10^{-9} M, final solution) and in the presence of the substrate gave k_2-values for the reaction between BuChE and sarin of 3.5 × 10^3 l $mole^{-1}$ sec^{-1}, respectively 8.9 × 10^3 l $mole^{-1}$ sec^{-1}. If sarin was added to the enzyme before the substrate, but in the presence of Tacrin, a protective effect of Tacrin was observed.

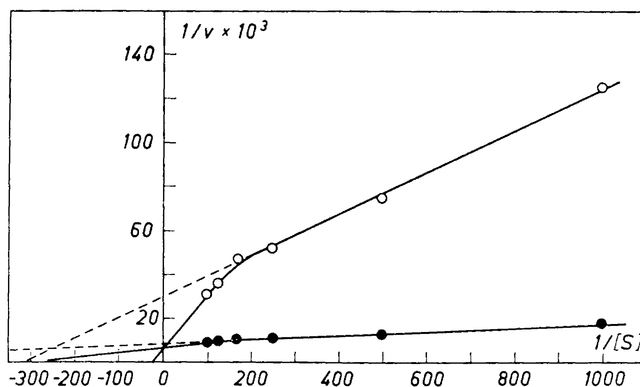

Fig. 1. Reciprocal of butyrylcholinesterase activity plotted against reciprocal of substrate concentration. ● = uninhibited enzyme, O = enzyme inhibited by 1.5 × 10^{-8} M Tacrin. All points represent mean values of two determinations.

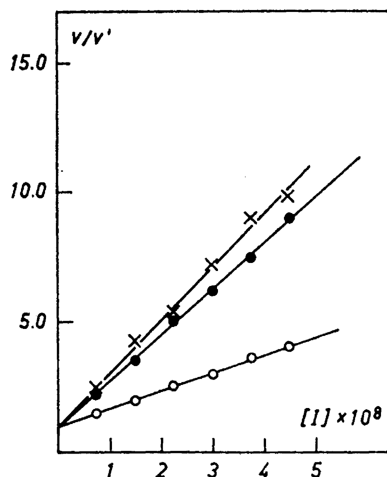

Fig. 2. Ratio of activity of uninhibited enzyme to inhibited enzyme plotted against the concentration of Tacrin. Concentration of acetylcholine iodide: ○ = 5×10^{-2} M, ● = 1×10^{-2} M, × = 5×10^{-3} M. All points represent mean values of two determinations.

The reversibility of the Tacrin inhibition was studied in dialysis experiments. The enzymes were dissolved in 0.1 M potassium chloride and inhibited with Tacrin (BuChE: 3×10^{-7} M, AChE: 5×10^{-7} M, final solution). The degree of inhibition was measured after a varying time of dialysis at + 4°C against 0.1 M potassium chloride. As is seen in Table 3, dialysis restored the enzyme activities. The activities were checked with uninhibited controls.

In vivo experiments. 1 mg Tacrin/kg body weight was injected *i.v.* in a dog. Bloodsamples were taken before the injection and 5, 15, 30, 120, 1 200 and 3 000 min after the injection. Plasma and erythrocytes were separated and the cholinesterase activity determined with three substrates. The result is seen in Fig. 3.

Table 2. Calculation of K_I according to $v/v' = 1 + [I] \frac{K_M}{K_I([S] + K_M)}$ (competitive inhibition), and $v/v' = 1 + [I] \frac{1}{K_I}$ (noncompetitive inhibition)

Series	[S]	K_I comp.	K_I noncomp.
1	5×10^{-2}	1.0×10^{-9}	14.8×10^{-9}
	1×10^{-2}	1.5×10^{-9}	5.6×10^{-9}
	5×10^{-3}	2.1×10^{-9}	5.0×10^{-9}
2	5×10^{-2}	0.95×10^{-9}	14.4×10^{-9}
	1×10^{-2}	1.4×10^{-9}	5.0×10^{-9}
	5×10^{-3}	2.0×10^{-9}	4.6×10^{-9}

Table 3. Dialysis of Tacrin-inhibited cholinesterase against 0.1 M potassium chloride.

Time of dialysis	Relative enzyme activity of			
	BuChE		AChE	
	control	inhibited enzyme	control	inhibited enzyme
Before dialysis	86, 86	0, 0	49, 45	13, 11
24 h of dialysis	83, 83	44, 45	—	—
48 h —,,—	81, 81	89, 91	35, 36	30, 30
96 h —,,—	—	—	25, 26	25, 25

It was observed that the dog 2 min after the injection salivated and showed increased lacrymation. Respiration was also increased. The pronounced respiration came and went several times during 20 min. After 30 min the dog was released and walked without difficulties.

DISCUSSION

In studies on the structure of the active center of the cholinesterases several results can be interpreted by assuming the presence of an anionic group in acetylcholinesterase but not in butyrylcholinesterase, or it can at least be assumed, that the anionic group is of more importance for the binding of substrates and inhibitors to AChE than to BuChE [9,10]. It is, *e.g.*, known that quaternary ammonium compounds have a greater inhibitory effect upon AChE than upon BuChE. Tacrin has no cationic group and is a better inhibitor of BuChE than AChE. This seems to be another confirmation of the above mentioned theory.

The experiments described in this paper show that Tacrin *in vivo* as well as *in vitro* acts as a reversible inhibitor of cholinesterases. With BuChE as enzyme, the compound seems to be only partly competitive with the substrate, preferably at higher concentrations of the substrate. Calculation of K_M from the left curve in Fig. 1 gives a value of 3.6×10^{-3} mole/l. The same value was obtained for butyrylcholinesterase acting on high concentrations of the sub-

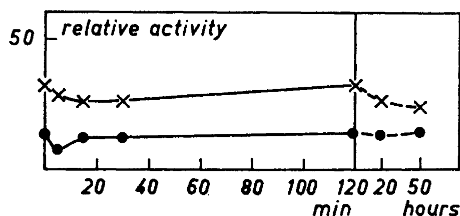

Fig. 3. Cholinesterase activity of dog erythrocytes plotted against time after injection of 1 mg Tacrin/kg body weight. Substrates: × = acetylcholine iodide, ● = acetyl-β-methylcholine iodide. The points represent mean values of two determinations.

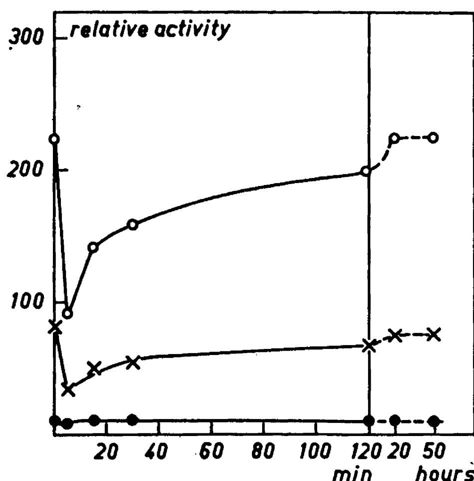

Fig. 4. Cholinesterase activity of dog plasma plotted against time after injection of 1 mg Tacrin/kg body weight. Substrates: × = acetylcholine iodide, O = butyrylcholine iodide, ● = acetyl-β-methylcholine iodide. The points represent mean values of two determinations.

strate, when pS-activity curves [11] were measured on the same enzyme preparation and the experimental values were treated as described earlier [12], *i.e.* when the preparation was supposed to contain two simultaneously acting cholinesterases.

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