Stereospecificity in the Reactions of Acetylcholinesterase.* (28354)

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The ability of acetylcholinesterase (AChE) to hydrolyze a great variety of substrates, and the readily measurable effects of small changes in substrate and inhibitor structure have made this enzyme a favorable material for the study of the molecular forces effective in the hydrolytic process. However, little attention has been given to the stereospecificity of AChE. Glick(1) used unpurified horse serum as a source of esterase with which to test acetyl-D-β-methylcholine (D-MeCh) and L-MeCh. The former was hydrolyzed at an extremely low rate; the latter not at all.

In a previous publication(2) it was suggested that D-MeCh might be a more suitable substrate for AChE than the commercially available DL-MeCh. It appeared of interest to submit this suggestion to experimental test with an active and highly specific preparation of AChE. Such test is reported here.

Experimental and results. Esterase activity was determined as described previously (3). The enzyme sources were: partially purified AChE from the electric organ of *Electrophorus*(4); serum esterase from the blood of female rats(5); and nerve bundles from the walking legs of lobsters. The results of experiments using D-MeCh, L-MeCh (6), DL-MeCh and ACh are given in Table I.

These results show no hydrolysis of L-MeCh by AChE, both the purified preparation and the nerve fiber homogenate, (this latter apparently a source of both AChE and non-specific esterases), and a high rate of hydrolysis of D-MeCh. Not only is L-MeCh not hydrolyzed, but it is an inhibitor of the enzymatic hydrolysis of D-MeCh. This can be seen by comparing the rates of hydrolysis of D-MeCh, DL-MeCh and DL-MeCh + L-MeCh. Although it is only a weak inhibitor the effect is of interest because in the generally available racemic DL-MeCh the concentration of the inhibitor is equal to the (initial) concentration of the true substrate. L-MeCh also inhibits the hydrolysis of ACh by AChE, but as the data show, apparently not the hydrolysis of ACh by rat serum esterase or by lobster nerve at a concentration of substrate where ACh is being hydrolyzed predominantly by non-specific esterase(7). The serum esterase hydrolyzes DL-MeCh at such a low rate, and even that possibly due to contamination from red-cell AChE, that the effect of L-MeCh on its hydrolysis was not examined further.

Since the inhibition by L-MeCh was rather weak it appeared relevant to test two other

TABLE I. Hydrolysis of ACh and the Stereoisomers of MeCh by 3 Sources of Esterases.

Esters	Conc (M)	μmoles hydrolyzed per hr
Electrophorus preparation		per mg lyophilized solid
D-MeCh	.03	506 ± 7*
DL-MeCh	.03	308 ± 2
DL-MeCh + L-MeCh	.03 + .03	186 ± 9
D-MeCh	.01	534 ± 5
DL-MeCh	.02	324 ± 20
DL-MeCh + L-MeCh	.02 + .01	261 ± 6
L-MeCh	.03	0 ± 0
ACh	.03	768 ± 27
ACh + L-MeCh	.03 + .03	428 ± 25
ACh	.01	1482 ± 14
ACh + L-MeCh	.01 + .01	1066 ± 52
Lobster nerve		per g fresh nerve
D-MeCh	.03	431 ± 2
DL-MeCh	.03	230 ± 7
DL-MeCh + L-MeCh	.03 + .03	161 ± 1
L-MeCh	.03	0 ± 0
ACh	.04	570 ± 45
ACh + L-MeCh	.04 + .04	550 ± 32
Rat serum		per ml serum
ACh	.03	52.8 ± 1.8
ACh + L-MeCh	.03 + .03	49.5 ± 2.8
DL-MeCh	.03	5.9 ± .1

* s.d. $= \sqrt{\frac{\Sigma d^2}{n-1}}$.

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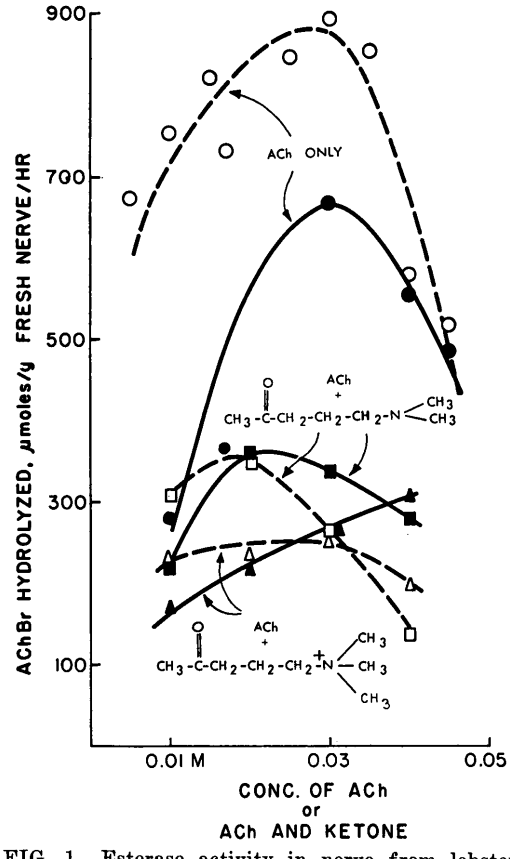

FIG. 1. Esterase activity in nerve from lobster walking leg: —— intact; – – – homogenized.

compounds closely related in structure to ACh, but where hydrolysis is impossible, namely, l-dimethylamino-4-pentanone and its quaternary (methiodide) analogue(8). The results of some experiments using these compounds are shown in Fig. 1. The 2 upper curves, drawn about points some of which were published previously(3) and some of which have been determined anew, show an optimal substrate concentration for lobster nerve around 0.03 M ACh and marked substrate inhibition thereafter. The 4 lower curves were obtained using l-dimethylamino-4-pentanone and its quaternary analogue as indicated. The formulas in Fig. 1 show the structural analogy to ACh. These 2 compounds, both ketones, were present at the same concentration as the substrate. Both compounds are weak inhibitors approximately comparable to choline(9). The extra methyl of the quaternary member contributes little to the binding as has been previously shown(9), and despite their presence the optimal ACh concentration is, again, 0.02-0.03 M. It also appears that at lower concentrations of substrate and inhibitor intact nerves are more inhibited, and at higher concentrations homogenized nerves are more inhibited, suggesting a non-uniform distribution of 2 or more kinds of esterase in the intact nerve.

The observations presented here may have practical implications with respect to the therapeutic use of MeCh and fundamental applications to the characterization of AChE and non-specific esterases and to the further study of the nature of the active site of AChE.

Summary. Acetyl-β-methylcholine is a racemic compound. Acetyl-D-β-methylcholine is hydrolyzed by acetylcholinesterase; the L-isomer is not. Acetyl-L-β-methylcholine is an inhibitor of the hydrolysis of the D-isomer and of acetylcholine by acetylcholinesterase. Therefore the generally available acetyl-β-methylcholine is composed of half substrate and half inhibitor. Two other structurally related compounds are also cholinesterase inhibitors when tested under conditions comparable to those obtaining in the use of acetyl-DL-β-methylcholine.

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