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Effect of tetraethylammonium ions on the affinity and phosphorylation or carbamylation constants of malaoxon, Tetram and Temik with acetylcholinesterase*

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DURING recent studies on the affinity and phosphorylation constants of malaoxon and acetoxon homologs,[1, 2] the findings suggested that the distance between the phosphorus atom and the carbonyl carbon was important for binding and for phosphorylation. When the distance between these two atoms approximated the distance between the esteratic and anionic site of erythrocyte acetylcholinesterase (2·5 to 4·5 Å),[3] both binding and phosphorylation occurred. This raised the question of whether the α-carbonyl carbon of the carbalkoxy phosphorus inhibitors bound at or near the anionic site, and could tetraethyl ammonium ions (TEA)[4, 5] be used to interfere with the affinity or binding of malaoxon.† This study was enlarged to include Tetram,† another organophosphorus inhibitor, and Temik,† an oxime carbamate.

Experimental

Enzyme. Bovine erythrocyte acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) was purchased from the Sigma Chemical Company (St. Louis, Mo.).

Substrate. Acetylcholine chloride (Ach) was obtained from the Sigma Chemical Company. Two substrate concentrations were used throughout this study. Thirty mM Ach plus 2% (by volume) *n*-butanol and 3·0 mM Ach alone were used at higher and lower inhibitor concentrations respectively.

Tetraethylammonium iodide. The quaternary ammonium salt was prepared in this laboratory by reacting an equimolar amount of ethyl iodide with triethylamine in ether. The salt was crystallized from an ethanol–ether solution, m.p. 301–2°.

Inhibitors. Malaoxon* and Tetram were prepared in this laboratory, while Temik was kindly donated by Union Carbide (Clayton, N.C.), m.p. 93–4°.

Measurement of inhibition. The procedure for the determination of the affinity (K_a), the phosphorylation rate (k_2) or the carbamylation rate (k_2) and the bimolecular rate constant (k_i) of the various inhibitors in the absence of TEA was essentially the same technique as that of Main and Iverson.[6] For measuring the effect of TEA in the presence of the inhibitors, 0·3 ml TEA and 0·3 ml of the enzyme solution were placed in one arm of the inhibition vessel and in the other was added 0·3 ml of the appropriate concentration of the inhibitor. The inhibition vessel was placed in a cold bath at 5°. After 10 min of incubation, the inhibitor was tipped into the enzyme–TEA mixture to start the inhibition for the desired time interval. The reaction was halted by flooding of the system with Ach. The same procedure was followed for assaying the residual enzyme activity as that without TEA. The concentrations of TEA were $1{\cdot}0 \times 10^{-2}$ M during the inhibition and $3{\cdot}0 \times 10^{-4}$ M during the substrate reaction. All of the inhibitions were carried out at 5°, pH 7·6, and the residual enzyme activity was measured in 50 ml substrate at 25°, pH 7·6, on a Radiometer pH-stat.

Results and discussion

Table 1 shows the effect that one concentration (1×10^{-2} M) of TEA had on the affinity and k_2 of malaoxon, Tetram and Temik. At least six different concentrations of the three inhibitors were used for the determination of the kinetic constants. The inhibitor concentrations ranged from 2·5 to 1×10^{-3} mM. Under all conditions, the plot of log v versus t was linear; therefore the same kinetics were applicable to determine the various constants, both in the presence and absence of TEA.

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† Tetram, an oxalate salt of Amiton, *O,O*-diethyl S-(2-diethylaminoethyl)phosphorothiolate; Temik, 2-methyl-2-(methylthio)propionaldehyde-*O*-(methyl-carbamoyl)oxime. Malaoxon, *O,O*-dimethyl S-(1,2-dicarbethoxy)ethyl phosphorothiolate.

TABLE 1. EFFECT OF TETRAETHYLAMMONIUM ION ON THE AFFINITY (K_a) AND THE PHOSPHORYLATION OR CARBAMYLATION CONSTANTS (k_2) FOR THE INHIBITION OF ACETYLCHOLINESTERASE WITH TETRAM, MALAOXON AND TEMIK

	Tetram alone	Tetram plus TEA	Malaoxon alone	Malaoxon plus TEA	Temik alone	Temik plus TEA
K_a (mM)	0·18 ± 0·01	0·46 ± 0·05	2·4 ± 0·15	7·8 ± 1·9	5·5 ± 0·6	11·0 ± 1·8
k_2 (min^{-1})	126 ± 6·4	8·0 ± 0·8	67·0 ± 2·6	8·3 ± 1·8	24·0 ± 2·6	17·4 ± 2·8
k_i (M^{-1} min^{-1})	7·1 × 10^5	0·17 × 10^5	28·0 × 10^3	1·1 × 10^3	4·3 × 10^3	1·6 × 10^3

In the presence of TEA, both the affinity and k_2 decreased, resulting in a 25-, 42- and 2·7-fold decrease in the overall inhibitory power (k_i) of malaoxon, Tetram and Temik respectively. The effect of TEA on the phosphorylation rate was of a much greater magnitude than its effect on the affinity. The k_2 of malaoxon in the presence of TEA showed an 8-fold decrease and the affinity was decreased 3·3-fold. The TEA ion affected the phosphorylation rate of Tetram even more than that of malaoxon. The k_2 decreased 16-fold and the k_i diminished about 42-fold. The affinity and carbamylation constants of Temik were affected only slightly. The k_2 was only 1·4-fold lower, while the affinity decreased 2-fold.

The data indicated that the presence of TEA significantly modified both the binding and phosphorylation or carbamylation constants of the three compounds studied. The findings did not clarify whether the α-carbonyl carbon bound at the anionic site, even though the affinity of malaoxon could be altered with TEA ion.

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Hepatic microsomal epoxidase in the cotton rat—Effect of dietary variables

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THE FUNCTIONAL integrity of hepatic microsomal membranes is known to be vital to the drug-metabolizing activities associated with this subcellular fraction.[1] Torula yeast diets can be used to produce a number of the symptoms of deficiency diseases, all having the common characteristic of a breakdown in cellular membrane structure and function. Some of these symptoms can be alleviated with vitamin