In vitro INHIBITION OF SOLUBLE BRAIN ACETYLCHOLINESTERASE BY ORGANOPHOSPHATES OF THE O-ETHYL-S-(2-DIALKYLAMINOETHYL)--METHYLPHOSPHONOTHIOLATE TYPE

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The rate of inhibition of soluble brain acetylcholinesterase from several animal species by four organophosphates of the O-ethyl-S-(2-dialkylaminoethyl)methylphosphonothiolate type was examined *in vitro*. It is shown that the bimolecular rate constant of inhibition depends on the structure of the group leaving from the organophosphate molecule.

Acetylcholinesterase belongs to enzymes playing an important role in the transfer of cholinergic nervous excitation. The enzyme is specifically inhibited by inhibitors of the alkylating type, such as organophosphates, carbamates, and sulfonates[1-5]. The product of this interaction is the phosphonylated, carbamylated, or sulfonylated enzyme. All these interactions involve the formation of a covalent bond between the esteratic site of the active center formed by the hydroxyl group of serine, an atom of phosphorus, carbon, or sulfur[3,7]. The kinetics of the interaction of acetylcholinesterase with organophosphates can be expressed by the following equation[7],

$$E + PX \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EPX \xrightarrow{k_2} EP + X,$$

where E is the enzyme, PX the organophosphates with leaving group X, EPX the reversible complex, EP the covalently phosphorylated enzyme, and k_1, k_{-1}, and k_2 the corresponding rate constants.

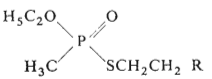
The properties of the phosphorylated enzyme (EP) depend exclusively on the character of the group bound and are independent on the structure of the leaving group (X). The leaving group, however, affects the rate of formation of temporary complex EPX and its conversion into the phosphorylated enzyme (EP) since the leaving groups depending whether they are bulky or not- can prevent the inhibitor molecule from orientation to the active surface of acetylcholinesterase[3,7] or, *vice versa*, facilitate this process.

The rate of inhibition of acetylcholinesterase by four inhibitors differing in the structure of the leaving group has been examined in this study.

EXPERIMENTAL

Material. Brains of white mice and rats, rabbits, guinea-pigs, dogs, calves, and man were used as a source of soluble acetylcholinesterase. The brains of small laboratory animals (rat, mouse, guinea pig, rabbit) and of dog were taken from animals which had been sacrificed by bleeding; calf brain was obtained from slaughtered animals, human brain from bioptic material (bleeding was the cause of death). In all cases the material was not taken later than 10 h after death. The brain tissue was sliced, the remaining blood washed off with physiological saline, and the tissue homogenized with 0·2M Tris-HCl buffer, pH 7·6, containing 1% of Triton (Koch-Light) to a 10% homogenate. The homogenates were centrifuged (60 min, 105 000 *g*, +4°C) and the clear supernatants were used as a source of the enzyme.

The activity of acetylcholinesterase was determined by the method of Ellman and coworkers[8] with 1 mM acetylcholine iodide (Lachema) as substrate and 1 mM 5,5'-dithio-bis-2-nitrobenzoic acid (Serva) as chromogen in 0·2M Tris-HCl buffer, pH 7·6, at 25°C. The change in absorbance at 412 nm was continuously recorded in a Vitatron recorder. Organophosphates which served as inhibitors had the following general formula



where R = NMe_2 (*I*), $^+NMe_3$ (*II*), NEt_2 (*III*), and Ni—Pr_2 (*IV*). The inhibition of the enzyme was continuously checked according to Hart and O'Brien[6] in the presence of acetylthiocholine. The interpolation of lines through the experimental points and the calculation of the rate constants were effected in a ZPA 600 computer.

RESULTS AND DISCUSSION

All the organophosphates examined are strong inhibitors of acetylcholinesterase. The values of the bimolecular rate constant are of the order of $10^5 - 10^6 M^{-1} min^{-1}$ (Table I). The rate of inhibition increases with the increasing size of the substituent on the amino group, both as regards human brain acetylcholinesterase and also rat brain acetylcholinesterase; the quaternarization of nitrogen seems to be without any effect on the inhibition rate.

Compound *IV* is the strongest inhibitor of all compounds examined and its interaction with acetylcholinesterase isolated from all species was studied. The differences in bimolecular rate constants are not great even though statistically significant differences (Student t-test) were found for the following pairs: dog-man, guinea pig-man, guinea pig-rabbit, and guinea pig-calf. It follows from the data obtained[9,10] that differences in the structure of the leaving group can affect the rate of the inhibition. Since the rate of inhibition increases with the increasing volume of the leaving group, this phenomenon cannot be accounted for exclusively by steric hindrances but by the existence of binding van der Waals forces between the methylene groups and

TABLE I

Bimolecular Rate Constants (k_2) of *in vitro* Interaction of Soluble Brain Acetylcholinesterase from Various Species with Various Organophosphates

The results are means of 6—8 determinations; confidence interval (P 95%).

Species	$k_2 . 10^{-6}$, $M^{-1} min^{-1}$			
	I	*II*	*III*	*IV*
Man	0·17 (0·1—0·25)	1·15 (0·81—1·49)	1·28 (0·64—1·9)	1·35 (0·85—1·8)
Calf	—	—	—	1·04 (0·47—1·6)
Dog	—	—	—	0·91 (0·66—1·15)
Rabbit	—	—	—	1·22 (0·84—1·59)
Guinea pig	—	—	—	0·68 (0·45—0·92)
Rat	0·38 (0·23—0·54)	1·06 (0·87—1·24)	1·0 (0·59—1·4)	1·26 (1·05—1·46)
Mouse	—	—	—	0·99 (0·57—1·41)

the hydrophobic site of the active center whose existence on the active surface of acetylcholinesterase has been demonstrated several times[11-14].

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