PHARMACOLOGY

THE IMPORTANCE OF THE POSITION OF THE QUATERNARY NITROGEN ATOM IN THE MOLECULE OF CERTAIN COMPOUNDS TO THEIR ABILITY TO REACT WITH TRUE CHOLINESTERASE AND PSEUDOCHOLINESTERASE

N. K. Fruentov

From the Laboratory of the Pharmacology and Biochemistry of Biologically Active Compounds (Head – Prof. M. Ya. Mikhel'son) of the I. M. Sechenov Institute of Evolutionary Physiology (Director – Academician the late L. A. Orbeli) of the AN SSSR, Leningrad

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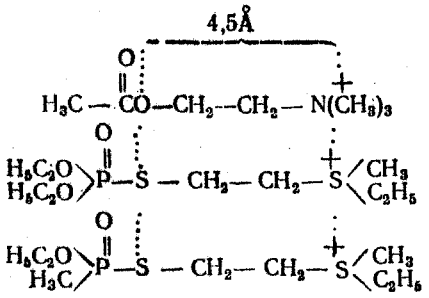
The development of a free positive charge on the nitrogen atom during the alkylation of the tertiary amines usually leads to enhancement of the biological action of the cholinolytic, cholinomimetic and anticholinesterase compounds (for the literature on this subject, see [1, 2]). The development of a positive charge on the sulfur or nitrogen atom enhances the anticholinesterase action of certain organic phorphorus compounds [3, 4, 5, 6, 8, 9]. If the structure of these compounds is compared with the structure of certain other compounds affecting the function of cholinergic structures, attention is drawn to the fact that the distance between the central atom of the cationic part of the molecule and its ester oxygen (sulfur) atom is identical both in the cholinesterase inhibitors under study and in acetylcholine.



Acetylcholine

Isosystox methylate

Preparation Gd-42*

In acetylcholine this distance (~ 4.5 Å)** must evidently correspond to the distance between the anionic and esterase points of the active center of the cholinesterase [10]. The strengthening of the biological activity of organic phosphorus compounds after the development of a charge in the molecule must be based on the acquisition by the compounds of the ability to become rapidly orientated on the surface of the cholinesterase. The

*Isosystox methylate was synthesized by T. A. Mastryukova, and preparation Gd-42 by N. N. Godovikova, in M. I. Kabachnik's laboratory (INEOS AN SSSR).

**Here and subsequently the distances (4.5Å and 6 Å) are given as the sums of the interatomic distances.

TABLE 1

Rate of Hydrolysis of Certain Tertiary Amines and Their Methiodides with Pseudocholinesterase

No.	Structure of preparation*	R	Distance R—O (in A)	Rate of hydrolysis of prep. with 1 mlot enzyme (in mM/hr)	Change in rate after acquisition of charge
1	$R-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-C(CH_3)_2\cdot C_6H_5$	$-N(CH_3)_2\cdot HCl$		$1\cdot10^{-3}$	
			4.5		×4.2
2		$-\overset{+}{N}(CH_3)_3\cdot\overset{-}{I}$		$4.2\cdot10^{-2}$	
3		$-N(C_2H_5)_2\cdot HCl$		$8.5\cdot10^{-3}$	
			4.5		×3.8
4		$-\overset{+}{N}(C_2H_5)_2CH_3\cdot\overset{-}{I}$		$3.2\cdot10^{-2}$	
5	$R-CH_2-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-C(CH_3)_2\cdot C_6H_5$	$-N(CH_3)_2\cdot HCl$		$9.5\cdot10^{-4}$	
			6.0		No hydrolysis occurs
6		$-\overset{+}{N}(CH_3)_3\cdot\overset{-}{I}$		0	
7		$-N(C_2H_5)_2\cdot HCl$		$9\cdot10^{-4}$	
			6.0		The same
8		$-\overset{+}{N}(C_2H_5)_2CH_3\cdot\overset{-}{I}$		0	

* The preparations were synthesized by G. T. Tatevosyan and S. Agbalyan in the Insitute of Fine Organic Chemistry of the AN Armenian SSR, Erevan (Director of the Institute: A. L. Mndzhoyan).

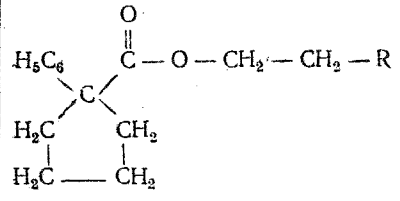
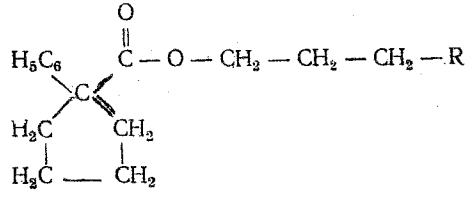
positively charged atom of the molecule is, as it were, anchored to the anionic point of the active center of the enzyme, and begins to revolve in a narrow orbit. The probability of collision between the phosphorylating group of the inhibitor and the esterase point of the active center of the cholinesterase, necessary for its phosphorylation, thus becomes increased.

If this hypothesis is correct, the strengthening of the power to react with cholinesterases by the development of a positive charge in the molecule of the inhibitor will probably depend on the distance from the atom of oxygen or sulfur) forming the ester bond to that in which the charge appeared. If this distance corresponds to the distance between the anionic and esterase points of the active center of the cholinesterase, maximum increase in activity may be expected.

TABLE 2

Anticholinesterase Action of Certain Tertiary Amines and Their Alkylates

No.	Structure of preparation*	R	Distance R⟶O (in A)	Cholinesterase			
				pseudo-		true	
				I_{50}(M)	change in action after alkylation	I_{50} (M)	change in action after alkylation
9	$(C_6H_5)_2CH-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-R$	$-N(C_2H_5)_2\cdot HCl$	4.5	$1\cdot10^{-4}$	× 48	$2\cdot10^{-3}$	1
10		$-\overset{+}{N}(C_2H_5)_3\cdot\bar{I}$		$2.1\cdot10^{-6}$		$1.9\cdot10^{-3}$	
11	$(C_6H_5)_2CH-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-CH_2-R$	$-N(C_2H_5)_2\cdot HCl$	6.0	$8.2\cdot10^{-6}$	: 1.6	$2.2\cdot10^{-3}$	: 5.9
12		$-\overset{+}{N}(C_2H_5)_2CH_3\cdot\bar{O}SO_2OCH_3$		$1.3\cdot10^{-5}$		$1.3\cdot10^{-2}$	
13	$C_6H_5(nC_3H_7)_2C-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-R$	$-N(CH_3)_2\cdot HCl$	4.5	$9.2\cdot10^{-4}$	× 115	$1.8\cdot10^{-3}$	× 3.9
14		$-\overset{+}{N}(CH_3)_3\cdot\bar{I}$		$8\cdot10^{-6}$		$4.6\cdot10^{-4}$	
15	$C_6H_5(nC_3H_7)_2C-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-CH_2-R$	—	6.0	$5.9\cdot10^{-4}$	: 1.3	$2.3\cdot10^{-3}$	: 1.5
16		$-\overset{+}{N}(CH_3)_3\cdot\bar{I}$		$7.4\cdot10^{-4}$		$3.4\cdot10^{-3}$	
17	$\begin{matrix}CH_3\\\|\\CH-CH_2-O-C_6H_4-\overset{O}{\overset{\|}{C}}-O-\overset{CH_3}{\underset{\|}{CH}}-\overset{CH_3}{\underset{\|}{CH}}-CH_2-R\\\|\\CH_3\end{matrix}$	$-N(C_2H_5)_2\cdot HCl$	6.0	$1.9\cdot10^{-5}$	1	$2.4\cdot10^{-4}$	: 3.4
18		$-\overset{+}{N}(C_2H_5)_2CH_3\cdot\bar{I}$		$1.9\cdot10^{-5}$		$8.4\cdot10^{-4}$	

19		—N $(C_2H_5)_2 \cdot HCl$	4.5	$1.2 \cdot 10^{-4}$	× 67	$2.5 \cdot 10^{-3}$	× 11
20		$-\overset{+}{N}(C_2H_5)_2\,CH_3 \cdot \overset{-}{S}O_4CH_3$		$1.8 \cdot 10^{-6}$		$2.2 \cdot 10^{-4}$	
21		—N $(C_2H_5)_2 \cdot HCl$	6.0	$1.5 \cdot 10^{-6}$	: 13.3	$2.4 \cdot 10^{-3}$	× 1.6
22		$-\overset{+}{N}(C_2H_5)_2\,CH_3 \cdot \overset{-}{S}O_4CH_3$		$2 \cdot 10^{-5}$		$1.5 \cdot 10^{-3}$	

*Synthesis of the preparations was as follows: No. 10 by N. I. Kudryashova (IEM, Leningrad); Nos. 11 and 12 by A. L. Mndzhoyan and O. L. Mndzhoyan; Nos. 13, 14, 15 and 16 by G. T. Tatevosyan and S. Agbadyan; Nos. 17 and 18 by V. D. Afrikyan and M. Grigoryan (Institute of Fine Organic Chemistry AN Armenian SSR, Erevan); No. 20 by N. V. Khromov-Borisov and A. M. Yanovitskaya (First Leningrad Medical Institute).

We had at our disposal a group of pseudocholinesterase substrates and a group of selective inhibitors of the enzyme, containing a nitrogen atom in their molecules, and we tried to use these preparations to show how the development of a positive charge at different distances from the oxygen atom forming the ester bond affected the power of the compounds to react with the enzyme: at a distance of about 4.5 Å in esters of alkylaminoethanol and at a distance of about 6 Å in esters of alkylaminopropanol.

Experiments with cholinesterase substrates. We investigated the rate of enzymic hydrolysis of 8 phenyldimethylacetic esters of choline, homocholine, and their diethylamine analogues with a purified and 40 times concentrated pseudocholinesterase of horse serum (Table 1). The determinations were made by an indicator method, namely by the rate of decolorization of phenol red by phenyldimethylacetic acid, given off in the process of hydrolysis of the compounds. Each sample was made up as follows: 0.2 ml of an aqueous solution of the cholinesterase preparation; 2.7 ml of bicarbonate buffer (pH = 7.9); 0.1 ml of a 0.025% solution of phenyl red in a borate buffer (pH = 8.0); 1 ml of a solution of the test preparation in a concentration of $1 : 10^{-2}$ M. The reactions were conducted at a temperature of 19-20°, in the chamber of a photoelectric colorimeter type FÉK-M, with a green color filter transmitting the band 5200-5400 Å (chamber 5.060 mm). Records were made of the changes in extinction (ΔE) in unit time t. Observations were continued until 7-10 reliable points had been obtained in a rectangular system of coordinates (ordinate — E, abscissa — time of experiment).

In order to determine the relationship between ΔE and the amount of phenyldimethylacetic acid (i.e., the amount of preparation decomposed), we performed two experiments in which the rate of hydrolysis of the compounds was determined concurrently by potentiometric titration and by the indicator method. This enabled the conversion coefficient for obtaining the amount of substrate hydrolyzed in mM/t from the value of $\Delta E/t$ to be determined.

It will be seen from Table 1, in which the results of the experiments are given, that methylation of the nitrogen atom situated at a distance of ~ 4.5 Å from the oxygen atom of the ester bond led in both cases to an approximately fourfold increase in the rate of hydrolysis. Conversely, in the two cases when the distance from the nitrogen atom to the oxygen atom of the ester bond was increased ~ 6 Å, methylation led to a complete cessation of detectable hydrolysis of the preparations. Hydrolysis of the preparations also ceased as the result of the addition of the cholinesterase inhibitors proserine and phosphacol to the test sample. This is evidence in favor of hydrolysis of the preparations being effected by cholinesterase itself.

Experiments with cholinesterase inhibitors. We determined the molar concentrations of a number of compounds (Table 2) which, in vitro, caused inhibition of the activity of true cholinesterase and pseudocholinesterase to the extent of 50% (I_{50}). Experiments were carried out by Hestrin's method [7]. The composition of each test sample was as follows: 1 ml of a 1.10^{-3} aqueous solution of acetylcholine chloride, 1 ml of the source of cholinesterase, 1 ml of an aqueous solution of the test compound and 1 ml of distilled water. The reaction of hydrolysis of acetylcholine was conducted at a temperature of 38 ± 0.25°. The enzymic hydrolysis of acetylcholine was terminated at the conclusion of the given exposure period (usually 120 seconds) by pouring 1 ml of a 15-20% solution of trichloroacetic acid into the flask.

As a source of true cholinesterase we used defibrinated ox blood, and as a source of pseudocholinesterase — horse serum. The concentration of acethydroxamic acid formed as a result of Hestrin's reaction was estimated in the FÉK-M photoelectric colorimeter with a 5.060 mm chamber, using a green color filter transmitting a waveband of 5200-5400 Å.

Table 2, which gives the results of this series of experiments, shows that all the preparations investigated were more effective as inhibitors of pseudocholinesterase and showed some variable degree of selectivity. The most informative results were therefore those obtained with this enzyme, especially as the action of the preparations on the two cholinesterases was affected differently by alkylation. In all cases when the distance between the nitrogen atom and the oxygen atom of the ester bond was 4.5 Å, alkylation led to enhancement of the power of the preparations to inhibit cholinesterase. If this distance increased to 6 Å, then after alkylation of the nitrogen atom, the anticholinesterase action, on the contrary, was slightly weakened or, in any case, remained unchanged.

The exception to this was difacil (trasentin), in which, after alkylation, the inhibitory action on true cholinesterase showed practically no increase (see Table 2, Nos. 9 and 10). In this connection it is important to mention that all the compounds studied inhibited true cholinesterase only very weakly and that their action in this enzyme was generally speaking little affected as a result of alkylation.

In the course of the work we checked the rate of hydrolysis of the preparations by the cholinesterases by means of the indicator method. The rate of their enzymic decomposition in our experimental conditions was very low and could not appreciably affect the results of the investigation of the anticholinesterase action.

The results obtained may be understood on the basis of the hypothesis that the distance between the anionic and esterase points of the active center in the molecules of the cholinesterases corresponds to the distance between the nitrogen atom carrying the positive charge and the oxygen atom of the ester bond in the acetylcholine molecule. The ability of the compounds to react with the cholinesterases may, therefore, be increased by the attachment of a positive charge to the molecule only when this charge is present in the molecule at an adequate distance from the oxygen atom of the ester bond.

SUMMARY

It thus follows that, in order to increase the power of cholinesterase substrates and inhibitors to react with these enzymes, great importance is attached not only to the development of a positive charge in the molecule of the compound, but also to the distance between the atom carrying the charge and the atom forming the ester bond in the molecule. In the group of esters of some organic acids and alkylaminoalcohols which were investigated, the appearance of the charge intensified this interaction only when the oxygen atom of the bond was at the same distance from the positively charged nitrogen atom as in the acetycholine molecule. With increase of this distance the appearance of the charge weakened the ability of the majority of the substances to react.

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