INHIBITION OF ACETYLCHOLINESTERASE BY BISQUATERNARY AMMONIUM SALTS CONTAINING HYDROPHOBIC RADICALS

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The introduction of hydrophobic adamantyl radicals into depolarizing myorelaxants normally changes their mode of action, converting them into nondepolarizing agents [7, 8]. This effect is clearly due to additional hydrophobic interactions of the compounds with the cholinoreceptors. In view of the general structural features in the structures of the cholinoreceptors and cholinesterases, it was of interest to establish the role of hydrophobic factors in the interactions of cholinotropic compounds with cholinesterases. At the same time, using hydrophobic radicals and varying their structure and position in the molecule, definite information on the topography and the role of hydrophobic zones in cholinesterases could be obtained.

The significance of hydrophobic interactions was examined using two series of compounds: 1) α,ω-bis-[N-methyl-N-(1-adamantyl)amino]alkane dimethiodides with a polymethylene chain length of from 4 to 11 units (Table 1), and 2) α,ω-bis-[N-methyl-N-(alkyl)amino]alkane dimethiodides, in which the alkyl radicals differed in hydrophobicity, the length of the methylene chain being from eight to ten units (Table 2).

Examination of anticholinesterase activity showed that the compounds inhibited cholinesterase (ACE) almost instantaneously, the magnitude of the effect being independent of the time of incubation with the enzyme. Consequently, these bisquaternary ammonium salts are reversible inhibitors of ACE.

None of the compounds tested had any effect on the Michaelis constant (K_m), the only effect of their presence being a reduction in the maximum hydrolysis rate of acetylcholine (V_{max}) on treatment with ACE, i.e., they behave as formally noncompetitive inhibitors of ACE. The effectiveness of inhibition (V_0/V_i) by bisquaternary ammonium salts is independent of the concentration of the substrate, and the inhibition constant (K_i) was calculated from the slope of the plot of V_0/V_i versus (I), using the equation $V_0/V_i = 1 + (I)/K_i$. In this case, the concentration of inhibitor inhibiting the rate of the enzymic reaction by 50% is numerically equal to the noncompetitive inhibition constant, i.e., $I_{50} = K_i$. In examining

TABLE 1. Acetylcholinesterase Activity of Bisquaternary Ammonium Salts $1\text{-Ad}(CH_3)_2\overset{+}{N}(CH_2)_n\overset{+}{N}(CH_3)_2\text{Ad-}1\cdot 2\bar{X}$

Compound	n	X	Range of inhibitor concentrations examined, M	$K_i - I_{50}^{**}$, M	Curariform activity of compound***
I	4	Br	$3{,}9\cdot10^{-6}$—$2{,}33\cdot10^{-4}$	$4{,}8\cdot10^{-5}$	
II [4]	5	I	$1{,}725\cdot10^{-4}$—$6{,}45\cdot10^{-4}$	$8{,}1\cdot10^{-5}$	0,8—1,2
III [4]	6	I	$7\cdot10^{-6}$—$1{,}89\cdot10^{-5}$	$9{,}5\cdot10^{-6}$	0,12—0,18
IV [4]	7	I	$1{,}04\cdot10^{-6}$—$1{,}59\cdot10^{-5}$	$1{,}85\cdot10^{-6}$	0,2—0,25
V [5]	8	I	$2{,}93\cdot10^{-7}$—$1{,}46\cdot10^{-6}$	$6{,}5\cdot10^{-7}$	0,09—0,12
VI* [2]	10	I	$2{,}6\cdot10^{-7}$—$1\cdot10^{-6}$	$5{,}7\cdot10^{-7}$	0,25—0,3
VII [2]	11	I	$3{,}06\cdot10^{-7}$—$1{,}25\cdot10^{-6}$	$5{,}9\cdot10^{-7}$	0,45—0,55

*Compound (VI) was decadonium (here and in Table 2).
**The I_{50} for proserine was $5.95\cdot10^{-8}$ M (here and in Table 2).
***Doses in mg/kg at which the compounds given intravenously cause blockage of neuromuscular transmission in cats.

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TABLE 2. Anticholinesterase Activity of Polymethylene Bisquaternary Alkylammonium Salts $(CH_3)_2\overset{+}{N}(CH_2)_n\overset{+}{N}(CH_3)_2 \cdot 2X^-$ (R, R)

Compound	n	R	X	Range of inhibitor concentrations examined, M	$K_i = J_{50}M$
VIII	8	Cyclohexyl	I	$8{,}45 \cdot 10^{-7}$—$4{,}01 \cdot 10^{-6}$	$3{,}9 \cdot 10^{-7}$
IX	8	Phenyl	I	$3{,}17 \cdot 10^{-7}$—$2 \cdot 10^{-6}$	$4{,}4 \cdot 10^{-6}$
V [5]	8	1-Ad—	I	$2{,}93 \cdot 10^{-7}$—$1{,}46 \cdot 10^{-6}$	$6{,}5 \cdot 10^{-7}$
X [1]	10	Cyclohexyl	I	$1{,}8 \cdot 10^{-6}$—$6 \cdot 10^{-7}$	$2{,}5 \cdot 10^{-7}$
XI	10	Phenyl	I	$2{,}6 \cdot 10^{-7}$—$1{,}03 \cdot 10^{-6}$	$1{,}5 \cdot 10^{-7}$
VI [2]	10	1-Ad—	I	$2{,}6 \cdot 10^{-7}$—$1 \cdot 10^{-6}$	$5{,}7 \cdot 10^{-7}$
XII [2]	10	$C_{10}H_{21}$	*	$1{,}5 \cdot 10^{-6}$—$8 \cdot 10^{-5}$	$2{,}3 \cdot 10^{-6}$

*X = CH_3OSO_3.

the effects of the structure of the compounds on their anticholinesterase activity, as shown by the k_i values, it was found that for the series of bisquaternary ammonium ions the relationship $pK_i = -\log K_i$ from the sum of the constants of the methylene groups between the quaternary nitrogen atoms is described by the equation: $pK_i = pK_i^0 + \varphi\pi_{CH_2 \cdot n}$ for values of n = 4-8, $\varphi = 1.1 + 0.2$ and $pK_i = \text{const}$.

The slope, equal to unity, shows that the interaction of polymethylene bisquaternary alkylammonium inhibitors with cholinesterase is accompanied by total transfer of the inhibitor into the enzyme phase, and is described by a model of the distribution of the compounds in the octanol—water system, on the basis of which Hansch et al. [9] constructed a scale of π-constants of hydrophobicity. The value of the bonding free energy increment $\Delta\Delta F = 2.3 RT\Delta \log K_i$ is in this case 0.7 kcal/mole for each CH_2 group. These results are in accordance with the hypothesis developed from a study of organophosphorus inhibitors, according to which the hydrophobic environment of the anionic site of the active center of ACE includes a "hydrophobic crown" and a binding region eight polymethylene groups long [3]. Further lengthening of the polymethylene chain in bisquaternary alkylammonium salts from n = 8 to n = 10 or 11 has no effect on the anticholinesterase activity of the compounds, apparently as a result of the limited extensibility of the sorptive region of the enzyme. In addition to the length of the polymethylene chain in bisquaternary alkylammonium salts, the volume and hydrophobicity of the radicals screening the quaternary ammonium nitrogens also have an effect on anticholinesterase activity. Changing from 1-adamantyl derivatives [(V) and (VI), Table 1] to the corresponding compounds containing the less steric phenyl [(IX) and (XI), Table 2] or cyclohexyl [(VIII) and (X), Table 2] radicals results in a change in anticholinesterase activity. For example, (VIII) inhibits ACE by a factor of 1.7, and (X) 2.3 times more than the corresponding 1-adamantyl analogs, and the cyclohexyl analog of decadonium (XI) was more active than the latter by a factor of 3.8. However, the cyclohexyl analog of (V) is less active by a factor of 1.6. Replacement of the 1-adamantyl radical in (VI) by n-decyl, which contains the same number of carbon atoms, results in fourfold decrease in activity, indicating the importance not only of the overall hydrophobicity of the radicals at the cationic center, but also their configuration, which in the case of the 1-adamantyl radicals is highly compact.

EXPERIMENTAL (CHEMISTRY)

Literature citations for methods of preparation of previously examined compounds together with their properties are given in Tables 1 and 2.

Bis-[N-methyl-N-(1-adamantyl)amino]butane Dimethobromide (I). A mixture of 1.8 g (0.01 mole) of N,N-dimethylaminoadamantane, 1.1 g (0.005 mole) of 1,4-dibromobutane, and 20 ml of benzene was heated for 12 h on a boiling water bath. The crystals which separated on cooling were filtered off, and washed with benzene and chilled alcohol to give 2.9 g (72%) of (I), mp 283-285°C (from alcohol, decomp.). Found, %: Br 26.91. $C_{28}H_{50}Br_2N_2$. Calculated, %: Br 27.37.

Bis-(N-methyl-N-phenylamino)octane Dimethiodide (IX). A mixture of 1.21 g (0.01 mole) of N,N-dimethylaniline, 1.83 g (0.005 mole) of 1,8-diiodooctane, and 10 ml of alcohol was heated for 5 h on a boiling water bath. A crystalline solid separated on cooling and stand-

ing, yield 2.3 g (75.6%), mp 195-198°C (decomp.). Found, %: I 41.38, N 4.52. $C_{24}H_{38}I_2N_2$. Calculated, %: I 41.30, N 4.60.

Bis-(N-methyl-N-phenylamino)decane Dimethiodide (X). Obtained similarly, yield 78%, mp 152-153°C (decomp.). Found, %: I 40.07, N 4.41. $C_{26}H_{42}I_2N_2$. Calculated, %: I 39.87, N 4.40.

Bis-(N-methyl-N-cyclohexylamino)octane Dimethiodide (XI). Obtained similarly by reacting N,N-dimethylaminocyclohexane with 1,8-diiodooctane. Yield 72%, mp 242-245°C (decomp.). Found, %: I 40.68, N 4.48. $C_{24}H_{50}I_2N_2$. Calculated, %: I 40.91, N 4.51.

EXPERIMENTAL (PHARMACOLOGY)

The enzyme preparation used was a freeze-dried ACE (EC 3:1:1:7) from human blood erythrocytes, specific activity 0.5 units/mg. The concentration of ACE in the working solution was $7.4 \cdot 10^{-4}$ mg/ml, and the substrate (acetylcholine chloride) concentration from $4 \cdot 10^{-5}$ to $6.5 \cdot 10^{-4}$ M. The inhibitors used were aqueous solutions of the bisquaternary alkylammonium salts. The rate of the enzymic reaction was measured by potentiometric titration at 38°C in 0.15 M sodium chloride in the presence of $8 \cdot 10^{-4}$ M phosphate buffer (pH 7.4). The values of the maximum rate of hydrolysis (V_{max}), the Michealis constant (K_M), and the inhibition constants (K_i) were obtained graphically by the Lineweaver–Burk method [6] before and after introduction of the inhibitors into the reaction mixture. Proserine (neostygmine) was used as a standard, the anticholinesterase activity being taken as the concentration of the inhibitor causing the residual activity of the ACE to be reduced by 50% on reaching the stationary state. For this purpose, to 1 ml of thermostated neostigmine solution of known concentration was added 0.2 ml of a solution of the enzyme ([E] = 10 mg/ml). At various time intervals, 0.2 ml samples were withdrawn from the mixture into a thermostated cell containing 15 ml of $5.7 \cdot 10^{-4}$ M aqueous acetylcholine solution containing 0.15 M sodium chloride (75-fold dilution of the reaction mixture). The residual activity of the ACE in the sample was calculated from the initial rate of enzymic hydrolysis of the acetylcholine, as determined by titration of the acid liberated with 0.02 M NaOH. The concentration of neostygmine causing 50% inhibition of the ACE activity in the stationary state (K_i) was then determined graphically.

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