ANTICHOLINESTERASE ACTIVITY OF MONOQUATERNARY AMMONIUM SALTS CONTAINING HYDROPHOBIC RADICALS

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The introduction of large hydrophobic radicals into the structures of cholinotropic compounds can have a substantial effect on their interactions with the corresponding choline receptors and change both their activities and the mechanism by which they act. Thus, the introduction of adamantyl radicals into a cholinomimetic series of mono- and bis-quaternary ammonium salts naturally transforms them into choline blockers — nondepolarizing myorelaxants [7].

In connection with the existence of common features in the structures of the active sites of choline receptors and acetylcholinesterase (ACE), there is considerable interest in elucidating how the hydrophobicity of the radicals attached to the quaternary nitrogen atom affects the anticholinestaerase activity (for a discussion of the significance of the structures of cationic sites to anticholinesterase activity see [9]).

First of all, we studied a series of tetramethylammonium derivatives in which one of the methyl groups was replaced by cyclic radicals having different degrees of hydrophobicity (R = cyclohexyl, phenyl, 1- or 2-adamantyl). For comparison, the anticholinesterase activities of salts of the corresponding amines $RNH_2 \cdot HCl$ were determined.

All of the studied ammonium salts are typical reversible inhibitors since the inhibition of ACE by these compounds does not depend on the time of incubation with the enzyme and occurs at once following the addition of inhibitor to an enzyme-substrate system.

In Table 1 data which were obtained by studying the effects of ammonium salts on the rates of hydrolysis of acetylcholine by ACE are presented. The majority of the compounds studied (I-VI, VII-XII) did not affect the maximum rate of hydrolysis (V_{max}) of acetylcholine by ACE, and the sole effect observed in their presence was an increase of K_M (Michaelis constant), which indicates that these compounds are classical competitive-type inhibitors. On the other hand, compounds XV and XVI reduce the maximum rate of hydrolysis of acetylcholine but do not affect K_M, i.e., these behave as classical noncompetitive inhibitors. The inhibition of acetylcholine hydrolysis by compounds VII and XIV is accompanied by an increase in K_M and a reduction of V_{max}, which is typical of mixed-type inhibition. As characteristies of the reactivities of reversible inhibitors we have presented the values of the inhibition constants K_i, which are indicative of the binding strength between the inhibitor and the enzyme.

It follows from Table 1 that the anticholinesterase activity is influenced by the volume and hydrophobicity (which varies in a parallel fashion with the number of carbon atoms) of the carbohydrate radicals attached to the quaternary nitrogen atom. Replacement of one of the methyl groups in the tetramethylammonium salt by a cyclohexyl group (compound IV) raises the hydrophobicity of the compound to the level of that of the tetraethylammonium salt II and thus results in the two compounds having comparable inhibition constants. It is noteworthy that the phenyltrimethylammonium salt VI has greater anticholinesterase activity than salt IV (the inhibition constant is a factor of 7 lower) in spite of the fact that the hydrophobicities of the two compounds are close to one another. This may be the result of additional ionic interactions due to partial charges arising in the substituted phenyl ring. Further increases in the hydrophobicities of the substituted ammonium salts studied brought about by introduction of the bornyl radical (compound IX) or, having approximately the same hydrophobicity, the 1- or 2-adamantyl radicals (compounds XI or XIII) did not substantially affect the anticholinesterase activity, possibly due to certain features of these compounds'

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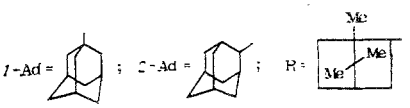
TABLE 1. Anticholinesterase Activities of N-Substituted Ammonium Salts Containing Hydrophobic Radicals

Alkylammonium salt**	Salt concentration, M	K_i, M	Type of inhibition
$Me_4\overset{+}{N}\cdot Cl^-$ (I)	$6{,}4\cdot10^{-4}$—$1{,}83\cdot10^{-3}$	$1\cdot10^{-3}$	Competitive
$Et_4\overset{+}{N}\cdot I^-$ (II)	$2{,}81\cdot10^{-4}$—$1{,}636\cdot10^{-3}$	$5.8\cdot10^{-4}$	»
Cyclo-$C_6H_{11}\overset{+}{N}H_2\cdot HCl$ (III)	$1{,}07\cdot10^{-3}$—$4{,}58\cdot10^{-3}$	$1.9\cdot10^{-3}$	»
Cyclo-$C_6H_{11}\overset{+}{N}Me_3\cdot I^-$ (IV) [10]	$1{,}51\cdot10^{-4}$—$1{,}285\cdot10^{-3}$	$6.6\cdot10^{-4}$	»
$PhNH_2\cdot HCl$ (V)	$2{,}95\cdot10^{-3}$—$1{,}325\cdot10^{-2}$	$1{,}07\cdot10^{-2}$	»
$Ph\overset{+}{N}Me_3\cdot I^-$ (VI)	$5{,}94\cdot10^{-5}$—$2{,}97\cdot10^{-4}$	$9.5\cdot10^{-5}$	»
$Ph\overset{+}{N}Et_3\cdot I^-$ (VII)	$4{,}13\cdot10^{-5}$—$1{,}59\cdot10^{-4}$	Competitive	Mixed
		$6{,}125\cdot10^{-5}$, Noncompetitive	
$RNH_2\cdot HCl$ (VIII) [11]	$2{,}64\cdot10^{-4}$—$2{,}107\cdot10^{-3}$	6.74 ± 10^{-4} $4\cdot10^{-4}$	Competitive
$R\overset{+}{N}Me_3\cdot I^-$ (IX) [11]	$1{,}56\cdot10^{-4}$—$5{,}51\cdot10^{-4}$	$2.5\cdot10^{-4}$	»
1-$Ad\overset{+}{N}H_2\cdot HCl$ (X) [2]	$1{,}10\cdot10^{-3}$—$5{,}52\cdot10^{-3}$	$2.6\cdot10^{-3}$	»
1-$Ad\overset{+}{N}Me_3\cdot I^-$ (XI) [5]	$4{,}5\cdot10^{-4}$—$1{,}22\cdot10^{-3}$	$6{,}4\cdot10^{-4}$	»
2-$Ad\overset{+}{N}H_2\cdot HCl$ (XII) [6]	$2{,}95\cdot10^{-3}$—$8{,}86\cdot10^{-3}$	$3{,}5\cdot10^{-3}$	»
2-$Ad\overset{+}{N}Me_3\cdot MeC_6H_4SO_3^-$ (XIII)	$1{,}45\cdot10^{-4}$—$1{,}307\cdot10^{-3}$	$6.8\cdot10^{-4}$	»
2-$Ad\overset{+}{N}Me_2Et\cdot MeC_6H_4SO_3^-$ (XIV)	$1{,}5\cdot10^{-4}$—$9{,}91\cdot10^{-4}$	Competitive	
		$9.3\cdot10^{-4}$, Noncompetitive	Mixed
		$2.95\cdot10^{-3}$	
2-$Ad\overset{+}{N}MeEt_2\cdot MeC_6H_4SO_3^-$ (XV)	$3{,}12\cdot10^{-4}$—$1{,}28\cdot10^{-3}$	$8{,}7\cdot10^{-4}$	Noncompetitive
2-$Ad\overset{+}{N}Et_3\cdot MeC_6H_4SO_3^-$ (XVI)	$4{,}07\cdot10^{-4}$—$1{,}88\cdot10^{-3}$	$1{,}2\cdot10^{-3}$	»

Note. Human erythrocyte ACE, 0.15 M NaCl, pH 7.4, 38°C, substrate — acetylcholine iodide.

stereoconfigurations. Within the series of ammonium salts containing the adamantyl radicals, one may note that the 1-Ad and 2-Ad derivatives XI and XIII are practically the same with respect to their anticholinesterase activities, i.e., the mode of attachment of the adamantyl radical to the ammonium nitrogen does not affect the value of K_i. Furthermore, upon increasing the total hydrophobicity of the adamantyltrialkylammonium salts by successive substitution of ethyl groups for methyl groups (compounds XIII-XVI) the anticholinesterase activity decreases and the type of inhibition observed changes from competitive (XIII) to mixed-type (XIV) to noncompetitive (XV and XVI). The reduction of anticholinesterase activity is evidently connected with the increase of the volume of the inhibitor molecule, which becomes noncomplimentary with the active surface of the enzyme.

The inhibition constants of all the quaternary salts examined were lower than those of the corresponding nonalkylated primary amines. This may be due to the stronger binding of the completely ionized quaternary salt during its interaction with the anionic sites of ACE.



There is also interest in explaining how the interactions of ACE with acetylcholine and its analogs are changed upon the introduction of the adamantyl radical into their structures. Earlier [4] we studied a series of acetylcholine derivatives and analogs containing adamantyl groups in various portions of the molecule. It was shown that, in contrast to acetylcholine, all of them are choline blockers.

Experiments indicated that the compounds examined were not hydrolyzed by human erythrocyte ACE at enzyme concentrations up to 0.15 mg/ml and also that the reversible inhibitors of ACE were of the classical competitive variety for the majority of compounds (XX-XXV). In connection with this the influence of the aminoester on the anticholinesterase activity was studied. The structural changes were implemented at the aminoalcohol and acidic portions of the aminoester molecules (Table 2).

TABLE 2. Anticholinesterase Activity of Acetylcholine Analogs Containing Hydrophobic Radicals

Structures of the aminoester salts	Ester concentration	K_i, M	Type of inhibition
$MeCOOCH_2CH_2\overset{+}{N}Me_2Ad\text{-}1\cdot MeC_6H_4SO_3^-$ (XVII) [4]	$3{,}17\cdot10^{-4}$—$1{,}59\cdot10^{-3}$	$2{,}45\cdot10^{-3}$	Noncompetitive
$MeCOOCH_2CH_2\overset{+}{N}Me_2CH_2Ad\text{-}1\cdot I^-$ (XVIII) [4]	$3{,}5\cdot10^{-4}$—$1\cdot10^{-3}$	$1{,}8\cdot10^{-3}$	»
$MeCOOCH(Ad\text{-}1)CH_2\overset{+}{N}Me_3\cdot I^-$ (XIX) [4]	$2{,}41\cdot10^{-4}$—$9{,}66\cdot10^{-4}$	Competitive $3{,}9\cdot10^{-4}$, Noncompetitive $2{,}25\cdot10^{-3}$	Mixed
$1\text{-}AdCOOCH_2CH_2\overset{+}{N}Me_3\cdot I^-$ (XX) [4]	$3{,}68\cdot10^{-5}$—$1{,}84\cdot10^{-4}$	$7\cdot10^{-5}$	Competitive
$1\text{-}AdCH_2COOCH_2CH_2\overset{+}{N}Me_3\cdot I^-$ (XXI) [4]	$8{,}72\cdot10^{-5}$—$6{,}07\cdot10^{-4}$	$2{,}95\cdot10^{-4}$	»
$1\text{-}AdCOOCH_2CH_2CH_2\overset{+}{N}Me_3\cdot I^-$ (XXII) [4]	$1{,}14\cdot10^{-4}$—$6{,}06\cdot10^{-4}$	$3{,}7\cdot10^{-4}$	»
$1\text{-}AdCOOCH_2CH_2CH_2CH_2\overset{+}{N}Me_3\cdot I^-$ (XXIII) [1]	$8{,}7\cdot10^{-5}$—$4{,}32\cdot10^{-4}$	$2{,}2\cdot10^{-4}$	»
$Cyclo\text{-}C_6H_{11}COOCH_2CH_2CH_2CH_2\overset{+}{N}Me_3\cdot I^-$ (XXIV) [1]	$1{,}41\cdot10^{-4}$—$1{,}125\cdot10^{-3}$	$6{,}05\cdot10^{-4}$	»
$PhCOOCH_2CH_2CH_2CH_2\overset{+}{N}Me_3\cdot I^-$ (XXV) [3]	$3{,}7\cdot10^{-4}$—$1{,}55\cdot10^{-3}$	$8{,}9\cdot10^{-4}$	»
$PhCOOCH_2CH_2CH_2CH_2\overset{+}{N}Me_2(Ad\text{-}1)\cdot I^-$ (XXVI) [1]	$2{,}92\cdot10^{-5}$—$1{,}1\cdot10^{-4}$	Competitive $5{,}85\cdot10^{-5}$, Noncompetitive $2{,}93\cdot10^{-4}$	Mixed

Legend. See note to Table 1.

The replacement of one of the methyl groups on the quaternary nitrogen atom of acetylcholine with an adamantyl or methyladamantyl group occurs such that the corresponding compounds XVII and XVIII are not substrates of ACE, but rather weak inhibitors (a high inhibition constant) of the classical noncompetitive variety. The replacement of a hydrogen atom by an adamantyl group at the α-carbon in the aminoalcohol portion of the acetylcholine molecule leads to an increase in the anticholinesterase activity for compound XIX; the type of inhibition is also altered in this way. Consequently, the location of the adamantyl group within the alkyl portion of the molecule influences the anticholinesterase activity.

The introduction of an adamantyl radical into the acidic portion of the acetylcholine molecule (Compound XX) results in a significant increase in the anticholinesterase activity (K_i is reduced by 35 times relative to compound XVII). If the adamantyl radical is separated from the ester group by one methylene linkage (compound XXI) the anticholinesterase activity is reduced by a factor of 4. Together with the presence of the adamantyl group in the acyl portion of the molecule, the anticholinesterase activity is evidently affected by the distance between the ester group and the quaternary nitrogen atom. As shown in Table 2, upon increasing the number of methylene groups in the aminoalcohol of XX from 2 to 3 and 4 (compounds XXII and XXIII respectively), the anticholinesterase activity is reduced by 3-5 times. One may conclude that compound XX, in which the adamantyl group is attached directly to an ester group which is itself separated from the quaternary nitrogen by two methylene groups, is the most complimentary to the active surface of ACE of the compounds studied.

For comparison with the adamantyl derivative XXIII, we examined compounds XXIV and XXV which contain less hydrophobic cyclohexyl and phenyl groups in the acid section of the amino-ester molecule. Such a substitution results in lower anticholinesterase activity for these compounds.

Thus, adamantyl analogs of acetylcholine are ACE inhibitors rather than substrates, with the more powerful inhibitors being those which contain the adamantyl radical in the acidic fragment of acetylcholine.

The obtained data should be considered in the search for compounds with cholinotropic and anticholinesterase activities.

EXPERIMENTAL METHODS

Literature references for the preparation and properties of those compounds examined which have been described in earlier works are given in Tables 1 and 2.

2-(N,N-Diethylamino)adamantane. A mixture containing 6 g adamantanone, 16.6 g N,N-diethyformamide, and 2 g formic acid was heated at 210-220°C in an oil bath. After cooling, water was added to the reaction mixture. The mixture was then acidified and the unreacted adamantanone (1.3 g) was extracted by ether. The aqueous layer was then made alkaline and the free base extracted into ether; the ether was evaporated off and the residue remaining was collected as fractions with a boiling point of 118°C/4 mm, n_D^{20} 1.4980. Yield: 3.8 g (59%). Hydrochloride: mp 133-134°C. Literature data of [13]: free base: bp 73-75°C/0.2 mm; hydrochloride (XII): mp 136°C.

2-(N,N-Diethylamino)adamantane p-Toluenesulfomethylate (XV). A mixture containing 2.1 g 2-(N,N-diethylamino)adamantane and 1.9 g p-toluenesulfonic acid methyl ester was heated at 120°C in a constant temperature bath for 7 h. After cooling the mixture was triturated with ethyl acetate. Yield: 3.1 g (77.5% of theoretical), mp 110-112°C (from alcohol/ethyl acetate). Found, %: N 3.64, S 7.86. $C_{22}H_{35}NO_3S$. Calculated: N 3.56%, S 8.13%.

2-(N,N-Diethylamino)admantane p-Toluenesulfoethylate (XVI). Obtained analogously, yield = 69%, mp 105-107°C. Found, %: N 3.53, S 7.64. $C_{23}H_{37}NO_3S$. Calculated, %: N 3.43, S 7.87.

2-(N,N-Dimethylamino)admantane p-Toluenesulfomethylate (XIII). Obtained analogously from 2-(N,N-dimethylamino)adamantane [6]. Yield = 79%, mp 175-176°C. Found, %: N 3.97, S 8.85. $C_{20}H_{31}NO_3S$. Calculated, %: N 3.83, S 8.77.

2-(N,N-Dimethylamino)adamantane p-Toluenesulfoethylate (XIV). Obtained analogously, yield = 72%, mp 115-117°C. Found, %: N 3.80, S 8.70. $C_{21}H_{33}NO_3S$. Calculated, %: N 3.68, S 8.44.

The enzyme used was a lyophilized preparation of ACE (EC 3.1.1.7) prepared from human erythrocytes by the Permski Scientific Research Institute of Vaccines and Sera having a specific activity of 0.5 U/mg. ACE concentrations in working solutions were $7.4 \cdot 10^{-2}$ mg/ml; substrate concentrations (acetylcholine iodide) were $4 \cdot 10^{-5}$-$6.7 \cdot 10^{-4}$ M. The inhibitors were used as aqueous solutions of the corresponding tertiary ammonium salts, the concentrations of which are given in Table 1.

Enzymatic reaction rates were determined by continuous potentiometric titration of the acid liberated during hydrolysis using a 0.02 M solution of NaOH at constant pH, temperature, and substrate concentration. Reactions were carried out at 38°C in 0.15 M NaCl in the presence of $8 \cdot 10^{-4}$ M phosphate buffer (Na_2HPO_4/KH_2PO_4 10:1.5), pH 7.4.

The value of V_{max} (maximum reaction rate) in the presence and absence of inhibitors was determined graphically by the Lineweaver–Burk method and I_V vs. $I_{[S]}$ coordinates. The type of enzyme inhibition was determined by two independent methods: Lineweaver–Burk and Dixon [8]. Inhibition constants (K_i) for competitive and noncompetitive inhibition were determined graphically by the Dixon plot. In the case of mixed-type inhibition, the competitive (K_i^{com}) and noncompetitive (K_i^{noncom}) inhibition constants were determined [12].

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