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REVIEW ARTICLE

On some principles of interaction of curare-like agents with acetylcholine receptors of skeletal muscles

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In the past few years the search for new substances has assumed a more purposeful character. The study of principles of interaction of pharmacological substances with receptors is significant for the creation of the theoretical basis for such research. This does not only touch upon the main functionally active groups of a compound, which determine its mode of action, but also upon other fragments of a molecule which affect the pharmacodynamics and pharmacokinetics of a compound (Barlow 1968; Kharkevich 1969, 1973, 1974, 1975; Kharkevich & Skoldinov 1970, 1971, 1974, 1976; Kier 1971; Triggle et al 1971; Anichkov 1974; Albert 1979.

The work done in this field was intensified by the research aimed at isolation and identification of different receptors. Considerable success has been achieved in relation to acetylcholine receptors (cf. Heidemann & Changeux 1979; Birdsall et al 1979 etc).

In this article the role of different types of interaction of curare-like agents with acetylcholine end-plate receptors is discussed.

Numerous experiments have shown that interaction of curare-like agents with the end-plate of skeletal muscles involves many types of polar and non-polar interatomic bonds (Table 1).

One of the most stable bonds is the *electrostatic bond* which in many cases is realized by ion-ion interaction. In addition, there may exist partial charges which appear as a result of displacement of electrons under the influence of polar substituents in aromatic systems or in systems with conjugated double bonds.

In ion-ion interactions the positive ions are the curare-like agents with their cationic centres (usually N^+). Acetylcholine receptors accordingly have anionic sites which take part in ionic interaction with 'cationic heads' of the compound. The number of cationic centres in the molecule of curare-like agents varies from one to several. Mono-onium compounds are usually of low activity as their interaction with acetylcholine receptors is less stable than that of poly-onium compounds. The high myoparalytic activity of some mono-onium salts is explained by the fact that ion-ion interaction is supplemented with other types of interatomic bonds.

The presence of several cationic centres provides more stable bonding with the acetylcholine receptor

Table 1. Possible types of interaction of curare-like agents with acetylcholine receptors.

Types of interaction	Approx. stability of bond kcal mol^{-1}	kJmol^{-1}	Decrease of bond's stability depending on the distance between atoms (r)*
Electrostatic (ionic)	5	21	r^{-2}
Ion-dipole	2–5	8–21	r^{-3}
Dipole-dipole	1–3	4–12	r^{-4}
Hydrogen bonds	2–5	8–21	r^{-4}
Van der Waals' (dispersion)	0·5	2	r^{-7}
Hydrophobic†	‡		

* Adopted from a series of sources (Barlow 1968; Kier 1971; Albert 1979; and others). The stability of chemical (covalent) bond—50–100 kcal mol^{-1} (210–420 kJmol^{-1}).

† The interaction of nonpolar molecules in aqueous medium is meant.

‡ 0·7 kcal (3kJ) per CH_2-group.

* Correspondence.

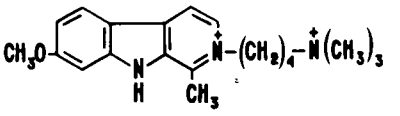
and a higher curare-like activity accordingly. Most curare-like agents belong to the group of bis-onium salts. The complementarity for bis-onium salts depends on many factors, such as: composition of the onium group, distance between cationic centres, structure of the interonium part of the molecule, flexiblility of the molecule and its conformation in the biophase at the moment of interaction with the acetylcholine receptor. In the series of polymethylene-bis-trimethylammonium derivatives (I) it was shown that in internitrogen distance two optima exist: with n = 10 and n = 18 (Paton & Zaimis 1949).

$$(CH_3)_3N^+ - [CH_2]_n - N^+(CH_3)_3 . 2X^- \qquad (I)$$

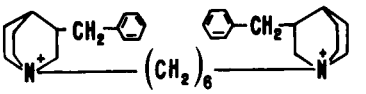
The criteria mentioned (for n = 10 in particular) are appropriate to many compounds. However, this rule is not universal. There are many curare-like agents in which the interonium distance varies within wide limits (Table 2). One of the possible reasons for such differences may be variability of distance between anionic centres of acetylcholine receptors. However, another possibility is that the ion-ion interaction of compounds with 'non-optimal' interonium distance occurs with one cationic centre only, and this seems more likely. The second quaternary nitrogen atom and the remaining part of the molecule interact with the receptors through other types of interatomic bonds. Thus, it is possible, that the second quaternary nitrogen atom contributes to an *ion-dipole bond*, or is bound to non-specific centres of full or partial negative charge. These types of interaction are possible in qualidilum and hexafluorenium where the quaternary nitrogen atoms are separated by hexamethylene chains. It is highly probable that in poly-onium compounds one pair of cationic centres with optimal interonium distance forms the ion-ion bond, and others (non-complementary to the anionic sites of the acetylcholine receptor) form the ion-dipole or other bonds.

For many compounds, esters for example (e.g. succinylcholine, diadonium), the *dipole-dipole bonding* is of considerable importance. In this case, the presence of permanent dipoles in the molecule of a compound and in the receptors is necessary in the first place. It is known, that the stability of dipole-dipole bonding depends on dipole moments, on the spatial orientation of each of the dipoles and on their distance apart. For esters, the permanent dipole is the carbonyl group, though the dipoles also form other groups with heteroatoms. However, the interaction of a constant dipole with an induced one is also possible. In this case an electric field of a polar molecule induces the generation of a dipole in a

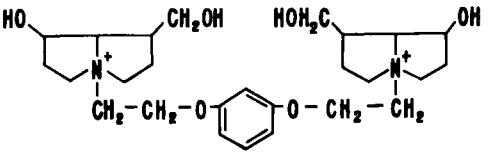
Table 2. Internitrogen distance(n) in some neuromuscular blocking agents (cations).



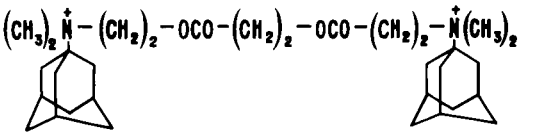
n = 4



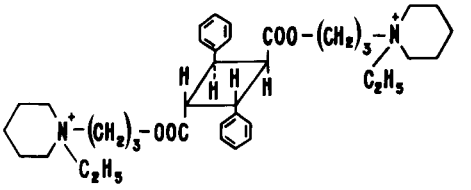
Qualidilum n = 6



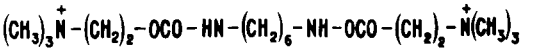
Diplacinium n = 9



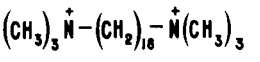
Diadonium n = 10



Anatruxonium n = 13



Carbolonium n = 16



n = 18

non-polar structure. The stability of the latter bond is low.

In the interaction of curare-like agents with acetylcholine receptors a certain role is played by *hydrogen bonds*, which appear between two negative atoms (most often between O and N) with the hydrogen atom as a binding link (O-H . . . O; O-H- . . . N; N-H . . . N; N-H . . . O). The hydrogen atom is covalently bound with one of the electronegative atoms. Oxygen and nitrogen are frequent components in the molecule of curare-like agents. It is quite natural that these forces of interaction can be effective only when the atoms connected by hydrogen are sterically optimally disposed, which means that they should be on the same line. Besides, it should be considered that this type of bonding appears only at close contact of the compound with the receptor as the hydrogen radius of action is small (Table 1).

In *van der Waals' bonds* the radius of action is even smaller. They appear only on close contact of the agent with the acetylcholine receptor, decreasing the attractive force to the sixth-seventh power of interatomic distance. Van der Waals' bondings are formed on the basis of interaction of instantaneously arising oscillating dipoles and induced dipoles. Taking into account the fact that these bonds are of the most universal character providing bonding with any atom, it is important to elucidate their role in the interaction of curare-like agents with acetylcholine receptors. The former, being large molecules, possess many possible contacts to form van der Waals' bonds. The additive effect of these bonds can provide sufficient stability to the drug-receptor complex.

Van der Waals' forces underlie the dispersion bonds and take part in so called *hydrophobic interactions*. In biological research, and especially in research connected with the problem of receptors, hydrophobic interactions are of primary importance as the reaction of the agent with the receptor is accomplished in aqueous medium. While the above mentioned types of interaction of the agents with acetylcholine receptors have been widely studied, the question of the role of hydrophobic interactions has been inadequately investigated and many of the studies that have been made have been with aromatic cyclics, linear and cyclic structures with heteroatoms and unsaturated bonds and other radicals, that do not permit inferences about the real role of hydrophobic bonds in the interactions of compounds with acetylcholine receptors of skeletal muscles. This fact provoked research in which special selection of compounds either with quite different hydrophobic properties or with gradual increase of hydrophobicity was made. For this purpose the non-polar saturated aliphatic (C_nH_{2n+1}) and alicyclic (cyclohexyl, bornyl, adamantyl) radicals were used. They can interact in aqueous medium with acetylcholine receptors mainly by hydrophobic bonds.

The role of adamantyl radicals was studied for mono- and bis-quaternary ammonium compounds. Firstly, it was noticed, that the insertion of adamantyl radicals in the trimethylammonium 'cationic-head' changed the mechanism of action of the compounds, transforming them from depolarizing into antidepolarizing drugs.

For the series of mono-quaternary ammonium compounds this effect was shown on the example of *N*-adamantyl analogues of tetramethylammonium, choline, acetylcholine, quaternary salts of alkamine esters of benzoic (II) and cinnamic (III) acids and their derivatives (p. 736).

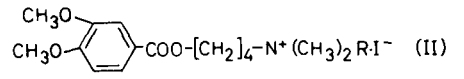
Similar results were obtained for bis-quaternary ammonium salts: *N*-adamantyl analogues of succinylcholine (IV) and decamethonium (V).

The change of mechanism of action of these *N*-adamantyl mono- and bis-quaternary ammonium compounds was followed by a pronounced decrease of their activity.

For the last two pairs of compounds (IV a,b; V a,b) physicochemical research was carried out (Drozhzin 1975) that permitted an estimation of the relation between their hydrophobicity and mechanism of action.

To assess the hydrophobicity of the compounds, several factors were tested, such as the surface activity (aqueous solution/air), the interphase activity (aqueous solution/benzoic solution of lypoproteins of nervous tissues), the interaction with polyelectrolyte (polyacrilic acid) and the effect on electrical conductivity of artificial phospholipid membrane (formed from a 2% solution of egg lecithin in n-heptane). In all the models tested, decadonium and diadonium were of higher activity than decamethonium and succinylcholine. These data prove that the antidepolarizing *N*-adamantyl derivatives are of higher hydrophobicity than their depolarizing trimethylammonium analogues.

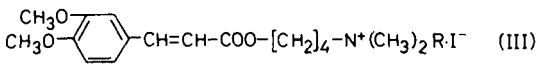
In all the above mentioned compounds the adamantyl radicals were inserted into the 'cationic head'. It is natural, that the attachment to quaternary nitrogen atoms of such a large radical as adamantyl changed the conditions of interaction with anionic centres of acetylcholine receptors. That is why alterations in the activity and mechanism of action of the compounds could have been associated with the change in the spatial interrelation between the



(II)

	Neuromuscular block*	Mechanism of action†
(a) R = CH_3	0·4–0·45	D
(b) R = (1-Ad)[1]	10–12	A

* Complete block of the transmission from sciatic nerve to the gastrocnemius muscle of the anaesthetized cat (minimal doses in mg kg^{-1}, i.v.). The sciatic nerve was stimulated by the supramaximal rectangular stimuli (0·5 ms; 1 Hz). The contractions of the gastrocnemius muscle were recorded. Each compound was tested on 6–8 cats.



(III)

	Neuromuscular block	Mechanism of action
(a) R = CH_3	0·03–0·05	D
(b) R = (1-Ad)	7–8	A

$[R(CH_3)_2N^+-[CH_2]_2-OCO-CH_2-]_2\cdot 2I^-$ (IV)

	Neuro-muscular block	Mechanism of action
(a) R = CH_3 (succinylcholine)	0·06–0·08	D
(b) R = (1-Ad) (diadonium)	0·25–0·35	A

$R(CH_3)_2N^+-[CH_2]_{10}-N^+(CH_3)_2R\cdot 2I^-$ (V)

(a) R = CH_3 (decamethonium)	0·03–0·04	D
(b) R = (1-Ad)[1] (decadonium)	0·25–0·3	A

† D-depolarizing; A-antidepolarizing. The mechanism of action was determined by estimation of the character of paralysis in pigeons, the effect on isolated frog rectus abdominis muscle, on the stimulating effect of carbachol and, the interaction with neostigmine in the experiments on neuromuscular transmission in anaesthetized cats.

[1] Structure of (1-Ad) see below.

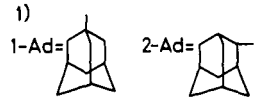
compounds' onium centres and the receptors' anionic structures. To analyse the data obtained mono- and bis-quaternary ammonium compounds with the functionally active *N*-trimethylammonium groups were synthesized. Adamantyl radicals were attached at different distances from the quaternary nitrogen atoms.

Thus, for example, the mono-quaternary ammonium compounds with the common structure VI were investigated.

$R^1-COO-CHR^2-[CH_2]_n-N^+(CH_3)_3\cdot I^-$ (VI)

R^1 = CH_3, (1-Ad),(1-Ad)–CH_2-

R^2 = H,(1-Ad)[1] n = 1,2,3



All of them with (1-Ad) in the R^1 or R^2 position, in contrast to their prototype acetylcholine, belong to antidepolarizing agents. Their curare-like activity is low. They evoke a flaccid paralysis in pigeons in the doses of 2–5 mg kg^{-1}.

Similar criteria were noted for bis-quaternary salts (VII).

$R^1\langle^{COO-CHR^2-CH_2-N^+(CH_3)_3}_{COO-CHR^2-CH_2-N^+(CH_3)_3}\cdot 2I^-$ (VII)

	Neuro-muscular block	Mechanism of action
(a) R^1 = $-(CH_2)_2-$; R^2 = (1-Ad)	11–13	A
(b) R^1 = (1-Ad)-CH<; R^2 = H	> 35	A
(c) R^1 = (1-Ad)<; R^2 = H	> 20	A

Thus, independently of the localization of the 1-adamantyl radical in the molecule, the trimethylammonium salts possess antidepolarizing action. These data show that the mechanism of action of the compounds at the insertion in their structure of adamantyl radicals is connected mainly with hydrophobic interactions but not with spatial factors.

In the examples given, an abrupt increase of hydrophobicity of the compounds was related to the insertion of a highly hydrophobic 1-adamantyl radical. To make a more detailed analysis of these criteria mono-(VIII) and bis-quaternary (IX) ammonium compounds with a gradual increase of hydrophobicity of the substituents on the quaternary nitrogen atoms were synthesized. This was achieved by extension of the aliphatic chain and increasing size of alicyclic radicals.

The increase of hydrophobicity by addition of carbon atoms to the radicals (R) at the quaternary nitrogen atom was followed by their being bound more stably with polyacrylic acid.

$RN^+(CH_3)_3\cdot X^-$ (VIII)

R = C_nH_{2n+1} (n = 2,4,6– . . . . 20); cyclohexyl, bornyl, (1-Ad)

X = I,Br

$R(CH_3)_2N^+-[CH_2]_{10}-N^+(CH_3)_2R\cdot 2I^-$ (IX)

R = C_nH_{2n+1} (n = 2,4,6– . . . . 20); cyclohexyl, bornyl, (1-Ad)

For mono-quaternary salts the change of the mechanism of action (the transformation of depolarizing agents into antidepolarizing ones) occurs at R = $nC_{10}H_{21}$ and R = bornyl or 1-Ad,* for bis-quaternary salts this change already occurs at R = nC_4H_9 and R = cyclohexyl.

* The structure of bornyl and adamantyl radicals also contains 10 carbogen atoms.

Consequently, the increase of hydrophobicity of the compounds to a certain extent provokes the change in their mechanism of action. It is most probable, that this change is due to the fact that the hydrophobic radical provides the additional points of fixation on the receptor protein. This stabilizes the structure of the receptor and prevents the depolarization.

For the above mentioned compounds the change of the mechanism of action was followed by a decrease in their myoparalytic activity. This may be caused by steric hindrance in ionic and other types of bondings which appear at the insertion of large hydrophobic radicals or by the absence of complementarity between the compounds' hydrophobic radicals and the hydrophobic sites of the acetylcholine receptor. Nevertheless the possibility exists of increasing the myoparalytic activity of compounds of this type. This was shown for the series of bis-quaternary polymethylene derivatives (X).

$$R(CH_3)_2N^+\text{-}[CH_2]_n\text{-}N^+(CH_3)_2R\cdot 2I^- \qquad (X)$$

	Neuromuscular block	Mechanism of action
(a) n = 5 R = CH_3	> 40*	A
(b) n = 5 R = (1-Ad)	0·8–0·12	A
(c) n = 6 R = CH_3	> 40*	A
(d) n = 6 R = (1-Ad)	0·12–0·2	A
(e) n = 8 R = CH_3	0·16*	M†
(f) n = 8 R = (1-Ad)	0·09–0·12	A

* Inhibition by 95% of the transmission from the sciatic nerve to the tibialis anterior muscle of the cat (Paton & Zaimis 1949).
† M-Partial agonist.

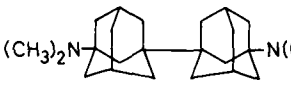
The importance of this series of compounds lies in the fact that besides the different distance between hydrophobic 1-adamantyl radicals attached to quaternary nitrogen atoms, the initial *N*-trimethylammonium compounds with n = 5 and 6 (Xa,b) possess an antidepolarizing type of action.

In all the compounds the replacement of *N*-methyl groups by *N*(1-adamantyl) radicals increases their myoparalytic activity. A more notable increase of curare-like activity is observed at n = 6 and 5.

Compound Xc where n = 6, is the known ganglion-blocking agent hexamethonium, but after the replacement of two *N*-methyl groups by 1-adamantyl there is a loss of ganglion-blocking properties and the compound (Xd) becomes an effective curare-like agent with activity that can be compared with tubocurarine chloride which provokes neuromuscular block at doses of 0·18–0·23 mg kg^{-1}. An analogous bis-tertiary salt (XI) also possesses a curare-like action (10–12 mg kg^{-1}, completely blocks neuromuscular transmission) though an anlogous bis-trimethylammonium salt is deprived of this activity.

$$(1\text{-Ad})CH_3N\text{-}[CH_2]_6\text{-}NCH_3(1\text{-Ad})\cdot 2HCl \qquad (XI)$$

At the insertion of two 1-adamantyl radicals in the central part of a molecule (XII) the interonium distance becomes very close to that of hexamethonium (Xc), Xd and XI.



(XII)

	Neuromuscular block	Mechanism of action
(a) X = CH_3I	0·7–0·9	A
(b) X = HCl	3–4	A

These two compounds possess a pronounced myoparalytic action, particularly the bis-quaternary salts (XIIa).

Thus the examples given prove that the hydrophobic radicals may increase the curare-like activity if they are complementary to the hydrophobic sites of acetylcholine receptors.

The data obtained testify to the important role of hydrophobic interaction in the mechanism of action and the activity of curare-like agents (Kharkevich & Skoldinov 1974, 1976; Kharkevich 1975). It is obvious, that the hydrophobic bonds increase the stability of interaction of curare-like agents with the receptor (in cases of their complementarity), and also stabilize the bondings of quaternary nitrogen atoms with anionic sites of the acetylcholine receptor. It is quite evident, that close contact of the agent with the receptor is of prior importance for van der Waals' forces. The slightest steric hindrance leads to reduction of the activity of the compounds.

In the presence of conjugated double bonds in a molecule of curare-like agents π-electron clouds appear that may induce supplementary interactions. That an acetylcholine receptor possesses the structures forming the *π-bonds* also cannot be excluded. This possibility should be taken into account for curare-like agents with aromatic rings and unsaturated heterocyclics or hydrocarbon chains as well. The examples given (XIII, XIV) support the assumed participation of π-bonds, although the benzol ring is planar and cyclohexane non-planar.

However, the replacement of an alicyclic radical by an aromatic one does not always cause an increase in the activity. The enhancement of the curare-like activity may be observed only in the case

$RN^+(CH_3)_3 \cdot I^-$ (XIII)

	Neuromuscular block
(a) R = cyclohexyl	> 1·5
(b) R = phenyl	0·25–0·3

$R\text{–}COO\text{–}[CH_2]_4\text{–}N^+(CH_3)_3 \cdot I^-$ (XIV)

(a) R = cyclohexyl	0·6–0·7
(b) R = phenyl	0·2–0·25

of a possible interaction of the π-electron clouds of the agent and the receptor.

It is natural, that π-bonds should function in parallel with other types of bonds (for example van der Waals' bonds). Therefore, the interaction of curare-like agents with acetylcholine receptors of skeletal muscle includes different types of interatomic bondings. Their cooperative effect conditions the activity, the duration and the mechanism of action of the agents which block the neuromuscular transmission.

As mentioned, the stability of the bonds considered depends on the *degree of approachment of the compound to the acetylcholine receptor*, which is determined by their complementarity. Therefore the spatial structure of a molecule plays the prior role in the action of curare-like compounds. This is true both of a molecule as a whole and of some of its components, in particular to cationic centres and interonium distances. Thus, for example, it was shown in the series of quaternary ammonium compounds with *N*-adamantyl radicals that the replacement in the 'cationic head' of the 1-adamantyl radical (1-Ad) by the 2-adamantyl radical (2-Ad) often leads to the reduction of curare-like activity (XV–XVI). In this case the hydrophilic-hydrophobic balance was the same for the whole compound. The only change that occurred was that of the spatial position of the adamantyl radical about the quaternary nitrogen atom which reduced the stability of hydrophobic bonds and indirectly of ionic bonds.

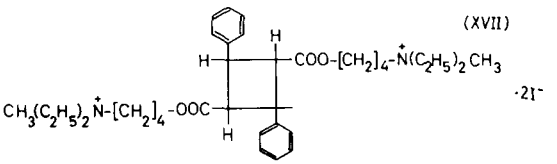
$R(CH_3)_2N^+\text{–}[CH_2]_8\text{–}N^+(CH_3)_2R \cdot 2I^-$ (XV)

	Neuromuscular block
(a) R = 1-Ad	0·09–0·12
(b) R = 2-Ad	0·6–0·8

$[R(CH_3)_2N^+\text{–}[CH_2]_2\text{–}OCO\text{–}CH_2\text{–}]_2 \cdot 2I^-$ (XVI)

	Neuromuscular block
(a) R = 1-Ad	0·35–0·45
(b) R = 2-Ad	2·0–2·5

As to the role of the spatial configuration of the interonium part of a molecule, the derivatives of stereoisomeric truxillic acids (α-, ϵ-,γ-) can be taken as an example. The stereoisomeric truxillic acids differ from one another only by the relation between the phenyl radicals and carboxylic groups about the plane of the cyclobutane structure (XVII).



(XVII)

Sterical configuration of truxillic acids	Neuromuscular block
(a) α-	0·25–0·35
(b) ϵ-	0·4–0·5
(c) γ-	0·6–1

The α-truxillic acid derivatives were the most active, those of γ-truxillic acid least active with the ϵ-truxillic salts having intermediate activity. It is possible, that in these series of compounds the hydrophobic interonium structure of α-truxullic acid derivatives is most complementary to acetylcholine receptors which provide favourable conditions for the close contact of the agent with the receptor and permits a more complete manifestation of different types of interatomic bonds.

Hence, there exists a considerable amount of information dealing with the principles of interaction of curare-like agents with acetylcholine receptors. The data obtained show that nearly all of the bondings (covalent bonds excluded) in different combinations take part in the interaction of curare-like agents with acetylcholine receptors. While the places and types of interaction of a molecule of a curare-like agent judging by their chemical structure are relatively clear, for acetylcholine receptors it is still necessary to draw a 'map' which should contain not only anionic sites, but also hydrophobic areas, localization of permanent dipoles and topography for hydrogen bondings. Beside their theoretical value these data will be of great practical importance as they will promote the synthesis of new curare-like agents with desirable properties.

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