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Architecture of the Neuronal Nicotinic Acetylcholine Receptor Ion Channel at the Binding Site of bis-Ammonium Blockers

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Abstract. Structure-activity relationships of 56 pentamethylenbis-ammonium compounds, the blockers of the neuronal nicotinic acetylcholine receptor (nAChR) ion channel, have been studied to estimate the cross-sectional dimensions of the channel pore. The cat superior cervical sympathetic ganglion *in situ* and isolated guinea pig ileum were used to evaluate the potency of the compounds to block ganglionic transmission. Minimum-energy conformations of each compound were calculated by the molecular mechanics method. A topographic model of the binding site of the blockers was proposed. It incorporates two narrowings, a large and a small one. The small narrowing is located between the large one and the cytoplasmic end of the pore. The cross-sectional dimensions of the large and small narrowings estimated from the dimensions of the blockers are 6.1 × 8.3 Å and 5.5 × 6.4 Å, respectively, the distance between the narrowings along the pore being approximately 7 Å. Most potent blockers would occlude the pore via binding to the channel at the levels of both narrowings. Less potent blockers are either too large or too small to bind to both narrowings simultaneously: large blockers would occlude the pore at the level of large narrowing, while small blockers would pass the large narrowing and occlude the pore at the level of small narrowing only. A comparison of the topographic model with a molecular five-helix bundle model of nAChR pore predicts *Serine* and *Threonine* rings to be the most probable candidates for the large and small narrowings, respectively.

Key words: Acetylcholine receptor — Ion channel — Open channel blockers — Structure-activity relationship — Molecular modeling

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Introduction

Nicotinic acetylcholine receptor (nAChR) ion channel may be blocked by various organic cations (Changeux & Revah, 1987), in particular, by bis-ammonium compounds (Lukomskaya & Gmiro, 1982*a;* Gurney & Rang, 1984). The blocking potency was shown to depend strongly on the structure of both ammonium groups and on the distance between them (Lukomskaya & Gmiro, 1982*a*). However, the location and topography of the binding site for bis-ammonium blockers remain unclear. Skok et al. (1984) demonstrated that the block of the nicotinic synaptic transmission by bis-ammonium compounds is due to the open channel block of the postsynaptic nAChRs. Therefore, the ganglion-blocking activity may be used as a rough estimate of the affinity of the blockers to their binding site in the pore. Analysis of conformation-activity relationships of the blocking drugs may help predict their binding site topography.

Earlier we studied conformation-activity relationships in a series of eleven bis-ammonium blockers of the neuronal nAChRs having the common structural formulae Et_3N^+-$(CH_2)_5$-$N^+R^1R^2R^3$ with the aim to estimate the dimensions of the pore at the level of binding of the variable trialkylammonium group. The dimensions corresponding to the best correlation between the blocking activity of the drugs and the total population of their pore-fitting conformations were found to be 6.1 × 8.3 Å (Zhorov et al., 1991). In this work, we studied an extended series of 56 bis-ammonium noncompetitive blockers of nAChR. The compounds considered include

pentaethonium derivatives Et_3N^+-$(CH_2)_5$-$N^+R^1R^2R^3$ and pentamethonium derivatives Me_3N^+-$(CH_2)_5$-$N^+R^1R^2R^3$. These two series demonstrate essentially different properties: the activity of pentaethonium blockers decreases whereas the activity of pentamethonium blockers increases with the dimensions of the variable ammonium group. The primary aim of this work was to create a topographical model of the binding site of the blockers which would explain the intriguing peculiarities in their structure-activity relationships. Based on the notion that the pore of nAChR is tapered from the extracellular to the cytoplasmic end (Changeux et al., 1992; Unwin, 1995) and on an analysis of structure-activity relationships of the blockers considered, we proposed that the binding site of the bis-ammonium blockers incorporates two narrowings, a large and a small one. Since most of the blockers are conformationally flexible, we calculated minimum-energy conformations of each compound and estimated the dimensions of the narrowings by using an approach proposed earlier (Zhorov et al., 1991). The proposed model helps explain the structure-activity relationships of the compounds considered. A comparison of the topographic model with modern data on nAChR channel structure suggests *Serine* and *Threonine* rings to be most probable candidates for the large and the small narrowings, respectively.

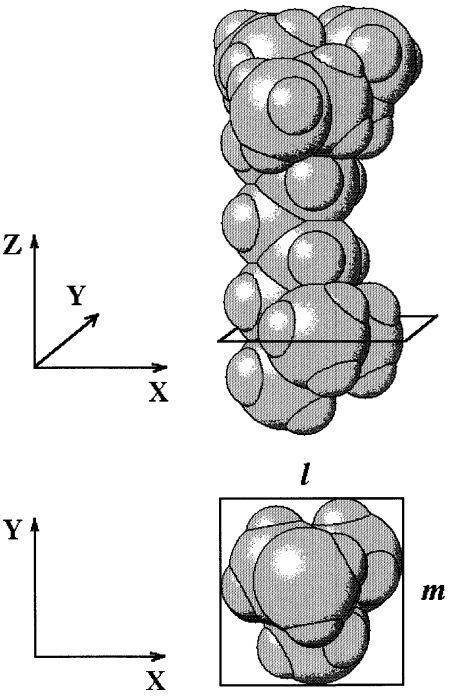


Fig. 1. The dimensions of the projection of a trialkylammonium group on the plane normal to the line between nitrogen atoms of bis-ammonium compound.

Materials and Methods

Determination of the Ganglion-Blocking Activity

The blocking effect of the compounds of ganglionic transmission was studied in cat superior cervical ganglion *in situ* by the method described by Lukomskaya and Gmiro (1982*b*). Contractions of nictitating membranes evoked by preganglionic electrical stimulation of cervical sympathetic nerve were recorded from the anaesthetized animals. Blocking drugs were injected into the lingual artery. ED_{50} values were estimated from dose-response curves. Activities of compounds were expressed relative to pentamethonium. ED_{50} value for pentamethonium taken as 1 is equal to 0.027 ± 0.003 μmol (n = 4).

The blockade of suberyldicholine-induced responses of enteric ganglia were studied in pieces of the guinea pig small intestine that were isolated and perfused with Tyrode solution at 37°C. Suberyldicholine was used since it activates nicotinic acetylcholine receptors without affecting muscarinic ones. Suberyldicholine and the blocking compound tested were applied in the perfusion saline. The intestine muscle contractions were recorded for estimation of IC_{50} value. The absence of antagonism with the muscarinic agonist 5-methylfurmetide was a criterion that the ganglion blocker did not affect the transmission from the nerve to the smooth muscle. The activities of blockers were also expressed relative to the penthamethonium, IC_{50} value of penthamethonium being 2.94 ± 0.42 μmol/l (n = 4).

Estimation of the Pore Dimensions from Conformation-Activity Relationships of the Blockers

The minimum-energy conformations of the blocking compounds were calculated as described elsewhere (Zhorov et al., 1991). A fully extended pentamethylene chain was used in the starting conformations (Rozengart & Zhorov, 1983). Minimum-energy conformations with energies up to 3.0 kcal/mol above the corresponding global minima were considered.

The cross-sectional dimensions $l_{ik} \times m_{ik}$ ($l_{ik} \leqslant m_{ik}$) of a trialkylammonium group of the compound k in the conformation i were calculated by finding a minimal-area rectangle described over the projection of the group at the plane normal to the axis between nitrogen atoms (Fig. 1). A profile of a narrowing in the pore was approximated by a rectangle with dimensions $a \times b$ ($a \leqslant b$). A probability T_k of the compound k to pass the narrowing is:

$$T_k = \sum [P_{ik} \cdot \sigma_{ik}],$$

where P_{ik} is a population of conformer i in the compound k, and

$$\sigma_{ik} = \begin{cases} 1 \text{ if } l_{ik} < a \text{ and } m_{ik} < b, \\ 0 \text{ if } l_{ik} > a \text{ or } m_{ik} > b. \end{cases}$$

Let us assume that in a series of compounds, the channel-blocking activity of (A_k) of the compound k is determined by a probability (T_k) of its variable cationic head to pass via a narrowing in the pore. Then it is possible to estimate the dimensions of the narrowing as those a and b which correspond to the maximal correlation coefficient between T_k and A_k (Zhorov et al., 1991).

Results and Discussion

Pharmacological Activity of the Blockers

The pharmacological activities of pentaethonium derivatives Et_3N^+-$(CH_2)_5$-$N^+R^1R^2R^3$ and pentamethonium de-

rivatives Me_3N^+-$(CH_2)_5$-$N^+R^1R^2R^3$ are given in Tables 1 and 2, respectively. The blocking potency of the compounds varies in a wide range and strongly depends on the structure of the ammonium groups. The distance between ammonium groups also affects the blocking potency. Compounds with 4–6 methylene groups between nitrogen atoms demonstrate the highest activity (Lukomskaya & Gmiro, 1982*a*). Therefore, only the compounds with two nitrogen atoms connected by a pentamethylene chain were investigated in the present work. As a rule, asymmetrical compounds (e.g., I–IV, VIII, XXXV–LII) are more potent than symmetrical (VII and LIII) ones. The activity of the blockers does not correlate strongly with their hydrophobicity nor with the ability to form hydrogen bonds. This suggests that the cross-sectional dimensions of the blocking molecules and their electrostatic and van der Waals interactions with the binding site are the major factors determining the ganglion-blocking activity of the compounds considered. To explain the structure-activity relationships of the blockers, we propose a qualitative topographical model of their binding site, determine certain geometrical characteristics of the model, and then compare the detailed topographical model with the molecular five-helix bundle model of the nAChR receptor pore.

A Topographic Model of the Binding Site

In the series of polymethylene-bis-ammonium channel blockers, the highest potency is exhibited by the compounds with two cationic heads of different size. Evidently, interaction of both heads with the corresponding nucleophillic groups in the channel are necessary for the effective blockade, the sterical requirements for the interaction with these two groups being essentially different (Lukomskaya & Gmiro, 1982*a;* Zhorov et al., 1991). There are evidences that the pore of nAChR is tapered from the extracellular to the cytoplasmic end (Changeux et al., 1992; Unwin, 1995). Hence, it may be suggested that the binding site for the potent bis-ammonium blockers is located in a tapered pore and incorporates two oval-shaped bracelets of different cross-sectional dimensions: a wide bracelet located more closely to the extracellular end of the pore and a more constricted bracelet located more closely to the cytoplasmic end of the pore, the distance between the planes of the bracelets corresponds to the distance between nitrogen atoms in pentamethonium derivatives (about 7 Å). A profile of a pore between parallel alpha-helices has repeating constrictions (at the levels of the residues facing the pore) and widenings (*see,* e.g., Oblatt-Montal et al., 1995). Therefore, nucleophillic groups responsible for the binding of the cationic heads of the blockers should be located at the narrowings. Both binding to and permeation through the nucleophillic narrowings should affect the activity of the blockers (Fig. 2).

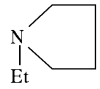
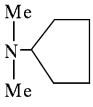
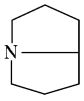
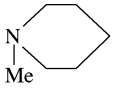
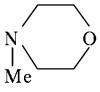
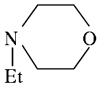
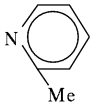
In the series of pentaethonium derivatives (Table 1), the blocking activity decreases with the increase of the variable cationic group (e.g., compounds II–V) while the blockers with two bulky cationic groups demonstrating low activity which only weakly depends on the structure (e.g., compounds VII, X–XIV). This suggests that the narrowing located more closely to the extracellular end of the pore is wide enough to let the medium-size ammonium group of the blockers to pass through and reach the more deeply located narrowing. At the same time, bulky triethylammonium groups of the blockers would occlude the wide narrowing and interact with it (Fig. 2*b*). A simultaneous interaction of both ammonium groups of the blockers with both nucleophillic groups of the binding site may explain high activity of such compounds. The large narrowing would retard either bulky variable ammonium groups of the compounds (VII), (X–XIV) to reach both nucleophillic groups simultaneously (Fig. 2a). Although these blockers are capable of occluding the channel pore via binding at the level of the large narrowing, the interaction of only one cationic head with nucleophillic group in the channel can not provide the effective block.

The potency of pentamethonium blockers whose trimethylammonium group would easily pass through the large narrowing increases with the size of the variable onium group (e.g., compounds XXXI–XXXVIII). This fact may be explained by the suggestion that the small narrowing at the cytoplasmic side of the pore retards the trimethylammonium group but lets through smaller groups (Fig. 2*c*). The trimethylammonium group of the blockers would be retarded at the narrowing and occlude the pore. Since only one ammonium group would interact with the binding site, pentamethonium compounds with the small variable cationic groups are low active.

Dimensions of the Large Narrowing

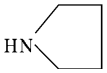
The activity of pentamethonium blockers (Table 1) should depend on the total population of their productive conformations in which the variable ammonium group is small enough to pass the large narrowing. Since hydrophobicity and donor-acceptor properties of the cationic groups would also affect the activity of the blockers, we have analyzed conformation-activity relationships in four independent groups (quaternary and nonquaternary blockers at sympathetic and parasympathetic ganglia). Of all four groups, the best correlation between the activity of the blockers and the total population of their productive conformations was found at the cross-sectional dimension of the narrowing 6.1 × 8.3 Å. The total populations of productive conformers P_1 corresponding to the narrowing dimensions 6.1 × 8.3 Å are

Table 1. Blocking activites of the compounds Et_3N^+-$(CH_2)_5$ - $N^+R^1R^2R^3$ and total population of their productive conformations

Compound	$NR^1R^2R^3$	Population P_1^a, %	Activity[b] at sympathetic	Ganglion parasympathetic
I	NMe_3	100.0	4.00	11.9
II	NMe_2Et	100.0	5.51	18.4
III	$NMeEt_2$	54.0	2.26	12.1
IV	$NMe_2Pr\text{-}i$	12.6	0.54	7.91
V	$NMe_2Pr\text{-}n$	6.0	0.14	0.37
VI	NMeEtPri-i	0.0	0.36	4.93
VII	NEt_3	0.0	0.09	0.15
VIII		95.5	2.60	7.95
IX		49.4	1.18	16.6
X		8.1	0.11	0.32
XI		0.0	0.05	0.13
XII		27.1	0.04	0.09
XIII		17.0	0.05	0.06
XIV		0.0	0.06	0.23
XV		0.0	0.51	6.22
XVI		0.0	0.12	0.91
XVII		92.4	2.33	11.6
XVIII		47.3	0.95	4.85
XIX		100.0	1.81	2.46
XX		95.5	0.54	
XXI	SMe_2	100.0	1.10	5.24
XXII	NH_3	100.0	0.50	1.31

Continued on next page

Table 1. *Continued*

Compound	$NR^1R^2R^3$	Population P_1^a, %	Activity[b] at sympathetic	Ganglion parasympathetic
XXIII	$NHMe_2$	100.0	2.31	4.48
XXIV	$NHEt_2$	79.6	0.82	3.13
XXV		100.0	0.84	2.87
XXVI	NH_2Bu-t	100.0	0.81	0.93
XXVII	NHMePr-i	98.5	0.75	2.16
XXVIII	NH_2Pri-i	100.0	0.43	0.76
XXIX	$NHPr_2$-i	41.6	0.11	0.75
XXX	NHMeBu-t	87.2	0.08	0.27

[a] Total population of conformations capable to pass via the narrowing of 6.1 × 8.3 Å.
[b] Relative to pentamethonium $Me_3N^+–(CH_2)_5–N^+Me_3$ whose absolute activity is 0.027 μmol at cat sympathetic ganglion (ED_{50}) and 2.94 μmol/l at guinea-pig small intestine enteric ganglia (EC_{50}).

given in Table 1. The following correlation coefficients were obtained:

0.91 for quaternary blockers at sympathetic nAChR,
0.81 for nonquaternary blockers at sympathetic nAChR,
0.96 for quaternary blockers at parasympathetic nAChR,
0.92 for nonquaternary blockers at parasympathetic nAChR.

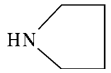
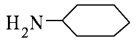
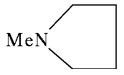
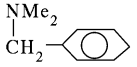
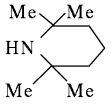
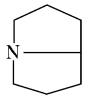
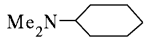
The optimal dimensions correspond to those obtained earlier with fewer blockers (Zhorov et al., 1991). The fact that the same dimensions were deduced using different experimental data proves the validity of the approach used. Although the dimensions of the quaternary and nonquaternary cationic groups optimal for the block are the same, the quaternary blockers exhibit essentially higher activity, probably, to their hydrophobicity.

A strong dependence of the activity of the blockers on their dimensions may be illustrated by several examples. A restriction of the conformational flexibility of two ethyl substituents in N^+Et_3 group in compound (VII) by their closing into pirrolidine ring (compound IX) increases a population of the productive conformers from 0 to 50% and, respectively, increases activity by an order of magnitude. A replacement of ethyl group in compound (VII) by methyl group (compound III) yields a similar effect. Compound (XVI) with π-pyridine ring does not have conformations capable to fit the rectangle of 6.1 × 8.3 Å (Fig. 3*b*) whereas its morpholine analogue (XVIII) does (Fig. 3*a*). This explains the fact that a substitution of methylene group in compound (XVI) by the oxygen atom in compound (XVIII) yields an 8-fold increase in activity (Table 1). Removing a methylene group in compound (XVIII) yields compound (XVII) whose activity is 2.5 times higher, evidently, due to a twofold increase in the population of the productive conformations (Table 1, Fig. 3*c*).

Dimensions of the Small Narrowing

If both cationic heads of a compound are small enough to pass the small narrowing, the compound would go beyond the binding site, demonstrating low blocking activity. The fact that compound (XLIII) with two trimethylammonium groups exhibits a moderate blocking potency suggests that the small narrowing retards the trimethylammonium group. Minimal-profile dimensions of the trimethylammonium group are 5.6 × 6.5 Å. Dimensions of the small narrowing should not be larger. They could be estimate more precisely by using a representative set of the blockers with one of the cationic groups whose cross-sectional dimensions are smaller than those of trimethylammonium group. Regretfully, only four compounds considered, namely (XXXI)–(XXXIV), satisfy this requirement. The fact that the blocking potency of these compounds increases with the dimension of the small cationic head was used to create the topographical model. However, the data on structure-activity relationships of these compounds cannot help estimate the dimensions of the small narrowing by using the correlation analysis since, along with the dimensions, both hydrophobicity of the compounds and their ability to form hydrogen bonds vary dramatically. As it was mentioned above, quaternary compounds are more potent than nonquaternary compounds with cationic heads of similar size. Therefore, we just postulated that the dimensions of the small narrowing are 5.5 × 6.4 Å, the figures slightly (0.1 Å) less than those in trimethylammonium group.

Table 2. Blocking activites of the compounds Me_3N^+-$(CH_2)_5$ - $N^+R^1R^2R^3$ and total population of their productive conformers

Compound	$N^+R^1R^2R^3$	Population P_2^a, %	Activity on sympathetic	Ganglion[b] parasympathetic
XXXI	NH_3	0.0	0.02	0.04
XXXII	NH_2Me	0.0	0.2	0.31
XXXIII	$NHMe_2$	33.8	0.7	0.87
XXXIV	NH_2Et	57.2	1.9	2.28
XXXV	NH_2Pr-i	100.0	5.5	16.0
XXXVI	NH_2Bu-t	100.0	3.0	19.2
XXXVII		100.0	2.85	
XXXVIII	NHMePr-i	100.0	5.5	9.93
XXXIX	NHMeBu-t	100.0	3.9	14.3
XL	$NHEt_2$	100.0	2.6	14.0
XLI	$NHPr_2$-i	100.0	6.7	22.1
XLII		100.0	3.0	8.4
XLIII	NMe_3	100.0	1.0	1.14
XLIV	NMe_2Et	100.0	1.36	5.1
XLV		100.0	2.86	4.5
XLVI	$NMeEt_2$	100.0	1.86	6.71
XLVII	NMe_2Pr-i	100.0	2.78	4.29
XLVIII		100.0	0.57	2.86
XLIX	NMeEtPr-i	100.0	3.44	
L	$NMePr_2$-i	100.0	3.57	
LI	NH_2-CH_2-Ad[c]	100.0	0.71	13.9
LII		100.0	1.29	21.4
LIII	NH_2-Ad[c]	100.0	1.07	42.9
LIV		100.0	2.5	40.5
LV		100.0	1.71	19.0
LVI		100.0	2.36	4.21

[a] The total populations of the conformers whose variable group is large enough to be retarded at the small narrowing of 5.5 × 6.4 Å.
[b] See footnote[b] to Table 1.
[c] Ad - adamantane

In productive conformations of pentamethonium derivatives, the variable cationic group should be large enough to be retarded at the small (5.5 × 6.4 Å) narrowing. The total populations P_2 of the conformations satisfying the requirement are given in Table 2. The compounds with $P_2 = 100\%$ essentially differ in activity, indicating that not only an ability of the compounds to be retarded at the narrowing determines activity of pentamethonium derivatives. It is noteworthy that the potency of the drugs increases with the bulkiness of their hydrophobic radicals.

Although variable cationic heads of compounds (XLIX) and (L) do not have conformations capable of passing the large narrowing, they demonstrate high activity. The reason is that the compounds would interact with both nucleophillic groups since the trimethyl-

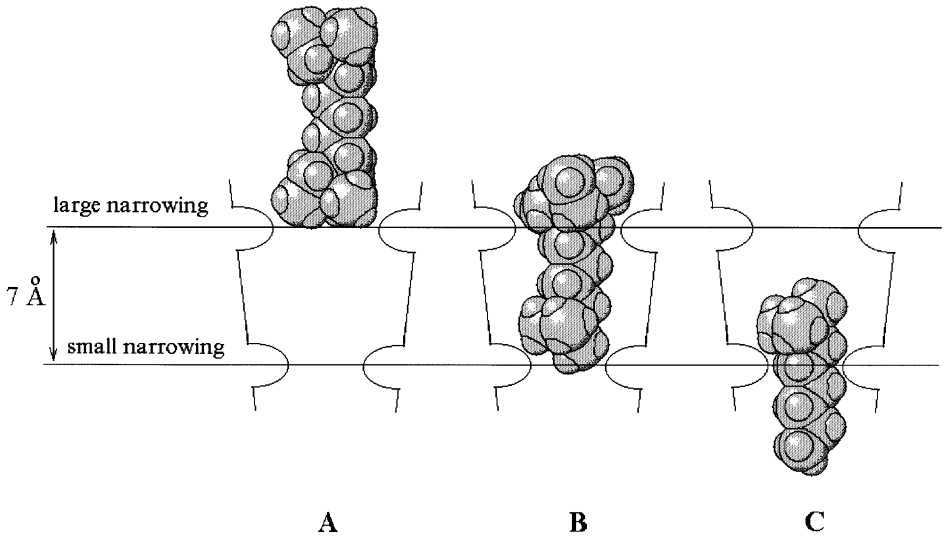


Fig. 2. A topographic model of the bis-ammonium blockers binding site in the pore of nAChR ion channel. (*A*) a blocking molecule with two bulky cationic heads is unable to reach the small narrowing and bind effectively. (*B*) both cationic heads of an asymmetric blocker interact with the corresponding narrowings providing high blocking potency. (*C*) one cationic head of a blocking molecule is small enough to pass through the small narrowing and, therefore, blocker can easily leave the binding site.

ammonium head would pass the large narrowing and reach the small narrowing. These compounds act in the same manner as the compounds (I) and (II) (*see* Table 1) which possess a bulky trimethylammonium group and a small group capable of passing through large narrowing. Compounds (XXXV)–(L) may reach the small narrowing by any cationic head.

Recently, Skok et al. (1995) studied conformation-activity relationships of bis-ammonium blockers to estimate cross-sectional dimensions of the neuronal nAChR pore. The authors deduced the smallest cross section to be 5.8 × 8.0 Å which essentially exceed our estimation. A reason may be that Skok et al. (1995) did not use the compounds with cationic head less than N^+Me_3, and, hence, were not able to probe the narrowing permeable for small cations only.

A Possible Location of the Large and Small Narrowings in the Pore of Neuronal nAChR

We created our model by using an analysis of conformation-activity relationships of bis-cationic blocking

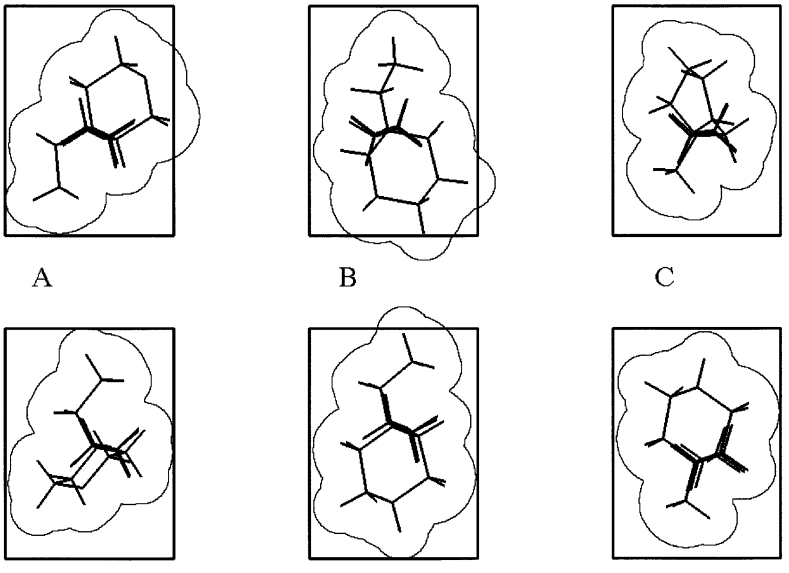


Fig. 3. Projections of the low-energy conformations of the variable cationic heads (compounds XVI, XVII and XVIII) on the plane normal to the axis passing through nitrogen atoms. Rectangle dimensions are 6.1 × 8.3 Å. (*A*) compound XVIII: the first conformer is productive, the second one is nonproductive. (*B*) compound XVI: both conformers are nonproductive. (*C*) compound XVII: both conformers are productive.

Table 3. Amino acid sequences of M2 segments of several nAChR

nAChR	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
α Torp. Cal	E	K	M	T	L	S	I	S	V	L	L	S	L	T	V	F	L	L	V	I	V
β Torp.Cal	E	K	M	S	L	S	I	S	A	L	L	A	V	T	V	F	L	L	L	L	A
γ Torp.Cal	Q	K	C	T	L	S	I	S	V	L	L	A	Q	T	I	F	L	F	L	I	A
δ Torp.Cal	E	K	M	S	T	A	I	S	V	L	L	A	Q	A	V	F	L	L	L	T	S
α Mouse	E	K	M	T	L	S	I	S	V	L	L	S	L	T	V	F	L	L	V	I	V
β Mouse	E	K	M	G	L	S	I	F	A	L	L	T	L	T	V	F	L	L	L	L	A
γ Mouse	Q	K	C	T	V	A	T	N	V	L	L	A	Q	T	V	F	L	F	L	V	A
δ Mouse	E	K	T	S	V	A	I	S	V	L	L	A	Q	S	V	F	L	L	L	I	S
α7 Chick	E	K	I	S	L	G	I	T	V	L	L	S	L	T	V	F	M	L	L	V	A

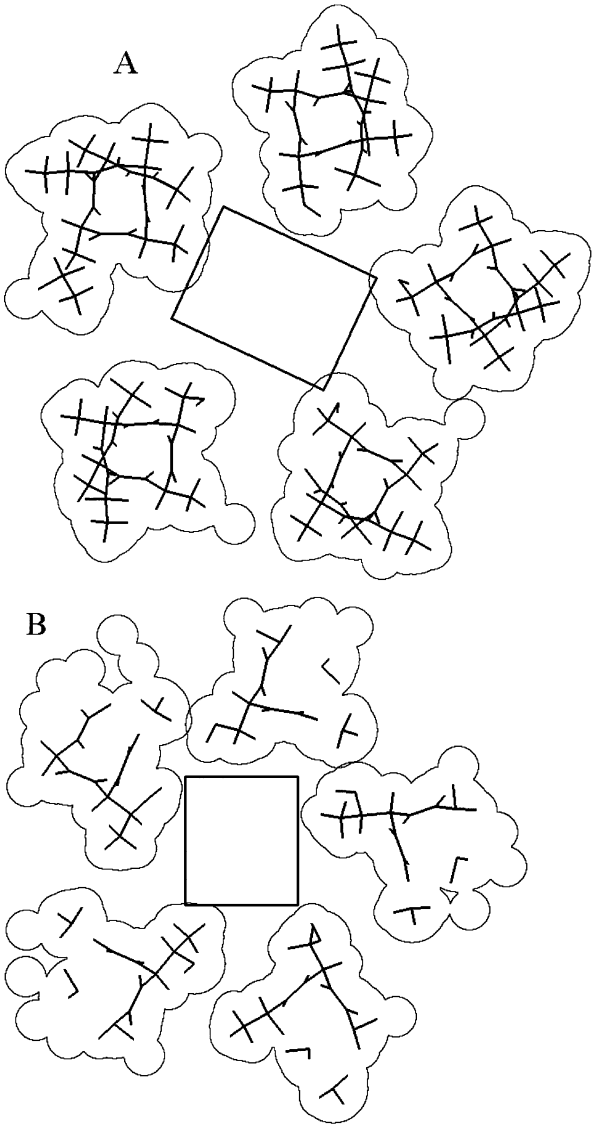


Fig. 4. Cross-sectional views of the model by Furois-Corbin and Pullman (1989). (*A*) at the level of the *Serine* ring, rectangle dimensions are 6.1 × 8.3 Å; (*B*) at the level of the *Threonine* ring, rectangle dimensions are 5.5 × 6.4 Å.

drugs. This approach provides only indirect information about the chemical nature of those groups that may represent the nucleophillic narrowings in the pore. Comparison of our model with the available data on the structure of the nAChR channel may help determine the location of the binding site and, hence, the chemical nature of the nucleophillic narrowings.

There is much evidence that the inner pore of the nicotinic acetylcholine receptor channel is formed by five M2 segments from each of the five subunits of the receptor oligomer. Photoaffinity labeling experiments and mutagenesis studies have demonstrated that amino acid residues located in M2 are responsible for the different pharmacological and functional properties, such as binding of noncompetitive blockers, ion permeability and desensitization, of the channel. The sequences of the M2 segments of some nAChR subunits are presented in the Table 3. [^{3}H]chlorpromazine labels M2 segment of the *Torpedo marmorata* nAChR in three positions: 4, 8 and 11 (Giraudat et al., 1986; Revah et al., 1990). The serine residues at position 8 are labeled by triphenylmethylphosphonium (Hucho, Oberthur & Lottspeich, 1986; Oberthur et al., 1986). Mutations at positions 8 and 12 affect the blocking potency of QX-222 on muscle nAChR (Leonard et al., 1988; Charnet et al., 1990). Mutations at positions 1, 15, 18 and 19 alter the calcium permeability of the neuronal nAChR channel (Bertrand et al., 1993). All these data are consistent with the α-helical organization of M2 segment (3.6 residues per turn of the helix) and indicate that the residues responsible for the binding of noncompetitive blockers and for the ionic permeability face the lumen of the channel (Changeux et al., 1992). Conservative residues (*see* Table 3) form *Intermediate* ring (position 1), *Threonine* ring (position 4), *Serine* ring (position 8), *Equatorial leucine* ring (position 11), *Valine* ring (position 15) and *Outer leucine* ring (position 18). The rings located near the synaptic side of the pore are formed by hydrophobic residues whereas the cytoplasmic side of the pore contains the *Intermediate*, *Threonine* and *Serine* rings with polar or charged residues.

It has been shown that mutations at the *Threonine*

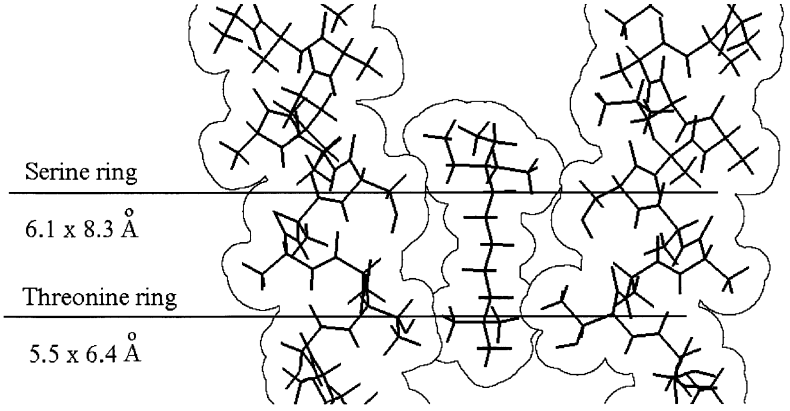


Fig. 5. A side view of the blocking compound (II) bound to the model of nAChR ion channel proposed by Furois-Corbin and Pullman (1989).

ring may restrict or enhance the ion flow depending on the volume of the substituting residue whereas mutations of the residues important for the binding of QX-222, a blocker known to interact with the *Serine* rather than *Threonine* ring (Charnet et al., 1990), do not affect the channel conduction (Imoto et al., 1991; Villarroel et al., 1991). These experiments indicate that the *Threonine* ring is the most constricted narrowing of the pore. All these data allow us to postulate that it is the *Threonine* ring which corresponds to the small narrowing in our model. The fact that the distance between nitrogen atoms in pentamethonium derivatives (7.2 Å) is close to the distance between adjacent turns of α-helix suggests that *Serine* ring corresponds to the large narrowing in our model. The hydroxyl oxygens in the *Serine* and *Threonine* rings would serve as the nucleophillic binding sites for the cationic heads of the blockers.

Furois-Corbin and Pullman (1987) proposed a three-dimensional model of *Torpedo Californica* nAChR channel basing on the known sequences of M2 segments and the results of chlorpromazine photoaffinity labeling experiments. We reproduced the model by Furois-Corbin and Pullman (1987) and compared the pore dimensions at the levels of *Serine* and *Threonine* rings with the dimensions of the narrowings obtained in our work. There is a remarkable agreement between these two models (Fig. 4). The results of direct docking of bis-ammonium blockers in the model of Furois-Corbin and Pullman are published elsewhere (Tikhonov et al., 1996). The energy profiles for the passage of the blocking molecules through a part of the channel involving the *Serine* and *Threonine* rings have few minima demonstrating different modes of ligand-receptor interaction. The deepest minimum corresponds to a state where both cationic heads of a blocker interact with the *Serine* and *Threonine* rings simultaneously. This type of binding is shown at Fig. 5.

Additional argument in favor of our model was obtained with QX-222, a classical blocker of nAChR. The lowest-energy conformation of QX-222 has the minimal-

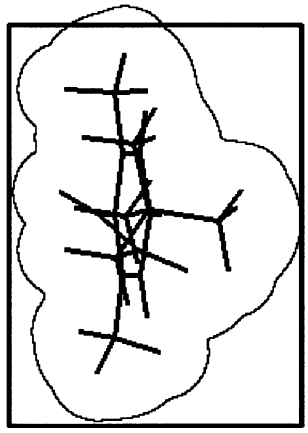


Fig. 6. The minimal profile projection of the lowest energy conformation of QX-222 exceeds the 6.1 × 8.3 Å rectangle. Therefore, the blocker cannot reach the *Threonine* ring.

profile dimensions slightly exceeding the large narrowing (*see* Fig. 6). Therefore, QX-222 should be retarded at the large narrowing (the *Serine* ring) and would not reach the *Threonine* ring. This prediction is in good agreement with the fact that mutations at position 4 (*Threonine* ring) do not affect QX-222 binding, whereas mutations at position 8 (*Serine* ring) and 12 significantly affect the activity of QX-222 (Charnet et al., 1990).

Whether our estimate of the small narrowing dimensions in the neuronal nAChR (5.5 × 6.4 Å) is valid for other subtypes of nAChRs? Muscle nAChRs are also blocked by bis-ammonium compounds, asymmetric blockers being much more potent than the symmetric ones (Tikhonov et al., 1996). However, different subtypes of nAChR exhibit essentially different sensitivity to bis-ammonium blockers, pentamethonium and pentaethonium being much more effective at the neuronal nAChR than at the muscle nAChR (Skok, Selyanko & Derkach, 1989; Zhorov et al., 1991). According to our model, the dimensions of trimethylammonium group are critical for the effective blockade of the neuronal

nAChR. Compounds with less spacious ammonium group would pass through the small narrowing and, hence would not interact effectively with the binding site. Low activity of pentamethonium blockers at the muscle nAChRs may be explained by suggesting that their small narrowing is larger than that in the neuronal nAChR. Experimental estimates of the minimal dimensions of the pore in the muscle nAChR vary essentially. The pore has been approximated by a rectangle of 6.5 × 6.5 Å (Dwyer, Adams & Hille, 1980), by a circle with a diameter of 7.4–8.1 Å (Lester et al., (1992) and by a circle with diameter ≈ 10 Å (Cohen, 1995). It should be noted that the dimensions of the permeating cations used by Dwyer et al. (1980) and by Lester et al. (1992) were estimated without taking into account their conformational flexibility. However, the pore in the muscle nAChR seem to be wider than that in the neuronal nAChR. This would explain different activity of bis-ammonium blockers at these two receptor subtypes.

The above analysis of the structure-activity relationships of bis-ammonium compounds allows us to outline main requirements for their effective noncompetitive block of neuronal nAChR receptor:

(i) a blocker should have a free access to the binding area from the synaptic cleft;

(ii) a blocker should meet some steric hindrances at the level of Serine and Threonine rings that would prevent its passage from the binding area into the more deeper part of the channel;

(iii) a blocker should possess a high complementarity to the binding site that would provide the effective hydrophobic and ion-dipole interactions of both ammonium groups with the corresponding nucleophillic narrowings in the pore.

Thus, the proposed topographical model of the bis-ammonium blockers binding site is in good agreement with the data on the structure and function of nAChR. The approach used in this work may help predict blocking activities of new bis-ammonium compounds. The dimensions of the pore estimated in this works may be useful for further experimental and theoretical studies of nAChR.

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