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The selectivity filter of a ligand-gated ion channel

The helix-M2 model of the ion channel of the nicotinic acetylcholine receptor

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Evidence from electrophysiology and biochemistry supports the hypothesis that the ion channel of the nicotinic acetylcholine receptor is formed by homologous amino acid sequences of all receptor subunits, called helices M2. A model of the ion channel is proposed and the selectivity filter is described as a ring of negatively-charged amino acid side chains [(1988) Nature 335, 645–648] which may undergo conformational changes upon permeation of the cation.

Acetylcholine receptor; Ion channel; Selectivity filter; Molecular modeling

1. INTRODUCTION

Ion channels in excitable membranes are complex proteins whose purpose it is to lower the energy barrier posed by the lipid bilayer to permeating charged particles. Operationally they are composed of two functional components [1]: (i) the gate which determines when and upon which trigger a channel is open; (ii) the selectivity filter which determines which ion may pass the channel.

The gate may be operated by changes of the electrical membrane potential or by a signal molecule. Voltage-gated and ligand-gated ion channels turned out to be structurally different. The primary structures suggest that the former are composed of one to four motifs of six transmembrane helices each, while the latter have probably only four α-helical membrane spanning sequences. Recently several ion channel proteins of both types have been cloned and sequenced, e.g. voltage-dependent sodium [2], potassium [3,4] and calcium [5] channels and ligand-gated nicotinic acetylcholine receptors [6], $GABA_A$ [7] and one subunit of the glycine receptors [8]. The sequence data provided by recombinant DNA techniques were supplemented by site-directed mutagenesis experiments, electrophysiological, ultrastructural (EM) and biochemical data. From this increasing amount of information, pictures of ion channels begin to emerge which allow discussion of the structure-function relationships of these key molecules in many cells.

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2. THE BIOCHEMISTRY OF THE NICOTINIC ACETYLCHOLINE RECEPTOR (AChR)

The AChR is presently the most thoroughly investigated membrane receptor protein [9–11]. It is the prototype of the type I receptors (in which the signal receiving and transducing moieties as well as the effector, the ion channel, are rigidly coupled integral components of one membrane molecule) and is available in large quantities from the electric tissue of *Torpedo* and *Electrophorus*. Its relative molecular mass is about 290 000, including about M_r 20 000 carbohydrate components. Its heteropentameric quaternary structure is $\alpha_2\beta\gamma\delta$. The subunit's primary structures have been deduced from cloned cDNAs encoding the respective subunit precursors. Based on these amino acid se-

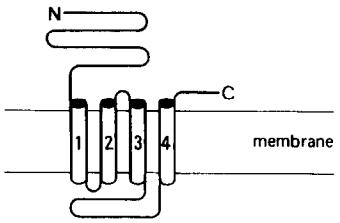

Fig. 1. Predicted transmembrane folding of type I receptors. This class of receptors, including the nicotinic acetylcholine receptor, is characterised by subunits having four transmembrane α-helices. Both the N- and the C-terminus of their polypeptide chains are located on the extracellular side of the membrane.

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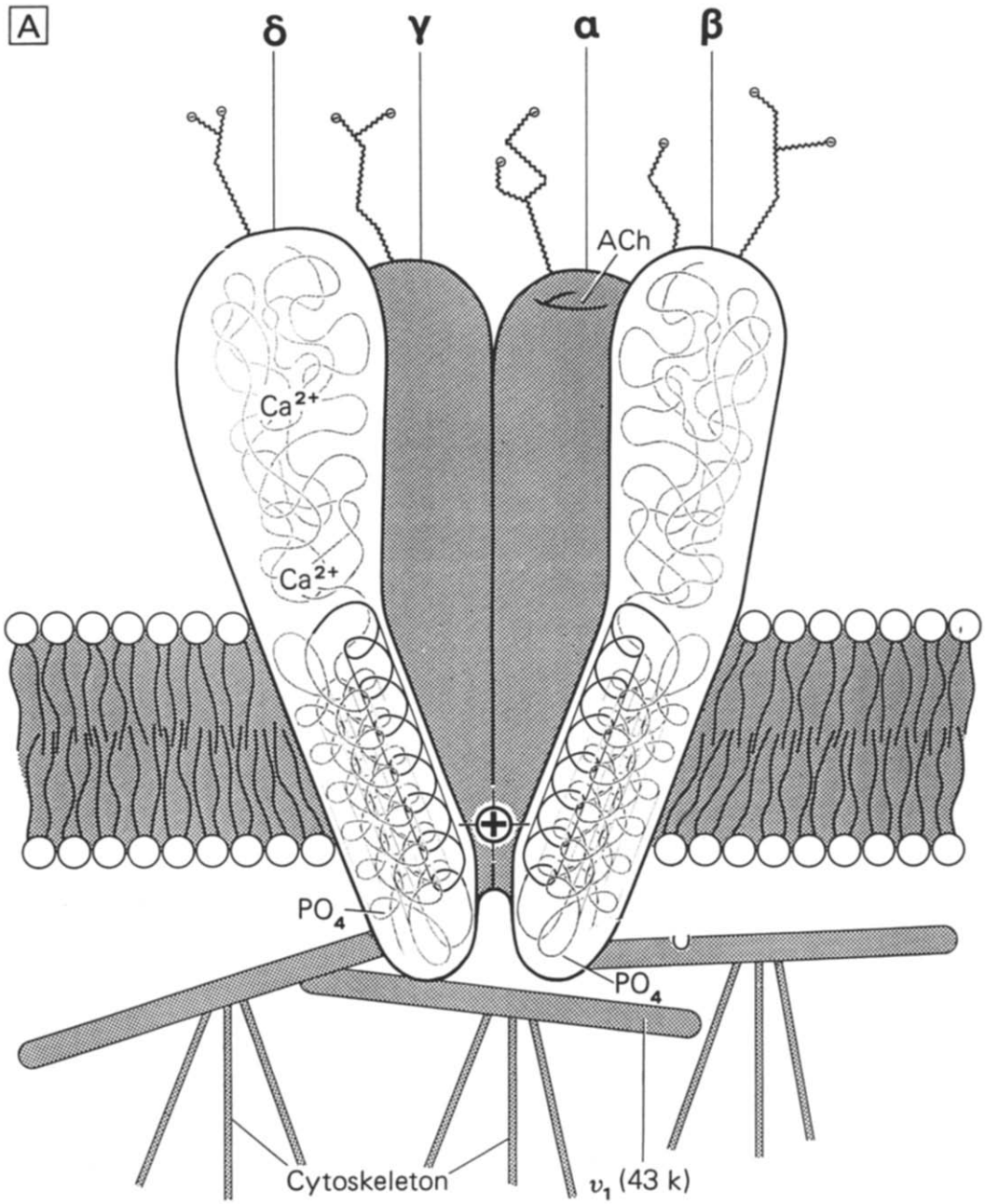


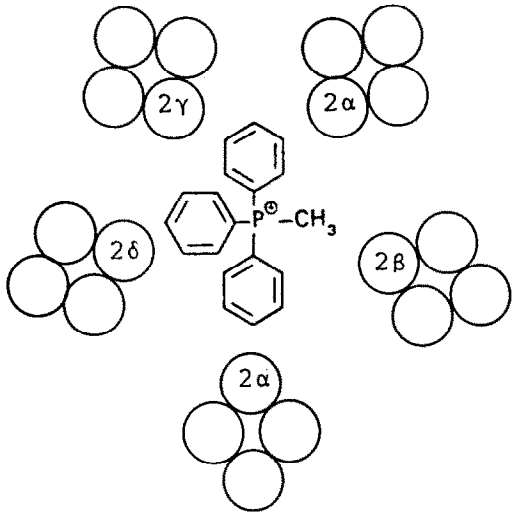
Fig.2. The helix M2-model of the ion channle of the nicotinic acetylcholine receptor (modified from [17]). (A) Longitudinal section; (B) cross-section at the level of the reaction sites with the channel-blocking affinity label triphenylmethylphosphonium ($TPMP^+$). Each of the five receptor subunits is depicted as four circles representing the four transmembrane helices.

quences, several alternative and controversial secondary structures have been predicted, among which those models that portray four membrane-spanning helices (called M1–M4) are the most widely accepted (fig.1, [12]).

All five receptor subunits span the membrane, all are glycosylated on the extracellular surface and all extend both their C- and N-terminal ends to this side of the plasma membrane. Possibly with the exception of the α-subunits, all subunits are phosphorylated multiply. The receptor contains large amounts of Ca^{2+}. The functional roles of these posttranslational additions to the receptor protein including its sialic and fatty acid moieties are at present unclear [11].

Like all receptors, the AChR is operationally composed of three parts: the receiving (R), transducing (T), and the effector part (E) [1]. R was located by affinity labeling on the α-subunits [13], and, more specifically and as far as the competitive antagonist α-bungarotoxin is concerned, on the amino acid sequence comprising residues 184–196 [14]. T has not been assigned to a specific domain of the receptor protein but has, rather,

B



been interpreted as the allosteric property of the total AChR [15,16]. E, the ligand-gated ion channel, has been investigated thoroughly with electrophysiological and biochemical methods. Some of its properties, as reviewed previouslly ([11] and the references therein), are the following: (i) the ion channel is selective for cations; (ii) concerning the type of cation, it is less selective. K^+ permeates only slightly better than Na^+. Divalent cations permeate too. The order of permeability as calculated from the reversal potentials of frog muscle is for monovalent ions Cs > Rb > K > Na > Li and for divalent ions Mg > Ca > Ba > Sr. From the permeability of different-size organic cations the maximum diameter of the open channel has been estimated to be 6.4 Å. This implies that cations could permeate in a partially hydrated form. (iii) H^+ blocks the channel. The dissociating protein side chain, which is possibly the cation-binding group, has a pK value of 4.8. It could be a carboxyl group. (iv) Opening and closing of the channel is an 'all or nothing' event; only open or closed channels are observed. The transition between these states occurs within 20 μs, the time resolution of the patch-clamp technique. Multiple and discrete conductance states (substates) of individual channels have been observed. (v) The mean open time of the channel depends on the temperature, the membrane potential, and on the agonist. It is 0.9 ms with carbamoylcholine, 2.4 ms with acetylcholine and 5.6 ms with suberyldicholine (11–15°C; 60–80 mV). (vi) The mean open time depends on the location of the channel in the cell membrane; extrasynaptic receptor channels have open times which are three to five times longer than those of endplate channels. (vii) The mean open time changes during ontogeny. With synapse maturation it decreases, a process not simply correlated with receptor density. (viii) The conductance of the open channel is 15–30 pS. About 10^4 ions permeate/ms. (ix) The channel is a water-filled pore. The permeating ions probably interact predominantly with water molecules rather than with protein side chains. (x) Myasthenia gravis, an autoimmune disease directed against the endplate and its receptors, alters the number, but not the properties of functional nAChR-ion channels.

Biochemically, the channel was shown to be formed by highly conserved and homologous sequences of the receptor subunits [17]. These so-called helix-M2 sequences are thought to line the channel, contributing to at least part of its wall.

3. THE HELIX-M2 MODEL

A structural model of the AChR-ion channel was proposed (fig.2) which has the following features [18,19]: (i) the channel has an entrance diameter of about 30 Å, which remains constant down to the level of the lipid bilayers; (ii) within the bilayer it narrows to about 11.5 Å at the reaction site of channel-blocking photolabels; (iii) towards the cytoplasm it narrows further to about 6.4 Å, which is the diameter of the largest organic cation able to permeate; (iv) the three diameters: 30 Å, 11.5 Å, and 6.4 Å are accomodated by a funnel-shaped model. The wall of the funnel is formed by the five helices M2 of the receptor subunits; (v) the postulated funnel is hydrophobic in its wide upper part, and hydrophilic in its narrow lower part; (vi) the selectivity filter is located close to the cytoplasmic end of the channel.

These features of the ion channel model stem from a variety of biochemical, electron microscopical and electrophysiological investigations. The model is strongly supported by the following findings.

Recombinant DNA technology in combination with patch-clamp electrophysiology of chimeric receptors constructed from different species expressed in *Xenopus* oocytes supported the special role of helix M2 [20]. Replacing this helix in the δ-subunit of the *Torpedo* receptor by the corresponding helix of the calf receptor resulted in an ion channel with the conductivity of calf AChR, although the vast majority of the protein was from *Torpedo*.

Site-directed mutagenesis in combination with patch-clamp electrophysiology (reviewed in [21]) pointed out the importance of individual amino acid residues within M2 as for example the rings of charged amino acids at both ends of the postulated M2 bundle (see below) and the serine residues labeled by photoaffinity labeling [22].

Planar lipid bilayers incorporate synthetic δ-M2 peptides forming channels with important characteristics of the native receptor channel [23]. In contrast, M1 peptides do not form channels. M1 was also considered to be a component of the AChR channel [24]. Of course,

channel formation by amphipathic peptides may as well be due to nonspecific perturbations of lipid bilayers because of their surfactant properties. Reconstitution experiments of this type therefore have to be interpreted with care. A 21 amino acid peptide having the sequence $(LSSLLSL)^3$ was shown to form similar channels despite its primary structure which is different from M2 [25].

Sequence comparison [18] emphasizes the importance of helix M2 for receptor function (fig. 3A,B). M2 shows only a few differences between α-, β-, γ-, and δ-subunits, and M2 is also highly homologous in e.g. chicken, mouse, *Torpedo* and bovine δ-subunits. Furthermore, in the anion-selective channels of the glycine and $GABA_A$ receptor M2 has also been implicated in channel forming [26,27]. M2 of AChR and of the glycine and GABA receptors show little homology, but both contain serine-OH and other polar groups. The difference is assumed to reflect the difference between cation and anion channels, while the similarity may point to the formation of a water-filled pore [28] supposed to strip off the hydration shell surrounding charged particles.

4. SELECTIVITY FILTER

Fig.3C shows the distribution of polar/charged and hydrophobic side chains within the hypothetical pore-forming helices M2. It is obvious that the lower thirds are more hydrophilic than the upper two thirds. This

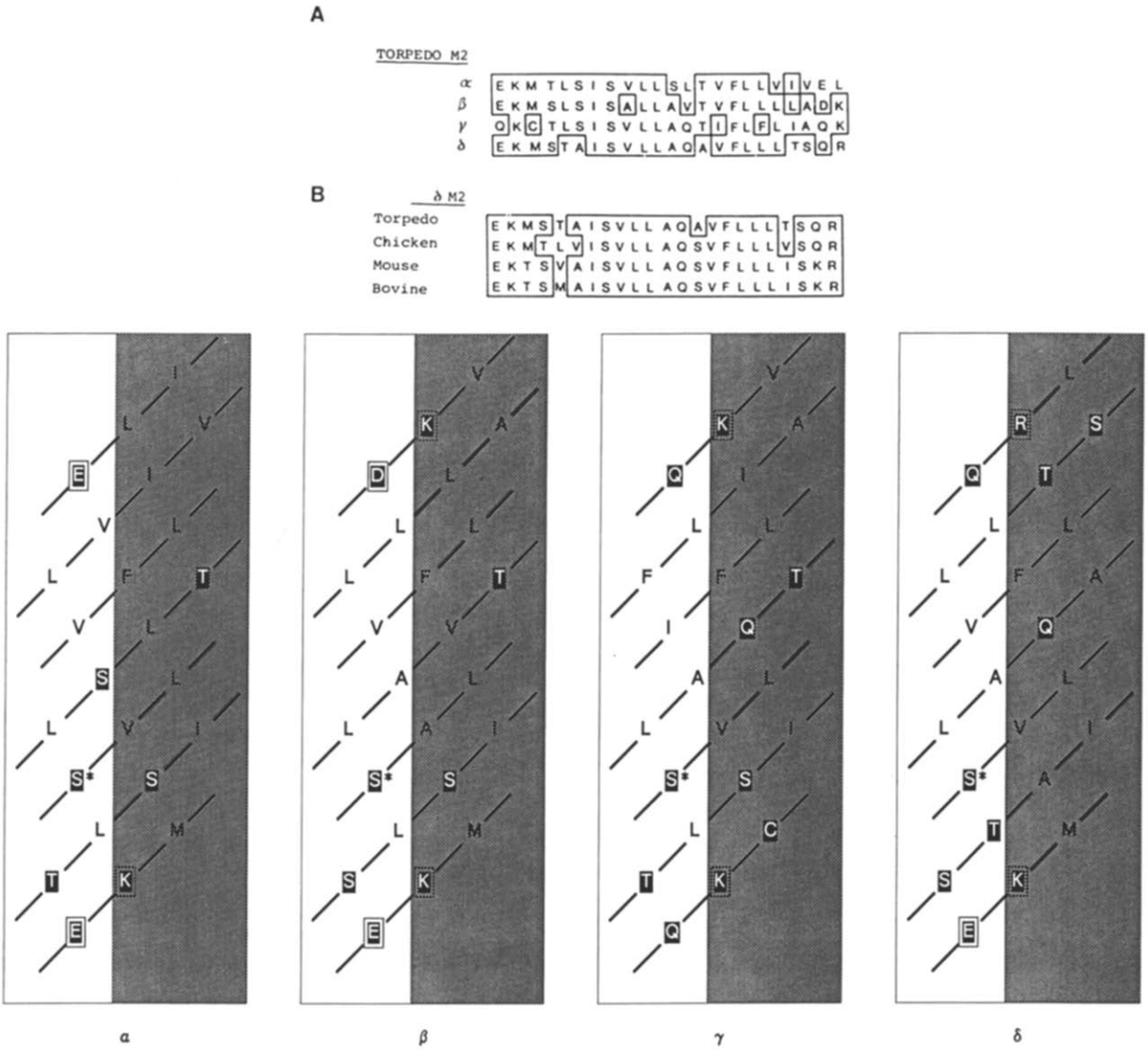


Fig.3. Amino acid sequences forming the helices M2. (A) Homology between M2 of the different subunits of nAChR from *Torpedo*. (B) Homologies of M2 helices of the δ-subunits of nAChR from different species. (C) The helices M2 from *Torpedo* AChR subunits, depicting the distribution of hydrophobic, polar (letters on solid squares) and charged (boxed letters on solid squares). The light part of each helix represents the surface oriented towards the lumen of the channel, shaded parts oriented towards the membrane. Asterisk labels the serine residues reacting with $TPMP^+$.

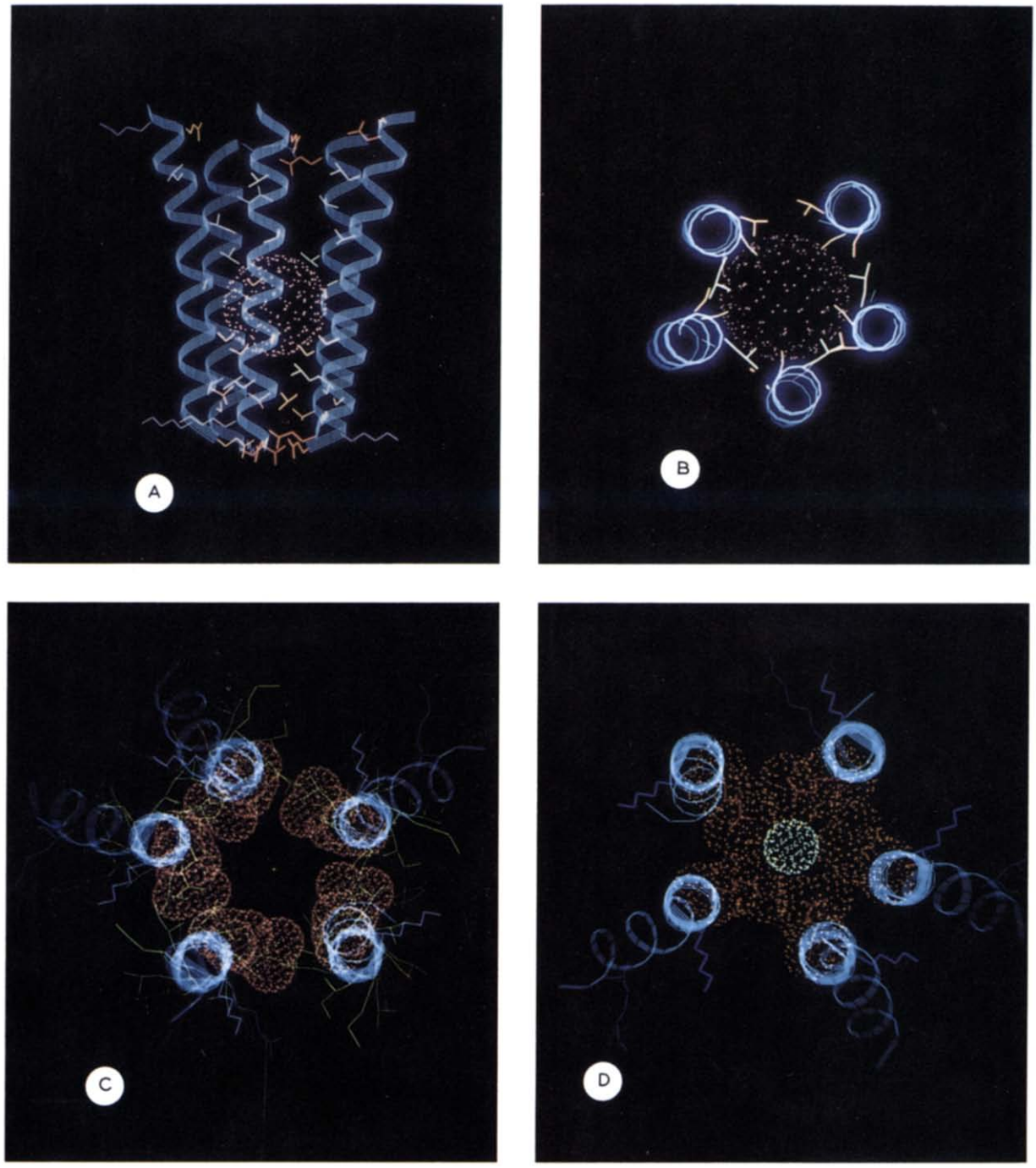


Fig.4. Computer modeling of the ion channel of the nAChR. (A) Side view showing the five M2 helices. The channel-blocking photoaffinity label $TPMP^+$ is represented as a sphere. Note the accumulation of amino acid side chains at the lower end of the helices. According to our hypothesis this is the location of the channel's selectivity filter. (B) Cross-section at the level of the reaction site of $TPMP^+$. (C) Channel's cross-section at the lower end of the five channel forming helices M2. We postulate that the Glu (and Gln) side chains located here (depicted as space filling red spheres) form the selectivity filter. Lysine side chains depicted in blue. (D) Selectivity is based on a conformational change of the side chains brought about by the permeating cation (green sphere, see text).

may be of significance for the structure of the selectivity filter which we postulate to be located close to the cytoplasmic end of the pore ([29] and this paper). With much structural information at hand it is tempting to model a hypothetical channel. Fig.4A shows a side view of the five M2 helices of the AChR subunits, arranged around a five-fold axis of symmetry. Fig.4B shows a cross-section at the binding site of a channel-blocking cation (where the diameter is supposed to be 11.5 Å), and in fig.4C a cross-section at the level of the Glu and

Gln residues underneath where the side chains of these residues seem to be of special importance as we have recently pointed out [29] and as was postulated on the basis of the site-directed mutagenesis experiments [30]. The diameter of the ring formed by the carboxylate (α, β, δ) and carboxylamide (γ) groups is approximately 7.5 Å, which is slightly wider than the 6.4 Å suggested by ion permeability experiments [31]. However, upon metal ion transfer through the channel the Glx side chains might undergo conformational changes from gauche(+)/*trans* to gauche(−)/*trans* for their X_1 and X_2 torsion angels (fig.4D). This confirmation is usually less populated but also permitted [32]. The diameter of the channel opening would then reduce to approximately 4.5 Å, a value slightly smaller than that observed in permeability experiments with organic cations. Of course, such a relatively close approach of Glu side chains would only be possible if their unfavorable charge interactions were to be neutralized by binding of the cation being transported. It is also conceivable that these negatively charged side chains form one of the many Ca^{2+}-binding sites of the AChR. The permeant sodium or potassium ions would then have to remove the cation bound at this site.

A similar situation has been found in the insulin hexamer where the carboxylate groups of the 6 Glu B13 side chains cluster around a central cavity making short contacts with each other [33]. Upon binding of Cd^{2+} or Ca^{2+}, these Glu side chains undergo conformational changes as has been found both by crystallography [34] and NMR spectroscopy [35].

It is obvious that such a ring of charged and bulky amino acid side chains could represent the bottleneck of the channel, the selectivity filter which is rate limiting for the diffusion of ions through the membrane. This assumption is supported by site-directed mutagenesis experiments in connection with patch-clamp analysis of mutated channels [26]. By replacing charged amino acids three rings of negative charges have been identified which affect channel conductance. One ring is formed by the side chains of α-Glu 262, β-Asp 268, together with the uncharged side chains of γ-Gln 271 and δ-Gln 276. This ring is located at the extracellular end of the helix-M2 funnel. A second ring located at the cytoplasmic end (it is actually located on the loops connecting helices M1 and M2) is formed by α-Asp 238, β-Asp 244, γ-Gln 246, δ-Gln 252. A third ring is assumed to form 'the narrowest part of the channel' and 'is therefore more important in determining the rate of ion transport than the two other anionic rings' [26]. It is formed by the negatively charged side chains of α-Glu 241, β-Glu 244, δ-Glu 255, and the polar side chain of Gln 250.

Finally, support for the selectivity filter being formed as proposed above comes from electron microscopy [36]. A constriction at about the location postulated on the basis of photoaffinity labeling experiments [17,37] has been observed, which is too narrow to be resolved by the electron microscope.

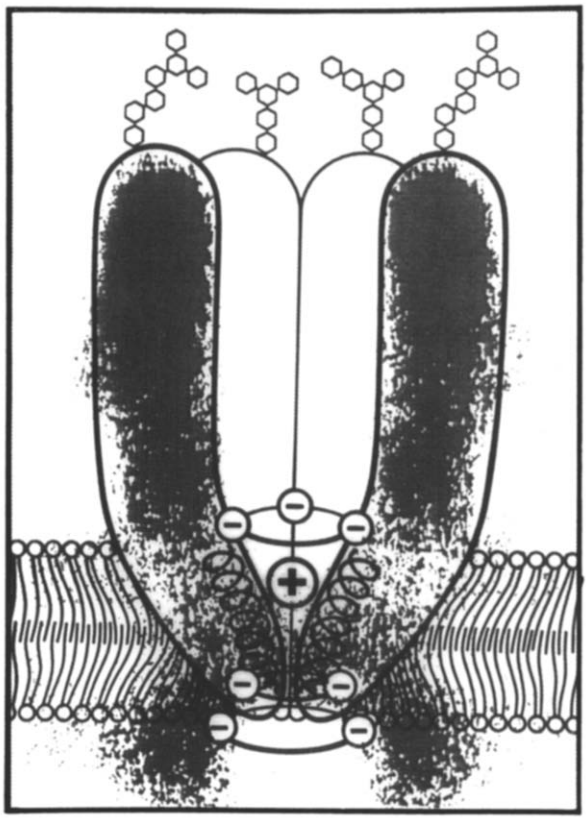


Fig.5. nAChR and its helix M2-ion channel. (Superposition of models derived from electron microscopy [36], electrophysiology [30], and biochemistry [17].)

In fig.5 the results from electron microscopy, electrophysiology and biochemistry are superimposed. The experimental evidence from the different approaches seems to support the hypothesis that the selectivity filter of the AChR-ion channel is formed by a ring of negatively charged amino acid side chains which may undergo conformational changes during permeation of the ion. This model does not exclude the possibility that other transmembrane sequences of the receptor protein besides M2 affect the ion channel. From energy profile calculations such effects have been deduced [38].

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