Minireview

Structure and action of the nicotinic acetylcholine receptor explored by electron microscopy

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Abstract The nicotinic acetylcholine (ACh) receptor is the transmitter-gated ion channel at the nerve/muscle synapse. Electron microscopical experiments on isolated postsynaptic membranes have determined the structure of this channel and how the structure changes upon activation. When ACh enters the ligand-binding domain it initiates rotations of the protein chains on opposite sides of the entrance to the membrane-spanning pore. These rotations are communicated to the pore-lining α-helices and open the gate – a constricting hydrophobic girdle at the middle of the membrane – by breaking it apart. The movements are small and involve energetically favourable displacements parallel to the membrane plane.

Key words: Acetylcholine receptor; Ion channel; Gate; Electron microscopy

1. Introduction

Nerve cells communicate rapidly with their target cells by releasing neurotransmitter in regions of close apposition known as the chemical synapse. The chemical transmitter binds to ion channels in the target cell membrane and opens them transiently, allowing selected ions to flow through and effect a change in membrane potential. The nicotinic acetylcholine (ACh) receptor, at the nerve/muscle synapse, is the best characterised transmitter-gated ion channel. Its structure is now known in near-atomic detail, and we understand in outline how the channel opens when the neurotransmitter, ACh, binds to it.

In contrast with the other proteins discussed at this symposium, our knowledge of the structure of the ACh receptor is based largely on data from electron images. Electron microscopy as a technique has unique advantages for the study of complex membrane proteins. The protein can be imaged in its native lipid surroundings and ionic environment, thus avoiding partial denaturation or refolding, as is likely to occur in the presence of detergent and/or exposure to unnatural salts. And transient events, like the opening of an ion channel, can be examined by reproducing the physiological process in vitro and freeze-trapping the reaction on the microscope grid.

The detailed structure of the receptor in the closed- and open-channel forms, and relevant developments in electron microscopy, have been described in several recent publications. Here I give a brief account of this background, and discuss the perceived principles by which the channel regulates ion flow across the membrane.

2. Imaging *Torpedo* membranes

Postsynaptic membranes from the (muscle-derived) electric organ of the *Torpedo* ray have been the source of material for the structural studies. The isolated membranes convert readily into long tubular crystals, having receptors and intervening lipid molecules arranged on a helical surface lattice [1,2]. This packing is similar to that found in vivo [2,3], and the dimensions of the tubes make them suitable for imaging in thin films of amorphous ice [4] and three-dimensional structure determination [5,6].

Radiation damage is a major limiting factor in electron microscopy, resulting in a 'permissible' dose which is too low to define with good statistics the details in a single molecule. However, the signal/noise ratio can be improved by averaging the information from the many identical molecules arranged precisely on a lattice [7,8], because the genuine features common to each molecule are then reinforced, while the statistical variations between them are smeared out.

Use of this averaging principle to enhance signal/noise is fundamental to all high-resolution electron microscopy, whether one is dealing with three-, two- or one-dimensional crystals, or with single particles. But additional practical aspects need to be taken into account to achieve optimal results. First, the images must be of the highest electron optical quality – as can be obtained only with a high voltage field emission electron source and an exceptionally stable cold stage [9]. Second, in averaging individual molecules, a high accuracy in alignment is required – necessitating the measurement and correction of distortions of the crystal lattice [10]. In practice, an averaged structure derived from 5×10^5 receptors (178 images of tubes) was sufficient to give a resolution of 4.6 Å [11]. About twice as many molecules were needed to achieve 4 Å [12].

3. Freeze-trapping transient events

How does the structure of the receptor change when it converts to the open-channel form? At the synapse, the chan-

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nels open within about 20 μs after the ACh has bound, and achieve close to 100% probability of being open until the ACh is degraded or until desensitisation takes place [13]. Desensitisation occurs within ~20 ms in the continued presence of ACh [14]. Thus to convert the receptor to the open-channel form and to retain this form so that it can later be imaged in the electron microscope, an extremely brief reaction with ACh is needed, followed by rapid freezing to trap the structural response.

We found that the short ACh receptor reaction time could be accomplished using an atomiser spray, combined with rapid freezing [15,16]. With this technique, a suspension of the tubular membranes is applied as a thin aqueous film on a microscope grid, and the grid is plunged into liquid nitrogen-cooled ethane such that a burst of spray droplets containing ACh impinges on it ~5 ms before it hits the ethane surface (Fig. 1A). The ACh mixes with the thin aqueous film over the 5 ms period, opening the channels over a time frame that is too short for a significant fraction of them to desensitise.

4. Architecture of the receptor

The ACh receptor is a 290 kDa, hetero-pentameric glycoprotein (subunit stoichiometry: α, γ, α, β, δ; [17,18]). It consists of a cation-selective membrane-spanning pore, framed on either side by a large N-terminal ligand-binding domain and a smaller intracellular domain, giving it a total length normal to the membrane plane of about 170 Å. The ligand-binding domain has two binding pockets for ACh, which are located in the α subunits at or near the α–γ and α–δ subunit interfaces and on opposite sides of the pore. The intracellular domain contains a central vestibule, having narrow lateral openings [11] which act as electrostatic filters for the ions [11,19]. The pore contains the gate, which opens when ACh occupies both binding pockets.

The receptor subunits in the ligand-binding domain are each organised around two sets of β-sheets packed into a curled β-sandwich and joined through a disulfide bridge [20,21]. The encircling chains together create a long, ~20 Å diameter central vestibule (Fig. 1B). The subunits in the membrane-spanning part are each made from four α-helical segments, and short connecting loops [12]. The helical segments are arranged symmetrically, forming an inner ring of helices which shape a narrow pore, and an outer shell of helices which coil around each other and shield the inner ring from the lipids. As shown in Fig. 1B, the protein chains of the ligand-binding domain overlie the outermost helices, leaving only the inner ones exposed directly to the ions.

5. Membrane-spanning pore

The inner pore-lining helices, M2, are 40 Å long (including portions outside the membrane [12]), and tilt inwards toward the central axis until they reach the middle of the membrane, where they are slightly kinked. At this level in the closed pore, the set of M2 helices come together separately from the set of outer helices, creating intervening water-filled spaces (Fig. 2A). These intervening spaces play an important role in the gating mechanism (see below), by providing room for the M2 helices to move relative to the outer lipid-facing protein wall [16].

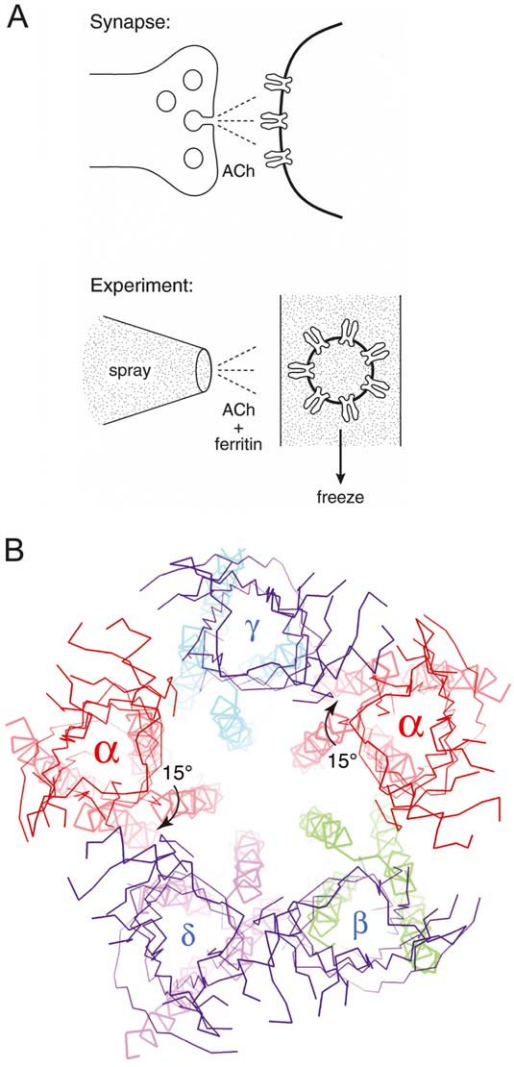

Fig. 1. Electron microscopy of the ACh receptor. A: Channel-opening event at the synapse (above) and the experiment to capture this event in vitro (below). In the experiment, the spraying of ACh-containing droplets onto an aqueous film containing isolated postsynaptic membranes mimics the release of neurotransmitter onto the target cell, and rapid freezing traps the channels in the open state. Ferritin marker particles, included in the spray solution, reveal the regions of the film where the droplets have impinged and spread, and so identify the membranes containing open channels. Reproduced from [16]. B: The polypeptide chains (α-carbon traces) fitted to the electron image density maps, as viewed from the synaptic cleft. The ligand-binding domain (uppermost) is composed mainly of β-sheet, while the domain forming the membrane-spanning pore (underneath) has an α-helical fold. Binding of ACh to the ligand-binding domain initiates rotational movements in the α subunits (arrows) which are communicated to the inner helices shaping the pore.

The closed pore has two special properties at the middle of the membrane. First, it is maximally constricted in this region due to the small separation of the M2 helices and to the presence of bulky hydrophobic side chains. Second, it is essentially symmetrical in this region, due to equal side-to-side hydrophobic interactions between equivalent surfaces of homologous residues. The contacts are at two levels: one involving leucine (at αL251) with the neighbouring alanine (or serine) side chains (at αS252), and the other phenylalanine (at

αF256) with the neighbouring valine (or isoleucine) side chains (at αV255).

These symmetrical side-to-side interactions bring together the side chains on the neighbouring helices to make a tight hydrophobic girdle around the pore (Fig. 2A; see also [8]). This girdle creates an energetic barrier to ion permeation across the lipid bilayer [22] because its ~6 Å diameter bore is too constricting for a sodium or potassium ion to pass through while retaining its first hydration shell, and the ion cannot readily lose part of this shell in the absence of polar surfaces that would substitute for water. Since there is no other such barrier or protein occlusion that would block the flow of ions, the girdle must correspond to the gate of the pore. Other ion channels, including the mechanosensitive channel MscL [23] and the inward rectifier potassium channel KirBac1.1 [24], also appear to have constricting rings of hydrophobic side chains forming the gate of the closed pore.

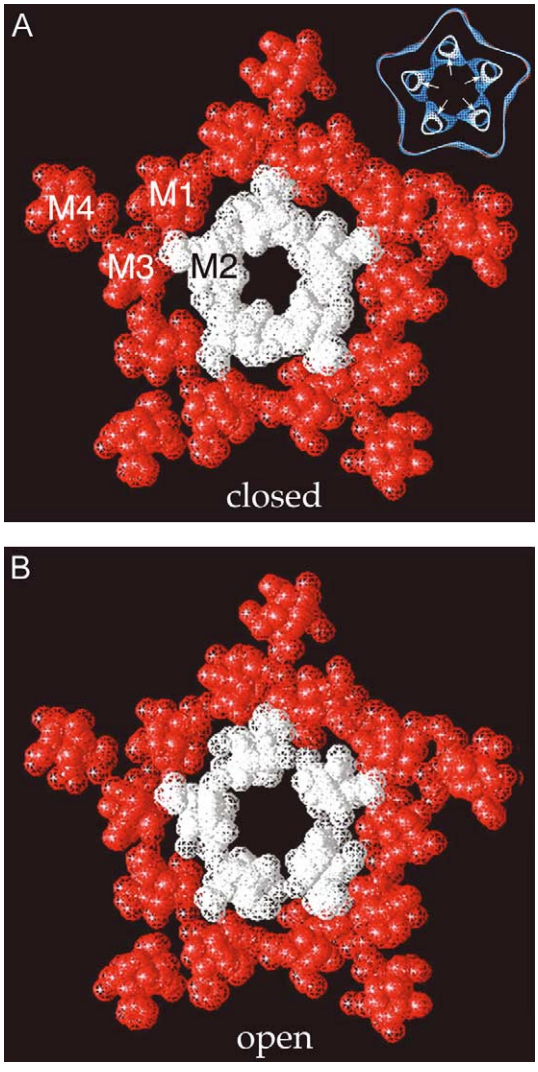

Fig. 2. Cross-sections at the gate in middle of the membrane, showing van der Waal's surfaces of the atoms encircling the closed (A) and the open (B) pore. The open pore is modelled by applying 15° clockwise rotations to each of the inner helices (coloured white). The change in structure, involving a widening of the pore by ~3 Å, is consistent with the changes observed experimentally at 9 Å resolution [16]; these changes are shown in the inset (blue: closed channel; white and brown: open channel). The view is from the synaptic cleft; individual helices, M1–M4, are identified on one of the subunits.

6. Activation involves rotations in the α subunits

Analysis of the tubular membranes that reacted briefly with ACh, as described above, yielded a 9 Å structure of the receptor in the activated, open-channel form [16]. An initial comparison of this structure with that of the closed channel showed that ACh initiates two interconnected events in the ligand-binding domain. One is a local disturbance in the region of the binding pockets, located about 35 Å from the membrane surface and about 50 Å from the gate. The other is an extended allosteric change, involving small axial rotations in the two α subunits, linking the local disturbance to the membrane.

AChBP, a soluble homo-pentameric ACh-binding protein [20], has a similar structure to that of the ligand-binding domain and made possible a more detailed description of the extended conformational change. We found that, to a good approximation, there are two alternative conformations of the receptor subunits: one characteristic of the two ligand-binding α subunits before exposure to ACh, and the other characteristic of the remaining three non-α subunits and the (ligand-bound) protomer of AChBP [21]. Substitution in the three-dimensional maps of α by the non-α subunits mimicked the changes seen on activation. This suggested that the conformational change is like that of an allosteric protein converting from the T state to the R state [21,25]. That is, the α subunits are distorted initially by their interactions with neighbouring subunits; the energy of ACh binding then overcomes these distortions, allowing the subunits to convert to the 'relaxed' non-α form and so making the whole assembly more symmetrical.

The conversion of the α subunits induced by ACh is accomplished through relative movements of the inner and outer parts of the β-sandwich around the disulfide bridge. Most significantly, the inner, pore-facing parts rotate by 15° about an axis oriented normal to the membrane plane.

7. The rotations open the pore

While the bulk of the ligand-binding domain overlies the outer shell of helices in the membrane, the short loops joining the first two β-strands of the inner sheets are positioned close to the ends of their respective apposing M2 helices. In the α subunits, valine side chains on these loops dock into the hydrophobic pockets made by the ends of these helices (Figs. 1B and 3).

This pin-into-socket interaction between the inner sheet and M2 helix of the α subunit represents the sole direct link made between the moving elements in the two domains, both of which rotate in the same sense when the pore opens [16,21]. The rotational movements of the inner sheets must therefore be communicated through this connection and along the pore-lining M2 helices to the gate at the middle of the membrane.

The freeze-trapping experiments had shown that pore widens by several Angstroms at the middle of the membrane when the receptor is activated (Fig. 2A, inset), and therefore that the hydrophobic girdle is broken apart by the rotational movements in the two α subunits. How does this occur? In principle, it is possible for the twisting of just two of the M2

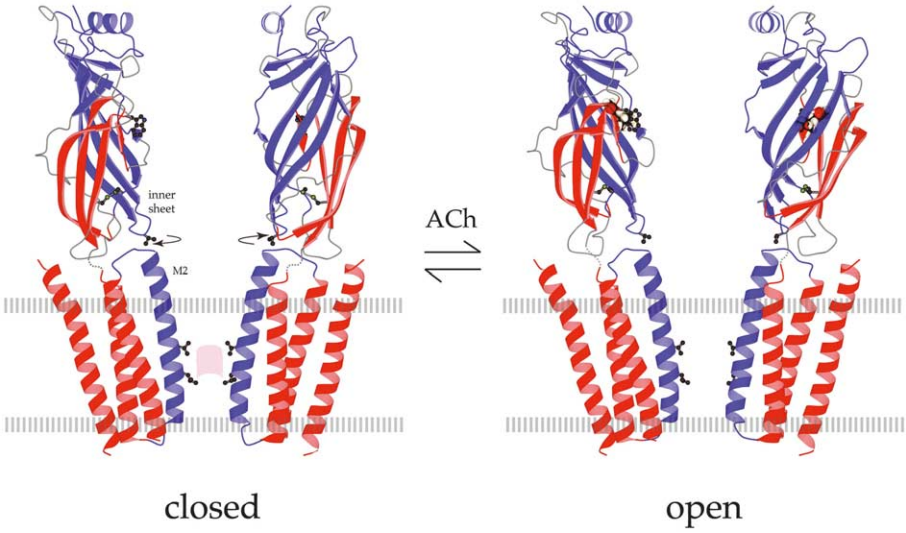

Fig. 3. Conformations of the closed (left) and open (right) channels, determined by fitting the polypeptide chains to the electron image density maps [12,16,21]. Binding of ACh opens the channel by initiating rotational movements (arrows) of the inner β-sheets of the α subunits in the ligand-binding domain. These movements are communicated to the inner (M2) helices lining the pore and break apart the gate – a hydrophobic girdle in the middle of the membrane – so that ions can flow through. A tryptophan side chain in the ligand-binding domain identifies the ACh-binding region; a valine side chain links the inner sheet to the inner helix; leucine and valine side chains on the inner helices make the gate (pink patch); the locations of the membrane surfaces are indicated by broken lines; the relevant moving parts are in blue. Most of the loop regions in the ligand-binding domain were not able to be fitted to the density map [21] and are modelled on the structure of AChBP [20].

helices, on opposite sides of the pore, to disrupt the girdle asymmetrically by perturbing four out of the five sets of stabilising interactions around the ring. However, the integrity of the girdle, like that of other symmetrical assemblies, is likely to depend on equal interactions between each of its components. Thus when only one of these components is sufficiently perturbed the neighbouring component will lose a set of interactions maintaining its position in the assembly, setting in train cooperative changes that cause the whole structure to switch.

Almost certainly, the M2 helices respond to the triggering action of the α subunits by 'collapsing' back concertedly against the outer protein wall. The evidence is as follows: First, the three-dimensional structure in the membrane has near-perfect five-fold symmetry, and this feature is equally characteristic of the receptor in the closed- and in the open-channel forms [16]. Second, the observed dimensional changes accompanying opening of the pore (Fig. 2A, inset) can only be modelled satisfactorily by applying equivalent 15° rotations to each of the M2 helices (Fig. 2B). Third, electrophysiological experiments on ACh receptors expressed in oocytes have shown that the opening sensitivity of the pore is increased by an equivalent amount, independent of the subunit involved, when the gate-stabilising residue at αL251 is substituted by a serine residue [26]. The α subunits are not special compared with the other subunits at this level in the receptor.

8. Conclusions

The structure and gating mechanism of the ACh receptor channel have been explored by electron microscopy of intact postsynaptic membranes. The analyses show that the protein subunits have a predominantly β-sheet fold in the ligand-binding domain and an α-helical fold in the pore domain (Fig. 3, left). The binding of ACh initiates rotations of the inner β-sheets of both α subunits. These rotations are communicated to the inner, pore-lining helices and cause them to switch cooperatively into an alternative configuration. The change in configuration destabilises the gate – a hydrophobic girdle formed by weak side-to-side interactions between the inner helices – and opens the pore (Fig. 3, right).

This mechanism to open the pore involves distinct structural changes associated with the different three-dimensional folds composing the two domains. The ligand-binding domain is a trigger, making efficient use of ACh to focus the relevant movements toward either side of the entrance to the pore. The pore domain is a robust on–off switch, using these movements to alter the relative stability of alternative configurations of helices around the pore. At the same time, the pore domain partitions the moving elements away from the influence of the surrounding lipids. Other ion channels involved in fast synaptic transmission may work by similar physical principles: these include members of the nicotinic superfamily – the serotonin, γ-aminobutyric acid, glycine and neuronal ACh receptors – and, less directly, members of the glutamate receptor superfamily and ATP-gated ion channels.

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