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REVIEW

Opened by a twist: a gating mechanism for the nicotinic acetylcholine receptor

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Introduction

Nicotinic acetylcholine receptors (nAChRs) play a central role in intercellular communications in the brain and at the neuromuscular junction. They are involved in nicotine addiction as well as in cognitive processes such as attention, access to consciousness, learning and memory and their pathologies include autism, schizophrenia, Parkinson and Alzheimer's disease (references in Changeux and Edelstein 2005). Understanding the functional organization of the nAChR at the atomic level should thus be a source of insights for the development of new drug therapies.

nAChRs are members of the cys-loop superfamily of ligand-gated ion channels. They are pentameric integral membrane proteins with a fivefold axis of pseudo-symmetry perpendicular to the membrane. Hetero- or homo-pentamers are based on the association of α1-10, β1-4, γ, δ and ε subunits which allows a large pharmacological variability and a diversity of control over activation and desensitization kinetics. The large number of subunits and of their combinations could also account for the diverse subunit distribution patterns, at the cellular and subcellular levels (Le Novere et al. 2002a).

Each subunit can be subdivided into two principal domains: extracellular and transmembrane. The extracellular domain (ECD) carries the acetylcholine (ACh) binding site at the boundary between subunits and the transmembrane ion pore domain (IPD) delineates an axial cation-specific channel (Corringer et al. 2000; Wilson and Karlin 2001). These topologically distinct domains are allosterically coupled to each other. Therefore, nAChRs possess the structural elements necessary to convert a chemical signal, typically a local increase of extracellular ACh concentration, into an electrical signal generated by the opening of the ion channel.

Electrophysiological analysis of nAChRs at the synapse shows that rapid release of ACh promotes fast opening of the channel, and that a prolonged application of nicotinic agonists and antagonists leads to a slow decrease of the response amplitude or "desensitization". Several kinetic models have been proposed for the physiological processes of activation and desensitization (Katz and Thesleff 1957) and for the structural transitions related to general mechanisms of allosteric transitions known to mediate signal transmission (see Changeux and Edelstein 2005; Edelstein and Changeux 1996; Perutz 1989). The concerted Monod–Wyman–Changeux (MWC) model (Monod et al. 1965) extended to the nAChR assumes that the receptor protein exists spontaneously in reversible equilibrium between a basal state (B), an active open channel state (A) stabilized by ACh and nicotinic agonists, and one or several high affinity desensitized state(s) with a closed channel (D) (see Changeux and Edelstein 2005; Edelstein and Changeux 1996).

This mini-revue presents insight on the structure and gating of nAChR, based on the structures obtained experimentally

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since 2001. Several aspects of the nAChR structure/function relationships are already described in recent reviews presenting more specifically the effect of lipids (Barrantes 2004), toxin binding (Dutertre and Lewis 2006), molecular dynamics (Xu et al. 2006b) and experimental data (Mourot et al. 2006; Sine and Engel 2006). Here, the focus is on the study of the gating mechanism by normal mode analysis initiated by our group (Taly et al. 2005, 2006) and subsequently developed by other groups (Amiri et al. 2005; Cheng et al. 2006a). First, available structures and modelling of the receptor are presented. Second, the models for the transition from the B to the A state (gating) using normal mode analysis are described. Finally, these results are compared to those obtained by experiments and using other modelling techniques.

Available structures and models constructed by homology modelling

Structures of the acetylcholine binding protein

The first structure available to investigate the structure and conformation of nAChRs was that of a protein from the fresh water snail *Limnea stagnalis* the acetylcholine binding protein (AChBP) (Brejc et al. 2001; Sixma and Smit 2003). This protein has been proposed to modulate the concentration of ACh in the mollusc's synapses (Smit et al. 2001). AChBP has a significant sequence homology with nAChRs ECD. The structure of AChBP has therefore been used to derive models of the ECD of nAChRs by comparative modelling (Le Novere et al. 2002b).

AChBP structures may also help in identifying nAChR allosteric changes. Following the analysis of all known AChBP structures it was observed that they can be classified into only two categories, either "open" or "closed" with respect to the conformation of the C loop (see Fig. 1a, b to localize the loops) which caps the binding site (Dutertre and Lewis 2006). Interestingly, all structures that should correspond to the resting state display an "open" C-loop whereas the others have a loop C in a "closed" conformation (Dutertre and Lewis 2006). The remainder of the structure is however rather unmodified.

Structure of the ion pore domain

The second source of information on the structure of the nAChRs has been derived from the study of the muscle

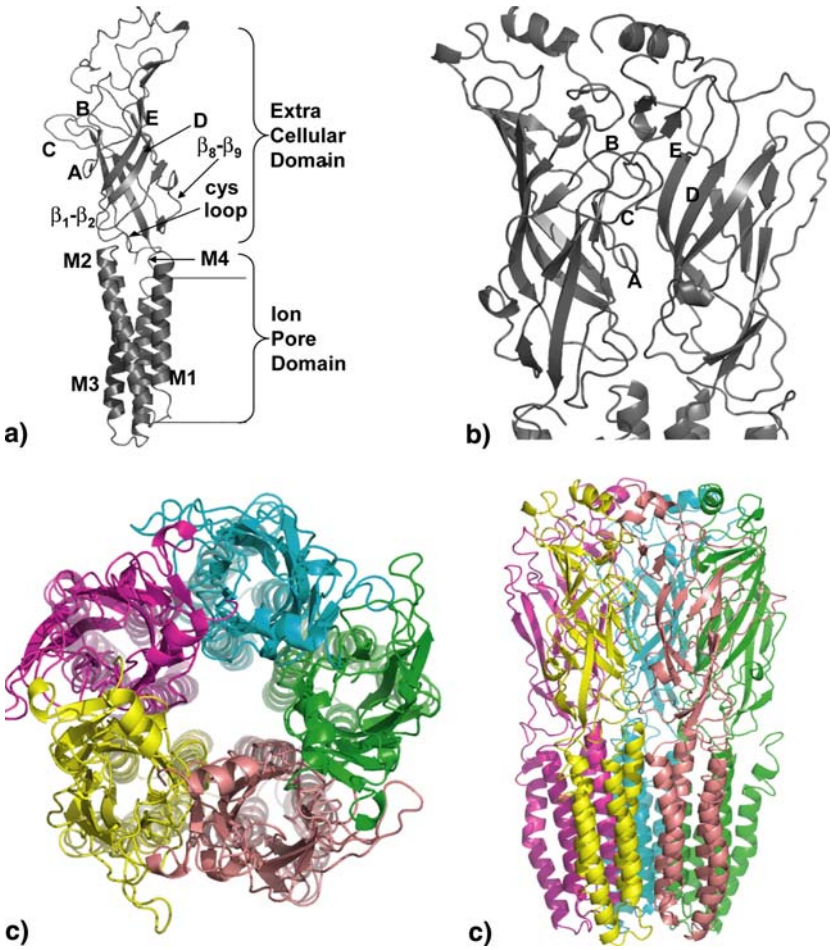

Fig. 1 Model of the α7 nicotinic acetylcholine receptor obtained by comparative modelling. **a** Structure of one subunit of the α7 nAChR model. **b** *Close view* of the binding site. For clarity only two monomers are represented. α7 nAChR pentamer in *top view* (**c**) and *side view* (**d**)

receptor from the electric ray *Torpedo californica*. The electric organ of *Torpedo* contains high amounts of muscle nAChR which made it possible to construct high resolution electron densities maps by electron microscopy (Unwin 2000, 2003). A model for the IPD of *Torpedo* nAChR was derived from electron microscopy images at 4.6 Å resolution and using the structure of AChBP in the position of the ECD (Miyazawa et al. 2003).

This structure allowed to study the dynamics of the IPD (Corry 2004, 2006; Hung et al. 2005; Saladino et al. 2005; Xu et al. 2005), the blockage of ions flow (Beckstein and Sansom 2006) as wells as the binding site of the local anaesthetics halothane (Vemparala et al. 2006) and the inhibitor chlorpromazine (Xu et al. 2006a). Furthermore, using the structure of AChBP as a template for the ECD and the structure of the muscular nAChR as a template of the IPD a model of the α7 nAChR has been constructed (Pons et al. 2004; Taly et al. 2005) (Fig. 1). The quality of the interface between the ECD and IPD was validated by the agreement of the model with the results of cross-linking experiments (Taly et al. 2005) and an exhaustive analysis of the possibilities in associating ECD and IPD (Amiri et al. 2005).

Structure of the muscular nAChR from Torpedo

The electron microscopy structure of *Torpedo* muscle receptor was latter refined at a 4 Å resolution (Unwin 2005) yielding a model of both the ECD and IPD of the *Torpedo* nAChR (Fig. 2). This experimentally derived model is very similar to that proposed for the α7 nAChR (compare Figs. 1c, d, 2, see also ref (Cheng et al. 2006a)). Indeed, the RMSD computed between Cα of equivalent residues is ~2.5 Å which is in the range of what is expected for the comparison of two proteins which share 30% identity (Cozzetto and Tramontano 2005).

The electron microscopy data was proposed to represent a basal state of the nAChR protein (Unwin 2005) although this interpretation remains a matter of controversy (Paas et al. 2003). Noteworthy, the structure of the C-loop of the α1 subunits is in an "open" conformation, while that of the other subunits is "closed" (Unwin 2005). Opening of the C-loop was previously shown to be necessary for the binding of α-neurotoxins (Fruchart-Gaillard et al. 2002; Le Novere et al. 2002b), which is generally thought to take place in the basal state (Bertrand et al. 1992; Middleton et al. 1999; Moore and McCarthy 1995). It has been shown very recently that *Torpedo* nAChR model (Unwin 2005) is compatible with the binding of Cbtx (Konstantakaki et al. 2007) which is in favour of a basal state structure.

The structures presented above have a resolution of 4 Å which is remarkable for a structure obtained by cryo-electron microscopy. However, this structure is not at atomic resolution but rather at medium resolution imposing limitations to the modelling studies based on it. The detailed position of amino-acids side chains is uncertain which prevents, at this stage, performing calculations that require high quality structures: electrostatics interactions and/or surface complementarity for docking, for example. Studies of the ions flows in the channel lumen are also difficult for the same reason. The effect of the medium resolution is, however, less dramatic for the studies presented below which are performed with a coarse-grained model. Therefore it can be

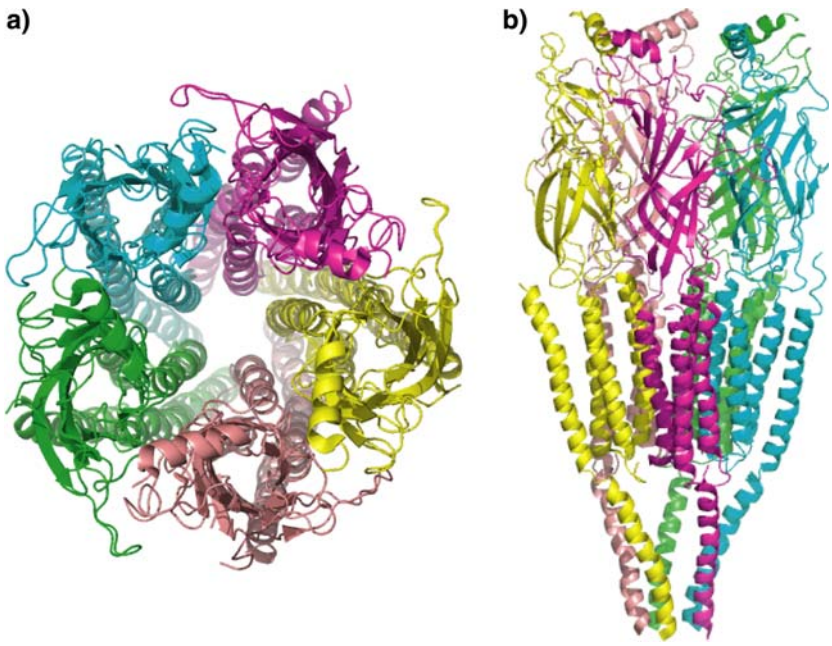

Fig. 2 Model of the *Torpedo* muscle nicotinic acetylcholine receptor obtained by cryo-electron microscopy (PDB code: 2BG9). The nAChR pentamer is viewed in *top view* (**a**) and *side view* (**b**)

anticipated that, although the structures should not be analyzed at the atomic level, the general trends should be correct.

The gating mechanism following normal mode analysis: a quaternary twist mode

Functional motions of proteins can be studied by coarse-grained normal mode analysis. Normal modes analysis approximates the surface of the conformational landscape and gives a decomposition of the movements into discrete modes. This is a method of interest as it is easily run on modern computers and the quality of predictions is sustained as they correlate to experimental observations (Bahar and Rader 2005).

The first study of the nAChR by normal mode analysis (Taly et al. 2005) was performed using the elastic-network model (Tirion 1996) as simplified by Hinsen (1998) who showed that it was possible to use C-α atoms only. Normal mode analysis using this approximation was shown to give a fair description of proteins flexibility (Bahar et al. 1997; Bahar and Rader 2005; Tama et al. 2002).

The first mode was selected because it produces a structural reorganisation compatible with channel gating: (a) an opening of the pore and (b) distributed changes in both the membrane and the extracellular domains, indicative of a coupling between the ACh binding site and the ion channel (Taly et al. 2005).

The first mode was found to be a concerted symmetrical quaternary twist motion of the protein with opposing rotations of the upper (extracellular) and lower (transmembrane) domains (Fig. 3; the same transition is shown in supporting movies 1 and 2). Still significant reorganizations are observed within each subunit, that involve their bending at the domain interface, an increase of the angle between the two beta-sheets composing the ECD, the internal beta-sheet (residues 1–87 and 94–125) being significantly correlated to the movement of the M2 alpha-helical segment (residues 238–265) (Taly et al. 2005). The internal beta-sheet and the M2 alpha-helical segment were proposed to form together the "internal" rigid block and the external beta-sheet (126–205) and the M1, 3-4 alpha-helical segments to form the "external" block. The interface between the two domains has also been found to be a zone of flexibility by another group using similar methods (Amiri et al. 2005).

An identical normal mode analysis was performed on a low-quality structure, generated in the process of modeling the structure, which is more similar to the *Torpedo* receptor. This test was performed to evaluate the influence of the fine three-dimensional structure on the normal mode analysis. The twist mode is essentially identical with that structure as well as with a new model (Taly et al. 2006).

The same kind of results have been obtained by another group (Cheng et al. 2006a). In that study the model of the structure was different, the energy model was more detailed (all atom) and the effect of the membrane was taken into account (Cheng et al. 2006a). Interestingly, this detailed study confirmed the existence of the twist mode. The fact that the twist mode is found with several setups

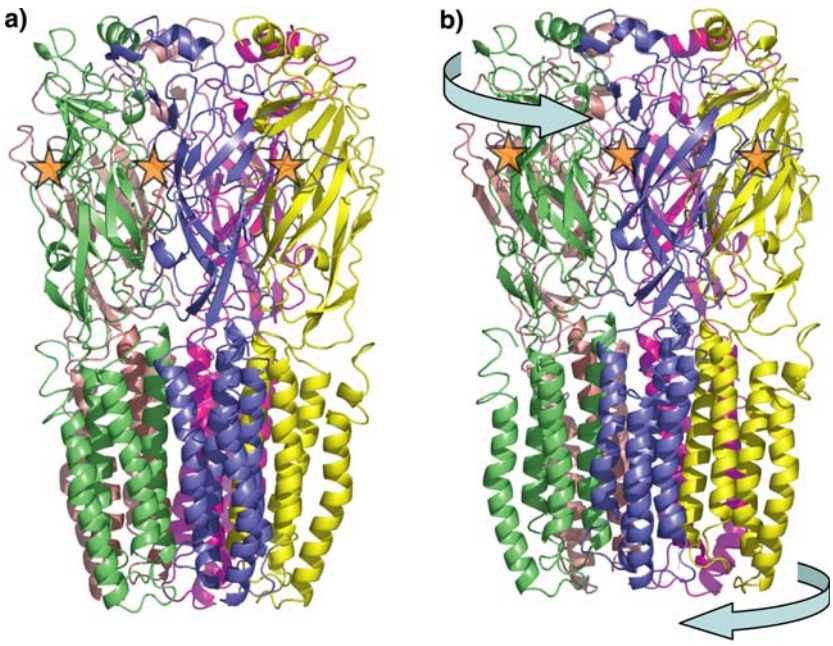

Fig. 3 **a** α7 nAChR model, in the closed-channel state, viewed from the membrane plane. A *different color* is used for each subunit. **b** Open-pore model obtained after exploration of the twist mode and energy minimization. The comparison of **a** and **b** demonstrates the quaternary twist motion between the structures (following the *arrows*). *Stars* were added to help localize the binding sites

supports the robustness of the results and the functional importance of the twist mode (Nicolay and Sanejouand 2006).

Comparison of the twist mode with experimental data

Accessibility of residue M1-213

The structures associated with the opening of the pore were tested for the change in accessibility of residue M1-213. This residue, positioned at the extracellular end of M1, has been shown to become accessible during the gating process (Zhang and Karlin 1997). Interestingly, an increase of accessibility for that particular residue was shown along the twist mode (Taly et al. 2005, 2006).

Electron microscopy

Using fast application of a high concentration of ACh followed by rapid freeze trapping of the protein, electron density maps at 9 Å resolution were recorded and proposed to represent the open conformation (Unwin 1995). Later, a rigid body fitting of AChBP was performed in the electron microscopy density maps obtained in the absence or presence of acetylcholine (Unwin et al. 2002). The subunit was divided in inner and outer β-sheet, the flexibility of the C-loop was not specifically addressed. Within the extracellular domain, strong asymmetry was observed for the closed conformation, and the two α-subunits were found to undergo a major structural reorganization upon activation, that mainly consist of a 15° tilt of the inner β-sheet, and that render the protein more symmetric. A similar increase in the angle between the internal and external β-sheets in the course of activation was observed in the twist model of gating (Cheng et al. 2006a; Taly et al. 2005).

It is possible to use normal mode analysis to fit a model into a low-resolution electron density from cryo-electron microscopy (Delarue and Dumas 2004; Tama et al. 2004a, b). This approach has been used on the electron densities obtained at 9 Å resolution in the resting (Unwin 1993) and the active state (Unwin 1995) (N. Unwin, personal communication). The twist mode was found to account for most of the change in electron density (M. Delarue and A. Taly, unpublished results).

X-ray analysis of AChBP

AChBP has been crystallized in the presence of both nAChR agonists and antagonists and it was observed that the main structural change lies in the C-loop. The twist mode involves motion in the entire ECD, which may be seen as a discrepancy between observations made in AChBP and nAChR. It is shown below that this apparent discrepancy is already partly solved by modelling studies.

Insights from a prokaryotic homologue

A prokaryotic homologue of the nAChR has been recently characterized (Bocquet et al. 2007). The main difference with eukaryotic ligand gated ion channels is that it lacks a cytoplasmic domain which suggests that this domain is not essential for the gating but rather participates in regulation and/or desensitization which in turn is in support of the modelling studies that ignore that domain. Interestingly, although it lacks a cysteine bridge, the cys-loop is conserved which is in agreement with its critical role in the gating.

Flexibility of the receptor

It has been shown that the flexibility obtained with coarse-grained normal mode analysis is in fair agreement with that found experimentally in the temperature factor observed for AChBP (Cheng et al. 2006a). The flexibility of specific secondary structure elements was also found to be in agreement with experiments (Cheng et al. 2006a). Analysis of the dynamic coupling of residues identified the interaction between the M2–M3 loop from the IPD and the cys-loop and $\beta1$–$\beta2$ loop from the ECD (Cheng et al. 2006a) in agreement with experiments which show that this zone participates crucially in the gating (Bouzat et al. 2004; Grutter et al. 2005a, b; Kash et al. 2003; Lummis et al. 2005).

Allosteric sites identified by normal mode perturbation scanning

To identify sites of the protein that can alter the gating mechanism represented by the twist mode, the protein model was modified in order to mimic the effect of binding a ligand or introducing a mutation (Taly et al. 2006). The cys-loop and β_8–β_9 loop which are both at the interface between the ECD and the IPD, have been identified for their capacity to stiffen the twist mode (i.e. to render it energetically less favourable) if modified, in agreement with experiments, as discussed above.

The five zones which have been identified to potentially soften the twist mode (i.e. to make it energetically more favourable) are grouped in one area which includes loops that form the binding site (B, C and E; see Fig. 1a, b for the position of loops) and two loops above the binding site. If an analogous effect occurred in actually binding a ligand, it would be in accord with the increase in entropy calculated for other systems (Grunberg et al. 2006) or the softening of the protein upon ligand binding (Balog et al. 2004).

Pathological mutations interfere with the twist mode

Several naturally occurring mutations of the nAChR promote a change in the gating properties of the receptor. A set of well-studied mutations of neuronal and muscular nAChRs are associated with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Steinlein 2004) and congenital myasthenia (Engel and Sine 2005) respectively.

The mutations were found to be localized at the interface between subunits and between rigid blocks (24 out of 27 mutations; see Table 2 in ref Taly et al. 2006 for the details of residues associated with the different rigid blocks) in statistically higher number than for the other residues ($\alpha < 0.02$). The interfaces between subunits and between dynamic domains within subunits are regions where significant changes are introduced by motion along the twist mode. The presence of mutations at these interfaces supports the conclusion that the quaternary and tertiary changes of the twist mode play a critical role in the gating mechanism.

A recent experimental study identified a pair of interacting residues which modify the gating properties when the interaction is affected by a mutation (Corradi et al. 2007). These residues are at the interface between the rigid blocks in agreement with the analysis proposed above.

Comparison with results from other simulation methods

In parallel to the analysis using normal mode analysis complementary information was obtained in studies using relatively long molecular dynamics and targeted molecular dynamics. Noteworthy they shed light on the role of the cys-loop.

Analysis of the gating mechanism through molecular dynamics

Long molecular dynamics simulations were performed (Law et al. 2005), which showed that the C-loop is highly flexible in the absence of ligand which is consistent with a resting state of the nAChR. The gating mechanism is proposed to be made of rotation and inward/outward motions of the helices which appear to be in agreement with the twist mode. The cys-loop was proposed to act as a stator to transmit the conformational change to the IPD which is similar to the notion that the interface between the ECD and IPD is associated with the hinge bending site of the twist mode.

Targeted molecular dynamics links C-loop closure to ion channel motions

A study using targeted molecular dynamics followed the conformational changes initiated in the nAChR due to the forced closure of the C-loop (Cheng et al. 2006b). Large movements were observed in the C-loop, F-loop and cys-loop. It was proposed that the loops at the interface participate strongly in the transmission of the conformational change from the C-loop to the IPD although the movements are rather small. Again, this observation is consistent with the notion that this region is a hinge.

Motions were observed in the IPD which are consistent with an opening of the pore and in agreement with the twist mode (Cheng et al. 2006b).

Conclusion

The twist mode has been found to be in agreement with numerous experiments: accessibility of residue M1-213, possibility to identify allosteric sites and analyse pathologic mutations, electron microscopy, and protein flexibility known from X-ray and mutation analysis. This mode of deformation of the protein therefore appears as a reasonable estimate of the gating mechanism.

For understanding of the finer details of the gating mechanism, much hope lies in the recent identification of a prokaryotic homologue of ligand gated ion channels (Bocquet et al. 2007; Tasneem et al. 2005). However, modelling is still expected to play an important role especially to further explore the apparent discrepancy between results obtained by X-ray analysis of AChBP and modelling studies of nAChR. Several studies propose that the movement in the loops connecting the ECD and IPD are limited (stator, hinge) but the details remain to be elucidated.

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