FEBS Letters 504 (2001) 118–125 FEBS 25111

Minireview

# Neuronal nicotinic receptors: from protein structure to function

Valérie Itier, Daniel Bertrand*

*Department of Physiology, CMU, 1 rue Michel Servet, CH-1211 Geneva 4, Switzerland*

Received 11 June 2001; accepted 26 June 2001

First published online 25 July 2001

Edited by Andreas Engel and Giorgio Semenza

**Abstract Neuronal nicotinic acetylcholine receptors are a prototype of ligand-gated channels that mediate transmission in the central and peripheral nervous system. Structure–function studies performed at the amino acid level are now unraveling the determinant residues either for the properties of the ligand-binding domain or the ionic pore. In this work we review, in the light of the latest finding, the structure–function relationship of these receptors and their implication in neurological diseases.** 

*Key words:* Neuronal nicotinic acetylcholine receptor; Ligand-gated channel; Transmission; Molecular structure

## 1. Introduction

First characterisation of the neurotransmission soon revealed the discontinuity of the electrical signal propagation and suggested the existence of a chemical compound that mediated transmission between neurones, or neurones and the effector cells. Acetylcholine (ACh) was the first identified neurotransmitter by the classical O. Loewi experiments on the heart muscle cells. Since then, it was demonstrated that cholinergic transmission could be divided in two groups according to the pharmacological sensitivity to muscarine or nicotine. Best studied at the vertebrate neuromuscular junction and in the fish electrical organ, nicotinic acetylcholine receptors (nAChRs) have been, for a long time, a model of ligand-gated channels.

Initial studies carried out at the neuromuscular junction have revealed that the nAChRs result from the assembly of five subunits in a pentamer with the stoichiometry $\alpha_2,\beta,\gamma,\delta$ [1]. This subunit composition corresponds to the *Torpedo* electrical organ or the embryonic neuromuscular junction receptor. When the endplate is innervated in the adult form, the γ subunit is replaced by the ε subunit. Forming both the ligand-binding site and the channel domain, assembly of these subunits was shown to faithfully reproduce the properties of the endplate receptor in reconstitution systems [2,3]. Paving the road to structure–function experiments these, initial studies have illustrated the importance of the correlation that exists between the amino acid residues and the receptor properties.

Given the strong dependence on nicotine intake, as seen for instance in tobacco smoke, it was supposed that nicotine effects should not be restricted to the neuromuscular junction endplate, but should also affect the central nervous system. Although it was shown that nAChRs mediate neurotransmission in ganglia, their existence and structure in the central nervous system remained elusive.

Following the identification of the genes encoding the neuromuscular receptor subunits, the cloning with low stringency soon revealed that sequences homologous to those of the muscle could be identified [4–6]. Mapping the expression of these homologous subunits revealed that they are expressed in the central nervous system [7–12]. So far, in vertebrate tissues, 12 nicotinic receptor subunits (α2–10; β2–4) have been cloned [12–14]

The identification of the neuronal nAChR genes gave a new and important impetus to the studies of neurotransmission. Interest in this field was further reinforced with the observation of the positive cognitive effects caused by nicotine, and the correlation existing between neurological diseases and the nAChRs (review in [15]).

In this work we focused on the neuronal nAChRs that are expressed in the central and peripheral nervous system with special attention on the structure–function relationship.

## 2. nAChR: pentameric glycoprotein

Historically, the nAChRs expressed by vertebrate neuromuscular junction and *Torpedo marmorata* or electrical eel organs were the first characterised. Because these tissues allowed extraction of a large fraction of highly rich receptors, these biological sources opened the way to biochemical experiments, and so, became the model of the nicotinic receptor investigations.

During the 1970s, the purification of the receptors from these tissues defined a glycoprotein of 290 kDa. The protein was then dissociated, using the SDS detergent technique, into four different types of subunits named α, β, γ and δ according to their increasing molecular weights (reviewed in [1]). Additional subdivision between subunits was made according to the presence of a cysteine doublet that was thought to participate in the ACh-binding site. Thus, when presenting two adjacent cysteines in their N-terminal part, muscle and neuronal subunits were classified in the α subunit group. To date,

*Corresponding author. Fax: (41)-22-702 54 05.
*E-mail address:* bertrand@cmu.unige.ch (D. Bertrand).

*Abbreviations:* ACh, acetylcholine; nAChR, nicotinic acetylcholine receptor; GABA, γ-aminobutyric acid; GluRCl, chloride permeable glutamate receptor; glyR, glycine receptor; α-Bgt, α-bungarotoxin; $5HT_3$, 5-hydroxytryptamine


PII: S0014-5793(01)02702-8

nine α (α2–α10) and three β (β2–β4) neuronal subunits encoding the nAChRs have been cloned [12,16].

Plot of the hydrophobicity of nAChR subunits revealed that they all presented four hydrophobic segments that were thought to form the transmembrane domains (TMI–TMIV). Other hallmarks are the long N-terminal extracellular part presenting glycosylation sites, a large intracellular domain (100–200 amino acids) between TMIII and TMIV presenting phosphorylation sites, and a short C-terminal domain (4–28 amino acids). The N-terminal hydrophilic part contains a disulphide-linked loop. The intracellular loop, comprised between the TMIII and TMIV segments, contains putative phosphorylation sites [17,18].

## 3. Receptor localisation

Mapping of the nAChRs in the brain yielded surprising results. It was observed that receptors are widely distributed, but that some receptors seem principally localised on terminal boutons. The emerging figure of nAChR subcellular pattern of localisation indicates a concentration of receptors on postsynaptic, presynaptic or even axonic areas [19,20]. In the view of the high calcium permeability of the neuronal nAChRs, different effects were predicted depending upon this subcellular localisation. Moreover, it is now widely documented that nAChRs can cause a significant calcium influx in the presynaptic bouton, and therefore modulate the neurotransmitter release [19–26]. Influence of the nAChRs on dopamine, glutamate, serotonin and GABA release was illustrated both in biochemical and electrophysiological experiments. For example, it is supposed that nicotine addiction is mediated by the influence of nAChRs on the dopamine release in the mesolymbic pathways. Experiments carried out with β2 knock-out mice have shown that these animals display a lower addiction to nicotine than their wild-type siblings [27]. These last studies justify the correlation between the nature of nAChR subtypes with their localisation.

## 4. Stoichiometry

While subunit composition at the neuromuscular receptors was established rather early, less was known concerning the neuronal nAChR composition. The minute amount of biological material renders biochemical experiments difficult, and putative stoichiometry must be derived from an ensemble of converging information. First, and as for the muscle receptor, the molecular weight of purified nAChRs is always in the 300 kDa range [28]. Since all subunits identified so far are less than 600 aa in length, every subunit is expected to weight approximately 40–70 kDa. Thus, neuronal nAChRs are expected to result from the assembly of five subunits. Immunoprecipitations, using antibodies directed against subunit specific epitopes, and in situ hybridisation indicate that the ganglionic receptors probably result from the assembly of α3 and β4 subunits, while the major brain receptor must contain α4 and β2 subunits [28,29]. Expression and identification of a small amount of the α5 subunit suggest that this subunit may further participate in the formation of a fraction of receptors [30]. Additional evidence indicate that alternative receptor assemblies, including α3 and β2 or other subunits, are also present in the central nervous system [31,32]. While it was clearly shown that many receptors result from heteromeric assembly of two or more distinct subunits, it was also shown that a fraction of the receptors are homomeric [16,33–36].

Reconstitution experiments performed in host systems such as *Xenopus laevis* oocytes and/or transfection in cell lines have clearly demonstrated that functional nAChRs can be obtained by expression of at least two subunits for heteromeric receptors (i.e. α4 and β2) and a single subunit for homomeric receptors (i.e. α7) [4–6,34,35,37–39]. In addition, combination of site-directed mutagenesis and single-channel studies have demonstrated the pentameric structure of α4β2 nAChRs, indicating that they are composed of two α4 and three β2 subunits [40]. Furthermore, it was reported that homomeric α7 receptors display five binding sites for the competitive antagonist methyllycaconitine, and therefore must also be pentameric [41]. The most recent report of the crystalline structure of the acetylcholine-binding protein (ACh-BP) isolated from snails further confirms the pentameric organisation of the N-terminal domain [42].

## 5. Binding site

The reconstitution of functional receptors isolated by biochemical purification of proteins from *Torpedo* electric organ constituted the first demonstration that these proteins can form both the ligand-binding site and the ionic pore. From investigations in the 1980s on the kinetics of ionic current through the single channel, it has been accepted that two agonist-binding sites exist. The occupancy of these two sites, in a positive cooperative way, stabilise the receptor channel in the open state [43–46]. Using the method of the fluorescence resonance energy transfer, between a receptor-bound fluorescent agonist and two membrane-fluorescent probes, the ACh-binding sites were estimated to be 25 Å below the extracellular apex of the nAChR. Later, high-resolution electron microscopy technique results were in agreement with the proposed location for the ACh-binding sites in putative 'pockets', at each of two α subunits in the pentameric receptor, and approximately 30 Å above the membrane surface [47–50].

With the availability of specific chemical compounds and the amino acid sequences of the different nAChR subunits, important advances concerning our knowledge of the ACh-binding site have been made. For instance, photoaffinity experiments carried out with the muscle-type receptor have shown that the ligand-binding site must be at the interface between the αs and their adjacent γ or δ subunits (Fig. 1) [51–54].

Six loops termed A–F have been identified in the formation of the ACh-binding site. The α subunit, which harbours the principal component of the binding site, comprises the A, B and C loops, while the complementary component of the adjacent subunit (γ, δ or ε for the muscle and β for the neuronal) comprises the D, E and F loops (Fig. 1). The important participation of tyrosine residues to the ACh-binding pocket has been identified by the combination of site-directed mutagenesis and electrophysiological investigation of the chick α7 receptor subtype [55]. Modelling of the putative three-dimensional structure of the nAChRs [56,57] all confirmed that the ACh-binding site must reside at the interface between two subunits and that the ligand must penetrate into a gorge to form appropriate chemical bridges and initiate the transduction. Modelling of the extracellular domains with the

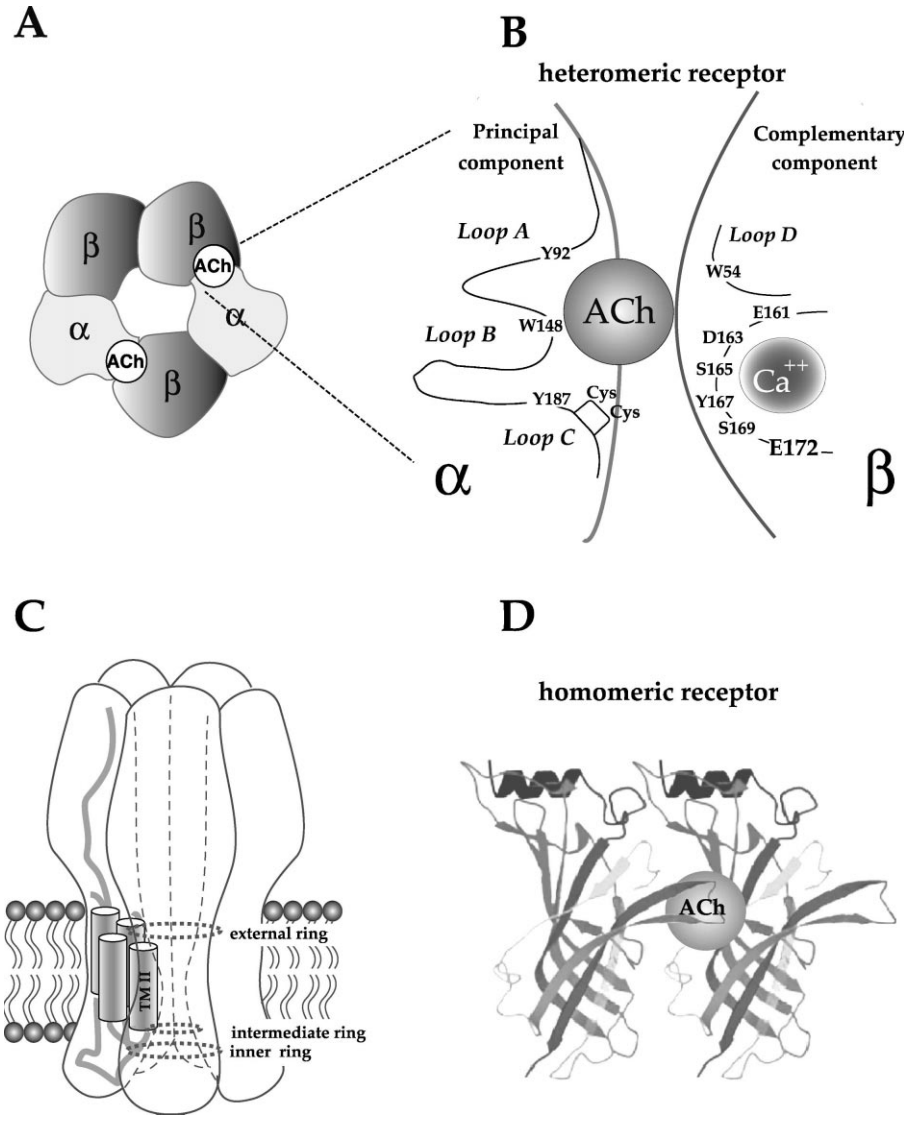


Fig. 1. Schematic representation of the nAChR. A: Heteropentameric complex of neuronal nicotinic α and β subunits (note the 2:3 ratio between the α and β). The ACh-binding site is localised at the interface of α and the adjacent subunit. B: Representation of the principal component (α subunit) with its three loops A, B, C and the two loops D, E from the complementary component (β subunit). Each loop is schematised with the principal amino acids identified at the chick α7 subunit. The E loop was shown to participate in the $Ca^{2+}$-binding region involved in the calcium potentiation. C: Side view of the topological pentameric organisation of subunits through the cell membrane with the schematic drawing of the four transmembrane domains (TMI–TMIV). D: Three-dimensional representation of the protein folding derived from the ACh-BP crystal (redrawn from [42]). The interface of two adjacent subunits showing more particularly the disulphide bridge of the C loop involved in the ACh-binding site.

known crystal structure of the copper-binding proteins was also attempted [58]. The very recent identification of ACh-BP, a molecule that efficiently binds ACh and that is secreted from the glial cells of snails, allowed the first crystallisation of a protein that resembles the N-terminal domain of the nAChRs. X-ray diffraction analysis of this structure confirmed the predictions made from the various models and the existence of the A, B, C, D and E loops in the formation of the binding pocket [42]. This crystal structure reveals two β sheet skeletons forming the gorge in which the ACh enters and binds to specific residues (Fig. 1D).

## 6. Receptor pharmacology

In the early work carried out at the neuromuscular junction and at ganglionic receptors, it was shown that significant pharmacological differences exist between these receptor subtypes. A major criteria initially used was the difference in sensitivity to the snake toxin α-bungarotoxin (α-Bgt) [59]. It was shown in reconstitution experiments that homomeric receptors α7, α8 and α9 are blocked by α-Bgt, whereas heteromeric receptors are insensitive to this toxin [16,34–36,60]. This observation shed new light in the understanding of the existence of a strong α-Bgt labelling in the rat brain [61]. Thus, it was postulated that α7-containing receptors must be expressed in the central nervous system and could participate in the neurotransmission. While the existence of homomeric α7 receptors in the brain was, for a long time, a matter of debate, a clear biochemical demonstration of the presence of such receptors was obtained from rat brain [62].

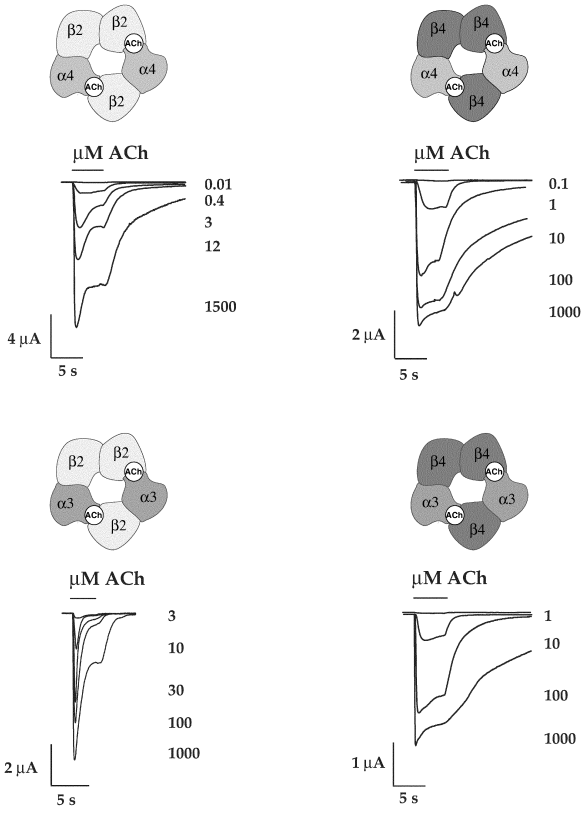


Fig. 2. Properties of the nAChRs are defined both by α and β subunits. Oocytes injected with different cDNA combinations were challenged with ACh in the voltage–clamp configuration. Cells were held at −100 mV and currents evoked by series of ACh concentrations were recorded and superimposed. Subunit composition is schematised on the top of each set of traces.

Because the ACh-binding site is at the interface between two adjacent subunits, it is evident that both must contribute to the binding-site properties. This is clearly illustrated when examining the ACh properties of different receptor combinations (Fig. 2). Exchange of α or β subunits causes marked changes at the current desensitisation and/or at the apparent ACh sensitivity. While discussion of the pharmacological properties of the nAChRs would require an entire review per se, it is interesting to note that nicotine versus ACh sensitivity could be distinguished using microchimeric constructions [63]. Bringing further insights in the understanding of the ACh-binding pocket, these experiments have again illustrated the importance of the A–E loops (Fig. 1).

## 7. Channel structure and permeability

The nAChRs belong to the superfamily of four transmembrane ligand-gated proteins, including γ-aminobutyric acid ($GABA_A$, $GABA_C$), glycine receptors (glyR), serotonin (5-hydroxytryptamine ($5HT_3$)) and the chloride permeable glutamate receptors (GluRCl). In addition to structural homologies with these receptors, the nAChRs display the same basic functional properties. Namely, upon binding of a specific neurotransmitter, the channel is quickly opened and ions freely diffuse following their gradient, and thereby mediate synaptic transmission. Fundamental differences exist, however, in the ionic selectivity between cationic (nAChRs, $5HT_3$) and anionic ($GABA_A$, $GABA_C$, glyR and GluRCl) permeable channels. In this section we shall review our knowledge about the mechanisms underlying the ionic selectivity of the nAChRs.

Electrophysiological and biochemical measurements performed at the neuromuscular junction nAChRs have shown that these receptors are permeable to monovalent cations, but display a rather low permeability to divalent cations. Measurements of the current–voltage relationship showed unequivocally that these receptors follow the Ohms law with the current amplitude being proportional to the electrical field applied across the membrane. Estimated by the displacement of the reversal potential in relation to the extracellular ionic composition, these channels display a pore size of about 6 Å [64,65] (reviewed in [66]). In contrast, neuronal nAChRs display two marked characteristics: (a) a strong voltage dependency (inward rectification) and (b) a large permeability to calcium [34,67–71]. Inward rectification and the high calcium permeability have been confirmed in both reconstituted and native receptors [72,73].

To better understand the properties of ligand-gated channels, and in particular of the nAChRs, it is necessary to examine the structure of the ionic pore. Initially, the channel structure was investigated by photolabelling using the open-channel blocker termed chlorpromazine. From the labelling of the second transmembrane segment of the four different subunits (α, β, γ, δ), it was concluded that this segment must form the wall of the ionic pore, and therefore defines its biophysical properties [74–76]. Indeed, it was soon confirmed that site-directed mutagenesis of amino acid residues at the inner or outer mouth of this putative aqueous pore had a significant effect on the ionic selectivity of the channel [2]. For instance, it must be recalled that, at the muscle receptor, mutation of a single amino acid within TMII was enough to reduce the inhibition caused by the lidocaine derivative QX-222 [77,78]. Another prediction derived from the chlorpromazine labelling is that TMII must form an α-helix. Modelling, derived either from sequence alignments or minimal energy computations, has supported this α-helix arrangement [56,58,79,80], as has high-resolution electron microscopy [48,50,81].

The identification of the homomeric α7 nAChR [35] permitted a simplification of the structure–function relationship studies. In these nAChRs, mutation of a single amino acid is enough to perform the mutation of the entire rings that confine the ionic pore. Using this protein as a prototype of ligand-gated channels, numerous findings have been made. Mutation of the chlorpromazine-labelled leucine residue that is conserved throughout the different nAChRs (chick α7 L247) caused marked and unexpected modification of the receptor functional properties. For instance, mutation L247T causes an increase of about 200-fold in ACh sensitivity, loss of desensitisation and changes in pharmacological profile with the competitive antagonists becoming agonists [82,83].

To understand the critical role played by this 'canonical' leucine residue, which is conserved throughout cationic and anionic receptors, it is necessary to recall the general putative topology of the channel. Presenting a wide opening of about 25 Å in the extracellular domain, the channel progressively becomes narrower and forms a constriction to become wider again in the intracellular domain (see Fig. 1). High-resolution electron microscopy images suggest that the L247 residue must be close to the selectivity filter, in a section of the channel that should display high conformation flexibility [50]. The

best explanation of the pleiotropic effects caused by the L247T mutation was proposed in the early work from Revah and collaborators who concluded that this mutation renders conductor one of the desensitised states. Thus, any action that stabilises the receptor in this desensitised state will evoke an inward current. Several predictions can be made from this proposition. For instance, at rest, it is known that a fraction of the receptor is in the desensitised state [1], thus it was predicted that the L247T mutant should display spontaneously open channels. This prediction was later confirmed in the chick α7 receptor [84]. Whereas it is not in the scope of this review to examine, in detail, all effects caused by this mutation, it is important to recall that similar findings were made for $5HT_3$, muscle nAChR, heteromeric nAChRs and $GABA_A$ [85–87].

### 7.1. Ionic selectivity

Determinants of ionic selectivity filter were analysed either by site-directed mutagenesis, designed on the basis of sequence comparison (i.e. [88]), or by cysteine scanning using cysteine-reactive probes (i.e. [89,90]). Cysteine scanning experiments have revealed that, in agreement with the putative α-helix structure, substitutions of the amino acid residues pointing toward the pore lumen render the channel sensitive to test reagents. In contrast, no reaction was observed when the mutations were effectuated at amino acids that were not supposed to face the pore [90]. The difference in blockade observed when the reagent was introduced from the extracellular or intracellular compartment was interpreted by these authors as reflecting a constriction of the channel in the vicinity of the intracellular mouth.

Sequence analyses of consensus amino acid residues facing the ionic pore between the different members of the four transmembrane domain ligand-gated channels revealed a striking homology, even between cationic and anionic channels. In view of this homology, substitution of the chick α7 residues with those found in $GABA_A$ or glyR was attempted. The power of this approach was shown with the conversion of the ionic selectivity of the chick α7 from cationic to anionic [88]. Moreover, in subsequent work, amino acids responsible for this ionic conversion were further analysed in more detail [91,92]. From these experiments, it was concluded that some amino acids were permissive, but that introduction of a proline or alanine residue between the inner and intermediate rings (see Figs. 1 and 3) was indispensable for the conversion

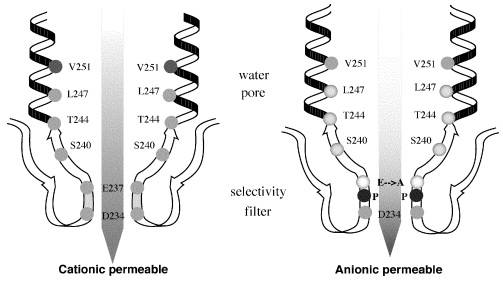


Fig. 3. Putative representation of the TMII segment in the pore-forming region. Wild-type amino acid and putative configuration is schematised in the left panel. The anionic permeable channel obtained by mutation of V251T, E237A and the proline insertion is represented in the right panel. Amino acids are indicated by their single-letter code and positions refer to the chick α7 numbering [88,92].

of the ionic selectivity. The basic principle underlying this ionic selectivity was further reinforced when determinant amino acids, proposed by Galzi et al., were mutated in the glyR and caused the conversion from anionic to cationic [93].

### 7.2. Calcium permeability and rectification

The chick α7 nAChR, highly permeable to calcium, can have the calcium permeation abolished with the exchange of a single amino acid residue in the second transmembrane domain [68]. It was shown that removal of a charged residue at the intermediate ring (E237, in the chick numbering) is sufficient to abolish the calcium permeability without significantly changing the ratio PK/PNa permeability. A second site was identified at the level of two adjacent leucines (L254 and L255), where mutation of either of these leucines into a threonine was sufficient to suppress calcium permeability. Interestingly, while a charged residue seems necessary at the intermediate ring for the calcium permeability, another mechanism must apply in the upper channel domain. Shortening of the lateral side chain is sufficient to prevent the calcium influx, apparently without modifying the monovalent cations permeation.

While most of site-directed mutagenesis experiments have been performed at the homomeric α7 receptor, basic principles involved in ionic selectivity have been verified on heteromeric receptors. The contribution of charged residues at the intermediate ring was also found to be determinant for the calcium permeability of the heteromeric α3β4 and α4β2 receptors [94]. Less permeable to calcium than the α7 receptor, these heteromeric receptors are equally affected by the substitution residue at the intermediate ring of the glutamate by an alanine, and removal of this charged residue affects both rectification and calcium permeability.

## 8. Conservation of functional domains

The identification of a large homology, and even identity between the members of the superfamily of four transmembrane ligand-gated channels, suggests that these receptors may have evolved from a common ancestor gene by duplication and selection. Developed by several authors [56], this concept implies that functional domains may have been conserved between the different members of the superfamily.

As a challenge to this hypothesis it was proposed that chimera may be produced either between members of a given receptor type (i.e. nAChRs) or even across near-by 'cousin' receptors (i.e. nAChRs and $5HT_3$). Constructions of microchimeras between different nAChR subunits represent an important tool for the investigation of a given subunit property. For instance, it was shown that homomerisation, which is a key feature of the α7 receptor, can be transmitted to chimeras constructed with the α3 subunit [95]. A further extension of this concept was demonstrated with the construction of a functional chimera between the α7 and $5HT_3$ receptor subunits [96]. Properties of this chimeric receptor are those of the α7 receptor with the ACh-recognition and -binding site, whereas the channel domain displays the properties of the $5HT_3$ receptor. Interestingly, it was later shown that the muscle α1 subunit can also be fused with the $5HT_3$ receptor, and resulted in a protein able to bind α-Bgt. Binding properties are conserved in this receptor, but nicotine or ACh failed to elicit current, indicating a lack of functional properties.

Since this initial work, a number of fusion proteins and further microchimerisation have been utilized to explore the microdomains of a given receptor.

## 9. nAChR: a prototype of allosteric protein

As an extension of their work, Changeux and co-workers introduced the concept that ligand-gated channels behave as an allosteric protein with multiple possible conformations [97]. According to the allosteric model, the receptor can spontaneously change state in the absence of ligand. Although any transition can occur, probability of channel opening is extremely low. Exposure to the neurotransmitter preferentially stabilises the receptor in the open configuration. In contrast, the induce fit model hypothesises that binding of the ligand causes a conformational change of the protein but, unless complex assumptions, no channel transition occurs in absence of ligand.

The 'multiple allosteric states' are described as four discrete B, A, I and D states. In the absence of an agonist the receptor is principally stabilised in the resting B (closed) state. The equilibrium constants are, however, such that a fraction of the receptor is always in the desensitised D (closed) state. At the neuromuscular junction, it was shown that approximately 20% of the receptors are in the desensitised state even in absence of the agonist (reviewed in [1]). Binding of the agonist preferentially stabilises the active A (open) state and then subsequently the intermediate I (closed) state and progressively, but with a slower time constant, the desensitised D (closed) state (see for review [63]). Interesting predictions derived from the allosteric model are that transition from one state to another can take place in the absence of ligand and that compounds affecting the transition constants can provoke either a potentiation or an inhibition of the receptor.

While in normal conditions spontaneous opening probability is extremely low at the neuromuscular junction [98], it was shown that mutation of the 'canonical' leucine (L247) in the chick α7 receptor was sufficient to cause channel opening in the absence of agonist [84]. In addition, positive or negative allosteric modulations of the receptors have been widely documented either for the homomeric α7 receptor [99] or the heteromeric α4β2 receptor [100,101]. Potentiation by extracellular calcium is indeed a good example of a positive allosteric modulation that was analysed at the amino acid level [102]. The observation that extracellular calcium causes a marked increase of the ACh-evoked current at the α7 nAChR, but blocks the $5HT_3$ receptor, leads to the proposal that microchimera construction should allow to identify the amino acid segments involved in the calcium recognition site. Furthermore, analysis of the N-terminal of the α7 receptor suggests the presence of calcium recognition elements near the ACh-binding site. A putative EF motif (observed in calmodulin proteins) was identified at the position 172 of the chick α7 receptor. Modification of these residues was shown to disrupt calcium potentiation at the chick α7 receptor. As a correlate, exchange of the corresponding $5HT_3$ receptor residues with those of α7 was sufficient to confer the calcium potentiation to the $5HT_3$ receptor [102]. Similarly, zinc modulation of heteromeric nAChRs showed that β4-containing receptors are potentiated by low zinc concentration, whereas β2-containing receptors were inhibited, and a putative zinc-binding sequence was identified [101].

## 10. Conclusions

First demonstrated at the neuromuscular junction, nicotinic receptors have been used for a long time as a model of the chemical synaptic transmission. With the discovery that neuronal nAChRs are widely expressed in the peripheral and central nervous system, and with their modulatory role in the neurotransmitter release, these receptors have received increased attention. Further interest in this field was manifested with the identification of associations between nAChRs and a variety of neurological disorders including Parkinson, Alzheimer, schizophrenia, neurophatic pain or epilepsy. Linkage between one form of genetically transmissible epilepsy and a mutation in the gene encoding the α4 subunit of the nAChR constituted the first important evidence for the contribution of these ligand-gated channels in neuronal network dysfunction.

Characterisation of mechanisms and properties at the molecular level underlying neurotransmission is one of the major challenges in today's biology. The important knowledge of the structure–function relationship of the nAChRs constitutes an invaluable tool for the studies of the four transmembrane domain ligand-gated channels family. The availability of a first crystal structure of the ACh-binding site is the latest important step that shall allow the development of new and better designed pharmaceutical compounds, and therefore open new roads for clinical treatments.

*Acknowledgements:* We thank S. Bertrand for discussion and continuous work. This work was supported by the Swiss National Science Foundation to D.B.

## References

[1] Changeux, J.P. (1990) in: Fidia Research Foundation Neuroscience Award Lectures (Changeux, J.P., Llinàs, R.R., Purves, D. and Bloom, F.E., Eds.), Vol. 4, pp. 21–168, Raven Press, New York.
[2] Imoto, K. et al. (1988) Nature 335, 645–648.
[3] Imoto, K., Konno, T., Nakai, J., Wang, F., Mishina, M. and Numa, S. (1991) FEBS Lett. 289, 193–200.
[4] Boulter, J., Evans, K., Goldman, D., Martin, G., Treco, D., Heinemann, S. and Patrick, J. (1986) Nature 319, 368–374.
[5] Boulter, J., Connolly, J., Deneris, E., Goldman, D., Heinemann, S. and Patrick, J. (1987) Proc. Natl. Acad. Sci. USA. 84, 7763–7767.
[6] Ballivet, M., Nef, P., Stalder, R. and Fulpius, B. (1983) Cold Spring Harbor Symp. Quant. Biol. 48, 83–87.
[7] Wada, K. et al. (1988) Science 240, 330–334.
[8] Wada, E., Wada, K., Boulter, J., Deneris, E., Heinemann, S., Patrick, J. and Swanson, L.W. (1989) J. Comp. Neurol. 284, 314–335.
[9] Wada, E., McKinnon, D., Heinemann, S., Patrick, J. and Swanson, L.W. (1990) Brain Res. 526, 45–53.
[10] Mcgehee, D.S. and Role, L.W. (1996) Curr. Opin. Neurobiol. 6, 342–349.
[11] Lindstrom, J., Anand, R., Peng, X., Gerzanich, V., Wang, F. and Li, Y. (1995) Ann. N.Y. Acad. Sci. 757, 100–116.
[12] Sargent, P.B. (1993) Annu. Rev. Neurosci. 16, 403–443.
[13] McGehee, D.S. and Role, L.W. (1995) Annu. Rev. Physiol. 57, 521–546.
[14] Devillers-Thiery, A., Galzi, J.L., Bertrand, S., Changeux, J.P. and Bertrand, D. (1992) NeuroReport 3, 1001–1004.
[15] Weiland, S., Bertrand, D. and Leonard, S. (2000) Behav. Brain. Res. 113, 43–56.
[16] Elgoyhen, A.B., Vetter, D.E., Katz, E., Rothlin, C.V., Heinemann, S.F. and Boulter, J. (2001) Proc. Natl. Acad. Sci. USA 98, 3501–3506.
[17] Swope, S.L., Moss, S.I., Raymond, L.A. and Huganir, R.L. (1999) Adv. Second. Messenger Phosphoprotein Res. 33, 49–78.

[18] Moss, S.J., McDonald, B.J., Rudhard, Y. and Schoepfer, R. (1996) Neuropharmacology 35, 1023–1028.
[19] Wonnacott, S., Drasdo, A., Sanderson, E. and Rowell, P. (1990) Ciba Found. Symp. 152, 87–101.
[20] Léna, C., Changeux, J. and Mulle, C. (1993) J. Neurosci. 13, 2680–2688.
[21] Mansvelder, H.D. and McGehee, D.S. (2000) Neuron 27, 349–357.
[22] Wonnacott, S., Kaiser, S., Mogg, A., Soliakov, L. and Jones, I.W. (2000) Eur. J. Pharmacol. 393, 51–58.
[23] Grady, S.R. et al. (2001) J. Neurochem. 76, 258–268.
[24] Dani, J.A. (2001) Biol. Psychiatry 49, 166–174.
[25] Liu, X., Onaka, T. and Yagi, K. (2001) NeuroReport 12, 641–643.
[26] Pidoplichko, V.I., Debiasi, M., Williams, J.T. and Dani, J.A. (1997) Nature 390, 401–404.
[27] Picciotto, M.R., Zoli, M., Rimondini, R., Lena, C., Marubio, L.M., Pich, E.M., Fuxe, K. and Changeux, J.P. (1998) Nature 391, 173–177.
[28] Lindstrom, J. (1997) Mol. Neurobiol. 15, 193–222.
[29] Deneris, E.S., Connolly, J., Boulter, J., Wada, E., Wada, K., Swanson, L.W., Patrick, J. and Heinemann, S. (1988) Neuron 1, 45–54.
[30] Balestra, B., Vailati, S., Moretti, M., Hanke, W., Clementi, F. and Gotti, C. (2000) Mol. Pharmacol. 58, 300–311.
[31] Vailati, S. et al. (1999) Mol. Pharmacol. 56, 11–19.
[32] Barabino, B., Vailati, S., Moretti, M., McIntosh, J.M., Longhi, R., Clementi, F. and Gotti, C. (2001) Mol. Pharmacol. 59, 1410–1417.
[33] Drisdel, R.C. and Green, W.N. (2000) J. Neurosci. 20, 133–139.
[34] Séguéla, P., Wadiche, J., Dineley-Miller, K., Dani, J.A. and Patrick, J.W. (1993) J. Neurosci. 13, 596–604.
[35] Couturier, S. et al. (1990) Neuron 5, 847–856.
[36] Gotti, C., Moretti, M., Maggi, R., Longhi, R., Hanke, W., Klinke, N. and Clementi, F. (1997) Eur. J. Neurosci. 9, 1201–1211.
[37] Ballivet, M., Nef, P., Couturier, S., Rungger, D., Bader, C.R., Bertrand, D. and Cooper, E. (1988) Neuron 1, 847–852.
[38] Buisson, B., Gopalakrishnan, M., Arneric, S.P., Sullivan, J.P. and Bertrand, D. (1996) J. Neurosci. 16, 7880–7891.
[39] Gopalakrishnan, M. et al. (1995) Eur. J. Pharmacol. Mol. Pharmacol. 290, 237–246.
[40] Cooper, E., Couturier, S. and Ballivet, M. (1991) Nature 350, 235–238.
[41] Palma, E., Bertrand, S., Binzoni, T. and Bertrand, D. (1996) J. Physiol. 491, 151–161.
[42] Brejc, K., van Dijk, W.J., Klaassen, R.V., Schuurmans, M., van Der Oost, J., Smit, A.B. and Sixma, T.K. (2001) Nature 411, 269–276.
[43] Colquhoun, D. and Sakmann, B. (1985) J. Physiol. 369, 501–557.
[44] Hess, G.P., Udgaonkar, J.B. and Olbricht, W.L. (1987) Annu. Rev. Biophys. Chem. 16, 507–534.
[45] Udgaonkar, J.B. and Hess, G.P. (1987) Biophys. J. 52, 873–883.
[46] Udgaonkar, J.B. and Hess, G.P. (1987) Proc. Natl. Acad. Sci. USA 84, 8758–8762.
[47] Tierney, M.L. and Unwin, N. (2000) J. Mol. Biol. 303, 185–196.
[48] Unwin, N. (1993) J. Mol. Biol. 229, 1101–1124.
[49] Unwin, N. (1995) Nature 373, 37–43.
[50] Miyazawa, A., Fujiyoshi, Y., Stowell, M. and Unwin, N. (1999) J. Mol. Biol. 288, 765–786.
[51] Galzi, J.L., Revah, F., Bouet, F., Ménez, A., Goeldner, M., Hirth, C. and Changeux, J.P. (1991) Proc. Natl. Acad. Sci. USA 88, 5051–5055.
[52] Changeux, J.P. et al. (1998) Brain Res. Rev. 26, 198–216.
[53] Karlin, A. and Akabas, M.H. (1995) Neuron 15, 1231–1244.
[54] Taylor, P., Malanz, S., Molles, B.E., Osaka, H. and Tsigelny, I. (2000) Pflug. Arch. 440, R115–R117.
[55] Galzi, J.L., Revah, F., Bessis, A. and Changeux, J.P. (1991) Annu. Rev. Pharmacol. Toxicol. 31, 37–72.
[56] Le Novere, N., Corringer, P.J. and Changeux, J.P. (1999) Biophys. J. 76, 2329–2345.
[57] Ortells, M.O., Barrantes, G.E., Wood, C., Lunt, G.G. and Barrantes, F.J. (1997) Protein Eng. 10, 511–517.
[58] Tsigelny, I., Sugiyama, N., Sine, S.M. and Taylor, P. (1997) Biophys. J. 73, 52–66.
[59] Ascher, P., Large, W.A. and Rang, H.P. (1979) J. Physiol. 295, 139–170.
[60] Gotti, C., Hanke, W., Maury, K., Moretti, M., Ballivet, M., Clementi, F. and Bertrand, D. (1994) Eur. J. Neurosci. 6, 1281–1291.
[61] Clarke, P.B.S., Schwartz, R.D., Paul, S.M., Pert, C.B. and Pert, A. (1985) J. Neurosci. 5, 1307–1315.
[62] Green, W.N. and Millar, N.S. (1995) Trends Neurosci. 18, 280–287.
[63] Corringer, P.J., Le Novere, N. and Changeux, J.P. (2000) Annu. Rev. Pharmacol. Toxicol. 40, 431–458.
[64] Adams, P.R. and Feltz, A. (1980) J. Physiol. 306, 283–306.
[65] Dwyer, T.M., Adams, D.J. and Hille, B. (1980) J. Gen. Physiol. 75, 469–492.
[66] Hille, B. (1992) Ionic Channels of Excitable Membranes, Sinauer Ass., Sunderland, MA.
[67] Bertrand, D., Ballivet, M. and Rungger, D. (1990) Proc. Natl. Acad. Sci. USA 87, 1993–1997.
[68] Bertrand, D., Galzi, J.L., Devillers-Thiery, A., Bertrand, S. and Changeux, J.P. (1993) Proc. Natl. Acad. Sci. USA 90, 6971–6975.
[69] Vernino, S., Amador, M., Luetje, C.W., Patrick, J. and Dani, J.A. (1992) Neuron 8, 127–134.
[70] Vernino, S., Rogers, M., Radcliffe, K.A. and Dani, J.A. (1994) J. Neurosci. 14, 5514–5524.
[71] Bertrand, D., Galzi, J.L., Devillers-Thiéry, A., Bertrand, S. and Changeux, J.P. (1993) Curr. Opin. Neurobiol. 5, 688–693.
[72] Adams, D.J. and Nutter, T.J. (1992) J. Physiol. 86, 67–76.
[73] Castro, N.G. and Albuquerque, E.X. (1995) Biophys. J. 68, 516–524.
[74] Giraudat, J., Dennis, M., Heidmann, T., Chang, J.Y. and Changeux, J.P. (1986) Proc. Natl. Acad. Sci. USA 83, 2719–2723.
[75] Heidmann, T. and Changeux, J.P. (1984) Proc. Natl. Acad. Sci. USA 81, 1897–1901.
[76] Revah, F., Galzi, J.L., Giraudat, J., Haumont, P.Y., Lederer, F. and Changeux, J.P. (1990) Proc. Natl. Acad. Sci. USA 87, 4675–4679.
[77] Leonard, R.J., Charnet, P., Labarca, C., Vogelaar, N.J., Czyzyk, L., Gouin, A., Davidson, N. and Lester, H.A. (1991) Ann. N.Y. Acad. Sci. 625, 588–599.
[78] Charnet, P., Labarca, C., Leonard, R.J., Vogelaar, N.J., Czyzyk, L., Gouin, A., Davidson, N. and Lester, H.A. (1990) Neuron 4, 87–95.
[79] Adcock, C., Smith, G.R. and Sansom, M.S. (1998) Biophys. J. 75, 1211–1222.
[80] Tikhonov, D.B. and Zhorov, B.S. (1998) Biophys. J. 74, 242–255.
[81] Unwin, N., Toyoshima, C. and Kubalek, E. (1988) J. Cell Biol. 107, 1123–1138.
[82] Bertrand, D. et al. (1992) Proc. Natl. Acad. Sci. USA 89, 1261–1265.
[83] Revah, F. et al. (1991) Nature 353, 846–849.
[84] Bertrand, S., Devillersthiery, A., Palma, E., Buisson, B., Edelstein, S.J., Corringer, P.J., Changeux, J.P. and Bertrand, D. (1997) NeuroReport 8, 3591–3596.
[85] Labarca, C., Nowak, M.W., Zhang, H., Tang, L., Deshpande, P. and Lester, H.A. (1995) Nature 376, 514–516.
[86] Yakel, J.L., Lagrutta, A., Adelman, J.P. and North, R.A. (1993) Proc. Natl. Acad. Sci. USA 90, 5030–5033.
[87] Pan, Z.H., Zhang, D., Zhang, X. and Lipton, S.A. (1997) Proc. Natl. Acad. Sci. USA 94, 6490–6495.
[88] Galzi, J.L., Devillers, T.A., Hussy, N., Bertrand, S., Changeux, J.P. and Bertrand, D. (1992) Nature 359, 500–505.
[89] Akabas, M.H., Kaufmann, C., Archdeacon, P. and Karlin, A. (1994) Neuron 13, 919–927.
[90] Wilson, G. and Karlin, A. (2001) Proc. Natl. Acad. Sci. USA 98, 1241–1248.
[91] Corringer, P.J., Bertrand, S., Galzi, J.L., Devillers-Thiery, A., Changeux, J.P. and Bertrand, D. (1999) Novartis Found. Symp. 225, 215–224.
[92] Corringer, P.J., Bertrand, S., Galzi, J.L., Devillers-Thiery, A., Changeux, J.P. and Bertrand, D. (1999) Neuron 22, 831–843.
[93] Keramidas, A., Moorhouse, A.J., French, C.R., Schofield, P.R. and Barry, P.H. (2000) Biophys. J. 79, 247–259.
[94] Haghighi, A.P. and Cooper, E. (2000) J. Neurosci. 20, 529–541.
[95] Garcia-Guzman, M., Sala, F., Sala, S., Campos-Caro, A. and Criado, M. (1994) Biochemistry 33, 15198–15203.

[96] Eiselé, J.L., Bertrand, S., Galzi, J.L., Devillers-Thiéry, A., Changeux, J.P. and Bertrand, D. (1993) Nature 366, 479–483.
[97] Edelstein, S.J. and Changeux, J.P. (1996) Experientia 52, 1083–1090.
[98] Jackson, M.B. (1994) Trends Biochem. Sci. 19, 369–396.
[99] Krause, R.M., Buisson, B., Bertrand, S., Corringer, P.-J., Galzi, J.L., Changeux, J.P. and Bertrand, D. (1998) Mol. Pharmacol. 53, 283–294.
[100] Valera, S., Ballivet, M. and Bertrand, D. (1992) Proc. Natl. Acad. Sci. USA 89, 9949–9953.
[101] Hsiao, B., Dweck, D. and Luetje, C.W. (2001) J. Neurosci. 21, 1848–1856.
[102] Galzi, J.L., Bertrand, S., Corringer, P.J., Changeux, J.P. and Bertrand, D. (1996) EMBO J. 15, 5824–5832.