Rational Understanding of Nicotinic Receptors Drug Binding

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FINAL

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Abstract: The atomic determination of the acetylcholine binding protein (AChBP), a molluscan cholinergic protein, homologous to the amino-terminal extracellular domain of nicotinic receptors (nAChRs), offers opportunities for the modeling of the acetylcholine binding site and its ligands. Recently, we constructed three-dimensional models of the N-terminal part of nAChR and docked in the putative ligand-binding pocket, different agonists (acetylcholine, nicotine and epibatidine) and antagonist (snake α-bungarotoxin). These hypothetical docking models offer a structural basis for rational design of drugs differentially binding to resting and active (or desensitized) conformations of the receptor site. These models thus pave the way to investigate, at the molecular level, the exciting challenge of the fast ion channel gating mechanisms by nicotinic agonists.

Key Words: Nicotinic acetylcholine receptor, structure activity relationship, allosteric transitions, drug design.

INTRODUCTION

The nicotinic acetylcholine receptors (nAChRs) are well-characterized transmembrane allosteric proteins, involved in fast ionic responses to acetylcholine (ACh) at the neuro-muscular junction and in the central and peripheral nervous system [1]. NAChRs belong to the Ligand-Gated Ion Channel superfamily (LGIC) [2] and are composed of five identical (homopentamers) or different (heteropentamers) polypeptide chains arranged symmetrically around an axis perpendicular to the membrane. The agonist binding sites are located at the interface between the amino-terminal extracellular domains of subunits, whereas the channel-framing elements are located within the membrane compartment [3]. The combinatorial assembly of vertebrate subunits (α1-10, β1-4, γ, δ, ε) generates a wide diversity of receptors, with various gating and binding properties.

Recently, the structure of a soluble homopentameric molluscan homologue of the amino-terminal domain of nAChR (AChBP for Acetylcholine Binding Protein) has been determined at the atomic level [4]. These data reveal the three-dimensional structure of a binding site for ACh and nicotinic ligands relevant to the interaction of drugs with the nicotinic receptor and their rational design "Fig. (**1A**)" and "Fig. (**1B**)".

ATOMIC STRUCTURE OF THE ACH-BINDING SITE: RATIONAL FOR DRUG DESIGN

Early investigations by photoaffinity labeling and site-directed mutagenesis experiments led to the identification of critical residues which form the ACh site in the *Torpedo* nAChR. Two components of the ACh-binding domain have been distinguished in nAChRs: a 'principal component' located on the α-subunit formed by the three loops A (Tyr93, α1 numbering), B (Trp149) and C (Tyr190, Cys192, Cys193 and Tyr198) and a 'complementary component' on the γ/δ subunits (β2/β4 subunits in neuronal heteromeric nAChRs) made up of loops D (Trp55, Glu57, γ numbering), E (Leu109, Tyr111, Tyr117, Leu119) and F (Asp174, Glu176) on the neighboring subunit "Fig. (**1C**)" and for review see ref. [3]. Moreover, beside these experimental approaches, theoretical studies on the tertiary folding of the nAChR subunit have predicted an immunoglobulin-like model of the amino-terminal extracellular domain of nAChR [3, 5]. The X-ray crystallographic analysis of AChBP at 2.7 Å resolution [4] revealed a striking resemblance between the AChBP structure and the anticipated structural organization of nAChR, thus *a posteriori* validating both the results of the experimental approaches and the computational predictions. First, all residues identified from biochemical experiments predicted to be part of the ligand-binding site were found at subunit boundaries in a box framing the putative ACh binding site in AChBP "Fig. (**1B**)". Second, AChBP is an all β protein, which forms actually an immunoglobulin-like fold.

Since AChBP is 26 % identical in sequence to the amino-terminal part of α7 nAChR, this protein represents an exceptional matrix for comparative structure modeling. Accordingly, we and other groups have constructed three-dimensional models of the extracellular domains of three main types of nicotinic receptors based on the crystal structure of AChBP [6-8]. We also performed semi-automatic dockings of agonists (ACh, nicotine and epibatidine) in the ligand-binding pocket of α7 with the program AUTODOCK, that finds favorable dockings in a protein-binding site, using both simulating annealing and genetic algorithms [6]. We found for those ligands positions consistent with photolabeling and mutagenesis experiments [6] "Fig. (**1B**)", enlarged view. For instance, the methyl groups substituting the ammonium of ACh are close to Tyr93, as suggested earlier [9], and the ammonium ion was able to establish a π-cation interaction with the aromatic residue Trp149 as

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proposed [10]. Moreover, the ester moiety of ACh is close to the vicinal Cys pair 192-193 and to Tyr198 as anticipated [11] "Fig. (**1B**)", enlarged view.

Hence, these *in silico* dockings may now help to understand the pharmacological differences observed between subtypes of nAChRs with different subunit composition and stoichiometries. For instance, epibatidine displays a higher affinity for the site located at the αγ interface than at the αδ interface of the mouse nAChR [12]. In our docking models, one position, γTyr117/δThr119 of loop E, could be critical in the pharmacological difference between the so-called high and low affinity binding "Fig. (**2**)". We propose that the tyrosine aryl faces the binding pocket and could reinforce the interaction with the ligand, and therefore decrease the free energy of the complex. The same observation can be done on neuronal nAChRs. Tyr117 of γ is a homolog of a phenylalanine in β2 (Phe118) and a glutamine in β4 "Fig. (**2**)". Accordingly, regardless of the α-subunit, the affinity for nicotine of receptors containing β4 is lower than one of the receptors containing β2. The loop E (residues 104-120) has already been suggested to be involved in this difference [13]. We propose that the phenylalanine would be involved in the high affinity for nicotine of the desensitized state of β2-containing receptors. In brief, those modeling experiments offer structural bases for rational design of drugs specifically interacting with defined nAChR subunit interfaces.

CONFORMATIONAL TRANSITIONS OF NACHRS

The ACh binding sites and the ion channel are located at approximately 30 Å distance within the nAChR protein molecule. Their interactions are thus indirect or "allosteric" and are mediated by conformational reorganizations of the tertiary and/or the quaternary structure of the oligomeric protein between different discrete states [1]. The molecular mechanisms of these allosteric transitions, governing the gating and desensitization of nAChRs, are currently under active investigation. Elucidation of these mechanisms would help to understand synaptic transmission at the molecular level but also to design drugs that would specifically target with a particular conformational state. The transitions of nAChR protein can be described by a minimal three-state model "Fig. (**3B**)" (four-state model in the case of the muscle-type receptor). In this model, the receptor molecule undergoes a conformational equilibrium between three main discrete and interconvertible quaternary states, which pre-exist to ligand binding: basal or activatable (B), active (A) and desensitized (D) "Fig. (**3B**)". The ion channel is open only in the A state whereas the D state, refractory to activation by agonist, corresponds to the desensitized state. In addition, these three conformations differ by (a) their affinities for acetylcholine, low (100 μM) in the B state, intermediate (1 μM) in the active state and high (10 nM) in the D state and, (b) their kinetics of interconversion, rapid (10-100 μs) towards A, intermediate (ms-s) towards I, and slow (s-min) towards D [14-17]. Under conditions of fast "phasic" transmission, the brief release of a high concentration pulse of ACh populates the active (A) state. At equilibrium, agonists and some antagonists stabilize the high affinity desensitized state.

Based on several independent arguments (high affinity of AChBP for nicotinic ligands and Hill coefficients that are either equal or below unity), it appears plausible, although still hypothetical, that AChBP has been crystallized in a conformation that spontaneously corresponds to the high affinity desensitized state of nAChR [18]. Indeed, this conformation represents a "compact" form of the quaternary structure of the pentameric oligomer. On the other hand, the closed basal state of nAChR may correspond to a less "compact" form of the quaternary structure, allowing the rapid interconvertion from the basal to the active state, in which the ion channel opens. Molecular dynamic simulations offer the possibility to test hypothetical models for these different states. We proposed, on the basis of molecular dynamic simulations of the complex of the α7 nAChR extracellular domain with snake α-bungarotoxin, a 3D organization of the ACh-binding pocket close to the conformation of the site displayed in the basal state [8]. The modeled structure is supported by three independent observations. First, the structure shows an "opening" of the binding site, thus allowing an easier access from the external surface of the pentamer for large snake venom toxins [8] that stabilize the basal "resting" state of nAChR [19, 20]. Second, the docking of small ligands (ACh, nicotine and epibatidine) in the modeled sites results in lower affinities for the basal state in agreement with what is experimentally observed [6]. As seen in "Fig (**3A**)," the two lobes formed by adjacent subunits are more separated in the basal state than in the desensitized state. Therefore, the site in the basal state displays less interactions with bound ligands leading to low affinity. Third, the "closure" of the ACh-binding pocket, accompanying the allosteric transition to the desensitized state of nAChR, is supported by dynamic photoaffinity labeling [21, 22]. In these studies, increasing of photolabeling of some adjacent subunits were reported during the time course of desensitization monitored either by a photo-sensitive antagonist [21] or agonist [22] and were interpreted as a reorganization of the quaternary structure of the nAChR at least in the ACh-binding area.

A parallel study has recently proposed a mechanism of activation based on comparison of electron microscopic data maps (at low resolution, 9 Å) of the extracellular domain of *Torpedo* nAChR with AChBP structure [23]. The authors suggested a modification of the tertiary structure of each α-subunits of *Tordepo* nAChR during the activation process.

Altogether, the aforementioned studies offer models of the reorganization of the molecular structure of nAChRs binding sites during activation and desensitization thus paving the way to the design of conformation specific drugs.

ALLOSTERIC PATHOLOGIES: CASE OF MYASTHENIC MUTANTS

Classically, receptor pathologies are viewed as resulting from "losses of function". Such genetic deficits have indeed been observed in the case of congenital myasthenic syndromes [24]. On the other hand, "gains of function" may also cause some allosteric pathologies of nAChRs. These physiological disorders are selectively mediated by molecular modifications of the allosteric properties of the receptor. For instance, autosomal dominant nocturnal frontal lobe epilespy (ADNFLE) is one of these neurologic

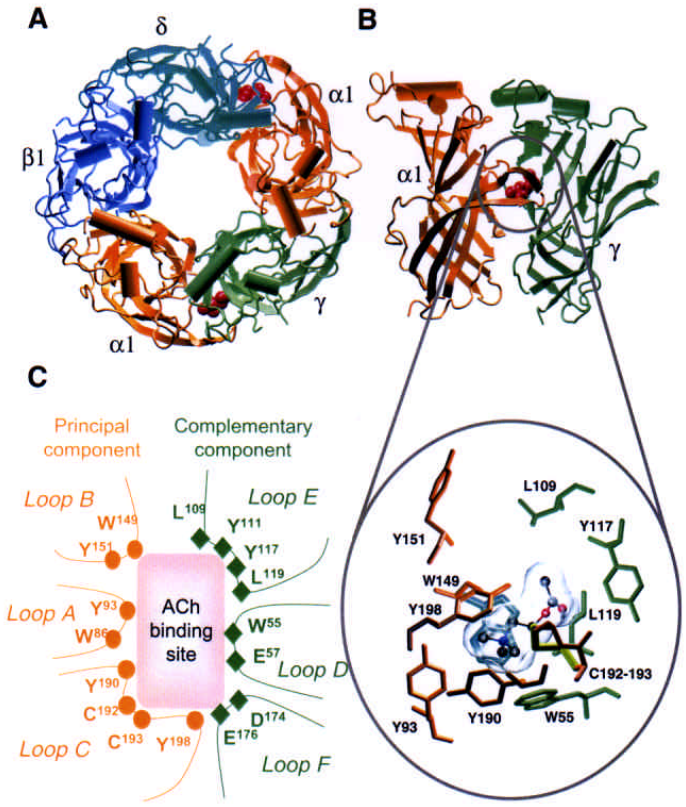

Fig. (1). Models of the extracellular domain of muscle-type nAChRs based on the crystal structure of AChBP. (A) View of the nAChR pentamer from the synaptic cleft along the fivefold axis reveals the anti-clockwise arrangements of the five subunits (α1, γ, α1, δ and β). (B) Side view of the α1-(orange) and γ- (green) subunits with a bound acetylcholine molecule (in red space filling) at the interface. An enlarged view of the putative binding site shows residues forming the acetylcholine binding site. (C) Residues identified by photoaffinity labeling and mutational analyses on *Torpedo* receptor (muscle-type receptor). Orange depicts residues contributing to the principal component (here, the α1 subunit) and green depicts residues contributing to the complementary component (here, the γ subunit). Numbering refers to α1 and γ- subunits.

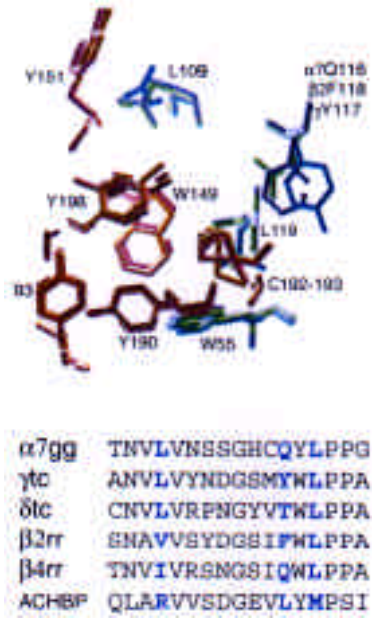

Fig. (2). Representation of the superposed agonist-binding sites between two α7-subunits (salmon and green), α4 (pink) and β2 (purple) and α1 (orange) and γ (blue). Numerals refer to α1 and γ otherwise stated. Aligned sequences of loop E region are also indicated.

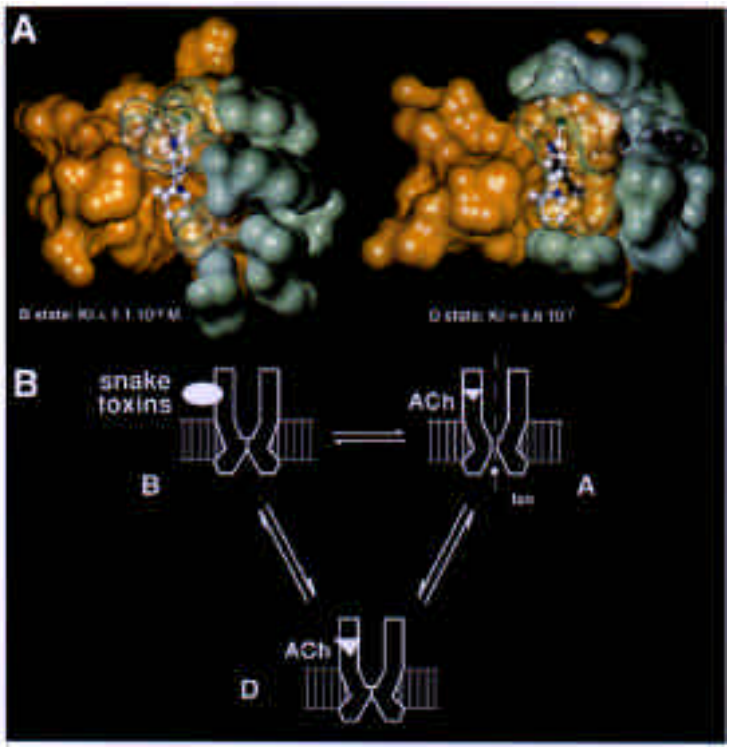

Fig. (3). Allosteric behavior of nAChRs. (A) Hypothetical acetylcholine binding site displayed in the basal state after dynamic simulation (left) and desensitized state (right) of the α7 extracellular domain [8]. For clarity, part of the acetylcholine-binding pocket is shown with the same color code as in Figure 1. Docked epibatidine is shown in both conformations with computed binding constants. (B) Minimal three-state model of nAChR. The receptor interconverts to different conformational states; basal or resting (B), active (A) in which the ion channel opens, and desensitized (D).

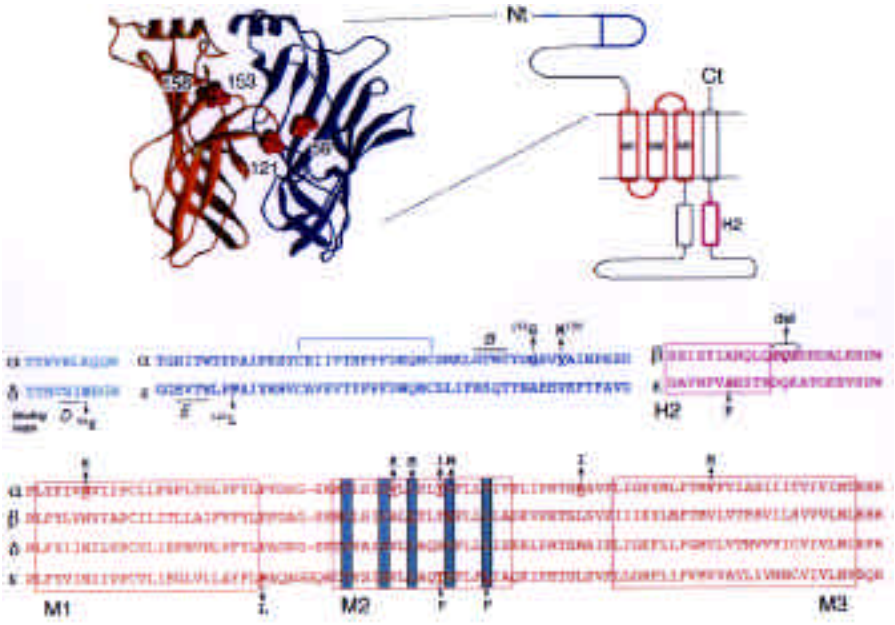

Fig. (4). Location of myasthenic mutants. A schematic representation of a nAChR subunit inserted in the membrane is shown. The drawing on the right indicate (in bold color) the protein domain concerned. The mutations are indicated by the single letter amino acid above/under the sequence with an arrow pointing from the wild-type highlighted residue. Underlined boldface residues indicate “gain of function” mutations. Acetylcholine binding loops (B, D and E) are also represented in italic. In the M2 transmembrane segment, blue boxes indicated residues that face the ion-conducting pathway. The 3D structure of two subunits of the extracellular domain of nAChR is also indicated on the left with myasthenic mutants in red space filling.

disorders. Despite its cause remains unclear, recent functional evidences now support that "gains of function" of a neuronal nAChR (α4β2) are the main cause of the disorder [25]. Allosteric "gain of function" mutations also occur in the case of myasthenic syndromes in muscles. Several of them were found dispersed in the sequence of nAChR "Fig. (**4**)" [26, 27]. Most of them are localized in the second transmembrane segment (M2) and face the lumen where the ions are conducted in the open state "Fig. (**4**)". Others are located in the amino-terminal domain of nAChR and can now be placed in the three-dimensional space. Strikingly, these mutations are located within the core of the subunit [Gly153Ser [28] and Val156Met [29]], whereas "loss of function" mutations are found at subunit boundaries [Pro121Leu [30] and Glu59Lys [31]] thus modifying the pattern of interactions between subunits "Fig. (**4**)".

Some of these myasthenic mutants (e.g. εThr264Pro) can be used to distinguish between classical alternative models of conformational transitions [32]. Two competing models have been suggested in the years 1965-66 [33, 34]. The sequential type model (KNF model) limits conformational states in the absence of ligand to one state, and the binding of the agonist to its specific site *induces* the conformational transition (induced fit) [34]. In addition, conformational transitions affect the monomers independently, resulting in multiple "intermediate" states. Alternatively, the concerted model (MWC model) allows *pre-equilibration* of the protein oligomer between discrete conformational states in the absence of ligand, and this latter selectively stabilizes the particular state for which it displays the highest affinity, thus shifting the equilibrium [33, 35]. Moreover, the conformation transition has been assumed to occur in a concerted fashion for all the protomers within the oligomer at the same time. In the absence of agonist, spontaneous transitions between states can occur, which are governed by the constants of the pre-existing conformational equilibrium. The observations that spontaneous channel openings occur in the wild-type [36] and mutant nAChRs [37], in particular in myasthenic mutants [32], rule out strict "induced fit" models. Additionally, the fact that myasthenic mutants are distributed at variable positions in the 3D organization of the molecule e.g. at subunit boundaries or within the core of each subunit "Fig. (**4**)", are consistent with the concerted model. Whatever their position in the 3D structure, these mutations are viewed as modifying the equilibrium constants between states rather than affecting at distance the intrinsic ligand-binding properties [32]. More recently, general models which incorporate multiple intermediate states were proposed to account for more complex behaviors of receptors [1, 38].

CONCLUSION

In conclusion, the three-dimensional structure of the extracellular domain of a homolog of nAChRs offer a structural basis for rational drug design. It is important to state that models of nAChR extracellular domain represent frozen "snapshots" of a particular state constrained by the crystal structure of AChBP. Therefore, drug design should also consider in its process conformational transitions of the receptor which allosterically modify the target site. Moreover, the atomic structure of the ion channel of nAChR is still unknown although its deciphering would be crucial to proper understanding of the dynamic of the protein. Elucidation of the three-dimensional structure of the ion channel in the closed and open conformations, as recently done for the calcium-gated potassium channel [39], should thus dictate the orientation of the extracellular domain of nAChR relative to the membrane. This would also help to resolve mechanisms involved in the gating and desensitization of nAChR, in order to understand synaptic transmission.

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REFERENCES

[1] Changeux, J.-P.; Edelstein, S. J. Allosteric receptors after 30 years. *Neuron* **1998**, *21*, 959-980.

[2] Le Novère, N.; Changeux, J.-P. The ligand gated ion channel database. *Nucleic Acids Res.* **1999**, *27*, 340-342.

[3] Corringer, P. J.; Le Novère, N.; Changeux, J.-P. Nicotinic receptors at the amino acid level. *Annu. Rev. Pharmacol. Toxicol.* **2000**, *40*, 431-458.

[4] Brejc, K.; van Dijk, W. J.; Klaassen, R.; Schuurmans, M.; van der Oost, J.; Smit, A. B.; Sixma, T. K. Crystal structure of AChBP reveals the ligand-binding domain of nicotinic receptors. *Nature* **2001**, *411*, 269-276.

[5] Le Novère, N.; Corringer, P. J.; Changeux, J.-P. Improved secondary structure predictions for a nicotinic receptor subunit: incorporation of solvent accessibility and experimental data into a two-dimensional representation. *Biophys. J.* **1999**, *76*, 2329-2345.

[6] Le Novère, N.; Grutter, T.; Changeux, J.-P. Models of the extracellular domain of the nicotinic receptors and of the agonist- and Ca^{2+} -binding sites. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3210-3215.

[7] Schapira, M.; Abagyan, R.; Totrov, M. Structural model of nicotinic acetylcholine receptor isotypes bound to acetylcholine and nicotine. *BMC Structural Biology* **2002**, *2*, 1-8.

[8] Fruchart-Gaillard, C.; Gilquin, B.; Antil-Delbeke, S.; Le Novère, N.; Tamiya, T.; Corringer, P. J.; Changeux, J.-P.; Ménez, A.; Servent, D. Experimentally based model of a complex between a snake toxin and the α7 nicotinic receptor. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3216-3221.

[9] Cohen, J. B.; Sharp, S. D.; Liu, W. S. Structure of the agonist-binding site of the nicotinic acetylcholine receptor. [^{3}H]acetylcholine mustard identifies residues in the cation- binding subsite. *J. Biol. Chem.* **1991**, *266*, 23354-23364.

[10] Zhong, W.; Gallivan, J. P.; Zhang, Y.; Li, L.; Lester, H. A.; Dougherty, D. A. From *ab initio* quantum mechanics to molecular neurobiology: a cation-pi binding site in the nicotinic receptor. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12088-12093.

[11] Grutter, T.; Ehret-Sabatier, L.; Kotzyba-Hibert, F.; Goeldner, M. Photoaffinity labeling of Torpedo nicotinic receptor with the agonist [^{3}H]DCTA: identification of amino acid residues which contribute to the binding of the ester moiety of acetylcholine. *Biochemistry* **2000**, *39*, 3034-3043.

[12] Prince, R. J.; Sine, S. M. Epibatidine activates muscle acetylcholine receptors with unique site selectivity. *Biophys. J.* **1998**, *75*, 1817-1827.

[13] Cohen, B.; Figl, N.; Quick, M.; Labarca, C.; Davidson, N.; Lester, H. Regions of beta2 and beta4 responsible for differences between the steady state dose-response relationships of the alpha3 beta2 and alpha3 beta4 neuronal nicotinic receptors. *J. Gen. Physiol.* **1995**, *105*, 745-764.

[14] Heidmann, T.; Changeux, J.-P. Fast kinetic studies on the interaction of a fluorescent agonist with the membrane-bound acetylcholine receptor from Torpedo marmorata. *Eur. J. Biochem.* **1979**, *94*, 255-279.

[15] Heidmann, T.; Changeux, J.-P. Interaction of a fluorescent agonist with the membrane-bound acetylcholine receptor from Torpedo

marmorata in the millisecond time range: resolution of an "intermediate" conformational transition and evidence for positive cooperative effects. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 889-896.

[16] Changeux, J.-P.; Devillers-Thiéry, A.; Chemouilli, P. Acetylcholine receptor: an allosteric protein. *Science* **1984**, *225*, 1335-1345.

[17] Boyd, N. D.; Cohen, J. B. Kinetics of binding of [^{3}H]acetylcholine and [^{3}H]carbamoylcholine to Torpedo postsynaptic membranes: slow conformational transitions of the cholinergic receptor. *Biochemistry* **1980**, *19*, 5344-5353.

[18] Grutter, T.; Changeux, J.-P. Nicotinic receptors in wonderland. *Trends Biochem. Sci.* **2001**, *26*, 459-463.

[19] Moore, M. A.; McCarthy, M. P. Snake venoms toxins, unlike smaller antagonists, appear to stabilize a resting state conformation of the nicotinic acetylcholine receptor. *Biochem. Biophys. Acta* **1995**, *1235*, 336-342.

[20] Middleton, R. E.; Strnad, N. P.; Cohen, J. B. Photoaffinity labeling the torpedo nicotinic acetylcholine receptor with [^{3}H]tetracaine, a nondesensitizing noncompetitive antagonist. *Mol. Pharmacol.* **1999**, *56*, 290-299.

[21] Galzi, J.-L.; Revah, F.; Bouet, F.; Menez, A.; Goeldner, M.; Hirth, C.; Changeux, J.-P. Allosteric transitions of the acetylcholine receptor probed at the amino acid level with a photolabile cholinergic ligand. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 5051-5055.

[22] Grutter, T.; Bertrand, S.; Kotzyba-Hibert, F.; Bertrand, D.; Goeldner, M. Structural reorganization of the acetylcholine binding site of the Torpedo nicotinic receptor as revealed by dynamic photoaffinity labeling. *Chem. Bio. Chem.* **2002**, *3*, 652-658.

[23] Unwin, N.; Miyazawa, A.; Li, J.; Fujiyoshi, Y. Activation of the nicotinic acetylcholine receptor involves a switch in conformation of the α subunits. *J. Mol. Biol.* **2002**, *319*, 1165-1176.

[24] Ohno, K.; Engel, A. G. Congenital myasthenic syndromes: genetic defects of the neuromuscular junction. *Curr. Neurol. Neurosci. Rep.* **2002**, *2*, 78-88.

[25] Bertrand, D.; Picard, F.; Le Hellard, S.; Weiland, S.; Favre, I.; Phillips, H.; Bertrand, S.; Berkovic, S. F.; Malafosse, A.; Mulley, J. How mutations in the nAChRs can cause ADNFLE epilepsy. *Epilepsia* **2002**, *43*, 112-122.

[26] Léna, C.; Changeux, J.-P. Pathological mutations of nicotinic receptors and nicotine-based therapies for brain disorder. *Curr. Opin. Neurobiol.* **1997**, *7*, 674-682.

[27] Engel, A. G.; Ohno, K.; Sine, S. *Congenital myasthenic syndromes*; Engel, A. G., Ed.; Oxford University Press. Oxford, United Kingdom, **1999**, pp 251-297.

[28] Sine, M. S.; Ohno, K.; Bouzat, C.; Auerbach, A.; Milone, M.; Pruit, J. N.; Engel, A. G. Mutation of the acetylcholine receptor alpha subunit causes a slow-channel myasthenic syndrome by enhancing agonist binding affinity. *Neuron* **1995**, *15*, 229-239.

[29] Croxen, R.; newland, C.; Beeson, D.; Oosterhuis, H.; Chauplannaz, G.; Vincent, A.; Newsom-Davis, J. Mutations in different functional domains of the human muscle acetylcholine receptor α subunit in patients with the slow-channel congenital myasthenic syndrome. *Hum. Mol. Genet.* **1997**, *6*, 767-774.

[30] Ohno, K.; Wang, H.-L.; Milone, M.; Bren, N.; Brengman, J. M.; Nakano, S.; Quiram, P.; Pruitt, J. N.; Sine, S. M.; Engel, A. G. Congenital myasthenic syndrome caused by decreased agonist binding affinity due to a mutation in the acetylcholine receptor ε subunit. *Neuron* **1996**, *17*, 157-170.

[31] Brownlow, S.; Webster, R.; Croxen, R.; Brydson, M.; Neville, B.; Lin, J.-P.; Vincent, A.; Newsom-Davis, J.; Beeson, D. Acetylcholine receptor δ subunit mutations underlie a fast-channel myasthenic syndrome and arthrogryposis multiplex congenita. *J. Clin. Invest.* **2001**, *108*, 125-130.

[32] Edelstein, S. J.; Schaad, O.; Changeux, J.-P. Myasthenic nicotinic receptor mutant interpreted in terms of the allosteric model. *C. R. Acad. Sci. Paris, Sciences de la vie/ Life Sciences* **1997**, *320*, 953-961.

[33] Monod, J.; Wyman, J.; Changeux, J.-P. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* **1965**, *12*, 88-118.

[34] Koshland, D. E., Jr.; Nemethy, G.; Filmer, D. Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry* **1966**, *5*, 365-385.

[35] Rubin, M. M.; Changeux, J.-P. On the nature of allosteric transitions: implications of non-exclusive ligand binding. *J. Mol. Biol.* **1966**, *21*, 265-274.

[36] Jackson, M. B. Spontaneous openings of the acetylcholine receptor channel. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 3901-3904.

[37] Bertrand, S.; Devillers-Thiéry, A.; Palma, E.; Buisson, B.; Edelstein, S. J.; Corringer, P. J.; Changeux, J.-P.; Bertrand, D. Paradoxical allosteric effects of competitive inhibitors on neuronal α7 nicotinic receptor mutants. *Neuro. Report* **1997**, *8*, 3591-3596.

[38] Duke, T. A. J.; Le Novère, N.; Bray, D. Conformational spread in a ring of proteins: a stochastic approach to allostery. *J. Mol. Biol.* **2001**, *308*, 541-553.

[39] Jiang, Y.; Lee, A.; Chen, J.; Cadene, M.; Chait, B. T.; Mackinnon, R. Crystal structure and mechanism of a calcium-gated potassium channel. *Nature* **2002**, *417*, 515-522.