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The Nicotinic Acetylcholine Receptor: The Founding Father of the Pentameric Ligand-gated Ion Channel Superfamily*

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A critical event in the history of biological chemistry was the chemical identification of the first neurotransmitter receptor, the nicotinic acetylcholine receptor. Disciplines as diverse as electrophysiology, pharmacology, and biochemistry joined together in a unified and rational manner with the common goal of successfully identifying the molecular device that converts a chemical signal into an electrical one in the nervous system. The nicotinic receptor has become the founding father of a broad family of pentameric membrane receptors, paving the way for their identification, including that of the $GABA_A$ receptors.

It has been 42 years since the isolation of the nicotinic acetylcholine receptor (nAChR)[2] from fish electric organ, the first ligand-gated ion channel and the first ion channel ever identified; 25 years since the first $GABA_A$ and glycine receptor subunits were cloned and sequenced and concomitantly their homology with the nAChRs recognized; and 5 years since the discovery that closely homologous ligand-gated ion channels are present in prokaryotes (1). In this minireview, I briefly retrace the main steps in the discovery of the nAChR, the titular head of this receptor superfamily.

The Concept of Receptor and the Chemical Identification of the Acetylcholine Receptor

The English physiologist John Newport Langley, working with neuromuscular preparations, proposed in 1905 that muscle tissue possesses "a substance that combines with nicotine and curare . . . receives the stimulus and transmits it." He called the muscle entity the "receptive substance." In the subsequent 50 years, the concept of pharmacological receptors inspired three main lines of research: first, the pharmacological approach aimed at characterizing the specificity of the receptor site by using novel chemical ligands (*e.g.* the distinction between nicotinic and muscarinic AChRs by Sir Henry Dale); second, the electrophysiological approach exemplified by Bernard Katz and John Eccles aimed at understanding the ionic responses to endogenous neurotransmitter signals; and third, the chemical tradition aimed at the chemical identification of the receptor molecule(s).

In the late 1960s, lipids, polysaccharides, proteins, and even nucleic acids were considered as potential receptors. The early independent efforts of Carlos Chagas, Eduardo de Robertis, and David Nachmansohn to identify the receptor for acetylcholine (ACh) in the electric organ of the fish *Electrophorus electricus* with radioactive ligands were abandoned because their tissue extracts lacked specificity (2). However, in the course of these studies, Nachmansohn recognized the extraordinarily rich content of nicotinic synapses in the electric organ (2). With Ernest Schoffeniels, he devised a method for preparing individual cells, or electroplaques, from the electric organ. This offered the opportunity to investigate, simultaneously, the electrophysiological, pharmacological, and biochemical characteristics of the response to ACh within the same biological system (2). At this time, there were also speculations that the enzyme acetylcholinesterase (AChE) and the physiological receptor site for ACh could reside on the same protein complex.

The introduction of new biochemical methods radically changed the field of receptor identification. One such method is affinity labeling, which relies on the use of compounds that are structural homologs of the neurotransmitter and also possess a highly reactive group. This combination allows for specific binding to the receptor site, and once bound, the probe covalently links to the protein. For instance, the molecule *p*-trimethylammonium benzenediazonium fluoroborate (TDF) carries a trimethylammonium group (as does ACh) as well as a reactive diazonium group (3). As anticipated, TDF interacted covalently with *E. electricus* electroplaque as an irreversible competitive antagonist, and curare protected against this covalent attachment (4). The method was subsequently improved upon with the synthesis of 4-(*N*-maleimido)phenyltrimethylammonium iodide, whereby the diazonium is substituted with a maleimide group (5). The latter selectively reacts with –SH groups exposed by treating the electroplaque membrane with dithiothreitol. However, at this stage, both the method of tissue preparation and the specificity of the compounds used were insufficient to allow for isolation of the receptor in its active form from the electric organ.

A second method that significantly advanced the field was the marked improvement of procedures for fractionation and purification of membrane fragments rich in AChE from *E. electricus* electric organs. Electron microscopic sections of these membrane fragments revealed that they formed closed vesicles (6). Inspired by the technique used with bacterial permeases (7), it became possible to measure radioactive Na^+ (or K^+) ion fluxes with these microsacs by using a simple filtration method (8, 106). The microsacs responded to nicotinic agonists with specificities closely resembling those recorded by electrophysiological methods employing intact electroplaques. The signal transduction by the neurotransmitter could be reproduced in a totally acellular system in the absence of energy supply and in a

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[2] The abbreviations used are: nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine; AChE, acetylcholinesterase; TDF, *p*-trimethylammonium benzenediazonium fluoroborate; α-BGT, α-bungarotoxin; GluCl, glutamate-gated chloride channel; DDF, *p*-*N*,*N*-dimethylammonium benzenediazonium difluoroborate; ELIC, *E. chrysanthemi* ligand-gated ion channel; GLIC, *G. violaceus* ligand-gated ion channel; TM, transmembrane segment.

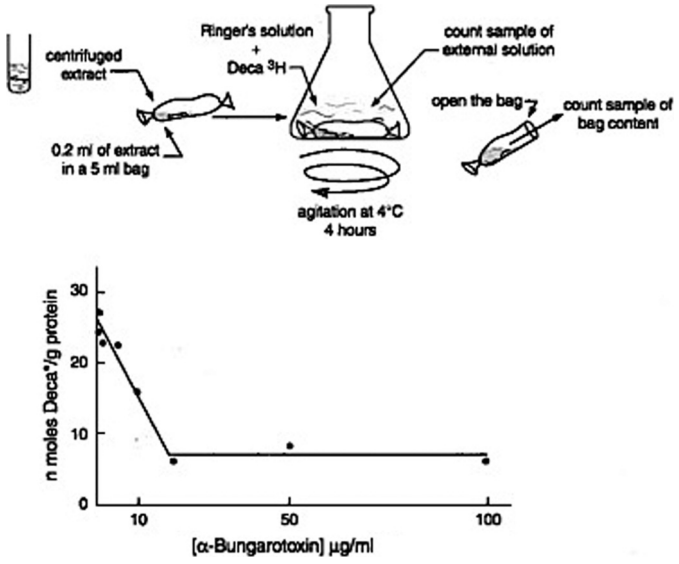

FIGURE 1. ***Upper*, binding method by equilibrium dialysis used for the identification of the nicotinic receptor. *Lower*, effect of the snake toxin α-BGT on binding of the nicotinic agonist [^{3}H]decamethonium (*Deca*).** This figure has been reprinted from Refs. 12 (*lower*) and 51 (*upper*).

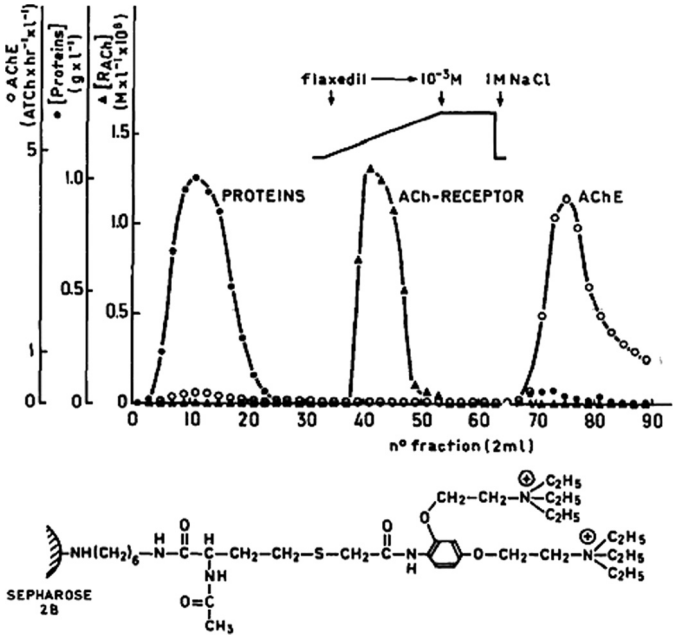

FIGURE 2. **Purification of the nAChR by affinity chromatography.** This figure has been reprinted from Ref. 17.

chemically defined environment. Thus, it became possible to study *in vitro* the chemistry of the physiological response to ACh and of the signal transduction mechanism involved (8, 106). The receptor molecule was evidently present in the purified membranes in a functional state. It was now possible to follow reversible binding to these purified membranes using the nicotinic agonist decamethonium as the radioactive ligand (by the method of equilibrium dialysis that Gilbert and Müller-Hill (9) used to identify the lac repressor) (Fig. 1). The detergent deoxycholate gently extracted the binding protein without denaturing it, and bound decamethonium was displaced by various nicotinic agonists and antagonists, including curare and Flaxedil in the order of their physiological effects (10). Since then, similar receptor binding assays have been used extensively to characterize the $GABA_A$ and glycine receptors (see the accompanying Classics).

Third, Chen-Yuan Lee, a Taiwanese pharmacologist, had found that a snake venom toxin, α-bungarotoxin (α-BGT), specifically blocks *in vivo* neuromuscular transmission in high vertebrates at the postsynaptic level without interacting with AChE (11). Aware of Claude Bernard's lesson to use toxic compounds as chemical lancets, I asked Lee, who unexpectedly visited me at Institut Pasteur, for a sample of the toxin. A few days later, I received it and immediately tried it in the three systems just mentioned. The result was remarkable (12): α-BGT blocked the electroplaque's electrical response *in vivo* and the microsac's ion flux response to nicotinic agonists *in vitro*; α-BGT also blocked the binding of radioactive decamethonium to the detergent extract (Fig. 1). This extract contained a protein, sensitive to Pronase digestion, that bound nicotinic agonists and the snake venom toxin in a mutually exclusive manner. This nicotinic receptor (nAChR) molecule was shown to be a high molecular weight hydrophobic protein that could be physically separated from AChE (12).

An α-toxin from *Naja nigricollis*, closely homologous to α-BGT, was then covalently coupled to Sepharose beads without losing its binding activity. Mixing the toxin beads with the membrane extract revealed that 75–100% of the nAChR protein bound to the toxin beads, whereas 85–100% of the AChE remained in the supernatant. The data (13) confirmed that AChE and the nAChR molecule were distinct protein entities. These studies also introduced Cuatrecasas' technique of affinity chromatography to the nAChR field. Many groups then became aware of these distinct methods (14–16). We (17, 18) and others (19) used alternative affinity columns with immobilized quaternary ammonium agonists or antagonists (Fig. 2), extending the use, by Miledi *et al.* (20), of radioactive ^{131}I-labeled α-BGT (which, according to them, selectively binds to the receptor in its resting state).

Another rather simple technological development that, retrospectively, had an important impact on nAChR research was the isolation of a novel generation of excitable microsacs exceptionally rich in nAChR (20–40% of total protein) prepared from homogenates of *Torpedo marmorata* electric organ (21), a finding that was readily confirmed by other groups. The nAChR-rich membranes made the structural and functional properties of the membrane-bound nAChR accessible to a variety of biochemical and biophysical methods, such as purification in large quantities (22), fluorescence spectroscopy (23), electron spin resonance (24), and x-ray diffraction (25).

Finally, the nAChR protein purified from *E. electricus* and the purified nAChR-rich membranes from *T. marmorata* were examined by electron microscopy and revealed ring-like particles (8–9 nm in diameter) with a hydrophilic core linked to a compact bundle (Fig. 3) (26). Made up of several (five to six) subunits, they formed closely packed two-dimensional assemblies in *T. marmorata* postsynaptic membranes (~8,000–12,000 μm^2) (Fig. 3) (26, 27). These nAChR images were the first ever of the structure of a neurotransmitter receptor. They were subsequently described in greater details by Nigel Unwin (reviewed in Ref. 28) and others. Similar pictures later became

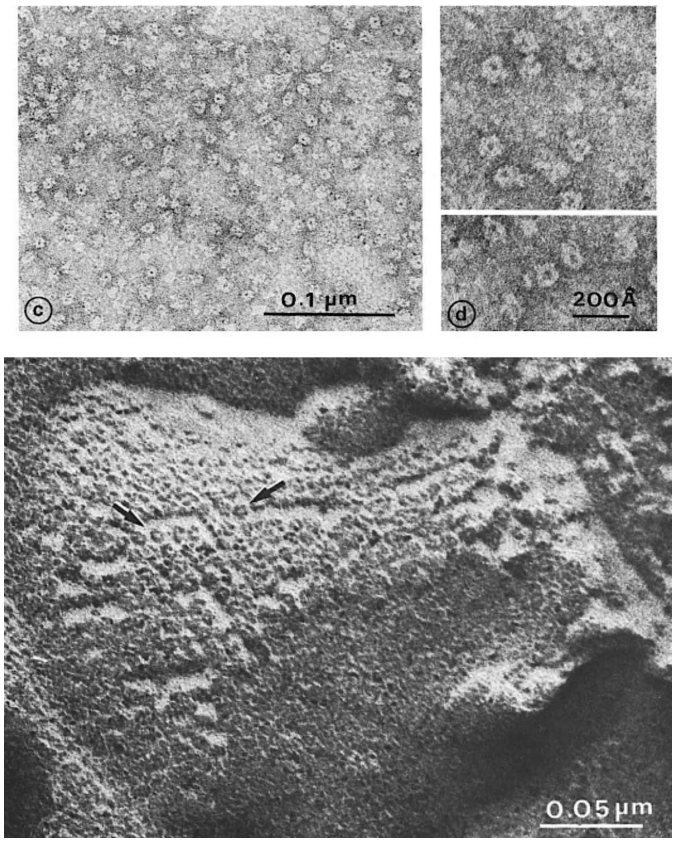

FIGURE 3. **First structural observation of the purified nicotinic receptor proteins from *E. electricus* (*upper*) and from purified subsynaptic membrane fragments from *T. marmorata* (*lower*).** This figure has been reprinted from Ref. 26.

available for the $GABA_A$ and glycine receptors (see the accompanying Classics).

The Pentameric Organization of the Nicotinic Receptor and the Complete Sequence of the Subunits

The amount of purified nAChR was sufficient to identify the subunit organization of the protein. A first study using partial cross-linking of the purified *E. electricus* nAChR revealed five well defined bands, suggesting a pentameric organization (29). The pentameric organization was rapidly confirmed by the teams of Karlin and Raftery, who, in addition, discovered that the nAChR molecule is composed of four distinct types of subunits with slight differences in molecular mass that assemble into a $2\alpha_1\beta_1\gamma_1\delta_1$ heteropentamer (30–33).

Nothing was known about the chemistry of the subunits. However, with the recently developed new technology of high resolution microsequencing, amino acid sequences could be determined from small quantities of protein. The sequence of 20 amino acids comprising the N-terminal domain of the α-subunit of the *T. marmorata* receptor was established in my laboratory (34). A chemical identity card of the receptor was made available, the first ever established for a neurotransmitter receptor. It was confirmed in the Raftery laboratory with the α-subunit of *Torpedo californica* (35) and extended to the N-terminal sequence of the four subunits, revealing a number of sequence identities among the subunits (36). Consistent with the Monod-Wyman-Changeux (1965) model (37), the nAChR protein was an authentic oligomer, but pseudosymmetrical, with a 5-fold axis of rotation perpendicular to the plane of the postsynaptic membrane.

Knowledge of the initial sequence data opened the nAChR field to recombinant DNA technologies. The teams of Shosaku Numa (38–40), Stephen Heinemann (41, 42), and Eric Barnard (43), as well as Anne Devillers-Thiéry, and Jérôme Giraudat (44, 45) in my laboratory, struggled to clone the complementary DNAs of the different subunits from electric organ and muscle and to establish their complete sequence. Experiments by Eric Barnard and Ricardo Miledi had demonstrated that messenger RNA extracted from the electric organ of *Torpedo* injected into *Xenopus* oocytes led to the synthesis and incorporation of functional AChRs into the membrane of the oocyte (46). Injection of the four mRNAs transcribed from the cloned cDNAs yielded functional nAChRs (47), confirming earlier biochemical experiments (48, 49) demonstrating that assembly of the four types of subunits suffices to recover a fully operational nAChR.

Examination of the complete cDNA sequences revealed several common structural domains along the sequences of the subunits that led to the first model of transmembrane organization of nAChR subunits (39, 40, 42, 45). It was proposed that the long hydrophilic N-terminal segment, four hydrophobic stretches, and a short hydrophilic segment were organized into an extracellular (synaptic) domain, four transmembrane α-helices, and an intracellular (cytoplasmic) domain. In 1986 and after, closely homologous sequences and the organization of the subunits, including a Cys loop, were found in neuronal nicotinic ACh receptors, including α7- and $\alpha4\beta2$-nAChRs (Ref. 50; reviewed in Ref. 51), $GABA_A$, glycine, $5\text{-}HT_3$, and glutamate-gated chloride channel (GluCl), thus creating the superfamily of pentameric receptors that is the subject of the accompanying Classics. The recent discovery of cationic orthologs in prokaryotes (52, 53) has extended the superfamily, plunging its evolutionary origins back 3 billion years (1).

Identification of the ACh-binding Sites

The actual tridimensional topology of the AChR protein and of the various sites it carries still could not be directly inferred from recombinant DNA technologies. Identification of the amino acids composing the ACh-binding site and the ion channel relied upon different technologies. The previously mentioned method of affinity labeling proved to be useful at this stage. A first result was obtained by Karlin's group using 4-(*N*-maleimido)phenyltrimethylammonium iodide (5), which labels the sulfhydryl groups of the ACh-binding site (see above). This led to the identification of a pair of adjacent cysteines (positions 192 and 193) located in the N-terminal domain of the α-subunit (54). Despite these results, the pharmacological specificity of the ACh-binding site remained unknown.

Our group demonstrated that the snake ^{3}H-labeled α-toxin itself, without additional modification, could be used as a photolabel. UV irradiation of the ^{3}H-labeled α-toxin-*Torpedo* receptor complex resulted in the incorporation of covalently bound radioactivity not only into the α-subunit but also into the γ- and δ-subunits (55). From this observation, it was concluded that the ACh-binding sites were located at the interface

between subunits (55) and were therefore non-equivalent. This was confirmed in subsequent functional studies.

The use of *p-N,N*-dimethylammonium benzenediazonium difluoroborate (DDF), an affinity probe similar to TDF (3, 4), provided additional important information (56). The dimethylammonium group of DDF created a resonant molecule that could be photoactivated by energy transfer from the protein. Indeed, eight amino acids were found labeled by DDF, six of them with an aromatic side chain, and all of them located in the long hydrophilic N-terminal domain of the α-subunit. These amino acids were distributed into three main loops, forming a sort of electronegative aromatic pocket in which the quaternary ammonium group of ACh was lodged (56–58), thus pointing to an analogy with the AChE-binding site, where π bonding is exhibited as well. These three loops, located on the α-subunit side of the binding site and referred to as the "principal component," were named A, B, and C (58), a nomenclature that has been adopted by the receptor community. In agreement with the snake ^{3}H-labeled α-toxin photolabeling data, the affinity probe DDF labeled the γ- and δ-subunits in addition to the α-subunit (51, 56–59). The various groups working on the receptor, including those of Arthur Karlin, Jonathan Cohen, and ourselves, further documented this notion and identified additional loops D, E, and F on the non-α-subunit side of the interface (Refs. 51 and 59 and references therein). These loops form a "complementary" component of the ACh-binding site on the γ- and δ-subunits. These biochemical data were supported by site-directed mutagenesis studies of the labeled amino acids identified in these studies (Refs. 51 and 59 and references therein).

Confirmation of the binding site organization has come from the crystal structure of a soluble snail protein that binds ACh, the ACh-binding protein, a close homolog of the nAChR extracellular domain (60) and of the full-length eukaryotic GluCl receptor (61) and the prokaryotic *Erwinia chrysanthemi* receptor (ELIC) bound with GABA (62) and ACh (as an antagonist) (Ref. 63; reviewed in Ref. 1).

Identification of the Ion Channel

By the early 1980s, no biochemical structure of any ion channel was known. The question was how to chemically identify the amino acids that line the pore through which ions flow. The quest (1974–1999) proved to be long and difficult (see Refs. 51 and 64). Pharmacological agents, such as local anesthetics, known for decades to block ion currents elicited by nicotinic agonists in an indirect noncompetitive manner, proved to be essential tools for chemical labeling the channel. The first experiments, performed with both *E. electricus* and *T. marmorata* receptor-rich membranes, demonstrated *in vitro* that, at pharmacologically active concentrations, the local anesthetics did not directly displace nicotinic ligands from the ACh-binding site but reversibly bound to a different allosteric site (65, 66). One of these compounds, chlorpromazine, displayed, in addition, the remarkable property of covalently linking to the receptor protein by simple UV irradiation. In receptor-rich membranes from *T. marmorata*, chlorpromazine labeled the four types of subunits of the nAChR (67), and precise quantitative measurements demonstrated that it bound to just one high affinity site per $2\alpha_1\beta_1\gamma_1\delta_1$ oligomer (68). The kinetics of access of chlorpromazine to this site increased by 100-fold when rapidly mixed with ACh under conditions expected to generate functional ion channels (69, 70). We proposed that chlorpromazine binds to a site located within the ion channel along the pseudosymmetry axis that becomes accessible to chlorpromazine when the ion channel opens. The conditions under which the channel could be specifically labeled were thus established.

It took more than a year to demonstrate that chlorpromazine labels serine 262, within the second transmembrane segment (TM2) of the δ-subunit (71), a finding that was rapidly confirmed by another group using the same protocol but with a different probe (72). Further identification of the chlorpromazine-labeled amino acids on the other subunits not only showed that the serines form a ring (73) but also revealed the adduct of other amino acids (leucines and threonines) located at a distance of three to four amino acids on both sides of the ring of serines (74). It was concluded that (*a*) the TM2 segments contribute to the channel walls, (*b*) these segments are folded into an α-helix, (*c*) the chlorpromazine-binding site is located at a near-equatorial position in the channel's pseudosymmetry axis; and (*d*) a positive reciprocal allosteric interaction exists between ACh and the chlorpromazine-binding sites.

In parallel site-directed mutagenesis experiments in which single channel recordings were carried out after reconstitution in *Xenopus* oocytes, a region located in the δ-subunit that comprises the putative TM2 segment and the adjacent bend portion between TM2 and TM3 was shown to be responsible for a conductance difference between *Torpedo* and bovine channels (75). Subsequent analysis (76) identified rings of negatively charged glutamine residues that were classified as external, intermediate, and cytoplasmic and that beautifully framed the amino acid clusters labeled by chlorpromazine, thus confirming their proposed location within the ion path (68–70). The teams of Henry Lester and Norman Davidson reached a similar conclusion (77).

Further studies identified amino acids that contribute to the ionic selectivity of the channel (78–80). A group of three residues was found to drive the conversion of the cationic selectivity of the ion channel into one of anionic selectivity (79, 80). For the first time, an excitatory receptor could be transformed into an inhibitory one. This finding, as well as the converse operation (from anionic to cationic), was reproduced with other receptors: $GABA_A$, glycine, GluCl, and 5-HT_3 (Ref. 51; see the accompanying Classics). A functional chimera was successfully constructed that joined the synaptic domain of α7-nAChR and the transmembrane domain of the 5-HT_3 receptor (81). Even combinations of prokaryotic and eukaryotic receptor domains were found functional (82). This demonstrates unambiguously a conservation of tertiary organization between members of the receptor superfamily. Finally, the high resolution x-ray data from prokaryotic ELIC and GLIC (from *Gloeobacter violaceus*) (83–85) are consistent with the biochemical data and EM structure (reviewed in Refs. 28 and 51) of the nAChR ion channel (1). They demonstrate further that the channel domain is topographically distinct from the neurotransmitter-binding domain and that the interaction between the neurotransmitter

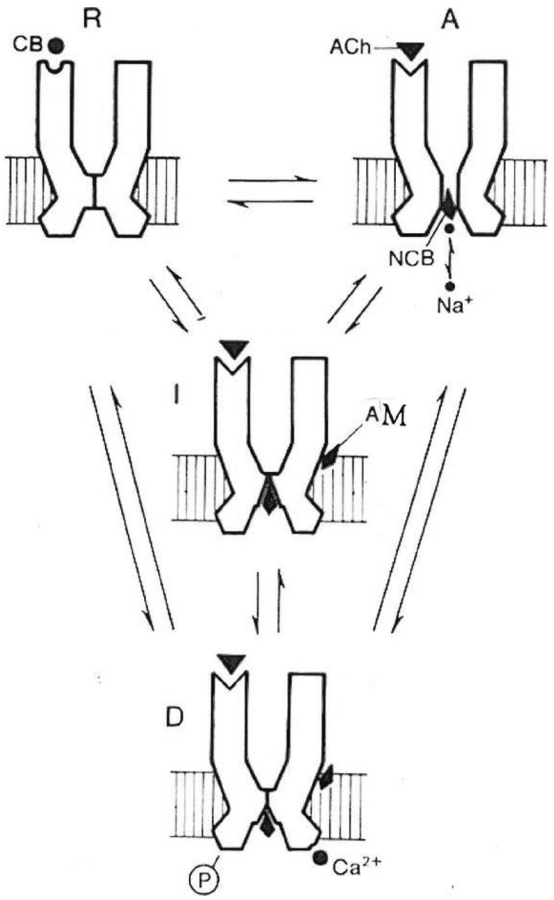

FIGURE 4. **Minimal four-state model for the allosteric transitions of the nicotinic receptor.** *CB*, competitive (orthosteric) blocker; *NCB*, noncompetitive (channel) blocker; *AM*, allosteric modulator; *P*, phosphorylation site. This figure has been reprinted from Ref. 64.

and the ion transport mechanism is an allosteric interaction (1, 37, 64, 86).

Allosteric Transitions of the Nicotinic Receptor: The Quaternary Twist Mechanism

Direct evidence for the conformational changes that mediate this interaction was still unavailable. Early rapid mixing experiments using snake ^{3}H-labeled α-toxin as a probe and receptor-rich membranes from *T. marmorata* revealed changes in conformation that took seconds to reach a high affinity state, possibly desensitized, from a low affinity resting state (87). Consistent findings were subsequently reported using muscle cells (88) and *Torpedo* membranes (89, 90, 107, 108). A refined kinetic analysis of the binding interaction of the fluorescent nicotinic agonist dansyl-C6-choline with receptor-rich membranes (91, 92, 109) and correlation with the *in vitro* measurement of ion transport through the ion channel (93) resulted in the demonstration of allosteric transitions between several conformational states: a resting closed channel state (R) stabilized by snake α-toxin and nicotinic antagonists; an active transient open channel state with low affinity for ACh and nicotinic agonists (A), and at least one desensitized and slowly accessible refractory state (D) with high affinity for both agonists and antagonists (in addition to a fast desensitized state (I)) (Fig. 4).

Moreover, under resting conditions, a sizeable fraction (~20%) of the receptor was found to be present in the high affinity desensitized state (91), and spontaneous channel openings of the muscle nAChR were recorded in the absence of ACh (94). This ruled out the induced fit mechanism to the benefit of the conformational selection (Monod-Wyman-Changeux) scheme (see Ref. 86). Still, the situation appeared more complex than for regulatory enzymes. There exists not only one but a cascade of discrete transitions between open and closed conformational states (Fig. 4) (see Refs. 1, 51, and 64).

Up until recently, little new information became available to help explain the structural transitions of the nAChR, except for *in situ* electron microscopy studies of the *Torpedo* receptor (95). *In silico* modeling from the available structural data brought novel insight into the conformational transitions of the receptor protein (96, 97). Normal mode analysis performed on a three-dimensional model of the α7-nAChR gave a breakdown of the protein movements into discrete modes. Among the first 10 lowest frequency modes, the first mode produced a structural reorganization that caused a wide opening of the channel pore resulting from a concerted and symmetrical transition (a quaternary twist motion of the protein) with opposing rotations of the upper (extracellular) and lower (transmembrane) domains and significant tertiary reorganizations within each subunit, in particular at the domain interface. The global quaternary twist motion accounted reasonably for the available experimental data on the gating process (97). Strong evidence emerged from the comparison studies of the x-ray structures of the prokaryotic receptors GLIC (from *G. violaceus*), which showed an open channel conformation, and ELIC, which displayed a closed channel conformation (83–85). Comparison of the two structures indicated that at least 29% of the quaternary twist transition model accounts for channel opening (83). Future developments include the molecular dynamics of the transition on a microsecond time scale (98).

Allosteric Modulatory Sites

The signal transduction process mediated by the nAChR is regulated by at least three main categories of allosteric "modulators," which bind to sites distinct from the neurotransmitter site and the ion channel. These modulators are thought to selectively shift the allosteric equilibrium in favor of either an active (positive modulators) or a resting/desensitized (negative modulators) conformation without competing with the neurotransmitter binding to the orthosteric sites (Refs. 64 and 86; Ref. 1 and references therein).

One group of modulators includes Ca^{2+}, which potentiates most neuronal nAChRs (99, 100) and binds to the extracellular domain below the ACh site at residues contributed from both sides of the subunit interface (96). Another includes Zn^{2+}.

A second important group consists of modulators, such as galantamine, that bind at "non-agonist" interfaces, which, in heteropentameric nAChRs, differ from the neurotransmitter-binding site and appear to be homologs of the benzodiazepine site on $GABA_A$ receptors (see observations made by Richard Olsen in the accompanying Classics).

Another group of allosteric modulators interacts with the transmembrane domain. The antihelminthic ivermectin was

originally discovered to behave as a strong positive modulator of α7-nAChR (101). Its action was altered by mutations within the TM2 domain (101). General anesthetics (both intravenous and volatile) negatively modulate excitatory nAChRs but positively enhance inhibitory GABA receptors. Photolabeling studies with $GABA_A$ receptors (see observations made by Richard Olsen and colleagues; see in the accompanying Classics) and x-ray structures of GLIC complexes with propofol or desfurane reveal a site within the upper part of the transmembrane domain of each subunit (102) with which nicotinic allosteric modulators may also interact in neuronal nAChRs (*AM* in Fig. 4) (103).

Allosteric modulatory sites have also been identified in the cytoplasmic loop that links TM3 and TM4 in all eukaryotic (but not prokaryotic) pentameric receptors. In nAChRs, several phosphorylation sites (104) that control desensitization in muscle and α7-nAChR also contribute to end plate localization by agrin-induced tyrosine phosphorylation of the cytoskeletal protein 43K-rapsyn (22, 51). The cytoplasmic domain of the α4-nAChR subunit also binds a variety of scaffold proteins that interact with cytoskeletal proteins and with G protein systems that are involved in intracellular signaling pathways (105).

Conclusion

Since the isolation of the nAChR and the discovery that $GABA_A$ and glycine receptor subunits are close orthologs of the nAChR, thereby founding the superfamily of pentameric ligand-gated ion channels, the whole field of pentameric receptors for neurotransmitters has blossomed, including the discovery of homologous receptors in prokaryotes. Several of these are the target of the most commonly used drugs, such as benzodiazepines, barbiturates, curare, and general anesthetics. The recent advances in the x-ray structures of several of these receptors (1) open new avenues for the rational design of pharmacological agents acting on the brain, in parallel with the abundant studies on the G protein-coupled receptors, which were identified several years later.

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