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Topical Review

Functional Architecture of the Nicotinic Acetylcholine Receptor: A Prototype of Ligand-gated Ion Channels

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I. The Nicotinic Acetylcholine Receptor: An Allosteric Protein

The nicotinic acetylcholine receptor (nAChR)[1] is involved in chemo-electrical transduction at the neuromuscular junction and at cholinergic synapses of the central nervous system. At the motor endplate, invasion of the motor nerve ending by an action potential causes the release in the synaptic cleft of a brief pulse of acetylcholine (ACh), whose local concentration reaches 0.1 to 1 mM (Katz & Miledi, 1977) for less than 1 msec (*see also* Clements et al., 1992 in the case of GluR). ACh diffuses through the cleft and binds to the nAChR present in the postsynaptic membrane, where it triggers the all-or-none opening of cation-selective ion channels through which Na^+/K^+ ions flow passively. When depolarization reaches a threshold, muscle contraction occurs. In the cleft, ACh concentration rapidly declines to background levels (10^{-9} M) as a consequence of diffusion and degradation by ACh esterase (Kuffler & Yoshikami, 1975; Katz & Miledi, 1977).

Key words: Nicotinic acetylcholine receptor—Channel gating—Allosteric transitions—Mutagenesis—Affinity labeling—Structure-function relationships

[1] Abbreviations: ACh: acetylcholine; nAChR: nicotinic acetylcholine receptor; NCB: noncompetitive blocker; GlyR: glycine receptor; $GABA_AR$: gamma-amino-butyric acid receptor; GluR: ionotropic glutamate receptor; $5HT_3R$: ionotropic serotonin receptor; NMDA: N-methyl-D-aspartic acid.

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The nAChR from fish electric organ and skeletal muscle is a heterologous transmembrane pentamer of about 300,000 daltons composed of four different subunits assembled with a stoichiometry $[2\alpha,\beta\gamma\delta]$ around an axis of pseudosymmetry perpendicular to the plane of the membrane (*reviews*: Karlin, 1991; Lukas & Bencherif, 1992; Galzi et al., 1991*b*; Unwin, 1993). The nAChR pentamer contains all the structural elements required for the physiological response: the binding sites for the cholinergic ligands, the ion channel and the mechanisms which ensure the various modes of coupling between these two categories of sites (Galzi et al., 1991*b*; Cockcroft et al., 1992; Ochoa et al., 1989, 1992). Electron microscopy of nAChR complexes with α bungarotoxin (Hamilton, McLaughlin & Karlin, 1977; Holtzman et al., 1982; Karlin et al., 1983; Bon et al., 1984; Kubalek et al., 1987) or with anti α subunit antibodies (Fairclough et al., 1983) reveals that in the oligomer the two α subunits are not adjacent. Yet, the arrangement of the other subunits in the pentamer is still debated (Unwin, 1993).

In nerve cells, only two types of subunits, α and β, (*reviews*: Heinemann et al., 1989; Schoepfer et al., 1989; Sargent, 1992) have been identified. Heterogeneity of quaternary organization in the nervous tissue is expected to be large since, so far, seven different α and three different β subunits have been identified (*reviews*: Role, 1992; Sargent, 1992). With some neuronal nAChR a $[2\alpha, 3\beta]$ stoichiometry has been reported (Anand et al., 1991; Cooper, Couturier & Ballivet, 1991; Whiting et al., 1991). However, in some cases, two different α subtypes may be present in a single nAChR molecule (Schoepfer et al., 1990; Conroy, Vernallis & Berg, 1992; Gotti et

al., 1992). If all possible hetero-oligomers did form, more than 1,000 combinations would be possible (*see discussion* in Role, 1992). Yet, expression studies in *Xenopus* oocytes have shown that only *one* subunit, the $\alpha 7$ or the $\alpha 8$ subunit, for example, may yield a functional nAChR (Couturier et al., 1990*b*; Schoepfer et al., 1990; S. and D. Bertrand, *unpublished results*).

Complete cDNA coding sequences for nAChRs have been established for *Torpedo* electric organ, or muscle and neuronal subunits from various species. They reveal a high degree of sequence identity with each other. Moreover, the sequences for other neurotransmitter-gated ion channels: Glycine (Grenningloh et al., 1987), $GABA_A$ (Schofield et al., 1987), $5HT_3$ (Maricq et al., 1991), Glutamate/Kainate and NMDA receptors (Gregor et al., 1989; Hollmann et al., 1989; Wada et al., 1989; Moriyoshi et al., 1991) display hydropathy profiles similar to those of the nAChRs suggesting that all possess a common transmembrane folding (Cockcroft et al., 1992). However, this view has recently been challenged for GluR (*review:* Seeburg, 1993). By analogy with nAChR they are thought to be pentameric proteins (Unwin, Toyoshima & Kubalek, 1988; Betz, 1990; Langosch, Thomas & Betz, 1988) and the minimal model of transmembrane organization which was proposed for *Torpedo* nAChR subunits (Claudio et al., 1983; Devillers-Thiéry et al., 1983; Noda et al., 1983) has been extended to all members of the superfamily.

It consists of: (a) a hydrophilic N-terminal domain oriented toward the synaptic cleft and carrying the glycosylated moities; (b) a compact hydrophobic region of about 70 amino acids subdivided into three uncharged segments long enough to span the membrane (referred to as MI, MII, MIII); (c) a hydrophilic domain oriented toward the cytoplasm which carries consensus sites for phosphorylation and (d) a short carboxy-terminal hydrophobic transmembrane segment (denoted MIV). In this minimal model, the N- and C-terminal ends are both oriented toward the synaptic cleft (McCrea, Popot & Engelman, 1987; DiPaola, Czajkowski & Karlin, 1989). According to the proposed model, the agonist binding sites are located on the N-terminal domain and the ion channel lies in the axis of pseudosymmetry of the molecule. Accordingly, the agonist site and the ion channel would be topographically distinct. Their distance evaluated in T · nAChR by energy transfer indeed lies in the range of 25–30 Å (Herz, Johnson & Taylor, 1989). Thus, indirect or "allosteric" interactions account for the opening of the ion channel by ACh.

Three classes of pharmacological ligands acting on nicotinic nAChR have been recognized: (1) the *agonists* such as ACh, nicotine, carbamylcholine, which elicit channel opening; (2) the *competitive antagonists,* such as the snake venom α-toxins or d-tubocurarine (in some systems, d-tubocurarine may behave as a partial agonist (Ascher, Marty & Neild, 1978); which block the postsynaptic response by binding to the cholinergic binding site; and (3) the *noncompetitive blockers* which either directly block ion flux by sterically plugging the ion channel or indirectly inhibit its function upon binding to regulatory sites distinct from the ACh binding sites (*review:* Changeux, 1981).

Upon prolonged exposure of nAChR to agonists, the ion channel closes spontaneously, a phenomenon known as *desensitization*. Katz and Thesleff (1957) proposed that, in addition to the resting and open channel conformations, ACh slowly (in the 100 msec to min time scale) and reversibly stabilizes a "refractory" closed state which exhibits a higher affinity for agonists than the resting and active states. Measurement of ACh binding *at equilibrium* with membrane-bound nAChR revealed, indeed, a very high affinity ($Kd = 10^{-8}$ M; Weber & Changeux 1974*a,b,c*; Boyd & Cohen, 1980; *review:* Changeux, 1981). On the other hand, $^{22}Na^+$ flux studies gave half-maximum values for "activation" in the 10 to 100 μM range (Kasai & Changeux, 1971; Neubig & Cohen, 1982; Heidmann et al., 1983*a*; Hess, Cash & Aoshima; 1983; Changeux, 1990). Such heterogeneity of binding constants was interpreted on the basis of the assumption that the nAChR is an allosteric protein which may exist under several discrete and interconvertible conformational states. A minimal model of four interconvertible states (R: resting, A: active, I: intermediate, and D: desensitized) derived from that of Katz and Thesleff (1957) for desensitization and that of Monod, Wyman and Changeux (1965) for allosteric transitions was proposed. These four states differ by: (a) their activation status: the ion channel opens only in the A state; the I and D states, which are refractory to activation by agonists, correspond to desensitized states; (b) their affinity for agonists, which is the lowest in the A state (100 μM) and increases for the I (1 μM) and D states (10 nM); (c) their kinetics of interconversion which are very fast toward the A state (10–100 μsec) and becomes slower toward the I (msec-sec) and D (sec-min) states (Heidmann & Changeux, 1979; Boyd & Cohen, 1980; Sakmann, Patlak & Neher, 1980; Feltz & Trautmann, 1982; Neubig & Cohen, 1982; Changeux, Devillers-Thiéry & Chemouilli, 1984; Changeux, 1990). Also, the cooperative binding of two molecules of agonists is required for the activation and desensitization processes (Colquhoun & Sakmann, 1985). Moreover, the pharmacology of the four allosteric states may differ: some

competitive antagonists stabilize the desensitized states of the receptor (Rang & Ritter 1970; Grünhagen & Changeux, 1976; Krodel, Beckman & Cohen, 1979; Weiland, Frisman & Taylor, 1979) as do most of the noncompetitive blockers (Heidmann, Oswald & Changeux, 1983*b*; Colquhoun & Sakmann, 1985; Ochoa et al., 1989, 1992). Finally, the proposed model of allosteric transitions posits that the several allosteric states may spontaneously exist prior to ligand binding. In the absence of ligand, a significant fraction (20%) of the nAChR is indeed already in the high affinity D state (Heidmann & Changeux, 1979).

II. The ACh Binding Sites

A Structural View of the ACh Binding Sites

The topology of the ACh binding sites on *Torpedo* nAChR was investigated with several photoaffinity labeling probes. Amino acids belonging to three different regions of the N-terminal hydrophilic domain of the α subunit were identified using DDF [(p(N,N)-dimethyl-aminobenzenediazonium fluoroborate; Goeldner & Hirth, 1980] labeling (Dennis et al., 1986, 1988; Galzi et al., 1990) and a *three loop* model of the agonist and competitive antagonist binding site on the α subunit was proposed (*review:* Galzi et al., 1991*b* and Fig. 1). In *loop A,* **Trp 86** and **Tyr 93** were identified using DDF, Tyr 93 being also labeled by acetylcholine mustard (Cohen, Sharp & Liu, 1991). In *loop B,* DDF was incorporated on **Trp 149** and **Tyr 151.** In *loop C,* DDF labeled **Tyr 190, Tyr 198** and the **Cys doublet 192–193** previously identified by Kao et al. (1984) by affinity labeling with MBTA [4-(N-maleimido)-benzene-trimethylammonium] and forming a rather rare viscinal disulphide bond in the native molecule (Mosckovitz & Gershoni, 1988). Tyr 190 was also labeled by the coral competitive antagonist lophotoxin (Abramson et al., 1988, 1989) and by d-tubocurarine (Pedersen & Cohen, 1990*a*). Moreover, nicotine at equilibrium labeled Tyr 190, Cys 192 and Tyr 198 (Middleton & Cohen, 1991).

Interestingly, DDF and other affinity ligands predominantly label aromatic residues. The presence of aromatic side chains in the binding site for quaternary ammonium ligands appears to be a general property shared by several choline-binding proteins including muscarinic receptors; AChE and antiphosphorylcholine antibody (Satow et al., 1986; Hibert et al., 1991; Sussman et al., 1991; Wess, Gdula & Brann, 1991). The electronegative character of aromatic residues is thought to be sufficient to complex the large organic ammonium ions exhibiting a diffuse positive charge (Galzi et al., 1991*b*).

The DDF-labeled amino acids are conserved at homologous positions in all known α subunits from muscle and neuronal receptors from all species, while being absent in non α subunits. The only exception, so far, is the neuronal $\alpha 5$ subunit which lacks Tyr 93 and 190. Yet, the $\alpha 5$ subunit does not form a functional nAChR when injected in *Xenopus* oocytes with any other neuronal subunits (Boulter et al., 1990; Couturier et al., 1990*a*), although it is associated *in situ* with $\alpha 3$ and/or $\alpha 4$ subunits (Conroy et al., 1992). On the other hand, Trp 149 and Tyr 93 are conserved in neuronal β subunits (references in Cockcroft et al., 1992) suggesting that these subunits may have a function in neural tissue distinct from that of the β, γ and δ subunits in muscle or electric organ nAChR. Such high degree of conservation noticed even in the α subunits which do not bind α bungarotoxin (from neuronal tissue, Luetje et al., 1990 or from snake, Neumann et al., 1989; Barchan et al., 1992) is consistent with their physiological role in ACh binding. The same three loops have been suggested to compose the two ACh binding sites, even though they display different pharmacological profiles (*see below*, and Galzi et al., 1991*b*). By electron microscopy, Unwin (1993) reported that each subunit contains three rods assumed to be α helices from their dimension. These rods would together create a cavity presumed by Unwin to be the agonist binding site. On the α subunits, such a cavity would be more open, and the slight difference in shape noticed between the two α subunits might possibly be responsible for their different pharmacological specificity (Unwin, 1993).

Unconventional agonistic effects of physostigmine (or eserine), an anti-ACh esterase agent on *Torpedo* nAChR, have been reported (Shaw et al., 1985; Albuquerque, Maelicke & Pereira, 1991). At low concentration, physostigmine activates ion fluxes even when the nAChR is in a desensitized conformation (Kuhlmann, Okonjo & Maelicke, 1991) and at elevated concentrations this compound behaves as an open channel blocker. Channel activation is not affected by several competitive antagonists (including α bungarotoxin and d-tubocurarine) but is blocked by the open channel blocker dibucaine and prevented by the antibody FK1 (Okonjo, Kuhlmann & Maelicke, 1991). Benzoquinonium, a competitive antagonist of the ACh site (Okonjo et al., 1991) exhibits effects that are similar to those of physostigmine (Schrattenholz et al., 1993). Photolabeling studies revealed that the binding site for physostigmine is located in the vicinity of loops A and

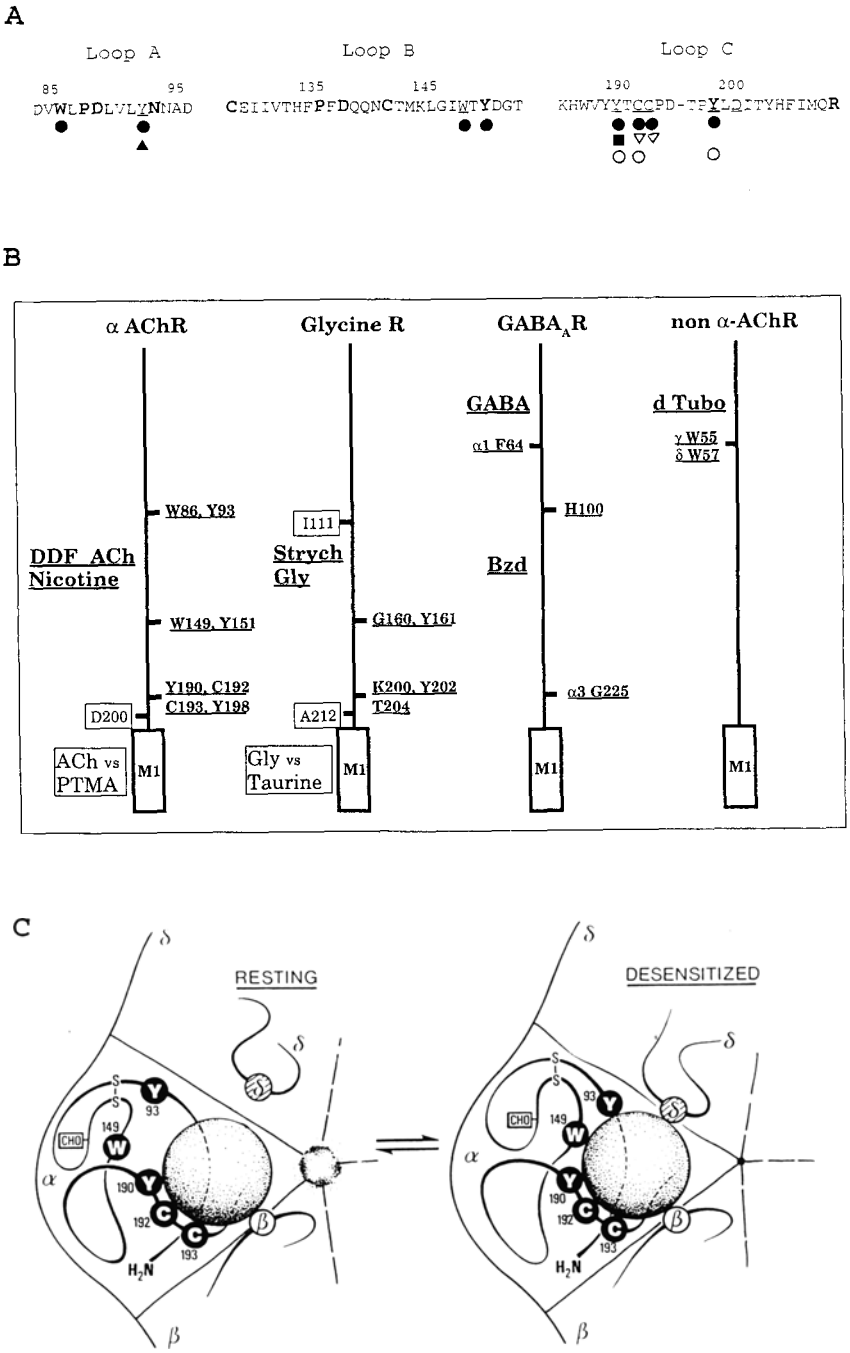


Fig. 1. The three loop model of the agonist binding site. (*A*) Sequence of the amino acids in loops A, B and C of the ligand binding site of *Torpedo* α subunit showing (i) the residues labeled by DDF (●), MBTA (▽), Acetylcholine mustard (▲), Lophotoxin and d-tubocurarine (■) and nicotine (○), underlined residues have been mutated (*see references in text*) and (ii) in bold, the "canonical" amino acids conserved in the superfamily of ligand-gated ion channels. (*B*) Schematic representation of the amino acid sequences of the N-terminal domains of the nAChR α-subunit, the GlyR α-subunits, the $GABA_AR$ α subunits and the non α-subunits of the nAChR showing that amino acids which are involved in agonist binding (underlined) and in allosteric transitions (boxed residues) occupy identical positions in those receptor sequences. DDF: p(N,N)-dimethyl-aminobenzene-diazonium fluoroborate; PTMA: phenyl-trimethylammonium; Strych: Strychnine; Gly: Glycine; GABA: gamma-amino-butyric acid; Bzd: Benzodiazepine; d Tubo: d-tubocurarine. (*C*) Model of the transitions occurring in the three loops of *Torpedo* α subunit N-terminal domain upon desensitization. DDF-labeled amino acids are shown in bold.

B; the labeled residue on *Torpedo californica* α subunit is Lys 125 (Tano et al., 1992) which is conserved on most α subunits but absent on non α subunits. These data suggest a second pathway of nAChR channel activation which may physiologically function as an allosteric control of receptor activity. This allosteric effect of physostigmine could possibly be comparable to that of barbiturates on $GABA_A$ receptors since barbiturates are able to enhance Cl^- currents in the absence of GABA (Sieghart, 1992).

A Functional View of the ACh Binding Sites

The functional significance of the chemically labeled amino acids was checked by mutagenesis. In *Torpedo* nAChR, replacement of Cys 192 or 193 residues by Ser in *loop C* modifies the affinity for agonists and abolishes the ACh-induced response (Mishina et al., 1985). Mutation of Tyr 190 into Phe (Tomaselli et al., 1991; O'Leary & White, 1992) increases by 50-fold the concentration of ACh required

for channel activation without significantly affecting α bungarotoxin binding.

The role of the other aromatic residues in *loops A, B and C* was investigated in chick neuronal $\alpha 7$ homoligomeric nAChR. Mutation of these amino acids into Phe (Y92F, W148F, Y187F, Galzi et al., 1991*a*) decreased the apparent affinity for the agonists ACh (10–100 fold) and nicotine (up to 350-fold for the mutant W148F), for the competitive antagonist dihydro-β-erythroidine and to a smaller degree the affinity for α bungarotoxin, while the time course and the voltage dependency of the ionic response and the inhibition by the open channel blocker QX222 remained unchanged. Replacement of Y92 or Y187 by Ser abolished the ionic response (Galzi et al., 1991*a*).

Contribution of the Non α Subunits to the ACh Binding Sites

The two α subunits of the *Torpedo marmorata* receptor (Klarsfeld et al., 1984) and that of mouse muscle nAChR (Merlie et al., 1983) are encoded by a single gene, but the two agonist binding sites are not strictly equivalent. They differ by their kinetics of binding and dissociation of α bungarotoxin (Weber & Changeux 1974*a, b, c*; Maelicke & Reich, 1976; Maelicke et al., 1977; Kang & Maelicke, 1980; Maelicke et al., 1989), by their affinities for the competitive antagonists d-tubocurarine (Neubig & Cohen, 1979; Pedersen & Cohen, 1990*a*) or decamethonium and by their different labeling sensitivity to affinity reagents such as bromoacetylcholine, MBTA (Karlin et al., 1976; Damle & Karlin, 1978; Delegeane & McNamee, 1980; Ratnam et al., 1986) or lophotoxin (Culver, Fenical & Taylor, 1984). Also some antibodies differentially block the binding of α bungarotoxin to one or the other α subunit (Watters & Maelicke, 1983; Gu, Silberstein & Hall, 1985; Whiting et al., 1985; Dowding & Hall, 1987).

For muscle and *Torpedo* nAChR, covalent labeling experiments (Oswald & Changeux, 1982; Pedersen & Cohen 1990*a*; Galzi et al., 1991*c*) and expression studies of pairs of subunits (Blount & Merlie, 1989) suggested that the most probable cause of the nonequivalence is that the binding areas span the boundaries between subunits. In agreement with a contribution of the non α subunits to the agonist binding site, the γ subunit can be specifically labeled by the photoaffinity reagent DDF (Langenbuch-Cachat et al., 1988). Strong evidence supports the notion that the γ subunit is involved in the high affinity site for d-tubocurarine and that the δ subunit contributes to the low affinity one (Blount & Merlie, 1989; Pedersen & Cohen, 1990*a*; Galzi et al., 1991*c*). Moreover, d-tubocurarine is incorporated upon UV irradiation into Trp residues 55 and 57 on the γ and δ subunits, respectively (Fig. 1*B*; Cohen et al., 1992*c*). Using stable cell lines which lack either the γ or δ subunit, Sine and Claudio (1991) concluded that both γ and δ subunits contribute to the regulation of the affinity and the cooperativity of nicotinic ligand binding to nAChR (*see also* Karlin, 1991). The pockets that Unwin (1993) assigns to the ACh binding sites in his electron microscopy images of *Torpedo* nAChR do not seem, however, located at the interfaces between subunits.

As already mentioned, only aromatic residues and no Asp or Glu residues have been identified in the ACh binding site. The presence of aromatic residues within the agonist binding site was noticed in many choline-binding proteins and it was thought that the electronegative character of these residues might be sufficient to complex the diffuse quaternary ammonium group of ACh. On the other hand, in the water soluble synthetic macrocycles which behave as chemical models of receptors able to bind choline or ACh (Dhaenens et al., 1984; Schneider, Güttes & Schneider, 1986; Sheppod, Petti & Dougherty, 1986) a crown of aromatic residues is circled by negative residues suggesting that long distance electrostatic interactions might occur. The presence of a negative subsite within 1 nm (Karlin, 1969) from the Cys doublet (Cys 192–193), was checked on *Torpedo* nAChR, using S-(2-[^{3}H]-glycylaminoethyl) dithio-2-pyridine (Czajkowski & Karlin, 1991). S-(2-[^{3}H]-glycylaminoethyl) dithio-2-pyridine is a bifunctional affinity reagent with a reactive SH group on one end and a glycylamino group on the other. This compound labeled predominantly a region of the δ subunit between amino acids 164 and 257. After systematically mutating each negatively charged aspartyl or glutamyl residue present in this region, Czajkowski, Kaufmann and Karlin, (1993) found two mutants, D180N and E189Q, for which the K_{app} for ACh was affected (respectively 227 and 36 μM as compared to 3 μM for the wild type nAChR). Since these two residues, δ-D180 and δ-E189, are conserved at equivalent position in γ, δ and ε subunits of all species but absent in β subunits the authors suggested, still without definite proof, that they could be part of the negative subsite at both the α-γ and α-δ ACh binding sites (Czajkowski et al., 1993).

In neuronal nAChR, both α *and* β subunits contribute to the pharmacological specificity of the receptor molecule. Expression of pairs of subunits ($\alpha 2$, $\alpha 3$ or $\alpha 4$ with either $\beta 2$ or $\beta 4$) in *Xenopus* oocytes has shown that the diverse receptor combinations display different pharmacological sensitivity to the nicotinic agonists ACh, nicotine, cytisine and DMPP (1,1-dimethyl-4-phenyl piperazinium). Under simi-

lar experimental conditions, clear differences do exist among different neuronal nAChR, but a strict comparison of apparent affinity values and rank order of potency of the different agonists is difficult since the values reported in the literature vary even for the same species (rat) (Cachelin & Jaggi, 1991; Luetje & Patrick, 1991; Whiting et al., 1991; Connolly, Boulter & Heinemann, 1992). Also between nAChR of different species, chick and rat, which share a high degree of sequence homology, apparent affinities for ACh strikingly differ. For $\alpha 3\beta 4$ and $\alpha 3\beta 2$, they are, respectively, 158 and 5.6 μM for chick (Couturier et al., 1990*b*) and 30 and 350 μM for rat (Cachelin & Jaggi, 1991). This variability could be due to uncontrolled stoichiometry of the coding sequences injected in oocytes (*see* Papke et al., 1989 for the nAChR; Pribilla et al., 1992 for the GlyR and Kleingoor et al., 1993 for $GABA_AR$), although no effect of the ratios of injected cDNA was detected for chick $\alpha 4\beta 2$ (S. and D. Bertrand, *unpublished results*). It could also be due to different experimental procedures, such as oocytes injected at a different stage of maturation, or to the contribution of Ca^{2+}-activated chloride channels (*see* Devillers-Thiéry et al., 1992 for nAChR and Bowie & Smart, 1993 for non NMDA GluR).

Selectivity for competitive antagonists is also dependent on the type of β subunit, $\alpha 3\beta 4$ oligomer being better inhibited by hexamethonium, mecamylamine and trimethaphan than $\alpha 3\beta 2$ oligomer (Cohen et al., 1992*a*). On the other hand, among the different combinations, only the $\alpha 3\beta 2$ oligomer is sensitive to neuronal bungarotoxin (Boulter et al., 1987; Wada et al., 1988; Duvoisin et al., 1989; Luetje et al., 1990). Experiments with chimeric molecules composed of $\beta 2/\beta 4$ hybrid subunits coexpressed with $\alpha 4$ subunit (in *Xenopus* oocytes) demonstrate that almost all the effects of the β subunits on agonist or competitive antagonist selectivity can be accounted for by the N-terminal domain (Cohen et al., 1992*a*; Figl et al., 1992).

The Organization of the ACh Binding Site Changes during the Course of Desensitization

As mentioned above, during transition from the resting (R) to the desensitized state (D), the affinity for ACh increases 10^4-fold. The structural modifications involved in the conformational transitions were investigated using DDF (Galzi et al., 1991*c*), while the fractional concentration of the receptor molecules in the D state was modulated by addition of the high affinity noncompetitive antagonist, meproadifen (Krodel et al., 1979; Heidmann et al., 1983). Preincubation of nAChR with meproadifen increased specific labeling of the α and δ subunits, decreased that of the γ subunit and did not affect that of the β subunit (*see* Fig. 1*C*). On the α subunit, labeling of the three loops was differentially affected upon desensitization. No change was observed in loop C, whereas labeling of loops A and B increased up to 6-fold (Galzi et al., 1991*c*). The increased labeling of loops A and B is consistent with a tighter binding of the ligand and thus a higher affinity of the D state. Also, cryomicroscopy experiments reveal quaternary rearrangements involving mainly γ and δ subunits after addition of carbamylcholine to the membrane-bound nAChR (Unwin et al., 1988). According to these authors, "under conditions which would promote receptor desensitization, the δ subunit becomes more inclined tangentially around the channel and the γ subunit is displaced slightly outward; the other subunits are not noticeably affected."

The Three Loop Model of the Agonist Binding Site and the Other Members of the Superfamily

The three loop model of the ligand binding site is supported by mutation experiments in other receptors of the superfamily. Mutation of His 100 (the homologue of Tyr 93 of *Torpedo* α subunit, Fig. 1*B*) on the α subunit of $GABA_AR$ modifies the apparent affinity for the benzodiazepine RO.15–4513 (Wieland, Lüddens & Seeburg, 1992; Korpi et al., 1993) and mutation of Gly 225 (homologue of Tyr 190, Fig. 1*B*) on the $\alpha 3$ subunit modifies the affinity for the benzodiazepine CL 218872 (Pritchett & Seeburg, 1991). On the *GlyR*, mutations of Gly 167 on the $\alpha 2$ subunit or of Gly 160 and Tyr 161 on the human $\alpha 1$ subunit (homologues of residues 150 and 151, Fig. 1*B*) modify the affinity for the competitive antagonist, strychnine (Kuhse, Schmieden & Betz, 1990; Vandenberg, Handford & Schofield, 1992*b*). Residues Lys 200, Tyr 202 and Thr 204 (homologues of residues 192, 194 and 196, Fig. 1*B*) were shown to be also involved in strychnine binding (Vandenberg et al., 1992*a*, *b*).

These functionally important amino acids are located in the postulated three loops of the ligand binding site in which some "canonical" amino acids are conserved in the superfamily. These are: *in loop A*, **Trp 86** (numbering refers to *Torpedo* α subunit; *see* Fig. 1*A*), **Pro 88, Asp 89** and **Asn 94;** *in loop B*, the two **Cys 128 and 142** [in *Torpedo* nAChR they form a Cys loop (Kao & Karlin, 1986; Kellaris & Ware, 1989) and mutation of any one abolishes α bungarotoxin binding (Mishina et al., 1985)], **Pro**

136, Asp 138, Tyr 151 (weakly labeled by DDF, Dennis et al., 1988 and replaced by a His in $5HT_3R$, Maricq et al., 1991); *in loop C* an aromatic residue **Tyr or Phe** at position **198** and **Arg 209.** In the superfamily, loops A, B and C may adopt a common main chain backbone structure and the pharmacological specificity of the different members of the ligand-gated ion channel receptor family would rely on the amino acid side chains.

Yet, other regions of the N-terminal domain also influence ligand binding. In the nAChR, mutation of Asp 200 to Asn in *loop C* decreases about 4-fold the apparent affinity for ACh (Fig. 1*B*; O'Leary & White, 1992). The two partial agonists of the nAChR, PTMA (phenyl-trimethylammonium) and TMA (tetramethylammonium) do not activate mutant D200N but behave as competitive antagonists. According to O'Leary and White (1992), "the mutations do not alter the affinity of the ligand binding site, but rather affect the coupling between the ACh site and the channel." Mutation on the $\alpha 1$ subunit of the heterooligomer $\alpha 1\ \beta 2\ \gamma 2$ $GABA_A$ receptor, of Phe-64 to Leu (homologue of γ-Trp 55 and δ-Trp 57 on *Torpedo* nAChR; Fig. 1B) conferred a 220-fold lower apparent affinity for the competitive antagonists bicuculline methiodide and SR95531 (which has a close structural similarity to the agonist GABA; Sigel et al., 1992). The authors postulate that a conformational change occurs in the extracellular domain of the $\alpha 1$ subunit upon mutation of that residue and that the conformational change is transferred to the other subunits, resulting in an altered apparent affinity for channel gating by GABA.

In the GlyR, $\alpha 1$ and $\alpha 2$ subunits differ by their affinities for the agonists taurine and β-alanine and the competitive antagonist strychnine. Construction of an $\alpha 1/\alpha 2$-subunit chimera and site-directed mutagenesis identified two positions as important determinants for taurine activation: residues Ile-111 on $\alpha 1$ subunit replaced by Val-118 on the $\alpha 2$ subunit and Ala-212 (homologue of Asp 200 on the α subunit of *Torpedo* nAChR, *see* Fig. 1*B*) on the $\alpha 1$ subunit replaced by Val-219 on the $\alpha 2$ subunit (Schmieden et al., 1992).

III. The Ion Channel

The Ion Channel Is Located in the Axis of Pseudosymmetry of the Molecule

The permeability response of the nAChR is blocked by a large number of ligands referred to as noncompetitive blockers (NCB), such as the toad toxin histrionicotoxin (Eldefrawi et al., 1977), the quaternary lidocaine derivative QX222 (Neher & Steinbach, 1978), the hallucinogen phencyclidine (Albuquerque et al., 1980; Kloog et al., 1980) or the anticonvulsant MK 801 (Clineschmidt et al., 1982). The last two compounds also block another member of the superfamily of ligand-gated ion channels, the NMDA receptor. All decrease the amplitude of the ion flux by entering the *open* channel and binding to a site located within it (Ascher et al., 1978; Adams 1981; Heidmann et al., 1983; Rapier et al., 1987; Ramoa et al., 1990). Consistent with this view, their blocking action is usually voltage sensitive, like that of QX222 (Neher & Steinbach, 1978; Cohen et al., 1992*b*) or that of chlorpromazine (P. Benoit and J.P. Changeux, 1993).

Equilibrium binding studies with several NCBs, including chlorpromazine, phencyclidine, histrionicotoxin and meproadifen reveal two categories of sites distinct from the ACh binding sites: one high affinity site, histrionicotoxin-sensitive and postulated to lie within the ion channel and 10–30 sites per nAChR molecule, not sensitive to histrionicotoxin and plausibly located at the lipid-protein interface (*review:* Heidmann et al., 1983*b*).

A Structural View of the Ion Channel

As in the case of the ACh binding area, affinity labeling experiments played an important role in the identification of the amino acids which delineate the ion channel. In the early studies, different NCBs and/or different *Torpedo* species (*T. ocellata, T. californica, T. marmorata*) were used, and specific labeling appeared on one or the other of the four subunits (Oswald et al., 1980; Oswald & Changeux, 1981; Haring et al., 1983; Muhn & Hucho, 1983). Chlorpromazine, when bound to its unique high affinity site, specifically labels *the four* subunits of *T. marmorata* nAChR and this labeling is enhanced by ACh under rapid mixing conditions (Heidmann & Changeux, 1984, 1986). These observations led as others to the proposal (Heidmann et al., 1983*b*) that the high affinity binding site for the NCBs is located in the axis of pseudosymmetry of the molecule within the ion channel. The amino acids photolabeled by [^{3}H]-chlorpromazine under *equilibrium conditions* in the presence of carbamylcholine (where a desensitized state is favored) are: α-Ser 248, β-Ser 254, β-Leu 257, γ-Thr 253, γ-Ser 257, γ-Leu 260 and δ-Ser 262 (Fig. 2*A, B*; Giraudat et al., 1986, 1987, 1989; Revah et al., 1990). All belong to the transmembrane MII segments of each subunit and the labeled Ser residues occupy equivalent positions on the four subunits. These Ser residues are

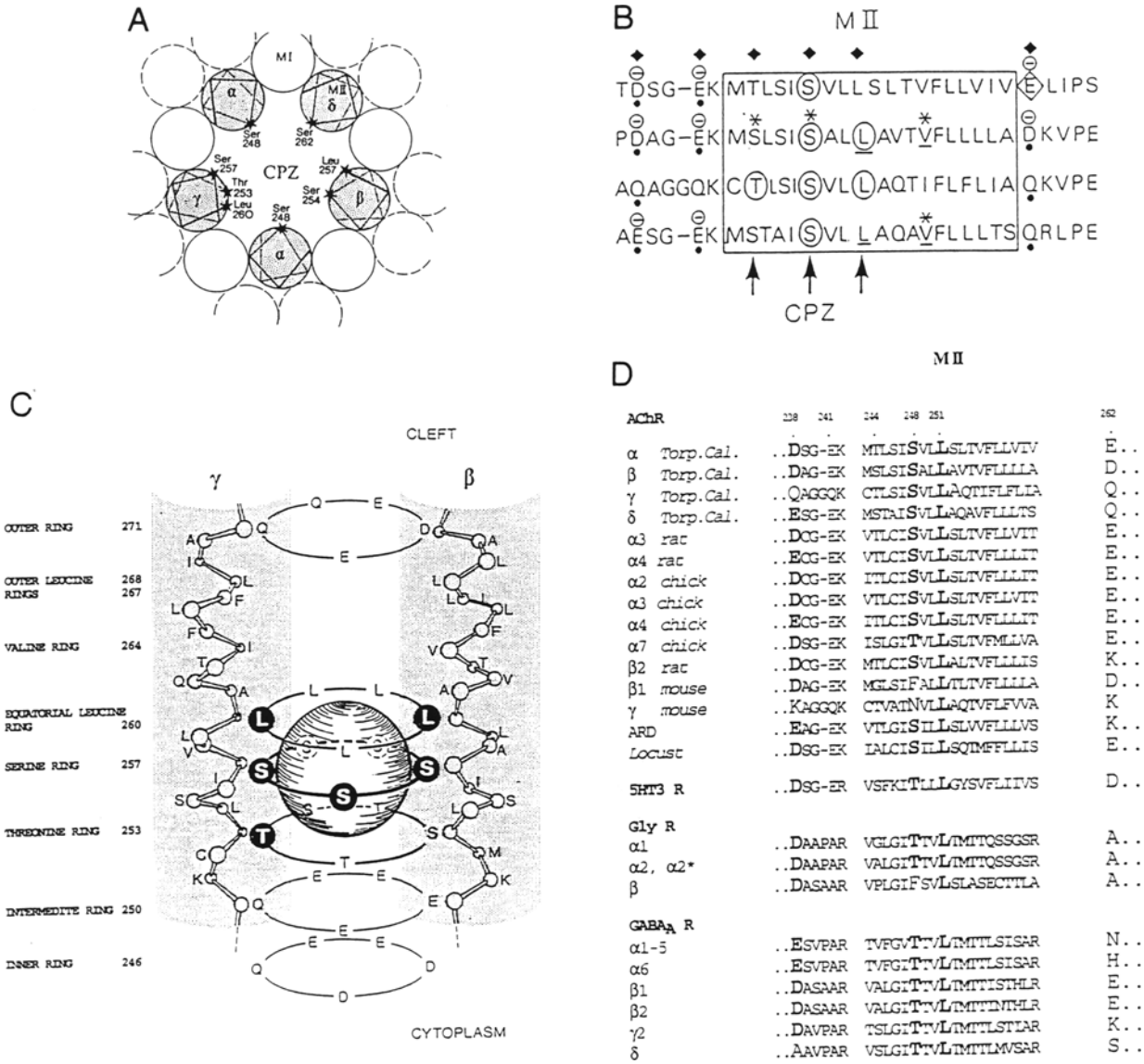


Fig. 2. The ion channel of *Torpedo* nAChR. (*A*) Top view of MII α helices from the five subunits showing the quasisymmetrical organization of the high affinity site for chlorpromazine within the ion channel. Those residues labeled by chlorpromazine are shown. (*B*) Location within MII segments of the five subunits of *Torpedo* nAChR of the amino acids labeled by chlorpromazine (CPZ), meproadifen mustard (◇), 3-(trifluoromethyl)-3-m-iodophenyl diazirine (TID, underlined), TID + carbamylcholine (*) and mutated (◆). (*C*) Side-view model of the high affinity site for chlorpromazine within the ion channel. The MII segments are arranged as transmembrane α helices, and superimposed rings of amino acids line the lumen of the ion channel (*see text*). (*D*) Sequence alignment of MII segments of the members of the superfamily. Bold residues indicate from the N-terminal to the C-terminal end conserved positions in the **Inner** ring, the **Serine** ring, the **equatorial Leucine** ring.

also labeled on α, β and δ subunits by trimethylphosphonium (Hucho, 1986; Oberthür et al., 1986). TID [3-(trifluoromethyl)-3-m-([^{125}I]iodophenyl) diazirine] specifically labels β-Leu 257, β-Val 261, δ-Leu 265 and δ-Val 269 in the absence of agonist. In the presence of agonist β-Ser 250, β-Ser 254, β-Leu 257, β-Val 261 become accessible to TID (White & Cohen, 1992) suggesting that the NCB binding site becomes wider when the nAChR is in a desensitized conformation. Meproadifen mustard, another NCB, labels α-Glu 262 at the C-terminal end of the nAChR (Pedersen & Cohen, 1990*b*). In addition, rapid labeling experiments with the NCB quinacrine azide, under conditions where the channel opens, reveal that amino acids from the N-terminal end of MI, α-Arg 209 and α-Pro 211 are labeled (Di Paola, Kao & Karlin, 1990; Karlin, 1991). These results indicate that the binding site for the ligand could be formed at least in part by the amino terminal end of the MI segment.

The lining of the ion channel by MII segments was also investigated with the help of recombinant DNA technology and expression in *Xenopus* oocytes (Imoto et al., 1986). In a chimera between the δ subunit of *Torpedo* (which displays a channel conductance of 90 pS) and that of bovine nAChR (which displays a 60 pS conductance), the conductance was shown to be determined by part of the MII segment (Imoto et al., 1986).

On the basis of the affinity labeling data, it was proposed (Hucho, 1986; Giraudat et al., 1986, 1987) that: (a) MII segments of each subunit are symmetrically packed and line the ion channel and (b) the labeling pattern of these segments (in the active or the desensitized conformations) is consistent with an α helix organization (*see*, however, Akabas et al., 1992). Mutation experiments (*see below*) have confirmed the functional role of MII segments and showed that the labeled amino acids belong to superimposed rings of homologous residues in the lumen of the channel referred to (from the N- to C-terminal end of the MII segment) as the *Threonine* ring, *Serine* ring, *equatorial Leucine* ring, *Valine* ring and *outer Leucine* rings (Fig. 2*C*). At both ends, the channel is framed by rings of negatively charged amino acids referred to as: the Inner and Intermediate rings at the N-terminal end and the Outer ring at the synaptic cleft (Imoto et al., 1988).

A Functional View of the Ion Channel

Site-directed mutagenesis experiments brought essential information about the functional role of these different rings of amino acids.

Mutations in the Three Rings of Negatively Charged Amino Acids

The *Inner* and *Outer* rings of negatively charged amino acids border the MII segment. Imoto et al. (1988) observed that at a low divalent ion concentration, removal of negative charges at these positions reduces both the inward and outward currents. The sidedness of the Mg^{2+} effect confirmed the orientation of the MII segment predicted by the model of transmembrane organization (Claudio et al., 1983; Devillers-Thiéry et al., 1983; Noda et al., 1983). Since Asp or Glu residues are also present in the Inner ring of the *anionic* GlyR and $GABA_AR$, the exact role of these negatively charged amino acids remains unclear.

Replacement of negative charges had, however, a more pronounced effect at the *Intermediate* ring. This ring according to Imoto et al. (1988) could be involved in the interaction of Mg^{2+}. Konno et al. (1991) claimed that this ring could "represent part of the physical correlate of the postulated selectivity filter in the nAChR channel." Mutations in this ring, in *T. californica* nAChR, affected the conductance for Cs^+ and Rb^+ ions (Konno et al., 1991) and the relative permeability for $Tris^+/Na^+$ (Cohen et al., 1992*b*) without changing the relative permeabilty for the physiological ions Na^+, K^+. Mutating the five positions of the Intermediate ring in the neuronal homo-oligomeric α7 nAChR from Glu to Ala (net charge = 0) yielded a functional receptor which was still permeable to monovalent cations but had lost its ability to conduct Ca^{2+} (Galzi et al., 1992; Bertrand et al., 1993*a*). This Intermediate ring thus seems to play a role in monovalent *vs.* divalent ion permeability.

Mutations of the Threonine Ring

Analysis of mutants at the Thr ring (Imoto et al., 1991; Villarroel et al., 1991, 1992) reveals that conductances are inversely related to the amino acid volume, the effects on conductances being the same for inward and outward currents (Villarroel et al., 1991). These data suggest that the Threonine ring could form the narrow part of the channel and select for the size of the ion. The relative contribution of each subunit to the channel constriction could, however, be different showing some asymmetry of the ion channel. According to Villarroel et al. (1992, in rat nAChR) and in agreement with the conclusions obtained on chimeric *Torpedo*-bovine receptors (Sakmann et al., 1985), the amino acid contributed by the δ subunit would be the major determinant in the channel constriction. However, according to Imoto et al. (1991) in *Torpedo* nAChR the γ subunit would have a predominant role. The sequence of this γ subunit, in all species, has an extra amino acid inserted at the N-terminal end of the MII segment which may create a local constriction of the ion channel. Indeed, deletion of the extra amino acid (Gly) in *Torpedo* nAChR increases channel conductance (Imoto et al., 1991).

In addition, for a given amino acid volume, the conductance is systematically higher when a Thr or Ser residue is present. It could be that the mild electronegative character of the polar groups may, by trapping the water molecules, reduce the size of the permeant ion and facilitate or even "catalyze" ion translocation through the channel (Changeux, 1990; Imoto et al., 1992).

Mutations of the Serine Ring

Ser residues on this ring were symmetrically labeled by chlorpromazine. Mutations of Ser to Ala (Leo-

nard et al., 1988) affected the residence time of the voltage-sensitive open channel blocker QX222 assumed to enter the pore (Neher & Steinbach, 1978; Cohen et al., 1992*b*) by decreasing the affinity for its site. Conversely, mutations of the Ala residues one ring adjacent to the equatorial Leu ring into Ser led to an opposite effect, the residence time for the NCB QX222 being increased. These data suggest that the polar head of QX222 may interact with the Ser ring, while the aromatic moiety would face the Ala residues (Charnet et al., 1990).

Several Rings Contribute to the Closure of the Ion Channel in the Desensitized States

Mutation of the equatorial Leucine ring in the homo-oligomeric $\alpha 7$ neuronal nAChR (position L247; Revah et al., 1991) lead to pleiotropic effects. In the L247T mutant: (a) voltage-sensitive channel block by QX222 was suppressed, supporting the notion that the Leu residue points toward the channel lumen; (b) desensitization of the permeability response was abolished; (c) apparent affinity for ACh (EC_{50}) was increased (from 115 μM in the wild type to 0.7 μM in the mutant); (d) the permeability response was raised in a time scale of seconds (Fig. 3 and Revah et al., 1991). One interpretation of these pleiotropic effects is that mutation of Leu into Thr renders one of the *high affinity, desensitized* states *conducting* (Revah et al., 1991; Devillers-Thiéry et al., 1992). In support of this conclusion, two levels of conductances were recorded at high ACh concentration (20 μM) in the L247T mutant: one of 45 pS, also observed for the wild type, and thus corresponding to the conductance of the "active" state and a new one of 80 pS which was *the only one* recorded at a low concentration of ACh (0.1 μM ACh) and thus characterized the new conducting state of the L247T mutant. As expected from the scheme of "allosteric" transitions, competitive antagonists (dihydro-β-erythroidine, d-tubocurarine or hexamethonium) which stabilize the desensitized states of *Torpedo* nAChR (Rang & Ritter, 1970; Grünhagen & Changeux, 1976; Krodel et al., 1979; Weiland et al., 1979) promoted a permeability response in the L247T mutant and activated the new 80 pS conductance which was blocked by α bungarotoxin (Bertrand et al., 1992).

Similar effects were observed when either the Ser ring (labeled by chlorpromazine, Giraudat et al., 1986) or the Val ring (labeled by TID, White & Cohen, 1992) were mutated on $\alpha 7$ homo-oligomeric nAChR. Mutants T244Q or D and V251T yielded functional nAChR conducting in one of the desensitized states and denoted as D* (Devillers-Thiéry et al., 1992). The D* state is thus characterized by: (1) a higher apparent affinity (EC_{50}) for agonists, (2) no desensitization of the permeability response; (3) a slow onset of the permeability response; (4) competitive antagonists which behave as agonists; (5) two levels of conductance.

These data indicate that three superimposed rings, two hydrophobic and one polar, contribute to the closure of the channel in the desensitized states.

Mutations in the Outer Rings of Leucine

nAChR are selective for Na^+ and K^+ but let Ca^{2+} ions flow through the ion channel, neuronal nAChRs being more permeable than muscle nAChR to Ca^{2+} (Mulle et al., 1992*a*, *b*; Vernino et al., 1992). A PCa/PNa of 0.3 has been reported for muscle nAChR (in Hille, 1992), of 0.93 in parasympathetic cardiac neurons (Fieber & Adams, 1991), of 2.5 in PC12 cells (Sands & Barish, 1991) and of 10–20 for the homo-oligomeric $\alpha 7$ nAChR (Séguéla et al., 1992; Bertrand et al., 1993*a*; Ferrer-Montiel & Montal, 1993). In neuronal $\alpha 7$ homo-oligomeric nAChR, Ca^{2+} permeability seems to be controlled at, at least, two sites. Site 1, as already mentioned, is the Intermediate ring of charged amino acids located at the N-terminal end of the MII segment and site 2 is located at the C-terminal end of the MII segment at the two adjacent outer rings of Leu (positions 254 and 255 in $\alpha 7$). Mutations of either Leu 254 or Leu 255 to Thr, Gly, Arg or Gln decrease the PCa/PNa from 10 to nearly zero (Bertrand et al., 1993*a*). Leu 254 could be the equivalent of the Gln/Arg position which was postulated to be important for the high Ca^{2+} permeability of GluR. When a Gln residue is present in GluR, channels are permeable to Ca^{2+}, when an Arg is present Ca^{2+} permeability is abolished (Hume et al., 1991; Mishina et al., 1991; Verdoorn et al., 1991; Burnashev et al., 1992*a*, *b*, *c*; Mori et al., 1992; Egebjerg & Heinemann, 1993; *reviewed in* Gasic & Heinemann, 1992). These two rings are thus involved in divalent ion permeation. Moreover, mutations at these two adjacent outer rings of Leu are accompanied by an increase in the apparent affinity for ACh with an augmentation of the cooperativity index and a diminished desensitization of the permeability response. These results are reminiscent of the effects occurring after mutation at the equatorial Leu ring. However, competitive antagonists in this case do not behave as agonists. Thus, it could be that either the desensitized I state becomes conducting upon mutation or that mutations do not modify the allosteric properties of the mutant but alter the coupling between the agonist binding site and the ion channel by changing the

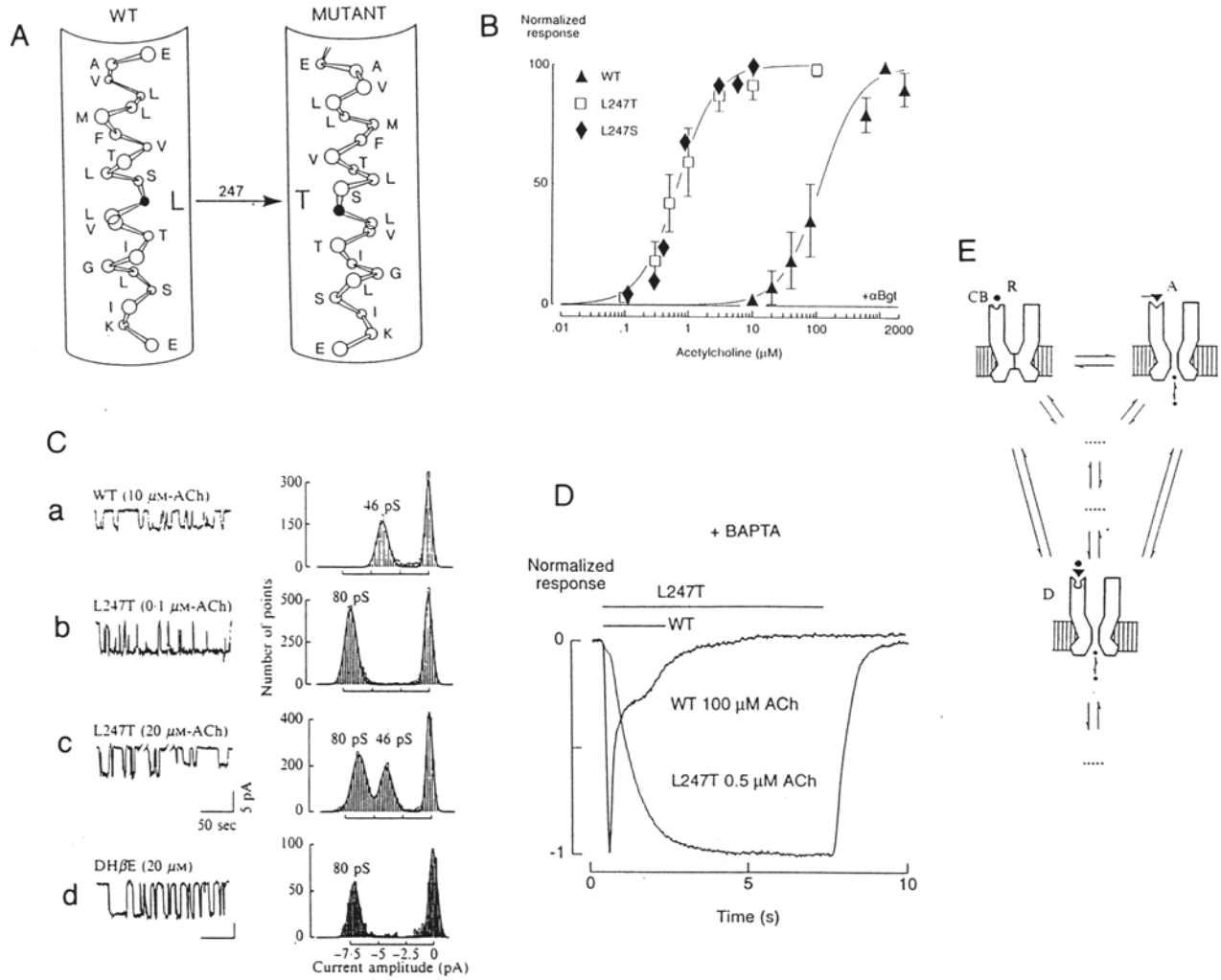
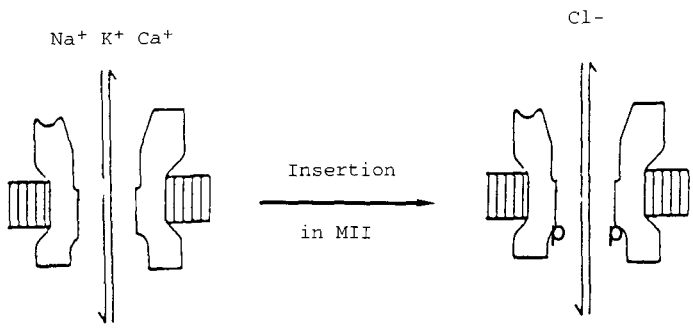


Fig. 3. Effects of mutation at the **equatorial Leucine** ring in homo-oligomeric $\alpha 7$ channel (modified from Revah et al., 1991 and Devillers-Thiéry et al., 1992). (*A*) Sequences of MII α helices from: right, $\alpha 7$ wild type (WT) nAChR; left, L247T mutant nAChR. (*B*) ACh dose-response curves for WT nAChR and mutants L247T and L247S. (*C*) Single channel conductances of WT and mutant L247T nAChR at a holding potential of -100 mV. Recordings from (*a*) WT nAChR (at 10 μM ACh) showing a single population of channels of 46 pS characterizing the "A" state. (*b*) Mutant L247T at an ACh concentration (0.1 μM) which does not activate the WT nAChR channel, showing the appearance of a new conducting state of 80 pS. (*c*) At a higher ACh concentration (20 μM), the new 80 pS conductance of the D* state is observed together with the 45 pS conductance state of the "A" state. (*d*) This new state of conductance is activated by dihydro-β-erythroidine (DHβE: a competitive antagonist of the WT nAChR). (*D*) Comparison of the normalized responses of WT and mutant L247T nAChR evoked by ACh at their EC_{50} concentrations (*see* Fig. 3*B*). Experiments were carried out in the presence of the Ca^{2+} chelator, BAPTA, injected into oocytes to block Ca^{2+}-activated chloride currents. (*E*) The effects of the L247T mutation are interpreted in the scheme of allosteric proteins as rendering one of the desensitized states, conducting. This new conducting state is denoted D*.

equilibrium constants between open and closed liganded states, for instance.

Conversion of Ionic Selectivity

As for the ligand binding site, the extensive sequence homology in the MII segment of the other members of the superfamily suggests that the different properties of these receptors may rely on a limited number of key amino acid side chains within a common backbone. However, an optimum alignment between cation- and anion-selective receptors required the insertion of one amino acid at the N-terminal end of the MII segment in the anionic-selective channels between the Inner and Intermediate rings of negatively charged amino acids. In view of this high degree of sequence homology and also since in some



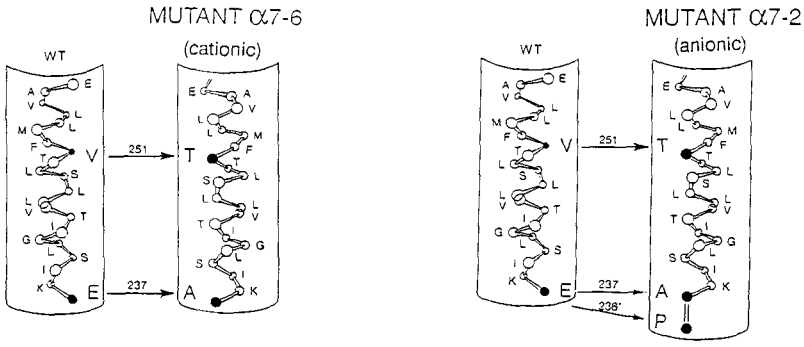


Fig. 4. Conversion of ionic selectivity of $\alpha 7$ homo-oligomeric nAChR from cationic to anionic (modified from Galzi et al., 1992). Left part: The double mutant E237A (Inner ring) and V251T (Valine ring), denoted $\alpha 7$-6, is a D* mutant selective for cations although its selectivity for Ca^{2+} is reduced as compared to that of the WT nAChR. Right part: When in mutant $\alpha 7$-6, a residue (either a proline or an alanine) is introduced between the Inner and the Intermediate rings (position 236'), the triple mutant $\alpha 7$-2 is now selective for anions.

invertebrates ACh-gated channels conduct anions (Tauc & Gerschenfeld, 1962; Kehoe, 1972; Ascher & Erulkar, 1983), it became conceivable to convert ion selectivity from cationic to anionic. In homo-oligomeric $\alpha 7$ neuronal nAChR exchange of those amino acids supposed to line the channel (in an α helical organization of the ion channel) by the corresponding ones of the anion-selective $\alpha 1$ subunit of the GlyR led to the multiple mutant which had the sequence: 236'P (inserted), E237A (Intermediate ring), S240G (Thr ring), V251T (Val ring), L254S, L255G (outer Leu rings), E258N (Outer ring) and which was now selective for anions (Galzi et al., 1992). Among the multiple mutated positions, only three were shown to be important for ion selectivity conversion: the V251T mutation which conferred a D* state (*see above*), the E237A mutation already mentioned and the *most critical* mutation was the insertion of a residue, which could either be a Pro or an Ala (*see* Fig. 4, mutant $\alpha 7$-2). Indeed, the triple mutant 236'P/A, E237A and V251T ($\alpha 7$-2) was selective for anions and the double mutant E237A and V251T ($\alpha 7$-6, Fig. 4) was selective for cations. These data suggest that the quaternary structure of the receptors and their ability to undergo conformational transitions are important parameters for determining the ion selectivity of their channels (Galzi et al., 1992).

The AChR Ion Channel is a Stratified Structure

These results suggest a stratified organization of the ion channel. Mutations in some layers alter in ion channel gating (the Serine ring, the equatorial Leucine ring, the Valine ring and the outer Leucine rings) while others modify ionic selectivity or promote ion transport. The layer whose mutation alters monovalent cation selectivity (the Serine ring) is distinct from those whose mutations modify monovalent *vs.* divalent ion selection (the Intermediate ring of negatively charged amino acids and the outer Leucine rings) and from the layer involved in cation *vs.* anion selectivity (between the Inner and the Intermediate rings of negatively charged amino acids) (Bertrand et al., 1993*b*).

The Three-dimensional Organization of the Ion Channel in the Superfamily of Ligand-gated Ion Channels

As mentioned, photoaffinity labeling and mutagenesis experiments of nAChR support a model in which the polypeptide segments which line the ion channel are coiled into α helices. By electron microscopy, Unwin (1993) observed in the map of *Torpedo*

nAChR inside the lipid bilayer *one* α helix which he assumes is the MII segment lining the pore, the other transmembrane segments being less organized structures or possibly β sheets. Unwin also noticed that the α helices are not straight, but bent near the middle of the bilayer (Unwin, 1993). According to Unwin (1993), the kink would be located at the level of the equatorial Leu ring and "transient cooperative rotations would bring the hydrophobic residues inward to block the pore."

The other members of the superfamily exhibit extensive sequence homologies in the MII segment with that of the nAChR (*see* Fig. 2). In particular, the "Inner" ring of negatively charged amino acids, the "Serine" ring where either a Ser or a Thr residue is present and the "equatorial Leu" ring are conserved. In view of the high degree of sequence homology, and although little information is available concerning the tertiary structure of these ion channels, it seems reasonable to assume that the MII segments line the ion channel and are organized in α helices. Indeed, MII segments of the GlyR when incorporated into black lipid layers elicited single channel events (Langosch et al., 1991). Also, in the homo-oligomeric α1 GlyR, picrotoxinin acts as a noncompetitive blocker, whereas in the hetero-oligomer, picrotoxinin inhibition is reduced 50–200 fold. Replacing in the hetero-oligomer the residues of the MII segment of β subunit by the equivalent ones of α subunit restored picrotoxinin sensitivity (Pribilla et al., 1992).

III. Conclusions

Our understanding of the functional architecture of the nAChR made important progress when the primary amino acid sequence became available through cDNA recombinant technologies. The high degree of sequence identity noticed between subunits was interpreted on the basis of an evolution from a common ancestor gene. Then, cloning of Glycine, $GABA_A$, 5 HT receptors further emphasized the homology with nAChR leading to the definition of a superfamily of ligand-gated ion channels which shares common principles of organization. These proteins are thought to be pentameric with a similar transmembrane organization.

The general backbone architecture of these molecules could be fairly similar. The agonist binding site of nAChR which is composed of three loops of the N-terminal domain contains a certain number of amino acids that are conserved at equivalent positions in the superfamily, stressing their importance in the structure of the site. Slight amino acid sequence differences in these domains between subunits of one family, at positions equivalent to those affinity-labeled in the nAChR, affect agonist and/or competitive antagonist recognition in the other members. The diversity of pharmacological properties of the different members of the superfamily and the different subunits of a given family could have arisen through rather simple genetic events.

The lining of the ion channel by MII segments appears plausible throughout the superfamily even though scarce data are available. Sequence alignment shows that a certain number of superimposed rings of amino acids is conserved. The "Inner" ring of negatively charged amino acids which is important in monovalent and divalent ions permeability, the Ser ring which seems to play a catalytic role in ion permeation, the equatorial Leu ring involved in the gating of the ion channel are conserved. Also, the possibility to convert ion selectivity from cationic to anionic by exchanging amino acids of the nAChR by those supposed to be equivalent in the GlyR strengthens a common scheme of organization of the channel. Finally, the observation of two levels of conductance in the mutant L247T is reminiscent of the properties of other members of the superfamily. Among the members of the GluR, NMDA and non NMDA, multiple conductance levels are observed which are preferentially associated with one of the several agonists (Nakanishi, Schneider & Axel, 1990; Patneau & Mayer, 1991). As in the mutant L247T, besides the active conformation, some other ones could be conducting. In view of these data, it is tempting to speculate that combinations of subunits which exhibit subtle amino acid variation may lead to receptors which display rather different physiological and pharmacological properties for the agonists, the competitive antagonists and/or the noncompetitive blockers.

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