Annu. Rev. Pharmacol. 1991. 31:37–72

FUNCTIONAL ARCHITECTURE OF THE NICOTINIC ACETYLCHOLINE RECEPTOR: From Electric Organ to Brain

Jean-Luc Galzi, Frédéric Revah, Alain Bessis, and Jean-Pierre Changeux

Unité Associée, Centre National de la Recherche Scientifique D 1284 Neurobiologie Moléculaire, Départment des Biotechnologies, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cédex 15, France

KEY WORDS: ligand-gated ion channel, allosteric protein, acetylcholine binding site

---

Acetylcholine (ACh) serves as a neurotransmitter in the brain and at the junction between the motoneuron and skeletal muscle. Its action at this site has been thoroughly studied (1). On rapid release from the motor nerve ending at a 0.1—1 mM concentration (2), ACh diffuses through the synaptic cleft and causes the all-or-none opening of ionic channels selective for sodium, potassium, and other small cations. If the resulting depolarization is above threshold, it leads to an action potential and to muscle contraction (reviewed in 3). In the brain, ACh also acts as an excitatory transmitter (reviewed in 4).

The identification of the molecule that converts the ACh signal into an electrical response, the nicotinic acetylcholine receptor (AChR), has been greatly facilitated by using as highly specific ligands (5) a combination of α toxins from snake venoms (6, 7) on eel or *Torpedo* electric organs. These provide exceptionally rich sources of cholinergic synapses and possess a receptor closely resembling that from the neuromuscular junction (8, 9).

0362-1642/91/0415-0037$02.00

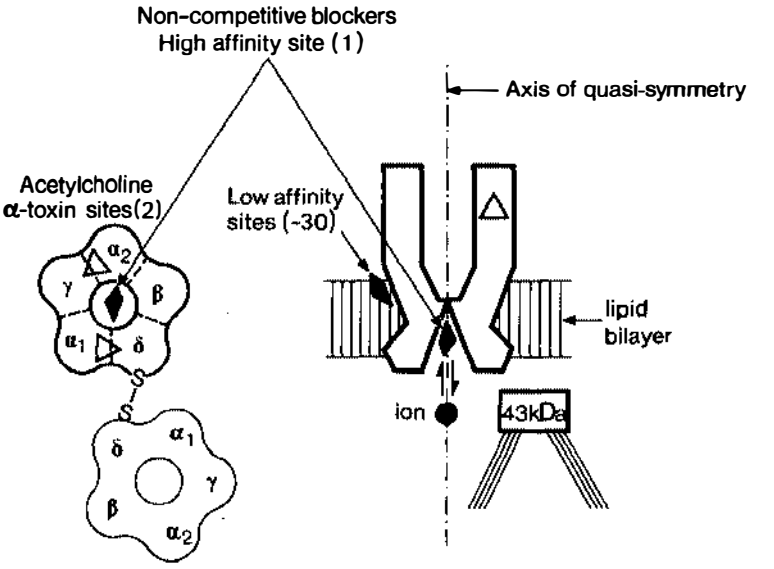

Figure 1 Schematic transmembrane organization of the AChR. **Left**: Possible arrangement of the AChR subunits around the axis of pseudosymmetry. This model takes the following considerations into account: *a*) the two α-subunits are not in contact with each other (24, 26, 27); (*b*) the δ-subunit, which is involved in the receptor dimer formation (266) is not the single subunit between the α-subunits (267); (*c*) as a result of covalent labeling of the ACh-binding sites (104, 118) and expression of subunit dimers (119), the γ- and δ-subunits are positionally homologous with respect to the α-subunits, which in turn are rotationally symmetrical (119). The two binding sites for agonists and competitive antagonists are mainly located on the α-subunits. **Right**: Transmembrane organization and allosteric sites of the AChR. The allosteric sites for the noncompetitive blockers are subdivided into two main categories. In this model, the unique high-affinity site, sensitive to histrionicotoxin or phencyclidine, is located on the axis of quasi-symmetry of the molecule. The multiple low-affinity sites are distributed at the boundary of the protein with the lipid bilayer.

In the electric organ and muscle, the receptor for acetylcholine, nicotine, or curare is a 290-kd transmembrane glycoprotein, composed of four distinct subunits assembled into a heterologous $\alpha_2\beta\gamma\delta$ pentamer (Figure 1). Reconstitution with the purified protein and subunit mRNA expression experiments in frog oocytes have shown that the $\alpha_2\beta\gamma\delta$ oligomer contains all the structural elements required for the physiological response: the agonist/competitive antagonist binding sites, the ion channel and the mechanisms that mediate fast coupling between them (reviewed in 9–14).

On electron micrographs, the purified and membrane-bound AChR from fish electric organ appears as a ringlike particle ~65 Å in diameter with a central 25 Å-wide depression and five peaks of electron density, assigned to the five subunits, arranged around an axis of pseudo-symmetry perpendicular

to the plane of the membrane (15–33). Viewed from the side, the AChR molecule looks like a 110 Å-long transmembrane cylinder protruding 55–65 Å into the synaptic cleft and 15–20 Å into the cytoplasm (32, 33).

Early attempts to characterize nicotinic AChR receptors in the nervous system were based on α-bungarotoxin binding to brain membranes. However, this toxin proved not to be a useful tool for such studies since it does not block ACh-evoked ion permeation in many systems nor does it bind to brain [^{3}H]nicotine or [^{3}H]ACh-binding sites (35–37). On the other hand, the pharmacological characterization of neuronal AChR has been facilitated by a new toxin, referred to as neuronal bungarotoxin, (38, 39) that blocks activation of the nicotinic AChR in several, but not all, neuronal preparations (4, 40–43; reviewed in 43). The purification, by affinity chromatography, using antibodies against *Torpedo* receptor, led to the partial characterization of chick and rat brain neuronal AChR (44–46; see also 47), which is composed of at least two groups of subunits, known as α- and non-α- (or β) subunits.

Finally, identification of the genes coding for the different subunits and electrophysiological measurements revealed the existence of multiple functional AChRs in the central nervous system. To date, their quaternary structures has not been entirely elucidated but could well have a heterologous pentameric structure (13, 48), like muscle AChR.

Primary Structure and Models of Transmembrane Organization of the Subunits.

Complete cDNA coding sequences have been established in *Torpedo californica* for the precursors of the α- (49), β- (50), γ- (51, 52) and δ-subunits (50), as well as the complete nucleotide sequence (53) for the α-subunit from *Torpedo marmorata*. Subsequently, DNA probes derived from *Torpedo* clones were used to isolate genes and cDNAs coding for muscle (54–56 and references below) and neuronal AChR subunits (54–60; reviewed in 61). The neuronal α-subunits (α2 to α5) possess adjacent cysteines analogous to cysteines 192–193 of muscle-type α-subunit (α1) that are missing in the β-subunits (β2 to β4) (see below).

Injection into *Xenopus* oocytes of mRNA transcribed from cDNAs coding for the AChR subunits further showed that the cloned genes code for functional subunits. Expression of cDNAs for the four *Torpedo* subunits yields acetylcholine-gated ion channels and α-toxin binding material (11, 62, 63). Omitting the α-subunit mRNA abolishes α-toxin binding and acetylcholine sensitivity. Furthermore, the neuronal β2-subunit substitutes for the muscle β1-subunit to form a functional muscle-type nicotinic receptor (66) and combines with three different neuronal α-subunits to form a functional receptor (65). Expression studies in *Xenopus* oocytes of different combinations of

neuronal AChR subunits also revealed that the functional and pharmacological properties of the multiple combinations of subunits obtained vary significantly (65, 66).

The aligned sequences of the various cloned receptor subunits appear to be strongly homologous and show similar hydrophobicity profiles, justifying a common subdivision of the homologous chains into: (*a*) a large hydrophilic amino-terminal domain of 210–220 amino acids; (*b*) a compact hydrophobic region of 70 residues subdivided into three segments of 19–27 uncharged amino acids (MI, MII, and MIII); (*c*) a second hydrophilic domain of variable length, generally much larger in the neuronal subunits; (*d*) a carboxy-terminal segment of 20 hydrophobic residues (MIV).

On the basis of these primary sequence data, several models of transmembrane organization common to the four homologous subunits in *Torpedo* have been proposed (10, 51–53, 67–73). Their common features are: (*a*) the orientation of the large hydrophilic domain towards the synaptic cleft; (*b*) the orientation of the small hydrophilic domain towards the cytoplasm; (*c*) the assignment of the four hydrophobic segments MI–MIV to transmembrane α-helices (by analogy with known membrane proteins such as bacteriorhodopsin and glycophorin A). All the proposed models assume that the acetylcholine binding site is located in the large hydrophilic domain on the α-subunit and that the walls of the ionic channel lie on the axis of quasi-symmetry of the AChR molecule and are delineated by homologous portions of each subunit. Differences arise over the number, orientation, and identity of the transmembrane segment(s).

In one group of models, each subunit chain spans the lipid bilayer four times and the carboxy- terminal end faces the synaptic cleft, one of the first three hydrophobic segments (51, 53) being the transmembrane component of an uncharged channel (Figure 2). Another model, based on periodicities in hydrophobicity, uses Fourier analysis (68) or transfer energy calculations (67) to postulate an additional fifth "amphipathic" helix, MA. This helix is formed at the expense of the cytoplasmic domain, thus reorienting MIV to expose the carboxy terminus to the cytoplasmic face. MA then delineates a charged channel (67, 68, 74). Finally, a model based on extensive immunochemical studies with poly- and/or monoclonal antibodies against peptides of defined sequence proposes that MIV is not transmembrane but cytoplasmic (71); hence, a transmembrane "hairpin" loop forms from the large hydrophilic domain and the region extending from amino acid 152 to 159 (71) or 156 to 179 (75) on *Torpedo* α-subunit is exposed to the cytoplasm. Tests for these models have been developed in several laboratories using different experimental approaches.

Covalent labeling of native AChR with photoactivatable arylazidophospholipids (76) supports the view that MIV is exposed to the lipid bilayer

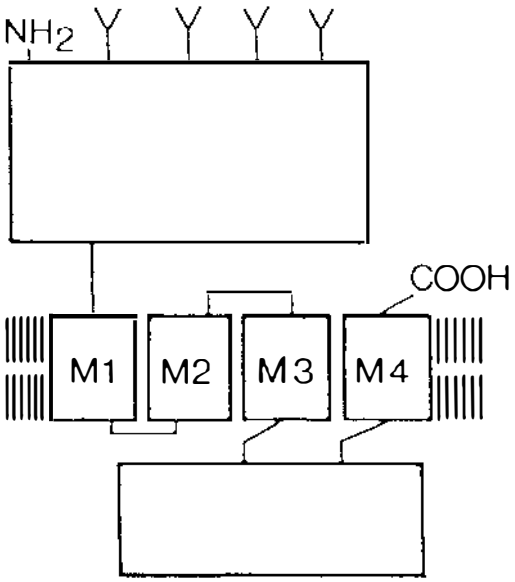

Figure 2 Model of transmembrane organization of the AChR subunits based on the hydrophobicity profile of the primary amino acid sequence (see text).

(see also 77). Using nonpermeant reducing agents on reconstituted vesicles (78) or native receptor-rich microsacs (79), it has been demonstrated that the disulfide bond linking δ-subunits in *Torpedo* AChR dimers is extracellular (see also 80). Given that the penultimate Cys residues of the δ-subunits are involved in the disulfide bond, the carboxy terminus thus faces the synaptic cleft (10). Furthermore, labeling of the region comprising the MA segment in the native receptor by pyridoxal phosphate and tritiated sodium borohydride (81) provided evidence for its location outside the membrane. Finally, the finding that a photoaffinity ligand of the acetylcholine binding site (82) labels the region of the α-subunit assigned to the cytoplasmic portion of the "hairpin" loop is incompatible with the folding postulated by Ratnam et al (71) (see below).

THE STRUCTURE OF THE ACETYLCHOLINE BINDING SITES

Pharmacological studies and ligand binding experiments have defined three classes of effectors of the nicotinic AChR: agonists, competitive antagonists, and noncompetitive blockers. Comparative analysis of muscle and neuronal AChR responses to these ligands reveals different pharmacological profiles.

For instance, the agonists, cytisine and 1,1 dimethyl-4-phenylpiperazinium, and the antagonists, neuronal bungarotoxin and neosurugatoxin, are potent effectors of neuronal AChRs only. Moreover, these receptors do not bind α-bungarotoxin (reviewed in 83, 84). The antagonist dihydro-β-erythroidine blocks the ACh-evoked responses in the prefrontal cortex (42), hippocampus (85), medial habenular nucleus (4), but not in the retinal ganglion (86). Also, d-tubocurarine, which acts as a potent competitive antagonist on muscle receptors, blocks the AChRs from medial habenular nucleus (4), hippocampus (85), but not from prefrontal cortex (42) or retinal ganglion (86).

In parasympathetic neurons (87), d-tubocurarine acts as a noncompetitive blocker, and the muscle AChR noncompetitive blocker mecamylamine (88) behaves as a competitive antagonist.

Thus, the difference in sensitivity to antagonist, as well as the different single-channel conductances (4, 89–91), are consistent with the existence of multiple subpopulations (at least three) of nicotinic AChRs.

Despite these pharmacological differences, all agonists and competitive antagonists exert their effects upon binding to sites that are mainly carried by the α-subunits (reviewed in 13, 14).

Two main ACh-binding sites exist per muscle (and possibly also neuronal) receptor molecule (92), and these two sites interact in a positive, cooperative manner (reviewed in 72, 93; see also 94). On *Torpedo* AChR, the positive cooperative interactions between ACh molecules take place between *nonequivalent* sites. Such nonequivalence was first suggested by the two-step kinetics of α-toxin binding and dissociation, and from a differential accelerating effect of cholinergic ligands upon the dissociation of the bound toxin (95–101). Furthermore, the equilibrium-binding curve discloses two distinct affinities for the antagonist [^{3}H]d-tubocurarine, but not for acetylcholine and carbamylcholine (102–104). Also, the affinity reagents MBTA or bromoacetylcholine attach to both sites with a different affinity (105–108) and similar data have been reported for the diterpenoid coral toxin lophotoxin (109). Some antibodies (monoclonal or from sera of *myasthenia gravis* patients) block one of the two binding sites for α-bungarotoxin (110–113) and of the twelve antibodies that block the binding of α-bungarotoxin to the receptor, six block the binding to one site, four to the other one, and two to both sites (114).

The origin of this non-equivalence is still not clear. Since the two α-subunits are encoded by a single gene in *Torpedo* (115) and mouse (116), they are most likely identical in primary structure. However, within the $\alpha_2\beta\gamma\delta$ oligomer, the two α-subunits cannot be equivalent in their mode of interaction with other subunits (see 14, 102 for discussion), and other subunits may possibly contribute domains for ligand binding (117, 118). Indeed, UV irradiation of the [^{3}H] α-toxin-receptor complex results in the covalent incorporation of radioactivity in the γ- and δ-subunits in addition to the α-

subunit (117). Furthermore, significant carbamylcholine-sensitive incorporation of N,N-(dimethylamino) benzenediazonium fluoroborate takes place in the γ-subunit (118). Also, [^{3}H]d-tubocurarine photoaffinity labels the α- and γ-subunits when bound to its high-affinity site and the α- and δ-subunits when bound to its low-affinity site (104). Recently, Blount & Merlie (119) expressed different pairs of mouse muscle α- and non α-subunits in fibroblasts and showed that the γ- and the δ-subunits associated efficiently with the α-subunit into complexes with different high-affinity binding sites for the competitive antagonist d-tubocurarine. The association of each α-subunit with either the γ- or δ-subunit may thus account for the nonequivalence of acetylcholine binding sites. Nevertheless, differences in posttranslational modifications such as glycosylation of the two α-subunits have been suggested (101, 120).

Experiments with mRNA injection into oocytes have provided evidence that the β2-subunit, combined with either α2-, α3-, or α4-subunits, can produce functional nicotinic receptors with pharmacological properties similar to those of neuronal AChRs (65). The nicotine-evoked permeability responses are blocked with neuronal bungarotoxin on the α3–β2 and α4–β2, but not α2–β2, combinations. Similarly, Duvoisin et al (66) have shown that the α3–β2, but not the α3–β4, combination is blocked by this toxin. These results suggest that, as with muscle receptors, the non-α-subunits of neuronal receptors also contribute to the binding site for cholinergic ligands. However, the subunit combinations corresponding to physiological neuronal receptors are still unknown, and the existence of pharmacologically nonequivalent (or equivalent) ACh-binding sites has not been demonstrated.

Identification of Amino Acids Composing the Acetylcholine Binding Sites

Despite this complexity, various experimental approaches have been employed to identify the amino acids that contribute to the ACh-binding area of *Torpedo* receptor. Kao et al (121) have demonstrated by peptide mapping and sequencing that the residues cysteine 192 and possibly 193, a tandem unique to the α-subunit, represent the sites of incorporation of the affinity label 4-(N-maleimido)-benzyltrimethylammonium (MBTA). This labeling, however, occurs exclusively on the *reduced* receptor. In the native receptor, cysteines 192 and 193 are linked by a disulfide bridge (122, 123). Subsequent studies based on the binding of snake α-toxins to α-subunit fragments (125–128), synthetic peptides (125, 129–131), deletion mutants (132), or α-subunit fragments expressed in *Escherichia coli* transformants (133), and site-directed mutagenesis experiments (74) confirm that the region containing cys 192 and cys 193 contributes to the interaction of cholinergic ligands and snake α-toxins with the α-subunit.

The characteristic Cys 192-Cys 193 doublet is also present on α-subunits of neuronal AChRs, which, however, do not bind α-bungarotoxin but only the neuronal bungarotoxin. Peptides corresponding to sequence 1–18 and 50–71 of the α3-subunits are among the main components of the binding site for neuronal bungarotoxin (134). However, as this toxin also interacts (with low affinity) with the Cys 192–Cys 193 region, the respective specificities of both α- and neuronal bungarotoxin remain unclear.

In none of the experiments carried out with the isolated α-subunit or its fragments was the high affinity for toxin binding recovered (nor, in most of the experiments, the competition by nicotinic ligands). This suggests that multiple domains of receptor primary structure participate in the functional organization of the α-toxin binding area (see 135).

A more detailed mapping of the ACh-binding sites has been obtained by the use of the photoaffinity probe *p*(N,N)-dimethyl-aminobenzene diazonium fluoroborate (DDF). In the dark DDF behaves as a reversible competitive antagonist of the electrical response of electroplaque of *Electrophorus electricus* and of the ACh-gated single-channel currents recorded in the C2 mouse cell line (118). DDF can be efficiently photoactivated by an energy transfer reaction between an excited tryptophan residue from the ligand-binding site and the photosensitive ligand, a procedure that improves the specificity of the labeling (136, 137). Under such irradiation conditions, DDF reacts with the AChR without prior reduction and labels the agonist/competitive antagonist binding site with a stoichiometry of 1 DDF incorporated per α-bungarotoxin binding site (118). The amino acids labeled by [^{3}H]DDF in a carbamylcholine-sensitive manner belong to three regions of the α-subunit (138). They were identified as *tyrosine 93, tryptophan 149, tyrosine 190,* and *cysteines 192 and 193* (Figure 3) (in addition, α-tryptophan 86, α-tyrosine 151, α-tyrosine 198 appeared to be weakly labeled) (82, 139). Both α-toxin and carbamylcholine decrease DDF labeling of the three regions in a parallel manner, supporting the conclusion that at least three loops of the NH_2-terminal large hydrophilic domain contribute to the acetylcholine binding site (82, 138, 139).

Several conclusions can be drawn from these data:

1. The data are consistent with the results of Kao et al (121) since the MBTA-labeled α-cysteines 192 and 193 are also labeled by DDF. Also, α-tyrosine 190, initially found to be labeled by DDF (82), covalently reacts with the coral competitive antagonist lophotoxin (140, 141). Moreover, DDF-labeled amino acids are conserved (Figure 4) at homologous positions in all α-subunits from muscle (reviewed in 142) and neuronal AChRs [such as in α2 (58, 143), α3 (58, 144), α4 (145)] from all species examined to date, including humans. An exception occurs in the sequence of the neuronal α5 clone, which does not contain the amino acids

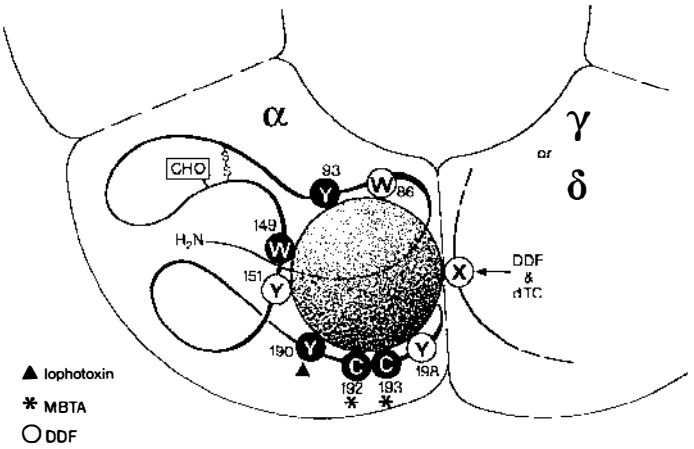

Figure 3 Model of the agonist/competitive antagonists binding site(s). Top view of a schematic model for the proposed folding of the amino-terminal extracellular segments of the α-chain in the AChR oligomer. The large sphere represents the space occupied by a molecule of the photoactivatable competitive antagonist DDF, in all possible orientations within its binding site. The polypeptide chain is folded in such a way that the [^{3}H]DDF labeled (O) amino acids (one letter code) are in contact with the sphere. Filled circles denote those residues clearly shown to be labeled and open circles those for which evidence indicative of labeling was obtained (82, 139). The amino acids labeled by [^{3}H]MBTA (*) (122) and [^{3}H]lophotoxin (▲) (141, 1989) are also indicated. Numbers refer to the positions within the sequence of the α-subunit of *Torpedo*. The X denotes as-yet unidentified amino acid(s) labeled by [^{3}H]DDF on the γ-subunit (118) and by [^{3}H]d tubocurarine (dTc) on the γ- and δ-subunits (104). The disulfide bond (S-S) linking α Cys-128 and α Cys-142 (122) and the site of N-linked glycosylation at Asn 141 (CHO) are indicated.

homologous to Tyr-93 and -190 and *does not* form functional AChR when expressed in *Xenopus* oocytes in association with any of the non-α-subunits cloned (146). None of the five predominantly labeled amino acids is conserved in electric organ or muscle β-, γ-, or δ-subunits. However α-tryptophan 149 and α-tyrosine 93 are both found at homologous positions in the so-called *non*-α-subunits from neuronal nicotinic receptor (58, 64, 66, 148), suggesting that these non-α-subunits may have a function in neural tissue, distinct from those of the β-, γ-, and δ-subunits in muscle and electric organ receptors. Such high conservation of the DDF-, lophotoxin-, and MBTA-labeled amino acids through evolution is consistent with the physiological role of these amino acids in ACh binding to the α-subunits.

2. Snake muscle AChR (149) as well as the cloned neuronal AChRs expressed in *Xenopus* oocytes (64, 143, 150) are resistant to α-bungarotoxin. This resistance may be explained by the absence of α-bungarotoxin binding to these AChR (41, 149). Yet, all contain the amino acids labeled by

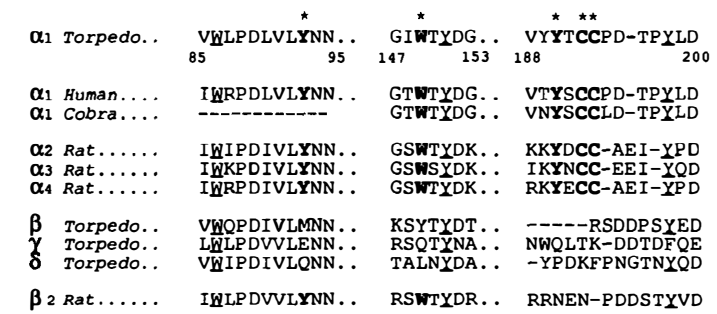

Figure 4 Comparison of the amino acid sequences containing the [^{3}H] DDF-labeled amino acids of AChR α-subunits of *Torpedo* (53) with the corresponding fragments of human muscle α_1 (54), snake muscle α (151), neuronal rat-α_2- (143), α_3- (144), α_4- (145), β_2- (64), and electric organ β-, γ- and δ-subunits (50) of nicotinic AChR. The amino acids clearly labeled on *torpedo* AChR (82, 139) are indicated in bold face type and those for which evidence indicative of labeling was obtained are underlined.

DDF, lophotoxin and MBTA at homologous positions in the sequence of their α-subunit (143–145, 151). These observations thus raise the possibility that the α-toxins, which competitively prevent the binding of small cholinergic ligands to muscle AChR, may interact with more amino acids than those strictly involved in the binding of agonists and competitive antagonists.

3. It has been widely held that carboxylate *anions* (side chains of aspartic and glutamic acids) in the ACh-binding site form a negative subsite responsible for the interaction with the cationic head group of ACh (reviewed in 152, 153). In fact, neither glutamyl nor aspartyl residues were among the [^{3}H]DDF- (82, 139) or [^{3}H] lophotoxin- labeled residues identified (141). Orientation and/or reactivity restrictions of these chemicals within the binding site may possibly lead to a preferential labeling of certain residues. Interestingly, on the other hand, these chemicals become predominantly incorporated into *aromatic* amino acids, in particular tyrosine residues α 93 and α 190 (and possibly α 151 and α 198). This finding is consistent with a model in which the electronegative character of the quaternary ammonium binding domain consists, at least in part, of the lone pair of electrons of the phenolic oxygen of these tyrosines, the sulfur atoms forming the α-cysteine 192–193 disulfide, and the nitrogen atom of tryptophan 149 (82, 139, 141). This view is also consistent with the observation that binding of methyl-substituted ammonium groups by macrocyclic compounds requires not only electrostatic but also hydrophobic interactions (154, 156; see also 156). Both these interactions can be provided by noncarboxylate-containing entities such as macrobicyclic polyphenoxides (157, 158).

4. Besides the cys 192–cys 193 doublet conserved in the AChR α-subunit sequences, two additional cysteine residues (cys 128 and cys 142 in the α-subunits) are found at homologous positions in *all* AChR subunits as well as in GABA and glycine receptors. These residues have been proposed to form a disulfide bridge in vivo, and their mutation to serine residues totally abolishes the response of AChR to ACh (74). In agreement with the data obtained with DDF on the α-subunit, they are most probably involved in the tertiary folding of the large amino terminal domain exposed to the synaptic cleft.

In conclusion, the ACh-binding site appears more complex than initially thought. It involves amino acids from several loops of the NH_2-terminal hydrophilic domain that are conserved at homologous positions in all muscle and neuronal α-subunit sequences. The non α-subunits (β, γ and δ in muscle, and β in neuronal receptors) also contribute as-yet unidentified domains to cholinergic ligand binding. Further investigations should elucidate the respective contributions of these non-α-subunits as a structural basis of the pharmacological differences noted between neuronal and muscle receptors.

IDENTIFICATION OF THE ION CHANNEL

The permeability response of muscle and neuronal AChR to agonists can be blocked by a heterogeneous group of pharmacological agents referred to as noncompetitive blockers. All decrease the amplitude of the permeability response to agonists without significantly affecting the agonist site (reviewed in 159, 160 for muscle AChR, and 86, 87, 161 for neuronal AChR).

Among these noncompetitive blockers, the frog toxin histrionicotoxin (162), the hallucinogen phencyclidine (163, 164) and the anticonvulsant MK 801 (165) not only block muscle AChR ionic permeability, but also that of neuronal AChR (86, 161) and N-methyl-D-aspartate receptor (166; reviewed in 167). This supports the concept of a receptor superfamily within which various antagonists and channel blockers may act on more than one member (86).

Equilibrium binding studies with a variety of noncompetitive blockers showed that these compounds bind to populations of sites topographically distinct from the ACh-binding sites but positively interacting with them in an allosteric manner (96, 168, 169, but see also 93). Further detailed studies with AChR-rich membranes of *T. marmorata* and tritiated derivatives of the noncompetitive blockers histrionicotoxin, phencyclidine, meproadifen, Triton X-100, chlorpromazine, and trimethisoquin led to the distinction of two main categories of allosteric sites for noncompetitive blockers on the AChR protein (159, 170): (*a*) a *high-affinity site,* sensitive to histrionicotoxin, and

present at a single-copy per receptor pentamer and (*b*) *low-affinity sites*, insensitive to histrionicotoxin, present in large numbers (10–30 sites per receptor molecule, and lipid-dependent), possibly located at the interface of the receptor with membrane lipids.

Identification of the Receptor Subunits Contributing to the High-Affinity Site for Noncompetitive Blockers

That the high-affinity site for noncompetitive blockers belongs to the $\alpha_2\beta\gamma\delta$ oligomer was definitively demonstrated in labeling studies with a radioactive photoaffinity derivative of the potent local anesthetic trimethisoquin, [^{3}H]5-azido trimethisoquin (171). This compound almost exclusively labels the δ-subunit from receptor of *T. marmorata* (171, 172) and this labeling is enhanced by carbamylcholine and inhibited by histrionicotoxin. Similar results were obtained with [^{3}H]perhydrohistrionicotoxin, [^{3}H]phencyclidine, [^{3}H]-trimethisoquin and [^{3}H]chlorpromazine by simple UV irradiation of their complex with the membrane-bound receptor from *T. marmorata* (174).

With quinacrine mustard (175), the labeling occurs primarily at the level of the α- and β-subunits of AChR of *T. californica*. Differences in the labeling pattern between the receptor proteins from *T. californica*, *T. marmorata* and *T. ocellata* were noted when triphenylmethylphosphonium (176) or azidophencyclidine (177) were used. Interestingly, with [^{3}H]-chlorpromazine, all four chains of the receptor of *T. marmorata* were labeled, and this labeling was enhanced by carbamylcholine and decreased by histrionicotoxin (178, 179). Consequently, the unique high-affinity site for noncompetitive blockers, to which all subunits may contribute, was proposed to lie in the axis of pseudo-symmetry of the AChR oligomer where the distance between subunits is minimal (159). Its location would thus resemble that of 2,3-diphosphoglycerate and several antisickling drugs in the hemoglobin molecule (180). Minor sequence variations may account for the variable labeling patterns noted between species of *Torpedo* (53). The differences observed would then result from the ability of the ligand to adopt different positions within a cleft lying on the axis of symmetry of the AChR, which binds both optical isomers of perhydrohistrionicotoxin (181).

The High-Affinity Binding Site for Noncompetitive Blockers is Located Within the Ion Channel

Electrophysiological measurements are consistent with the notion that some NCBs (such as QX 222), under defined conditions, enter the open channel in a diffusion-controlled manner and sterically block ion flux (160, 182, 183; reviewed in 93). In particular, the physiological effects of the compounds used in labeling experiments, chlorpromazine, and phencyclidine, were explored in detail at concentrations (10–200nM) close to their dissociation

constants (100 nM) for the high-affinity site (184). Chlorpromazine and phencyclidine shorten mean burst times of acetylcholine-activated single channels from myotubes of the mouse cell-line C_2, data consistent with the "slow channel block" mechanism described by Neher & Steinbach for high-affinity noncompetitive blockers. These observations and the labeling experiments with chlorpromazine, in particular, led to the further proposal that the high-affinity site for noncompetitive blockers is located *within* the ion channel, on the axis of quasi-symmetry of the AChR where ions cross the membrane (159), according to the models of transmembrane organization. Consistent with such a scheme, channel-permeant cations competitively inhibit the binding of the noncompetitive blockers ethidium (185) to this high-affinity site (186).

Further evidence comes from investigation of the interactions of noncompetitive blockers with their high-affinity site in the time-scale of the transient opening of the ion channel using rapid mixing photolabeling equipment (187–191). The rate of covalent attachment of [^{3}H]chlorpromazine to the high-affinity site resolved in the 10 msec-sec time-scale increases 100–1000-fold ($K_{on} = 10^7\ M^{-1}s^{-1}$) when acetylcholine is added (188) in a concentration range (Kapp 30 μM) close to that required for activation of the ion channel in in vitro experiments (103, 179, 188, 192, 193). This effect is blocked by competitive antagonists such as d-tubocurarine, flaxedil, and α toxin. The rate of [^{3}H]chlorpromazine incorporation declines upon prolonged exposure of the receptor to acetylcholine, with time-course and concentration-dependence characteristics (188, 189) closely related to those reported for the rapid desensitization of the ion flux response of native membranes of *T. californica* (194) or reconstituted microsacs (195). Though the kinetics of covalent attachment of other noncompetitive blockers might differ quantitatively (191, 196), the characteristics of this rapid labeling process support the notion that, under the conditions tested, [^{3}H]chlorpromazine binds to its high-affinity site without restriction to diffusion, and covalently reacts with this site while the channel opens (188, 189).

The MII Segment is a Component of the Ion Channel

The amino acids photolabeled by [^{3}H]chlorpromazine under equilibrium conditions with AChR-rich membranes were identified by peptide mapping and sequencing experiments (197–200). First, the δ-subunit was purified and digested with trypsin. Sequence analysis of the fraction of tryptic peptides containing the specifically labeled material before and after CNBr subcleavage and repurification unequivocally demonstrated that δ Ser 262 is labeled by [^{3}H]chlorpromazine (197). Labeling of δ Ser 262 is blocked by phencyclidine. Residue δ Ser 262, which lies within the hydrophobic segment

MII, is thus part of (or in close vicinity to) the high-affinity site for noncompetitive blockers (197).

Similar experiments were repeated with the other subunits and sequence analysis resulted in the identification of Ser 254 and Leu 257 for the β-subunit (198), Ser 248 for the α-subunit (199) and Thr 253, Ser 257 and Leu 260 for the γ-subunit (200) as residues labeled by [^{3}H]chlorpromazine in a sensitive manner by phencyclidine (Figure 5).

The labeled serines on all AChR subunits have homologous positions and belong to the hydrophobic stretch MII of the AChR. Interestingly, this MII sequence is highly conserved between subunits and throughout evolution from *Torpedo* to brain. Following the finding with chlorpromazine, Oberthür et al (201) using triphenymethyl-phosphonium (TPMP) have also identified δ Ser 262 as the labeled amino acid. In another report, Hucho et al (202) have obtained evidence that also points to Ser 248 and Ser 254 as the amino acids labeled by triphenylmethylphosphonium on the α- and β-subunits, respectively.

Cohen and coworkers (203, 204) used another noncompetitive blocker to show that a mustard derivative of meproadifen affinity labels the α-subunit amino acid Glu-262. Unlike chlorpromazine and triphenylmethylphosphonium, meproadifen mustard labels an amino acid located at the border of the MII segment in the region linking MII and MIII. This result might be explained by the chemical reactivity of the nitrogen mustards, which require nucleophilic groups to form covalent bonds. No such reactive groups are present within the MII sequence. One possibility is that meproadifen mustard reversibly binds to the same high-affinity site as chlorpromazine but covalently reacts with the closest available nucleophilic amino acids.

Homologous regions of different receptor subunits thus contribute to the unique high-affinity site for noncompetitive blockers within the ion channel, a finding consistent with the proposal (159) that this site is located in the axis of quasi-symmetry of the receptor molecule. Moreover, the MII segments are not homologous with the regions labeled by affinity reagents and photoaffinity of the acetylcholine binding site on the α-subunits, a result consonant with the concept that the ACh-binding site and the high-affinity site for channel blockers are structurally distinct (9, 96).

The contribution of segment MII to the ion channel has subsequently received support from electrophysiological experiments carried out with functional AChRs channels expressed in *Xenopus* oocytes (11). Receptor proteins of *T. californica* and calf exhibit different intrinsic ion channel conductances and the domain of the receptor δ-subunit responsible for the observed differences in conductance has been identified by replacing the δ-subunit mRNA by various chimeric δ-subunit mRNAs from *Torpedo* x calf. The data suggest that MII and the segment located between MII and MIII are involved

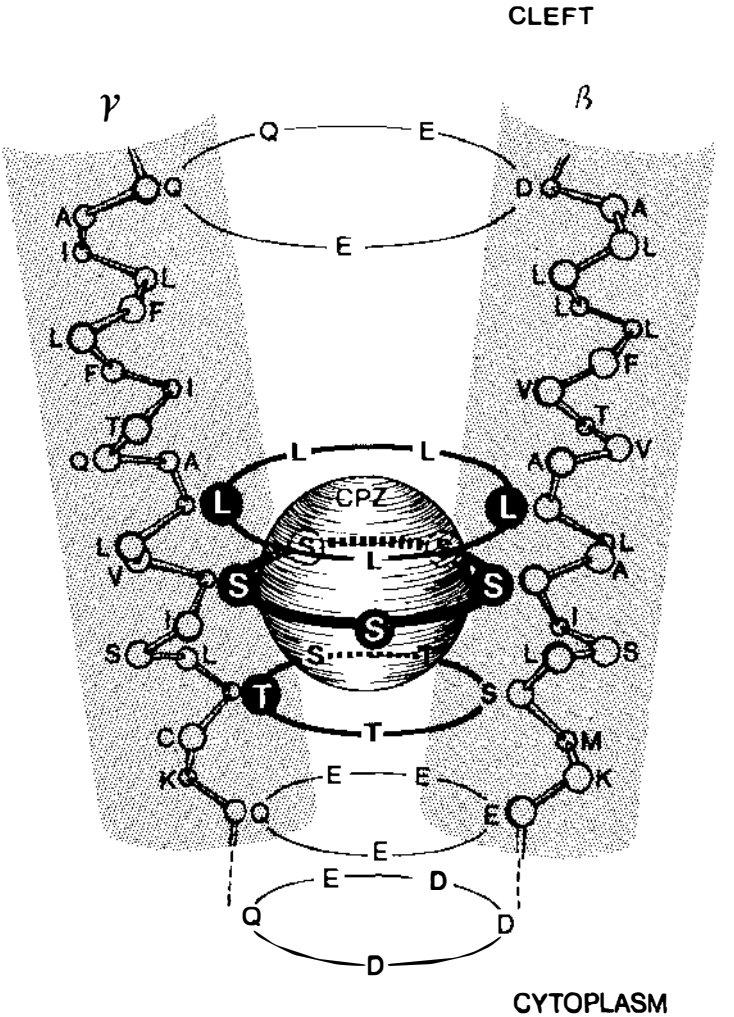

Figure 5 Model of the high-affinity site for chlorpromazine within the AChR ion channel taken from Revah et al (200). The MII segments, arranged as transmembrane α-helices, are quasi-symmetrically organized around the central axis of the molecule and are tilted with respect to this axis (216). Segments MII of the β- and γ-subunits are depicted in more detail. The scheme displays the α carbons of the considered amino acids (Standard one letter code).

The different materialized rings of amino acids are from the cleft to cytoplasm: (*a*) the "outer charged ring" (Q,E,D . . . corresponding to α Glu 261 and homologs) mutated by Imoto et al (208), (*b*) the three rings of amino acids labeled by chlorpromazine (filled circles) (197–200), and triphenylmethylphosponium (202); the serine (S) ring of mouse receptor has been mutated by Leonard et al (206); and (*c*) the "intermediate" and "inner" charged rings mutated by Imoto et al (208). The sphere represents the space occupied by a molecule of chlorpromazine in all possible orientations within its binding site.

in determining the conductance difference noted between *Torpedo* and calf channel (205). In subsequent studies, the physiological importance of the ring of serines homologous to α Serine 248 of *Torpedo* (197–200) was clearly demonstrated by site-directed mutagenesis and expression in *Xenopus* oocytes of mouse receptor subunit mRNAs (206, 207). Receptors with serine-to-alanine mutations displayed a selective decrease in outward single channel potassium currents, and the data on the voltage dependence and rectification suggest that the mutated residues are located within the ion channel, near its cytoplasmic end. In addition, serine-to-alanine mutations in this ring decrease the residence time (and thus the affinity) of the channel blocker QX 222 (206), whereas serine- (or threonine-) to-alanine mutation in the ring homologous to α serine 252 of *Torpedo* leads to a "stronger and longer binding" (207). These results are consistent with the notion that the polar end of QX 222 binds to the polar ring formed by the [^{3}H]chlorpromazine-labeled serines, which provide a waterlike region.

In another study (208), point mutations were introduced into the receptor subunit cDNAs of *Torpedo* to alter the net charge of the three rings of negatively charged or glutamine residues α-Asp 238, α-Glu 241, and α-Glu 262 and homologs that frame the MII segment. Under conditions of low divalent ion concentration, these mutations were accompanied by changes of channel conductance for monovalent cations. On the basis of the sidedness of Mg^{2+} effects, the anionic ring between MII and MIII [α-Glu 262 and homologs] was located on the extracellular side, the two others between MI and MII [α-Asp 238 and homologs] and [α-Glu 241 and homologs], on the cytoplasmic side. Thus, the segment MII spans the membrane and the site for [^{3}H]chlorpromazine and triphenylmethylphosphonium binding in MII is framed by the anionic rings within the membrane, in agreement with the models of Giraudat et al (198) and Hucho et al (202).

The Distance Between the Acetylcholine Receptor Site and the High-Affinity Site for Channel Blockers

The spatial relationship between the agonist binding sites and the high-affinity site for channel blockers was explored with the use of the fluorescent agonist "dansyl C_6 choline" (209) or the competitive antagonist bis (choline)-*N*-(4-nitrobenzo-2-oxa-1,3-diazol-7-yl)-iminodipropionate (210) (BCNI). The fluorescence emitted by these compounds, when bound to the agonist site, was monitored with ethidium bromide as an energy acceptor, bound to the high-affinity site for channel blockers (211). The distance between the two categories of sites was estimated to be between 21–35 Å for the BNCI-ethidium pair and 22–40 Å for the dansyl C_6 choline-ethidium pair. These measurements and the known molecular dimensions of the receptor protein led Herz et al (185) to propose that the agonist binding sites are located

approximately 20 Å above the membrane plane. In this hypothesis, the high-affinity site for noncompetitive blockers in the axis of symmetry of the molecule would be located near the extracellular surface of the membrane bilayer. The proposed organization is not consistent with (*a*) the results of electron microscopy of toxin-labeled receptor (22, 26) or intersite distance measurements using fluorescent toxins (212), suggesting that these ligands are located on the synaptic apex of the receptor, and (*b*) with data indicating that the NCB site is near or within the transmembrane domain of the receptor molecule (see above). However, a scheme whereby different noncompetitive blockers may adopt different positions within the axial cleft of the AChR may account for this organization (see 180).

Nevertheless, these studies clearly demonstrate that the interaction between the ACh-binding sites and the high-affinity site for noncompetitive blockers (within the ion channel) takes place between topographically distinct (and distant) sites and is thus a typical allosteric (214) interaction (see 9).

MODEL FOR THE ACETYLCHOLINE-GATED ION CHANNEL

The Acetylcholine-Gated Ion Channel is an "Uncharged Channel" Lined by Homologous MII α-Helices

As discussed above, models inferred from the primary structure of the transmembrane organization of the AChR subunits have direct implications for the organization of the ion channel. In each case the ion channel is assumed to lie in the axis of quasi-symmetry of the receptor oligomer and its walls made up of homologous segments of the subunits. Convergent results from affinity labeling studies (197–204) and site-directed mutagenesis experiments (205–208) point to the hydrophobic MII segment as a critical component of the channel. The common properties of the acetylcholine gated ion channels of electric organ, muscle, and neuronal receptors, and the sequence homologies between MII segments (Figure 6) suggest that the ion channels are similarly organized in all these receptors.

To test whether a purely hydrophobic bundle may serve as an ion channel, Furois-Corbin & Pullman (215) computed the "energy profile" of a sodium ion placed in successive planes of a strictly hydrophobic channel made up of stable bundles of polyalanine helices. They found that the interaction energy is favorable all along the structure. Furthermore, polar (noncharged) residues such as serines help the passage of the cation through the channel. The notion that the MII segment spans the membrane as an α-helix has received support from the [^{3}H]chlorpromazine-labeling data and site-directed mutagenesis. The respective position on the MII sequence of the three [^{3}H]chlorpromazine-labeled amino acids of the γ-subunit and the two on the β subunit, is

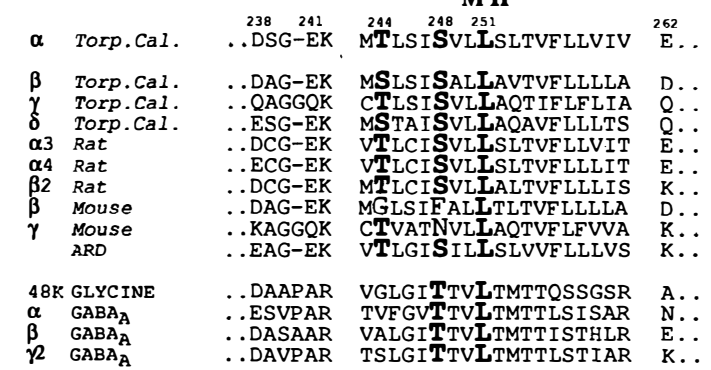

Figure 6 Comparison of the MII amino acid sequences from various subunits of ligand-gated ion channels. The amino acids sequences of the AChR subunits of *Torpedo* (51) are compared to the corresponding MII regions of rat neuronal α_3- (144), α_4- (145), mouse muscle β- and γ- (250, 268), and *Drosophila* acetylcholine receptor ARD (269) subunits of nicotinic AChR.

The alignment with the glycine receptor 48K (221), $GABA_A$ receptor α-, β,- (219) and γ_2-subunits (220) are also shown.

consistent with the organization of MII into an α-helix (Figure 5). In such a configuration, the α carbons of the labeled amino acids are aligned on the same meridian on adjacent turns of the helix (see 198, 200), and also aligned with α Glu 241 (and homologs) but not with α Lys 242, a disposition consistent with the results of site-directed mutagenesis experiments (208; see also 207).

However if chlorpromazine is assumed to reach its high-affinity site through the extracellular side of the receptor molecule,[1] then the helices must be tilted (216; see also 202), forming a truncated conical structure widest, at the top. In a close contact configuration of the MII helices, a narrow part would be at the bottom and would be wide enough to let cations flow (216). This narrow region, which contains one main cation-binding site (217, 218), has been estimated by measuring streaming potentials to be 3–6 Å long (218), a value corresponding to approximately one α-helix turn. These results are consistent with the notion that permeation is limited by one main binding site within the transmembrane ion path that can hold only one cation at a time.

Permeation Barriers Along the Ion Path

As mentioned above, mutations within several rings of homologous amino acids alter the ion transport properties of the ion channel: (*a*) the three rings located outside MII of negatively charged and glutamine residues homologous to [α-asp 238] (inner charged ring) and [α-glu 241] (intermediate charged ring) on the cytoplasmic side of MII, and to [α-glu 262] (outer charged ring)

on the synaptic side of MII; (*b*) two rings of hydrophilic residues within MII homologous to [α Thr 244] and [α ser 248] (hydrophilic rings) and one ring of hydrophobic residues homologous to α Leu 251. The question still remains, however, whether all these rings contribute to the physiological transport of ions through the channel. The comparison of the MII amino acid sequences (Figure 6) from the known muscular and neural nicotinic receptors and of the neural GABA (219, 220), glycine (221), and kainate (222–224) receptors, together with the results of the site-directed mutagenesis experiments (208) suggest the following interpretations:

(a) The [α asp 238] and homologs and [α Glu 262] and homologs "inner" and "outer charged" rings The amino acids of these rings are conserved (as aspartic acid, glutamic and/or glutamine) at homologous positions in all known neuronal receptor subunits. In the GABA and glycine receptors, where the transported ion is negatively charged, despite MII not having been shown to contribute to the transport of ions, the inner ring remains negatively charged and the outer ring, negative or neutral. Even though another alignment may be found, the outer and inner charged rings seem to be more directly involved in the regulation of ion *access* to the channel than in their actual *transport* through the membrane (see also 225).

(b) The [α-Glu 241] and homologs "intermediate" ring Of all the rings studied, the intermediate ring is where the strongest effect on conductance takes place after mutation (208) and where amino acid permutations have been reported to modify ionic selectivity (Imoto et al, unpublished data). This negatively charged ring is conserved in all neuronal nicotinic receptors but is absent from GABA or glycine receptor MII where it is replaced by either an alanine or arginine ring, depending on the alignment. Thus, the intermediate ring may be viewed as the site probing for the charge of the permeating ion.

(c) The serine and threonine "hydrophilic rings" These "hydrophilic" clusters of serines and threonines are located within the MII segment and not at its borders and could act as "cation-binding macrocycles" (226). In an attempt to synthesize artificial ion channels, Jullien & Lehn (227) built a macrocycle (or "chundle") containing six ether oxygens that formed an "annulus" to which they attached eight long polyether chains with a negative charge at the end. Carmichael et al (228) and Nakano et al (229) synthesized similar compounds but with different side-arms assumed to form a "rigid channel wall", which display authentic ion transport properties. Even though such model systems are far from the "natural" AChR channel, they all point to a plausible functional role for serines and analogs in ion complexation and transport.

Interestingly, the "ring of serines" [homologous to α ser 248] is conserved

through all neuronal nicotinic receptor subunits. The amino acid at homologous position is a serine in the frog (223) and chick (222) kainate subtype of glutamate receptors and a threonine in the glycine and GABA receptors. Similarly, the second annulus of polar amino acids, the "ring of threonines" homologous to α Thr 244, is conserved in AChR subunit sequences. Mutations in this ring, which is, however, not conserved in the GABA and glycine receptors, dramatically affect the conductance of mouse AChR (207). Therefore, the presence of internal hydrophilic rings seems to be more significant than the charged rings, and may play a more fundamental role in the general process of ion permeation. The hydrophilic rings may simply constitute the narrowest region of the transmembrane channel, which stops the translocation of the channel blockers. However, their hydrophilic character may be related to the state of hydration of the transported ion. In the glutamate-NMDA receptor, Ca^{++} ions penetrate the channel whereas Mg^{++} ions, which do not, behave as channel blockers (230, 231). This different behavior has been ascribed to variations in the speed at which Mg^{++} and Ca^{++} ions exchange the water molecules of their inner shell of hydration (reviewed in 232). Even though the channel of the nicotinic receptor may display different properties (see 217, 233), the "hydrophilic rings" may contribute to the exchange of water molecules surrounding the transported ion and both probe the size of the dehydrated (or partially hydrated) ion and facilitate (or even "catalyze") its translocation (93).

(d) The leucine ring This ring of chlorpromazine-labeled leucines homologous to α Leu 251 is conserved in all known subunits of AChR, glycine, and GABA. The role of such bulky and hydrophobic residues in ion transport remains unclear and has not been investigated by site-directed mutagenesis. These leucine residues could, however, play a role in the gating mechanism of the channel by sterically inhibiting ion flux in the closed conformation of the channel (234; see also 235).

Further research combining, for instance, site-directed mutagenesis and measurements of permeability ratios and/or streaming potentials, should define the respective physiological roles of the "charged and hydrophilic" rings more closely.

Gating of the Ion Channel

Theoretical work on bundles of five helices including bulky hydrophobic amino acids (leucines and/or alanines) (215, 236, 237) at the interface between the helices has described several modes of packing for such "optimized" pentagonal prisms. Energy minimization calculations suggest four quite stable structures, similar in energy, where the relative positions of the helices are significantly different. These calculations illustrate how the size

and shape of an axial "channel" could be modulated by tilting and/or sliding of the helices during "asymmetrical" transitions of the pentameric bundle. The analogy with the AChR is not straightforward since the α-helices in these model bundles point in opposite directions around the "channel". In contrast, in the "natural" receptor such disposition may exist within a subunit, but not around the walls of the channel, which are assumed to be made up of parallel homologous helices. Nevertheless, these models, allow asymmetrical motions of rigid α-helices within receptor subunits as documented in globular proteins (see 226, 238).

Based on labeling data obtained with the noncompetitive blocker quinacrine azide, Di Paola et al (239) have suggested that the hydrophobic segment MI of the α-subunit[2] of the receptor is involved in the coupling between the ACh-binding site and the ion channel (see also 240). One striking feature of the MI segment is that it contains a highly conserved proline residue in ACh, glycine, and GABA (and possibly kainate) receptors. Since prolines cannot form hydrogen bonds, the structure of helical rods is disrupted. Their presence in transmembrane domains has been proposed to provide flexibility to otherwise relatively rigid structures (241, 242), a fact that might be related to the gating of ion channels (243, 244). One possibility might be that the flexibility of the MI segment allows the MII helices lining the ion channel to adopt different orientations (or positions) with respect to each other and thus lead to different functional states of the channel.

In addition, besides the local changes that allow ion flux, more complex modifications of the quaternary structure of the receptor may be involved in channel gating:

1. Unwin et al (31) used cryoelectron microscopy of crystallized membranes of *T. marmorata* to detect differences in the arrangement of receptor subunits, before and after equilibration with a desensitizing agonist. Seen from the surface and in the presence of carbamylcholine, one and, to a large extent a second, subunit appear to fall away from the pentagonal symmetry as a consequence of an approximately 10° difference of inclination tangential to the receptor axis. This tilt is particularly pronounced in the synaptic domain distributed over the lipid bilayer. The identification of these two subunits as δ and γ presumes a clockwise $\alpha,\beta,\alpha,\gamma,\delta$ arrangement of the subunits around the receptor (30, 245). This relative disposition is under debate (see 104, 119). Though the conformational changes observed concern the resting and desensitized AChR, they illustrate what may occur during "gating" of the ion channel by ACh (see below).
2. Photolabeling studies with 3-trifluoromethyl-3-(m-[^{125}I]-iodophenyl)diazirine ([^{125}I]TID), a hydrophobic, photoactivatable probe, revealed a differential incorporation of the label into all subunits (the γ-subunit

incorporating approximately four times as much as each of the other subunits). The agonist carbamylcholine decreases the labeling of all subunits with (246) or without (247) a change in subunit-labeling stoichiometry.

3. Nicotinic receptors of cat and *Torpedo* desensitize at different rates. Coinjection of a large excess of one subunit mRNA of *Torpedo* with cat denervated muscle total mRNA into *Xenopus* oocyte shows that the hybrid protein desensitizes with a rate determined by the γ-subunits (248). The particular role of the γ-subunit is further supported by site-directed mutagenesis experiments. Mutation of cysteine 451 (located in the MIV segment of γ-subunit of *Torpedo*) to a serine or a tryptophan (249) decreases by 50% the activity of the receptor expressed in *Xenopus* oocytes by reducing the single channel opening frequency and changing the duration of its opening. Also, upon [^{3}H]CPZ photolabeling of the AChR, of all subunits the γ-subunit incorporates the lowest amounts of radioactivity in the desensitized state (159), and the highest in the open-channel configuration. It also exhibits kinetics of labeling by [^{3}H]chlorpromazine different from those of other subunits (189).

Thus, the γ-subunit may play a critical role in the conformational transitions leading to the desensitized state. Consistent with such a role, note that the sequence of MII from the γ-subunit displays unique characteristics: in *Torpedo,* the aspartic and glutamic acids of all charged rings are replaced by uncharged glutamines (51); in the mouse (57, 250) lysines are present in the outer and inner charged rings, glutamine in the intermediate charged ring and asparagine replaces the "universal" serine of the hydrophilic ring. In other words, the γ-subunit appears as an "inert" or even "blocking" subunit in which tighter participation to the walls of the channel may restrict ion permeation and thus create a desensitized conformation (see 31). Further structural work should help identify in more detail how the tertiary and/or quaternary structure responsible for the allosteric coupling between ligand binding sites and ion channel are reorganized (see 226).

Other important facts about the molecular mechanism involved in channel gating include (*a*) the all-or-none opening and closing of the ionic channel elicited by acetylcholine (89, 251–256); (*b*) the independence of the channel conductance of both the nature of the agonist (257) and its concentration; and (*c*) the spontaneous occurrence in the absence of agonist of channel openings that are identical, within experimental error, to those activated by agonists (94, 258, 259). These observations are consistent with the occurrence of concerted transitions between discrete states according to the two-state allosteric model (260).

Several possibilities (Figure 7) may be envisioned for the structural corre-

lates of the gating process (see 93): (*a*) global all-or-none "twist" of the molecule (see 261), compatible with the scheme proposed by Furois-Corbin & Pullman (216). According to this scheme, the five transmembrane subunits would rigidly tilt tangentially to the axis of quasi-symmetry of the molecule, thereby changing the diameter of the central cavity. (*b*) Global *all-or-none "bloom"* of the molecule. The gating would consist of an opening- or closing-up of the molecule (like an umbrella), which would be more important at the synaptic than cytoplasmic side of the membrane. In the closed resting state, amino acid side chains from the MII (or neighboring) helices would plug the lumen of the channel. In the open state, the channel would open by the vertical tilt, with the bottom of the molecule remaining fixed. Thus, the ion selectivity determined by this ring would not change but a "gate" located in the upper COOH-terminal part of the channel would open.

It is difficult to distinguish between these models at this stage. A high resolution picture of the three-dimensional organization of the receptor is needed and its structural transitions in relation with ligand binding must be identified. A detailed comparison of the data obtained for AChR and for globular regulatory proteins of known X-ray crytallographic structure (such as hemoglobin, aspartate transcarbamylase, or phosphorylase b (226)) might elucidate structural rules common to all these allosteric proteins as well as the rules that characterize ligand-regulated ion channels.

CONCLUSION

The structural and functional studies of the AChR of *Torpedo* and muscle have provided extensive data on their quaternary organization, on the molecu-

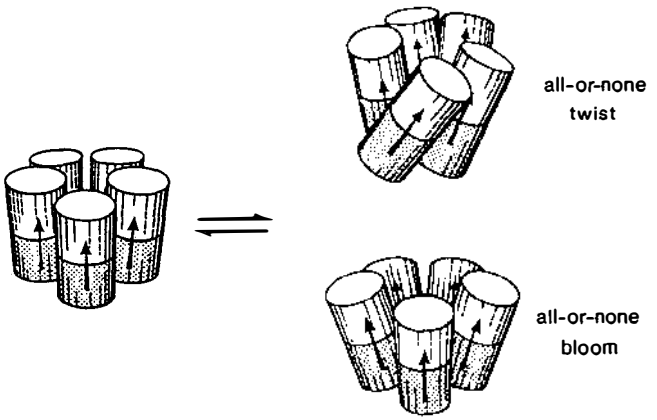

Figure 7 Two models of allosteric transition of the acetylcholine receptor protein possibly engaged in the gating of the ion channel (see text).

lar mechanisms of the interaction between their various ligand-binding sites and on the regulation of ion permeation through the associated channel. The proposed models derived from these data show predictive properties that may apply to the neuronal AChR and to the other ligand-gated ion channels. For instance, the combination of site-directed mutagenesis and labeling experiments have allowed the interactions between a permeant ion and the walls of the channel at the amino acid level to be analyzed. It is now tempting to predict some of the properties of a channel from primary amino acid sequences, and to extend the interpretations and models derived from electric organ and muscle AChRs to the whole superfamily of ligand-gated ion channels (AChR, GABA, glycine, and kainate subclass of glutamate receptors). For instance, all these receptors may display a similar organization of the ionic channel and similar allosteric coupling mechanisms between agonist binding sites and ionic channel[3]. They may also possess additional allosteric sites with pharmacological specificities distinct from those of the agonist binding sites. Such sites may, for instance, be responsible for the pharmacological action of barbiturates, benzodiazepines, and β-carbolines for the GABA receptor (reviewed in 262), or of glycine for the glutamate-NMDA receptor (263). However, minor variations in the structure of the subunits and their multiple combinations (for example, see 61, 264, 265) may confer to the different members of the superfamily important differences in ligand-binding properties or ion channel function and distinctive regulatory properties, such as the parameters of the fast and slow allosteric transitions and, in particular, their sign: sensitization vs desensitization.

Complete understanding of how the ligand-gated ion channels function as membrane-bound allosteric proteins will require their three-dimensional structure to be completely deciphered at the atomic level. A key step toward this goal will be to obtain receptor crystals suitable for X-ray diffraction analysis.

ACKNOWLEDGMENTS

We thank Christophe Mulle, Ralph Laufer, and Catherine Vidal for their suggestions and helpful advice. This work was supported by research grants from the Association Française contre les Myopathies, the Collège de France, the Centre National de la Recherche Scientifique, the Ministère de la Recherche, Institut National de la Santé et de la Recherche Médicale (Contract 872 004) and the Direction des Recherches Etudes et Techniques (Contract 87/211). F.R. was supported by Foundation Roux and J.L.G. was supported by the Association Française contre les Myopathies.

Literature Cited

1. Katz, B. 1966. *Nerve Muscle and Synapse*. New York: McGraw Hill
2. Katz, B., Miledi, R. 1977. Transmitter leakage from motor nerve endings. *Proc. R. Soc. London, Ser. B* 196:59–72
3. Henderson, C. 1990. Making mouse muscle move. *Trends Neurosci.* 13:39–41
4. Mulle, C., Changeux, J.-P. 1990. A novel type of nicotinic receptor in the rat central nervous system characterized by patch-clamp techniques. *J. Neurosci.* 10:169–75
5. Changeux, J.-P., Kasai, M., Lee, C. Y. 1970. The use of a snake venom toxin to characterize the cholinergic receptor protein. *Proc. Natl. Acad. Sci. USA* 67:1241–47
6. Lee, C. Y., Chang, C. C. 1966. Modes of actions of purified toxins from elapid venoms on neuromuscular transmission. *Mem. Inst. Butantan Sao Paulo* 33:555–72
7. Lee, C. Y., Tseng, L. F. 1966. Distribution of *Bungarus multicintus* venom following envenomation. *Toxicon* 3:281–90
8. Nachmansohn, D. 1959. In *Chemical and Molecular Basis of Nerve Activity*. New York: Academic 235 pp.
9. Changeux, J.-P. 1981. The acetylcholine receptor: An "allosteric" membrane protein. *Harvey Lect.* 75:85–254
10. Popot, J. L., Changeux, J.-P. 1984. Nicotinic receptor of acetylcholine: structure of an oligomeric integral membrane protein. *Physiol. Rev.* 64:1162–239
11. Mishina, M., Kurosaki, T., Tobimatsu, T., Morimoto, Y., Noda, M., et al. 1984. Expression of functional acetylcholine receptor from cloned cDNAs. *Nature* 307:604–8
12. Hucho, F. L. 1986. The nicotinic acetylcholine receptor and its ion channel. *Eur. J. Biochem.* 158:211–26
13. Lindstrom, J., Schoepfer, R., Whiting, P. 1987. Molecular studies of the neuronal nicotinic acetylcholine receptor family. *Mol. Neurobiol.* 1:281–39
14. Karlin, A. 1980. Molecular properties of nicotinic acetylcholine receptors. In *Cell Surface Reviews*, ed. G. Poste, G. L. Nicolson, C. W. Cotman, 6:191–260. Amsterdam: North Holland
15. Cartaud, J., Benedetti, L., Cohen, J. B., Meunier, J. C., Changeux, J.-P. 1973. Presence of a lattice structure in membrane fragments rich in nicotinic receptor protein from the electric organ of *Torpedo marmorata*. *FEBS Lett.* 33:109–13
16. Cartaud, J., Benedetti, L., Sobel, A., Changeux, J.-P. 1978. A morphological study of the cholinergic receptor protein from *Torpedo marmorata* in its membrane environment and in its detergent-extracted purified form. *J. Cell Sci.* 29:313–37
17. Cartaud, J., Sobel, A., Rousselet, A., Devaux, P. F., Changeux, J.-P. 1981. Consequences of alkaline treatment for the ultrastructure of the acetylcholine-receptor-rich membranes from *Torpedo marmorata* electric organ. *J. Cell. Biol.* 90:418–26
18. Nickel, E., Potter, L. T. 1973. Ultrastructure of isolated membranes of *Torpedo* electric tissue. *Brain Res.* 57:508–17
19. Reed, K. R., Vandlen, R., Bode, J., Duguid, J., Raftery, M. A. 1975. Characterization of acetylcholine receptor-rich and acetylcholine esterase-rich membrane particles from *Torpedo californica* electroplax. *Arch. Biochem. Biophys.* 167:138–44
20. Ross, M. J., Klymkowsky, M. W., Agard, D. A., Stroud, R. M. 1977. Structural studies of a membrane-bound acetylcholine receptor from *Torpedo californica*. *J. Mol. Biol.* 116:635–59
21. Schiebler, W., Hucho, F. 1978. Membranes rich in acetylcholine receptor: characterization and reconstitution to excitable membranes from exogeneous lipids. *Eur. J. Biochem.* 85:55–63
22. Klymkowsky, M. W., Stroud, R. M. 1979. Immunospecific identification and three-dimensional structure of a membrane-bound acetylcholine receptor from *Torpedo marmorata*. *J. Mol. Biol.* 128:319–34
23. Zingsheim, H. P., Neugebauer, D. C., Barrantes, F. J. 1980. Structural details of membrane-bound acetylcholine receptor from *Torpedo marmorata*. *Proc. Natl. Acad. Sci. USA* 77:952–56
24. Zingsheim, H. P., Neugebauer, D. C., Frank, J., Hänicke, W., Barrantes, F. J. 1982. The arrangement and structure of the membrane-bound acetylcholine receptor studied by electron microscopy. *EMBO J.* 1:541–47
25. Brisson, A. D. 1978. Acetylcholine receptor: protein structure. In *Proc. 9th Int. Conf. Electron Microsc.* 2:180–81
26. Kistler, J., Stroud, R. M. 1981. Crystalline arrays of membrane-bound acetyl-

choline receptor. *Proc. Natl. Acad. Sci. USA* 78:3678–82
27. Bon, F., Lebrun, E., Gomel, J., Van Rappenbusch, R., Cartaud, J., et al. 1984. Image analysis of the heavy form of the acetylcholine receptor from *Torpedo marmorata*. *J. Mol. Biol.* 176:205–37
28. Brisson, A., Unwin, P. N. T. 1984. Tubular crystals of acetylcholine receptor. *J. Cell Biol.* 99:1202–11
29. Brisson, A., Unwin, P. N. T. 1985. Quaternary structure of the acetylcholine receptor. *Nature* 315:474–77
30. Kubalek, E., Ralston, S., Lindstrom, J., Unwin, N. 1987. Location of subunits with the acetylcholine receptor by electron image analysis of tubular crystals from *Torpedo marmorata*. *J. Cell Biol.* 105:9–18
31. Unwin, N., Toyoshima, C., Kubalek, E. 1988. Arrangement of the acetylcholine receptor subunits in the resting and desensitized states, determined by cryoelectron microscopy of crystallized *Torpedo* postsynaptic membranes. *J. Cell Biol.* 107:1123–38
32. Toyoshima, C., Unwin, N. 1988. Ion channel of acetylcholine receptor reconstructed from images of postsynaptic membranes. *Nature* 336:247–50
33. Mitra, A. K., McCarthy, M. P., Stroud, R. M. 1989. Three-dimensional structure of the nicotinic acetylcholine receptor and location of the major associated 43-kD cytoskeletal protein, determined at 22 Å by low dose electron microscopy and X-ray diffraction to 12.5 Å. *J. Cell. Biol.* 109:755–74
34. Patrick, J., Stallcup, W. B. 1977. Alpha-bungarotoxin binding and cholinergic receptor function on a rat sympathetic nerve line. *J. Biol. Chem.* 252:8629
35. Patrick, J., Stallcup, W. B. 1977. Immunological distinction between acetylcholine receptor and the alpha-bungarotoxin-binding component on sympathetic neurons. *Proc. Natl. Acad. Sci. USA* 74:4689–92
36. Marks, M. J., Collins, A. C. 1982. Characterization of nicotine binding in mouse brain and comparison with the binding of α-bungarotoxin and quinuclidinyl benzilate. *Mol. Pharmacol.* 22: 554–64
37. Clarke, P. B. S., Schwartz, R. D., Paul, S. M., Pert, C. D., Pert, A. 1985. Nicotinic binding in rat brain: autoradiographic comparison of [^{3}H]acetylcholine, [^{3}H]nicotine, and [^{125}I]-α-bungarotoxin. *J. Neurosci.* 5:1307–15
38. Chiappinelli, V. A. 1983. Kappa toxin: a probe for neuronal nicotinic receptor in the avian ciliary ganglion. *Brain Res.* 277:9–21
39. Loring, R. H., Chiappinelli, V. A., Zigmond, R. E., Cohen, J. B. 1984. Characterization of a snake venom neurotoxin which blocks nitotinic transmission in the avian ciliary ganglion. *Neuroscience* 11:989–99
40. Ravdin, P. M., Berg, D. K. 1979. Inhibition of neuronal acetylcholine sensitivity by a toxin from *Bungarus multicintus* venom. *Proc. Natl. Acad. Sci. USA* 75:2072–76
41. Halvorsen, S. W., Berg, D. K. 1986. Identification of a nicotinic acetylcholine receptor on neurons using an α-neurotoxin that blocks receptor function. *J. Neurosci.* 6:3405–12
42. Vidal, C., Changeux, J.-P. 1989. Pharmacological profile of nicotinic acetylcholine receptors in the rat prefrontal cortex: an electrophysiological study in a slice preparation. *Neuroscience* 29:261–70
43. Loring, R. H., Zigmond, R. E. 1988. Characterization of neuronal nicotinic receptors by snake venom neurotoxins. *Trends Neurosci.* 11:73–78
44. Whiting, P. J., Lindstrom, J. M. 1986. Purification and characterization of a nicotinic acetylcholine receptor from chick brain. *Biochemistry* 25:2082.
45. Whiting, P. J., Lindstrom, J. 1986. Pharmacological properties of immunoisolated neuronal nicotinic receptors. *J. Neurosci.* 6:3061–69
46. Whiting, P. J., Lindstrom, J. 1987. Purification and characterization of a nicotinic acetylcholine receptor from rat brain. *Proc. Natl. Acad. Sci. USA* 84:595–99
47. Abood, L. G., Langone, J. J., Bjercke, R., Lu, X., Banerjee, S. 1987. Characterization of a purified nicotinic receptor from rat brain by using idiotypic and anti-idiotypic antibodies. *Proc. Natl. Acad. Sci. USA* 84:6587–90
48. Schoepfer, R., Whiting, P., Luther, M., Keyser, K., Karten, H., Lindstrom, J. 1989. Structure of muscle and neuronal nicotinic acetylcholine receptors. In *Molecular Biology of Neuroreceptors and Ion Channels*, ed. A. Maelicke. NATO ASI Ser. H32:37–53. Berlin/Heidelberg: Springer-Verlag
49. Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Furutani, Y., et al., 1982. Primary structure of alpha-subunit precursor of *Torpedo californica* acetylcholine receptor deduced from cDNA sequence. *Nature* 299:793–97

50. Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., et al. 1983. Primary structures of beta and delta-subunit precursors of *Torpedo californica* acetylcholine receptor deduced from cDNA sequences. *Nature* 301:251–55
51. Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., et al. 1983. Structural homology of *Torpedo californica* acetylcholine receptor subunits. *Nature* 302:528–32
52. Claudio, T., Ballivet, M., Patrick, J., Heinemann, S. 1983. Nucleotide and deduced amino acid sequences of *Torpedo californica* acetylcholine receptor gamma-subunit. *Proc. Natl. Acad. Sci. USA* 80:1111–15
53. Devillers-Thiéry, A., Giraudat, J., Bentaboulet, M., Changeux, J.-P. 1983. Complete mRNA coding sequence of the acetylcholine binding alpha subunit of *Torpedo marmorata* acetylcholine receptor: A model for the transmembrane organization of the polypeptide chain. *Proc. Natl. Acad. Sci. USA* 80:2067–71
54. Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Tanabe, T., et al., 1983. Cloning and sequence analysis of calf cDNA and human genomic DNA encoding α-subunit precursor of muscle acetylcholine receptor. *Nature* 305:818–23
55. LaPolla, R. J., Mayne, K. M., Davidson, N. 1984. Isolation and characterization of a cDNA clone for the complete protein coding region of the δ subunit of the mouse acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 81:7970–74
56. Nef, P., Mauron, A., Stalder, R., Alliod, C., Ballivet, M. 1984. Structure, linkage, and sequence of the two genes encoding the δ and γ subunits of the nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 81:7975–79
57. Boulter, J., Evans, K. L., Martin, G., Gardner, P. D., Connolly, J., et al. 1986. Mouse muscle acetylcholine receptor molecular cloning of α, β, γ and δ subunit cDNA's and expression in *Xenopus laevis* oocytes. *Soc. Neurosci. Abstr.* 12:40.2, 146
58. Nef, P., Oneyser, C., Alliod, C., Couturier, S., Ballivet, M. 1988. Genes expressed in the brain define three distinct neuronal nicotinic acetylcholine receptors. *EMBO J.* 7:595–601
59. Couturier, S., Nef, P., Oneyser, C., Ballivet, M. 1989. The gene family encoding neuronal acetylcholine receptors: structure of the avian alpha 2 and alpha 3 genes. See Ref. 48, pp. 31–36
60. Cauley, K., Agranoff, B. W., Goldman, D. 1990. Multiple nicotinic acetylcholine receptor genes are expressed in goldfish retina and tectum. *J. Neurosci.* 10:670–83
61. Heinemann, S., Boulter, J., Deneris, E., Connolly, J., Gardner, P., et al. Brain and muscle nicotinic acetylcholine receptor: a gene family. See Ref. 48, pp. 13–30
62. Mishina, M., Takai, T., Imoto, K., Noda, M., Takahashi, T., et al. 1986. Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. *Nature* 321:406–11
63. Claudio, T., Green, W. N., Hartman, D. S., Hayden, D., Paulson, H. L., et al. 1987. Reconstitution of functional acetylcholine-receptor channels in mouse fibroblasts. *Science* 238:1688–94
64. Deneris, E. S., Connolly, J., Boulter, J., Wada, E., Wada, K., et al. 1988. Primary structure and expression of β2: a novel subunit of neuronal nicotinic acetylcholine receptors. *Neuron* 1:45–54
65. Papke, R. L., Boulter, J., Patrick, J., Heinemann, S. 1989. Single-channel currents of rat neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes. *Neuron* 3:589–96
66. Duvoisin, R. M., Deneris, E. S., Patrick, J., Heinemann, S. 1989. The functional diversity of the neuronal nicotinic acetylcholine receptors is increased by a novel subunits: β4. *Neuron* 3:487–96
67. Guy, H. R. 1984. Structural model of the acetylcholine receptor channel based on partition energy and helix packing calculations. *Biophys. J.* 45:249–61
68. Finer-Moore, J., Stroud, R. M. 1984. Amphipathic analysis and possible formation of the ion channel in an acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 81:155–59
69. McCarthy, M. P., Earnest, J. P., Young, E. F., Choe, S., Stroud, R. M. 1986. The molecular neurobiology of the acetylcholine receptor. *Annu. Rev. Neurosci.* 9:383–413
70. Criado, M., Hochschwender, S., Sarin, V., Fox, J. L., Lindstrom, J. 1985. Evidence for unpredicted transmembrane domains in acetylcholine receptor subunits. *Proc. Natl. Acad. Sci. USA* 82:2004–8
71. Ratnam, M., Le Nguyen, D., Rivier, J., Sargent, P. B., Lindstrom, J. M. 1986. Transmembrane topography of nicotinic acetylcholine receptor: immunochemical tests contradict theoretical predictions based on hydrophobicity profiles. *Biochemistry* 25:2633–43
72. Changeux, J.-P., Devillers- Thiéry, A., Chemouilli, P. 1984. Acetylcholine re-

ceptor: an allosteric protein. *Science* 225:1335–45
73. Guy, R. H., Hucho, F. 1987. The ion channel of the nicotinic acetylcholine receptor. *Trends Neurosci.* 10:318–21
74. Mishina, M., Tobimatsu, T., Imoto, K., Tanaka, K., Fujita, Y., et al. 1985. Location of functional regions of acetylcholine receptor alpha-subunit by site-directed mutagenesis. *Nature* 313:364–69
75. Pedersen, S. E., Bridgman, P. C., Sharp, S. D., Cohen, J. B. 1990. Identification of a cytoplasmic region of the Torpedo nicotinic acetylcholine receptor α-subunit by epitope mapping. *J. Biol. Chem.* 265:569–81
76. Giraudat, J., Montecucco, C., Bisson, R., Changeux, J.-P. 1985. Transmembrane topology of acetylcholine receptor subunits probed with photoreactive phospholipids. *Biochemistry* 24:3121–27
77. Blanton, M.P., Wang, H. H. 1990. Photoaffinity labeling of the *Torpedo californica* nicotinic acetylcholine receptor with an aryl azide derivative of phosphatidylserine. *Biochemistry* 29: 1186–94
78. McCrea, P. D., Popot, J. L., Engelman, D. M. 1987. Transmembrane topography of the nicotinic acetylcholine receptor delta-subunit. *EMBO J.* 6:3619–26
79. DiPaola, M., Czajkowski, C., Karlin, A. 1989. The sidedness of the COOH terminus of the acetylcholine receptor δ-subunit. *J. Biol. Chem.* 264:1–7
80. Dunn, S. M. J., Conti-Tronconi, B. M., Raftery, M. A. 1986. Acetylcholine receptor dimers are stabilized by extracellular disulfide bonding. *Biochem. Biophys. Res. Commun.* 139:830–37
81. Dwyer, B. P. 1988. Evidence for the extramembranous location of the putative amphipathic helix of acetylcholine receptor. *Biochemistry* 27:5586–92
82. Dennis, M., Giraudat, J., Kotzyba-Hibert, F., Goeldner, M., Hirth, C., et al. 1988. Amino acids of the *Torpedo marmorata* acetylcholine receptor α subunit labeled by a photoaffinity ligand for the acetylcholine binding site. *Biochemistry* 27:2346–57
83. Wonnacott, S. 1987. Neurotoxin probes for neuronal nicotinic receptors. In *Neurotoxins and Their Pharmacological Implications,* ed. P. Jenner, p. 209. New York: Raven
84. Steinbach, J. H., Ifune, C. 1989. How many kinds of nicotinic acetylcholine receptor are there? *Trends Neurosci.* 12:3–6
85. Alkondon, M., Albuquerque, E. X. 1990. Nicotinic acetylcholine receptor (nAChR) ion channel currents in rat hippocampal neurons. *Soc. Neurosci. Abstr.* In press
86. Ramoa, A. S., Alkondon, M., Aracava, Y., Irons, J., Lunt, G. G., et al. 1990. The anticonvulsant MK-801 interacts with the peripheral and central nicotinic acetylcholine receptor ion channels. *J. Pharmacol. Exp. Ther.* 254:71–82
87. Ascher, P., Large, W. A., Rang, H. P. 1979. Studies on the mechanism of action of acetylcholine antagonists on rat parasympathetic ganglion cells. *J. Physiol.* 295:139–70
88. Varanda, W. A., Aracava, Y., Sherby, S. M., VanMeter, W. G., Eldefrawi, M. E., Albuquerque, E. X. 1985. The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. *Mol. Pharmacol.* 28:128–37
89. Neher, E., Sakmann, B. 1976. Noise analysis of drug-induced conductance voltage clamp currents in denervated frog muscle fibres. *J. Physiol.* 258:705–29
90. Lipton, S. A., Aizenman, E., Loring, R. H. 1987. Neural nicotinic acetylcholine responses in solitary mammalian retinal ganglion cells. *Pflügers Arch.* 410:37–43
91. Aracava, Y., Deshpande, S. S., Swanson, K. L., Rapoport, H., Wonnacott, S., et al. 1987. Nicotinic acetylcholine receptors in cultured neurons from the hippocampus and brain stem of the rat characterized by single channel recording. *FEBS Lett.* 222:63–70
92. Reynolds, J. A., Karlin, A. 1978. Molecular weight in detergent solution of acetylcholine receptor from *Torpedo californica. Biochemistry* 17:2035–38
93. Changeux, J.-P. 1990. Functional architecture and dynamics of the nicotinic acetylcholine receptor: an allosteric ligand-gated ion channel. *Fidia Res. Found. Neurosci. Award Lectures,* Vol. 4. New York: Raven. pp. 21–168
94. Jackson, M. B. 1988. Dependence of acetylcholine receptor channel kinetics on agonist concentration in cultured mouse muscle fibres. *J. Physiol.* 397: 555–83
95. Weber, M., Changeux, J.-P. 1974. Binding of *Naja nigricollis* 3H-alpha-toxin to membrane fragments from *Electrophorus* and *Torpedo* electric organs. 1. Binding of the tritiated alpha-neurotoxin in the absence of effector. *Mol. Pharmacol.* 10:1–14
96. Weber, M., Changeux, J.-P. 1974. Effect of the cholinergic agonists and

antagonists on the binding of the tritiated α-neurotoxin. *Mol. Pharmacol.* 10:15–34

97. Maelicke, A., Reich, E. 1976. On the interaction between Cobra alpha-neurotoxin and the acetylcholine receptor. *Cold Spring Harbor Symp. Quant. Biol.* 40:203–10
98. Maelicke, A., Fulpius, B. W., Klett, R. P., Reich, E. 1977. Acetylcholine receptor responses to drug binding. *J. Biol. Chem.* 252:4811–30
99. Kang, S., Maelicke, A. 1980. Fluorescein isothiocyanate-labeled alpha-cobratoxin: Biochemical characterization and interaction with acetylcholine receptor from *Electrophorus electricus*. *J. Biol. Chem.* 256:7326–32
100. Maelicke, A., Plümer-Wilk, R., Fels, G., Spencer, S. R., Engelgard, M., 1989. The limited sequence specificity of anti-peptide antibodies may introduce ambiguity in topological studies. See Ref. 48, pp. 321–26
101. Conti-Tronconi, B. M., Tang, F., Walgrave, S., Gallagher, W. 1990. Non-equivalence of α-bungarotoxin binding sites in the native nicotinic receptor molecule. *Biochemistry* 29:1046–54
102. Neubig, R. R., Cohen, J. B. 1979. Equilibrium binding of [^{3}H] tubocurarine and [^{3}H] acetylcholine by *Torpedo* postsynaptic membranes: stoichiometry and ligand interactions. *Biochemistry* 18:5464–75
103. Neubig, R. R., Cohen, J. B. 1980. Permeability control by cholinergic receptors in *Torpedo* post synaptic membranes: Agonist dose response relations measured at second and millisecond times. *Biochemistry* 19:2770–79
104. Pedersen, S. E., Cohen, J. B. 1990. d-tubocurarine binding sites are located at α–γ and α–δ subunit interfaces of the nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 87:2785–89
105. Karlin, A., Weill, C. L., McNamee, M. G., Valderrama, R. 1976. Facets of the structure of acetylcholine receptor from *Electrophorus* and *Torpedo*. *Cold Spring Harbor Symp. Quant. Biol.* 40:203–10
106. Damle, V. N., Karlin, A. 1978. Affinity labeling of one of two α-neurotoxin binding sites in acetylcholine receptor from *Torpedo californica*. *Biochemistry* 17:2039–45
107. Deleglane, A. M., McNamee, M. G. 1980. Independent activation of the acetylcholine receptor from *Torpedo californica* at two sites. *Biochemistry* 19:890–95
108. Ratnam, M., Gullick, W., Spiess, J., Wan, K., Criado, M., Lindstrom, J. 1986. Structural heterogeneity of the alpha-subunits of the nicotinic acetylcholine receptor in relation to agonist affinity alkylation and antagonist binding. *Biochemistry* 25:4268–75
109. Culver, P., Fenical, W., Taylor, P. 1984. Lophotoxin irreversibly inactivates the nicotinic acetylcholine receptor by preferential association at one of the two primary agonist sites. *J. Biol. Chem.* 259:3763–70
110. Watters, D., Maelicke, A. 1983. Organization of ligand binding sites at the acetylcholine receptor: a study with monoclonal antibodies. *Biochemistry* 22:1811–19
111. Mihovilovic, M., Richman, D. P. 1984. Modification of alpha-bungarotoxin and cholinergic ligand-binding properties of *Torpedo* acetylcholine receptor by a monoclonal anti-acetylcholine receptor antibody. *J. Biol. Chem.* 259:15051–59
112. Gu, Y., Silberstein, L., Hall, Z. W. 1985. The effects of a myasthenic serum on the acetylcholine receptors of C2 myotubes: 1. Immunological distinction between the two toxin-binding sites of the receptor. *J. Neurosci.* 5:1909–16
113. Whiting, P., Vincent, A., Newsom-Davis, J. 1985. Monoclonal antibodies to *Torpedo* acetylcholine receptor. Characterisation of antigenic determinants within the cholinergic binding site. *Eur. J. Biochem.* 150:533–39
114. Dowding, A. J., Hall, Z. W. 1987. Monoclonal antibodies specific for each of the two toxin-binding sites of *Torpedo* acetylcholine receptor. *Biochemistry* 26:6372–81
115. Klarsfeld, A., Devillers-Thiéry, A., Giraudat, J., Changeux, J.-P. 1984. A single gene codes for the nicotinic acetylcholine receptor alpha-subunit in *Torpedo marmorata*: Structural and developmental implications. *EMBO J.* 3:35–41
116. Merlie, J. P., Sebbane, R., Gardner, S., Lindstrom, J. 1983. cDNA clone for the alpha-subunit of the acetylcholine receptor from the mouse muscle cell line BC3H-1. *Proc. Natl. Acad. Sci. USA* 80:3845–49
117. Oswald, R. E., Changeux, J.-P. 1982. Crosslinking of alpha-bungarotoxin to the acetylcholine receptor from *Torpedo marmorata* by ultraviolet light irradiation. *FEBS Lett.* 139:225–29
118. Langenbuch-Cachat, J., Bon, C., Goeldner, M., Hirth, C., Changeux, J.-P. 1988. Photoaffinity labeling by aryldiazonium derivatives of *Torpedo mar-*

morata acetylcholine receptor. *Biochemistry* 27:2337–45
119. Blount, P., Merlie, J. P. 1989. Molecular basis of the two nonequivalent ligand binding sites of the muscle nicotinic acetylcholine receptor. *Neuron* 3:349–57
120. Conti-Tronconi, B. M., Hunkapiller, M. W., Raftery, M. A. 1984. Molecular weight and structural nonequivalence of the mature α subunits of *Torpedo californica* acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 81:2631–34
121. Kao, P. N., Dwork, A. J., Kaldany, R. R. J., Silver, M. L., Wideman, J., et al. 1984. Identification of the alpha-subunit half-cystine specifically labeled by an affinity reagent for the acetylcholine receptor binding site. *J. Biol. Chem.* 259:11662–65
122. Kao, P. N., Karlin, A. 1986. Acetylcholine receptor binding site contains a disulfide cross-link between adjacent half-cystinyl residues. *J. Biol. Chem.* 261:8085–88
123. Mosckovitz, R., Gershoni, J. M. 1988. Three possible disulfides in the acetylcholine receptor α-subunit. *J. Biol. Chem.* 263:1017–22
124. Wilson, P. T., Gershoni, J. M., Hawrot, E., Lentz, T. L. 1984. Binding of alpha-bungarotoxin to proteolytic fragments of the alpha-subunit of *Torpedo* acetylcholine receptor analyzed by protein transfer on positively charged membrane filters. *Proc. Natl. Acad. Sci. USA* 81:2553–57
125. Wilson, P. T., Lentz, T. L., Hawrot, E. 1985. Determination of the primary amino acid sequence specifying the α-bungarotoxin binding site on the α subunit of the acetylcholine receptor from *Torpedo californica*. *Proc. Natl. Acad. Sci. USA* 82:8790–94
126. Neumann, D., Gershoni. J. M., Fridkin, M., Fuchs, S. 1985. Antibodies to synthetic peptides as probes for the binding site on the alpha-subunit of the acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 82:3490–93
127. Neumann, D., Barchan, D., Safran, A., Gershoni, J. M., Fuchs, S. 1986. Mapping of the alpha-bungarotoxin binding site within the α subunit of the acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 83:3008–11
128. Oblas, B., Singer, R. H., Boyd, N. D. 1986. Location of a polypeptide sequence within the alpha-subunit of the acetylcholine receptor containing the cholinergic binding site. *Mol. Pharmacol.* 29:649–56
129. Radding, W., Corfield, P. W. R., Levinson, L. S., Hashim, G. A., Low, B. W. 1988. α-toxin binding to acetylcholine receptor α 179–191 peptides: intrinsic fluorescence studies. *FEBS Lett.* 231:212–16
130. Mulac-Jericevic, B., Atassi, M. Z. 1986. Segment alpha-182-198 of *Torpedo californica* acetylcholine receptor contains a second toxin-binding region and binds anti-receptor antibodies. *FEBS Lett.* 199:68–74
131. Ralston, S., Sarin, V., Thanh, H. L., Rivier, J., Fox, J. L., Lindstrom, J. 1987. Synthetic peptides used to locate the α-bungarotoxin binding site and immunogenic regions on alpha-subunits of the nicotinic acetylcholine receptor. *Biochemistry* 26:3261–66
132. Barkas, T., Mauron, A., Roth, B., Alliod, C., Tzartos, S. J., Ballivet, M. 1987. Mapping the main immunogenic region and toxin-binding site of the nicotinic acetylcholine receptor. *Science* 235:77–80
133. Gershoni, J. M. 1987. Expression of the α-bungarotoxin binding site of the nicotinic acetylcholine receptor by *Escherichia coli* transformants. *Proc. Natl. Acad. Sci. USA* 84:4318–21
134. McLane, K. E., Tang, F., Conti-Tronconi, B. M. 1990. Localization of sequence segments forming an K-bungarotoxin-binding site on the α3 neuronal nicotinic receptor. *J. Biol. Chem.* 265:1537–44
135. Maelicke, A., Fels, G., Conti-Tronconi, B. M. 1989. Evidence for multipoint attachment of ligands to the nicotinic acetylcholine receptor. See Ref. 48, pp. 353–59
136. Goeldner, M. P., Hirth, C. G. 1980. Specific photoaffinity labeling induced by energy transfer: application to irreversible inhibition of acetylcholinesterase. *Proc. Natl. Acad. Sci. USA* 77:6439–42
137. Goeldner, M. P., Hirth, C. G., Kieffer, B., Ourisson, G. 1982. Photosuicide inhibition—a step towards specific photoaffinity labelling. *Trends Biochem. Sci.* 7:310–12
138. Dennis, M., Giraudat, J., Kotzyba-Hibert, F., Goeldner, M., Hirth, C., et al. 1986. A photoaffinity ligand of the acetylcholine binding site predominantly labels the region 179–207 of the α-subunit on native acetylcholine receptor from *Torpedo marmorata*. *FEBS Lett.* 207:243–49
139. Galzi, J. L., Revah, F., Black, D., Goeldner, M., Hirth, C., Changeux, J.-P. 1990. Identification of a novel amino acid α Tyr 93 within the active site of the acetylcholine receptor by photoaffinity

labeling: additional evidence for a three-loop model of the acetylcholine binding site. *J. Biol. Chem.* 265:1043●-37

140. Abramson, S. N., Culver, P., Klines, T., Guest, Y. L., Gutman, L., Taylor, P. 1988. Lophotoxin and related coral toxins covalently label the α-subunit of the nicotinic acetylcholine receptor. *J. Biol. Chem.* 263:18568–73
141. Abramson, S. N., Li, Y., Culver, P., Taylor, P. 1989. An analog of lophotoxin reacts covalently with Tyr 190 in the α-subunit of the nicotinic acetylcholine receptor. *J. Biol. Chem.* 264:12666–72
142. Stroud, R. M., Finer-Moore, J. 1985. Acetylcholine receptor structure, function and evolution. *Annu. Rev. Cell Biol.* 1:317–51
143. Wada, K., Ballivet, M., Boulter, J., Connolly, J., Wada, E., et al. 1988. Functional expression of a new pharmacological subtype of brain nicotinic acetylcholine receptor. *Science* 240:330–34
144. Boulter, J., Evans, K., Goldman, D., Martin, G., Treco, D., et al. 1986. Isolation of a cDNA clone coding for a possible neural nicotinic acetylcholine receptor α-subunit. *Nature* 319:368–74
145. Goldman, D., Deneris, E., Luyten, W., Kochhar, A., Patrick, J., Heinemann, S. 1987. Members of a nicotinic acetylcholine receptor gene family are expressed in different regions of the mammalian central nervous system. *Cell* 48:965–73
146. Boulter, J., O'Shea-Greenfield, A., Duvoisin, R. M., Connolly, J. G., Wada, E., et al. 1990. α3, α5, and β4: three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. *J. Biol. Chem.* 265:4472–82
147. Deneris, E. S., Boulter, J., Swanson, L. W., Patrick, J., Heinemann, S. 1989. β_3: a new member of nicotinic acetylcholine receptor gene family is expressed in brain. *J. Biol. Chem.* 264:6268–72
148. Isenberg, K. E., Meyer, G. E. 1989. Rapid communication: Cloning of a putative neuronal nicotinic acetylcholine receptor subunit. *J. Neurochem.* 52:988–91
149. Burden, S. J., Hartzell, H. C., Yoshikami, D. 1975. Acetylcholine receptors at neuromuscular synapses: phylogenetic differences detected by snake α-neurotoxins. *Proc. Natl. Acad. Sci. USA* 72:3245–49
150. Boulter, J., Connolly, J., Deneris, E., Goldman, D., Heinemann, S., Patrick, J. 1987. Functional expression of two neural nicotinic acetylcholine receptors from cDNA clones identifies a gene family. *Proc. Natl. Acad. Sci. USA* 84:7763–67
151. Neumann, D., Barchan, D., Horowitz, M., Kochva, E., Fuchs, S. 1989. Snake acetylcholine receptor: cloning of the domain containing the four extracellular cysteines of the α subunit. *Proc. Natl. Acad. Sci. USA* 86:7255–59
152. Luyten, W. H. M. L. 1986. A model for the acetylcholine binding site of the nicotinic acetylcholine receptor. *J. Neurosci. Res.* 16:51–73
153. Barnard, E. A., Darlison,, M. G., Seeburg, P. 1987. Molecular biology of the $GABA_4$ receptor: the receptor/channel superfamily. *Trends Neurosci.* 10:502–9
154. Dhaenens, M., Lacombe, L., Lehn, J. M., Vigneron, J. P. 1984. Binding of acetylcholine and other molecular cations by a macrocyclic receptor molecule of speleand type. *J. Chem. Soc. Chem. Commun.*, pp. 1097–99
155. Lehn, J. M. 1985. Supramolecular chemistry: receptors, catalysts, and carriers. *Science* 227:849–56
156. Kieffer, B., Goeldner, M., Hirth, C., Aebersold, R., Chang, J. Y. 1986. Sequence determination of a peptide fragment from electric eel acetylcholinesterase, involved in the binding of quaternary ammonium. *FEBS Lett.* 202:91–96
157. Schneider, H. J., Güttes, D., Schneider, U. 1986. A macrobicyclic polyphenoxide as receptor analogue for choline and related ammonium compounds. *Angew. Chem. Int. Ed. Engl.* 25:647–49
158. Schneider, H. J., Güttes, D., Schneider, U. 1988. Host-guest complexes with water-soluble macrocyclic polyphenolates including induced fit and simple elements of a proton pump. *J. Am. Chem. Soc.* 110:6449–54
159. Heidmann, T., Oswald, R. E., Changeux, J.-P. 1983. Multiple sites of action for non competitive blockers on acetylcholine receptor-rich membrane fragments from *Torpedo marmorata*. *Biochemistry* 22:3112–27
160. Adams, P. R. 1981. Acetylcholine receptor kinetics. *J. Membr. Biol.* 58:161–74
161. Rapier, C., Wonnacott, S., Lunt, G. G., Albuquerque, E. X. 1987. The neurotoxin histrionicotoxin interacts with the putative ion channel of thenicotinic acetylcholine receptors in the central nervous system. *FEBS Lett.* 212:292–96
162. Eldefrawi, A. T., Eldefrawi, M. E.,

Albuquerque, E. X., Oliveira, A. C., Mansour, N., et al. 1977. Perhydrohistrionicotoxin: a potential ligand for the ion conductance modulator of the acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 74:2172–76

163. Kloog, Y., Kalir, A., Buchman, O., Sokolovsky, M. 1980. Specific binding of [^{3}H]phencyclidines to membrane preparation. Possible interaction with the cholinergic ionophore. *FEBS Lett.* 109:125–28
164. Albuquerque, E. X., Tsai, M. C., Aronstam, R. S., Eldefrawi, A. T., Eldefrawi, M. E. 1980. Sites of action of phencyclidine. II. Interaction with the ionic channel of the nicotinic receptor. *Mol. Pharmacol.* 18:167–78
165. Clineschmidt, B. V., Williams, M., Witoslawski, J. J., Bunting, P. R., Risley, E. A., Totaro, J. A. 1982. Restoration of shock-suppressed behaviour by treatment with (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cycloheptene-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic, and apparent anxiolytic properties. *Drug Dev. Res.* 2:147–63
166. Foster, A. C., Fagg, G. E. 1987. Taking apart NMDA receptors. *Nature* 329: 395–96
167. Albuquerque, E. X., Alkondon, M., Lima-Landman, M. T., Deshpande, S. S., Ramoa, A. S. 1988. Molecular targets on noncompetitive blockers at the central and peripheral nicotinic and glutamatergic receptors. In *Neuromuscular Junction,* ed. L. S. Sellin, R. Libelius, S. Thesleff, 13:273–300. New York: Elsevier
168. Cohen, J. B., Weber, M., Changeux, J.-P. 1974. Effects of local anesthetics and calcium on the interaction of cholinergic ligands with the nicotinic receptor protein from *Torpedo marmorata. Mol. Pharmacol.* 10:904–32
169. Cohen, J. B., Boyd, N. D. 1979. Conformational transitions of the membrane bound cholinergic receptor. In *Catalysis in Chemistry and Biochemistry,* ed. B. Pullmann, O. Ginsburg. Dordrecht, Holland: Reidel
170. Heidmann, T., Changeux, J.-P. 1981. Stabilization of the high affinity state of the membrane-bound acetylcholine receptor from *Torpedo marmorata* by noncompetitive blockers. *FEBS Lett.* 131:239–44
171. Waksman, G., Oswald, R., Changeux, J.-P., Roques, B. 1980. Synthesis and pharmacological activity on *Electrophorus electricus* electroplaque of photoaffinity labelling derivatives of noncompetitive blockers di- and tri-methisoquin. *FEBS Lett.* 111:23–38
172. Oswald, R., Sobel, A., Waksman, G., Roques, B., Changeux, J.-P. 1980. Selective labeling by [^{3}H–]trimethisoquin azide of polypeptide chains present in acetylcholine receptor rich membranes from *Torpedo marmorata. FEBS Lett.* 111:29–34
173. Saitoh, T., Changeux, J.-P. 1980. Phosphorylation *in vitro* of membrane fragments from *Torpedo marmorata* electric organ. *Eur. J. Biochem.* 105: 51–62
174. Oswald, R., Changeux, J.-P. 1981. Ultraviolet light-induced labeling by noncompetitive blockers of the acetylcholine receptor from *Torpedo marmorata. Proc. Natl. Acad. Sci. USA* 78:3925–29
175. Kaldany, R. R. J., Karlin, A. 1983. Reaction of quinacrine mustard with the acetylcholine receptor from *Torpedo californica:* Functional consequences and sites of labeling. *J. Biol. Chem.* 258:6232–42
176. Muhn, P., Hucho, F. 1983. Covalent labeling of the acetylcholine receptor from *Torpedo* electric tissue with the channel blocker [^{3}H] triphenylmethylphosphonium by ultraviolet irradiation. *Biochemistry* 22:421–25
177. Haring, R., Kloog, Y., Kalir, A., Sokolovsky, M. 1983. Species differences determine azido phencyclidine labeling pattern in desensitized nicotinic acetylcholine receptors. *Biochem. Biophys. Res. Commun.* 113:723–29
178. Oswald, R. E., Changeux, J.-P. 1981. Selective labeling of the delta-subunit of the acetylcholine receptor by a covalent local anesthetic. *Biochemistry* 20:7166–74
179. Heidmann, T., Bernhardt, J., Neumann, E., Changeux, J.-P. 1983. Rapid kinetics of agonist binding and permeability response analysed in parallel on acetylcholine receptor rich membranes from *Torpedo marmorata. Biochemistry* 22: 5452–59
180. Perutz, M. F., Fermi, G., Abraham, D. J., Poyart, C., Bursaux, E. 1986. Hemoglobin as a receptor of drugs and peptides: X-ray studies of the stereochemistry of binding. *J. Am. Chem. Soc.* 108:1064–78
181. Spivak, C. E., Maleque, M. A., Takahashi, K., Brossi, A., Albuqueque, E. X. 1983. The ionic channel of the nicotinic acetylcholine receptor is unable to differentiate between the optical antipodes of perhydrohistrionicotoxin. *FEBS Lett.* 164:189
182. Neher, E., Steinbach, J. H. 1978. Local

anaesthetics transiently block currents through single acetylcholine-receptor channels. *J. Physiol.* 277:153–76

183. Adams, P. R. 1977. Voltage jump analysis of procaine action at frog end-plate. *J. Physiol.* 268:291–318
184. Changeux, J.-P., Pinset, C., Ribera, A. B. 1986. Effects of chlorpromazine and phencyclidine on mouse C2 acetylcholine receptor kinetics. *J. Physiol.* 378: 497–513
185. Herz, J. M., Johnson, D. A., Taylor, P. 1989. Distance between the agonist and noncompetitive inhibitor sites on the nicotinic acetylcholine receptor. *J. Biol. Chem.* 264:12439–48
186. Herz, J. M., Erlanger, T. P., Kolb, S. J. 1989. Channel permeant cations block noncompetitive inhibitor binding to the nicotinic acetylcholine receptor. *Soc. Neurosci. Abstr.* 15–387.1:969
187. Cox, R. N., Kaldany, R. R., Brandt, P. W., Ferren, B., Hudson, R. A., Karlin, A. 1984. A continuous-flow, rapid mixing, photo-labeling technique applied to the acetylcholine receptor. *Anal. Biochem.* 136:476–86
188. Heidmann, T., Changeux, J.-P. 1984. Time-resolved photolabeling by the noncompetitive blocker chlorpromazine of the acetylcholine receptor in its transiently open and closed ion channel conformations. *Proc. Natl. Acad. Sci. USA* 81:1897–901
189. Heidmann, T., Changeux, J.-P. 1986. Characterization of the transient agonist-triggered state of the acetylcholine receptor rapidly labeled by the noncompetitive blocker [^{3}H]chlorpromazine: additional evidence for the open channel conformation. *Biochemistry* 25:6109–13
190. Muhn, P., Fahr, A., Hucho, F. 1984. Photoaffinity labeling of acetylcholine receptor in millisecond time scale. *FEBS Lett.* 166:146–50
191. Fahr, A., Lauffer, L., Schmidt, D., Heyn, M. P., Hucho, F. 1985. Covalent labeling of functional states of the acetylcholine receptor: Effects of antagonists on the receptor conformation. *Eur. J. Biochem.* 147:483–87
192. Dunn, S. M. J., Raftery, M. A. 1982. Activation and desensitization of *Torpedo* acetylcholine receptor: Evidence for separate binding sites. *Proc. Natl. Acad. Sci. USA* 79:6757–61
193. Dunn, S. M. J., Raftery, M. A. 1982. Multiple binding sites for agonists on *Torpedo californica* acetylcholine receptor. *Biochemistry* 24:6264–72
194. Hess, G. P., Pasquale, E. B., Walker, J. W., McNamee, M. G. 1982. Comparison of acetylcholine receptor-controlled cation flux in membrane vesicles form *Torpedo californica* and *Electrophorus electricus:* chemical kinetic measurements in the millisecond region. *Proc. Natl. Acad. Sci. USA* 79:963–67
195. Walker, J. W., Takeyasu, K., McNamee, M. G. 1982. Activation and inactivation kinetics of *Torpedo californica* acetylcholine receptor in reconstituted membranes. *Biochemistry* 21: 5384–89
196. Cox, R. N., Kaldany, R. R. J., DiPaola, M., Karlin, A. 1985. Time-resolved photolabeling by quinacrine azide of a noncompetitive inhibitor site of the nicotinic acetylcholine receptor in a transient, agonist-induced state. *J. Biol. Chem.* 260:7186–93
197. Giraudat, J., Dennis, M., Heidmann, T., Chang, J. Y., Changeux, J.-P. 1986. Structure of the high-affinity binding site for noncompetitive blockers of the acetylcholine receptor: Serine-262 of the delta subunit is labeled by ^{3}H chlorpromazine. *Proc. Natl. Acad. Sci. USA* 83: 2719–23
198. Giraudat, J., Dennis, M., Heidmann, T., Haumont, P. T., Lederer, F., Changeux, J.-P. 1987. Structure of the high-affinity binding site for noncompetitive blockers of the acetylcholine receptor: ^{3}H chlorpromazine labels homologous residues in the beta and delta chains. *Biochemistry* 26:2410–18
199. Giraudat, J., Galzi, J. L., Revah, F., Changeux, J.-P., Haumont, P. Y., Lederer, F. 1989. The noncompetitive blocker [^{3}H]chlorpromazine labels segment M2 but not segment MI of the nicotinic acetylcholine receptor alpha-subunit. *FEBS Lett.* 253:190–98
200. Revah, F., Galzi, J. L., Giraudat, J., Haumont, P. Y., Lederer, F., Changeux, J.-P. 1990. The noncompetitive blocker [^{3}H]chlorpromazine labels three amino acids of the acetylcholine receptor γ subunit: implications for the α helical organization of the MII segments and the structure of the ion channel. *Proc. Natl. Acad. Sci. USA* 87:4675–79
201. Oberthür, W., Muhn, P., Baumann, H., Lottspcich, F., Wittmann-Liebold, B., Hucho, F. 1986. The reaction site of a noncompetitive antagonist in the delta-subunit of the nicotinic acetylcholine receptor. *EMBO J.* 5:1815–19
202. Hucho, F. L. Oberthür, W., Lottspeich, F. 1986. The ion channel of the nicotinic acetylcholine receptor is formed by the homologous helices MII of the receptor subunits. *FEBS Lett.* 205:137–42
203. Pedersen, S. E., Cohen, J. B. 1990. [^{3}H]-meproadifen mustard reacts with

glu-262 of the nicotinic acetylcholine receptor (AChR) α-subunit. *Biophys. J.* 57:126a

204. Dreyer, E. B., Hasan, F., Cohen, S. G., Cohen, J. B. 1986. Reaction of [^{3}H]meproadifen mustard with membrane-bound Torpedo acetylcholine receptor. *J. Biol. Chem.* 261:13727–34
205. Imoto, K., Methfessel, C., Sakmann, B., Mishina, M., Mori, Y., et al. 1986. Location of a delta-subunit region determining ion transport through the acetylcholine receptor channel. *Nature* 324:670–74
206. Leonard, R. J., Labarca, C. G., Charnet, P., Davidson, N., Lester, H. A. 1988. Evidence that the M2 membrane-spanning region lines the ion channel pore of the nicotinic receptor. *Science* 242:1578–81
207. Charnet, P., Labarca, C., Leonard, R. J., Vogelaar, N. J., Czyzyk, L., et al. 1990. An open-channel blocker interacts with adjacent turns of α-helices in the nicotinic acetylcholine receptor. *Neuron* 2:87–95
208. Imoto, K., Busch, C., Sakmann, B., Mishina, M., Konno, T., et al. 1988. Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature* 335:645–48
209. Waksman, G., Changeux, J.-P., Roques, B. 1980. Structural requirements for agonist and noncompetitive blocking action of acylcholine derivatives on *Electrophorus electricus* electroplaque. *Mol. Pharmacol.* 18:20–27
210. Bolger, M. B., Dionne, V., Chrivia, J., Johnson, D. A., Taylor, P. 1984. Interaction of a fluorescent acyldicholine with the nicotinic acetylcholine receptor and acetylcholinesterase. *Mol. Pharmacol.* 26:57–69
211. Herz, J. M., Johnson, D. A., Taylor, P. 1987. Interaction of noncompetitive inhibitors with the acetylcholine receptor. The site specificity and spectroscopic properties of ethidium binding. *J. Biol. Chem.* 262:7238–47
212. Johnson, D. A., Voet, J. G., Taylor, P. 1984. Fluorescence energy transfer between cobra alpha-toxin molecules bound to the acetylcholine receptor. *J. Biol. Chem.* 259:5717–25
213. Johnson, D. A., Brown, R. D., Herz, J. M., Berman, H. A., Andreasen, G. L., Taylor, P. 1987. Decidium: a novel fluorescent probe of the agonist/antagonist and noncompetitive inhibitor sites on the nicotinic acetylcholine receptor. *J. Biol. Chem.* 262:14022–29
214. Monod, J., Changeux, J.-P., Jacob, F. 1963. Allosteric proteins and cellular control systems. *J. Mol. Biol.* 6: 306
215. Furois-Corbin, S., Pullman, A. 1988. Theoretical study of potential ion-channels formed by bundles of α-helices. Partial modelling of the acetylcholine receptor channel. In *Transport Through Membranes: Carriers, Channels and Pumps*, ed. A. Pullman, pp. 337–57. Dordrecht, Holland: Reidel
216. Furois-Corbin, S., Pullman, A. 1989. A possible model of the inner wall of the acetylcholine receptor channel. *Biochim. Biophys. Acta* 984:339–50
217. Dani, J. A. 1988. Ionic permeability and the open channel structure of the nicotinic acetylcholine receptor. See Ref. 215, pp. 297–319
218. Dani, J. A. 1989. Open channel structure and ion binding sites of the nicotinic acetylcholine receptor channel. *J. Neurosci.* 9:884–92
219. Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A. et al. 1987. Sequence and functional expression of the $GABA_A$ receptor show a ligand-gated receptor super-family. *Nature* 328:221–27
220. Shivers, B. D., Killisch, I., Sprengel, R., Sontheimer, H., Köhler, M., et al. 1989. Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. *Neuron* 33:327–37
221. Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., et al. 1987. The strychnine binding subunit of the glycine receptors shows homology with nicotinic acetylcholine receptors. *Nature* 328:215–20
222. Gregor, P., Mano, I., Maoz, I., McKeown, M., Teichberg, V. I. 1989. Molecular structure of the chick cerebellar kainate-binding subunit of a putative glutamate receptor. *Nature* 342: 689–92
223. Wada, K., Dechesne, C. J., Shimasaki, S., King, R. G., Kusano, K., et al. 1989. Sequence and expression of a frog brain complementary DNA encoding a kainate-binding protein. *Nature* 342: 684–89
224. Hollmann, M., O'Shea-Greenfield, A. Rogers, S. W., Heinemann, S. 1989. Cloning by functional expression of a member of the glutamate receptor family. *Nature* 342:643–48
225. Dani, J. A. 1989. Site-directed mutagenesis and single-channel currents define the ionic channel of the nicotinic acetylcholine receptor. *Trends Neurosci.* 12:125–28
226. Perutz, M. F. 1989. Mechanisms of cooperativity and allosteric regulation in

proteins. *Q. Rev. Biophys.* 22:139–236
227. Jullien, L., Lehn, J. M. 1988. The "Chundle" approach to molecular channels: synthesis of a macrocycle-based molecular bundle. *Tetrahedron Lett.* 29:3803–6
228. Carmichael, V. E., Dutton, P. J., Fyles, T. M., James, T. D. Swan, J. A., Zojaji, M. 1989. Biomimetic ion transport: a functional model of a unimolecular ion channel, *J. Am. Chem. Soc.* 3:767–69
229. Nakano, A., Xie, Q., Mallen, J. V., Echegoyen, L., Gokel, G. W. 1990. Synthesis of a membrane-insertable, sodium cation conduction channel: kinetic analysis by dynamic ^{23}Na NMR. *J. Am Chem. Soc.* 112:1287–89
230. Ascher, P., Nowak, L. 1988. Quisqualate and kainate activated channels in mouse central neurons in culture. *J. Physiol.* 399:227–45
231. Ascher, P., Nowak, L. 1988. The role of divalent cations in the responses of central neurons to N-methyl-D-aspartate. *J. Physiol.* 399:247–66
232. Ascher, P. 1988. In *Frontiers in Excitatory Amino Acid Research*, ed. E. A. Cavalheiro, J. Lehman, L. Tursk, pp. 151–57. New York: Liss
233. Hille, B. 1984. *Ionic Channel of Excitable Membranes.* Sunderland, MA: Sinauer
234. Unwin, N. 1989. The structure of ion channels in membranes of excitable cells. *Neuron* 3:665–76
235. Millhauser, G. L. 1990. Reptation theory of ion channel gating. *Biophys. J.* 57:A124
236. Furois-Corbin, S., Pullman, A. 1986. Theoretical studies of the packing of α-helices of poly (L-alaninc) into transmembrane bundles. Possible significance for ion transfer. *Biochim. Biophys. Acta* 860:165–77
237. Furois-Corbin, S., Pullman, A. 1987. Theoretical study of potential ion-channels formed by a bundle of alpha-helices: effect of the presence of polar residues along the channel inner wall. *J. Biomol. Struct. Dyn.* 4:589–97
238. Chothia, C., Lesk, A. M. 1985. Helix movements in proteins. *Trends Biochem. Sci.* 10:116–18
239. DiPaola, M., Kao, P. N., Karlin, A. 1990. Mapping the alpha subunit site photolabeled by the noncompetitive inhibitor ^{H}-quinacrine azide in the active state of the nicotinic acetylcholine receptor. *J. Biol. Chem.* 265:11017–29
240. Karlin, A., Kao, P. N., DiPaola, M. 1986. Molecular pharmacology of the nicotinic acetylcholine receptor. *Trends Pharmacol. Sci.* 7:304–8
241. Campbell, I. D. 1988. The structure and dynamics of membrane spanning helices by high resolution NMR and molecular dynamics. See Ref. 215, pp. 91–101
242. Brandl, C. J., Deber, C. M. 1986. Hypothesis about the function of membrane-buried proline residues in transport proteins. *Proc. Natl. Acad. Sci. USA* 83:917–21
243. Läuger, P. 1987. Dynamics of ion transport systems in membranes. *Physiol Rev.* 67:1296–331
244. Edmonds, D. T. 1985. The alpha-helix dipole in membranes—A new gating mechanism for ion channels. *Eur. Biophys. J.* 13:31–35
245. Chatrenet, B., Tremeau, O., Bontems, F., Goeldner, M. P., Hirth, C. G., Menez, A. 1989. Topographical studies of toxin-antibody and toxin-acetylcholine receptor complexes using photoactivable toxin derivatives. *Proc. Natl. Acad. Sci. USA* 87:3378–82
246. McCarthy, M. P., Stroud, R. 1989. Changes in conformation upon agonist binding, and nonequivalent labeling of the membrane-spanning regions of the nicotinic acteylcholine receptor subunits. *J. Biol. Chem.* 264:10911–16
247. White, B. H., Cohen, J. B. 1988. Photolabeling of membrane-bound *Torpedo* nicotinic acetylcholine receptor with the hydrophobic probe 3-trifluoromethyl-3 (m-[^{125}I] iodophenyl diazirine. *Biochemistry* 27:8741–51
248. Sumikawa, K., Miledi, R. 1989. Change in desensitization of cat muscle acetylcholine receptor caused by coexpression of *Torpedo* acetylcholine receptor subunits in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* 86:367–71
249. Li, L., Schuchard, M., Palma, A., Pradier, L., McNamee, M. G. 1990. Functional role of the cysteine 451 thiol group in the M4 helix of the γ subunit of *Torpedo californica* acetylcholine receptor. *Biochemistry* 29:5428–36
250. Yu, L., LaPolla, R. J., Davidson, N. 1986. Mouse muscle nicotinic acetylcholine receptor gamma subunit: cDNA sequence and gene expression. *Nucleic Acids Res.* 14:3539–55
251. Katz, B., Miledi, R. 1971. Further observations on acetylcholine noise. *Nature* 232:124–26
252. Katz, B., Miledi, R. 1973. The effect of atropine on acetylcholine action at the neuromuscular junction. *Proc. R. Soc. London Ser. B* 184:221–26
253. Katz, B., Miledi, R. 1973. The characteristics of "end-plate noise" produced by different depolarizing drugs. *J. Physiol.* 230:707–17
254. Anderson, C. R., Stevens, C. F. 1973.

Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *J. Physiol.* 235:655–91
255. Neher, E., Sakmann, B. 1976. Single channel currents recorded from membrane of denervated frog muscle fibers. *Nature* 260:799–802
256. Colquhoun, D., Sakmann, B. 1985. Fast events in single-channel currents activated by acetylcholine and its analogues at the frog muscle endplate. *J. Physiol.* 369:501–57
257. Gardner, P., Ogden, D. C., Colquhoun, D. 1984. Conductances of single ion channels opened by nicotinic agonists are indistinguishable. *Nature* 309:160–62
258. Jackson, M. B. 1984. Spontaneous openings of the acetylcholine receptor channel. *Proc. Natl. Acad. Sci. USA* 81:3901–4
259. Jackson, M. B. 1989. Perfection of a synaptic receptor: kinetics and energetics of the acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 86:2199–203
260. Monod, J., Wyman, J., Changeux, J.-P. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* 12:88–118
261. Unwin, P. N. T., Zampighi, G. 1980. Structure of the junction between communicating cells. *Nature* 283:545–49
262. Fischer, J. B., Olsen, R. W. 1986. Biochemical aspects of GABA/Benzodiazepine receptor function. In *Benzodiazemine/GABA Receptors and Chloride Channels: Structural and Functional Properties*, pp. 241–59. New York: Liss
263. Johnson, J. W., Ascher, P. 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 325:529–31
264. Levitan, E. S., Schofield, P. R., Burt, D. R., Rhee, L. M., Wisden, W., et al. 1988. Structural and functional basis for $GABA_A$ receptor heterogeneity. *Nature* 335:76–79
265. Pritchett, D. B., Sontheimer, H., Gorman, C. M., Kettenmann, H., Seeburg, P. H., Schofield, P. R. 1988. Transient expression shows ligand gating and allosteric potentiation of $GABA_A$ receptor subunits. *Science* 242:1306–8
266. Hamilton, S. L., McLaughlin, M., Karlin, A. 1977. Disulfide bond cross-linked dimer in acetylcholine receptor from *Torpedo californica*. *Biochem. Biophys. Res. Commun.* 79:692–99
267. Karlin, A., Holtzman, E., Yodh, N., Lobel, P., Wall, J., Hainfeld, J. 1983. The arrangement of the subunits of the acetylcholine receptor of *Torpedo californica*. *J. Biol. Chem.* 258:6678–81
268. Buonanno, A., Mudd, J., Merlie, J. P. 1989. Isolation and characterization of the β and epsilon subunit genes of mouse muscle acetylcholine receptor. *J. Biol. Chem.* 264:7611–16
269. Hermans-Borgmeyer, I., Zopf, D., Ryseck, R. P., Hovemann, B., Betz, H., Gundelfinger, E. D. 1986. Primary structure of a developmentally regulated nicotinic acetylcholine receptor protein from *Drosophila EMBO J.* 5:1503–8
270. Kuhse, J., Schmieder, V., Betz, H. 1990. A single amino acid exchange alters the pharmacology of neonatal glycine receptor subunit. *Neuron*. In press

NOTES ADDED IN PROOF:

[1]Recent unpublished studies with a mouse cell line suggest that chlorpromazine blocks the channel more efficiently from the inside than from the outside of the membrane.

[2]The amino acids labeled by quinacrine azide have been identified as α-Arg 209 and α-Pro 211 (M. Di Paola, A. Karlin, personal communication).

[3]It was recently shown on the glycine receptor that amino acid α-617 plays an important role in strychnine binding (270). Interestingly, this amino acid is homologous to AChR α-150 (according to superimposition of the Cys 128–Cys 142 loop) which is next to Trp α-149 labeled by [^{3}H]DDF. This region may thus play a common role in ligand binding in both receptors.