Physiological Mini-Reviews

Edited by the Argentine Physiological Society.

Vol. 1, N° 5, December 2005.

http://www.mini.reviews.safisiol.org.ar

Physiological Mini-Reviews

Physiological Mini-Reviews is a scientific journal, publishing brief reviews on "hot" topics in Physiology. The scope is quite broad, going from "Molecular Physiology" to "Integrated Physiological Systems". As indicated by our title it is not our intention to publish exhaustive and complete reviews. We ask to the authors concise and updated descriptions of the "state of the art" in a specific topic. Innovative and thought-provoking ideas are wellcome.

Annual suscriptions rates are (see the electronic version for payment instructions):
a) Printed (Institutions): 120 U$S (Air mail.)
b) Printed (Individuals): 100 U$S (Air mail. Including Safis Annual fee.)
c) Electronic (Individuals-.PDF): 30 U$S (Including Safis Annual fee.)
d) Electronic (Institutions-.PDF): 50 U$S

Preparation and Submission of manuscripts:
"Physiological Mini-Reviews" will have a maximum of 2500 words, 30 references and 4 figures. Material will be addressed to scientific people in general but not restricted to specialist of the field. For citations in the text and reference list see Cereijido et al. Vol 1, N° 1. Final format will be given at the Editorial Office. Most contributions will be invited ones, but spontaneous presentations are welcome. Send your manuscript in Word format (.doc) to:
mini-reviews@safisiol.org.ar

Advertising:
For details, rates and specifications contact the Managing Editor at the Journal address e-mail:
mini-reviews@safisiol.org.ar

The "Sociedad Argentina de Fisiología" is a registered non-profit organization in Argentina. (Resol. IGJ 763-04)

NEUROTRANSMITTER-GATED ION CHANNELS AT FAST CHEMICAL SYNAPSES: FROM STRUCTURE TO PATHOLOGY.

Cecilia Bouzat
Instituto de Investigaciones Bioquímicas de Bahía Blanca,
Universidad Nacional del Sur-CONICET,
Bahía Blanca, Argentina.
(inbouzat@criba.edu.ar)

The human brain is a vast and complicated network, where billions of nerve cells use signals to communicate with each other. Signals must be rapid and precise. Within a neuron, this speed is achieved using electrical signals that are conducted along extended, cable-like processes. At chemical synapses, these electrical signals are first converted into chemical signals by the release of neurotransmitter into a narrow synaptic gap after depolarization of the presynaptic cell. Neurotransmitter-gated ion channels at the postsynaptic cell, which act as complete signal-transduction systems, reconvert signals from chemical to electrical in under one millisecond. After the transmitter is released and binds to an extracellular region of the receptor, the channel opens to excite or inhibit the train of postsynaptic action potentials. Just as important, the channel closes within a few milliseconds as the transmitter dissociates to terminate the synaptic event. Chemical synaptic transmission offers the advantages of signal amplification, reversal of signal polarity and greater potential for modulation, all important properties for higher brain function.

Pentameric neurotransmitter-gated ion channels (LGICs) play key roles in chemical synapses throughout the nervous system, and include receptors activated by acetylcholine (ACh), γ-aminobutyric acid, glycine and serotonin (5-HT) (LeNovere and Changeux, 2001; Lester et al., 2004). They are known as the Cys-loop receptors because all family subunits contain in their amino-terminal, extracellular halves, a pair of disulfide-bonded cysteines, which are separated by 13 residues. Their vital role in converting chemical recognition into an electrical impulse makes these receptors prime loci for learning, memory and disease processes, as well as targets for clinically relevant drugs. Over the past 30 years, LGICs have been the major targets of the most commonly prescribed drugs, such as neuromuscular blockers, barbiturates and benzodiazepines. In the last years an ever increasing number of human and animal diseases have been found to be caused by defective ion channel function of Cys-loop receptors.

In vertebrates, the Cys-loop superfamily consists of two families of cation-selective channels: the nicotinic (AChR) and the 5-hydroxytryptamine type 3 receptors ($5HT_3$); and two families of anionic channels, the γ-aminobutyric acid receptor ($GABA_A$ and $GABA_C$) and the glycine (GlyR) receptors. The repertoire of invertebrate LGIC is larger, including a GABA-gated cation channel (Beg and Jorgensen, 2003), serotonin- and ACh-gated anion channels (Putrenko et al., 2004 JBC), and histamine-gated channels (Zheng et al., 2002). The selectivity of the channels for cations or anions governs the sign of the current, and in turn, the type of response: inhibitory, for anionic channels because they hyperpolarize the cell, and excitatory, for cationic channels because they induce membrane depolarization by allowing a net influx of Na^+ ions into the cell.

The AChRs have received arguably the most attention of all members of the family. They have been object of attention since Claude Bernard investigated the action of the Central American arrow poison, curare. The muscle AChR was the first to be identified and purified, and the first to be characterized biochemically and electrophysiologically.

The AChR is widely distributed throughout the animal kingdom, from nematodes to human (Le Novere and Changeux, 1995). They are expressed in many regions of the central and peripheral nervous system. In muscle, AChR plays a major role in neuromuscular transmission, initiating the action potential which ends in muscle contraction. This receptor is the target of competitive blockers, such as curare, and other muscle relaxants used in surgery. In nematodes, muscle AChRs are targets for anthelmintic chemotherapy (Jones and Satelle, 2003). In neuronal tissues, AChRs contribute to a wide range of brain activities and influence a number of physiological functions (Gotti and Clementi, 2004). ACh is probably the oldest signaling molecule, and appeared very early in evolution before nervous system. It is present in bacteria, algae, protozoa and plants, and AChRs are also present in various non-neuronal tissues, such as blood cells (De Rosa et al., 2005), keratinocytes, lung cells (Gotti and Clementi, 2004).

The $5HT_3$ receptor is found in the central and peripheral nervous system. It is known to be involved in sensory processing, nociception, emesis, cardiovascular regulation, gut function (Hoyer et al., 2002; Yang et al., 2003). Selective antagonists are used as antiemetic agents during antineoplasic therapy.

The $GABA_A$ and GlyR receptors are involved in the inhibition in the central nervous system, with the $GABA_A$ distributed throughout the CNS and the GlyR found predominantly in the brainstem and spinal cord. The activity of GABA receptors is allostercially enhanced by benzodiazepines, barbiturates, intravenous anesthetics, alcohols, steroids and volatile anesthetics; and it is blocked by picrotoxin. GlyR are targets of the plant alkaloid strychnine, which by acting as competitive antagonists leads to agitation, muscle spasms, and convulsions. Therefore, developing therapeutic agents against GlyR may have significant utility as muscle relaxants and analgesic agents (Connolly and Wafford, 2004).

OVERALL STRUCTURE

Cys-loop receptors are composed of five identical (homopentamers) or different (heteropentamers) polypeptide chains arranged around an axis perpendicular to the membrane **(Fig. 1)**. The analysis of the evolutionary relationships within the superfamily suggests that the ancestor receptor was probably homo-oligomeric and appeared 2500 million years ago (Ortells and Lunt, 1995; LeNovere and Changeux, 1995). Homomeric receptors are the structurally simplest type of Cys-loop receptor. They diverged least from the common ancestral Cys-loop receptor and likely share functional features found among all members of the superfamily. Subunits are classified as α or non-α, with the α subunits containing a CC motif in the binding site. A wide number of α and non-α subunits have been cloned for all members of the superfamily (Ligand-gated ion channel database, http://www.ebi.ac.uk/compneur-srv/LGICdb/cys-loop.php).
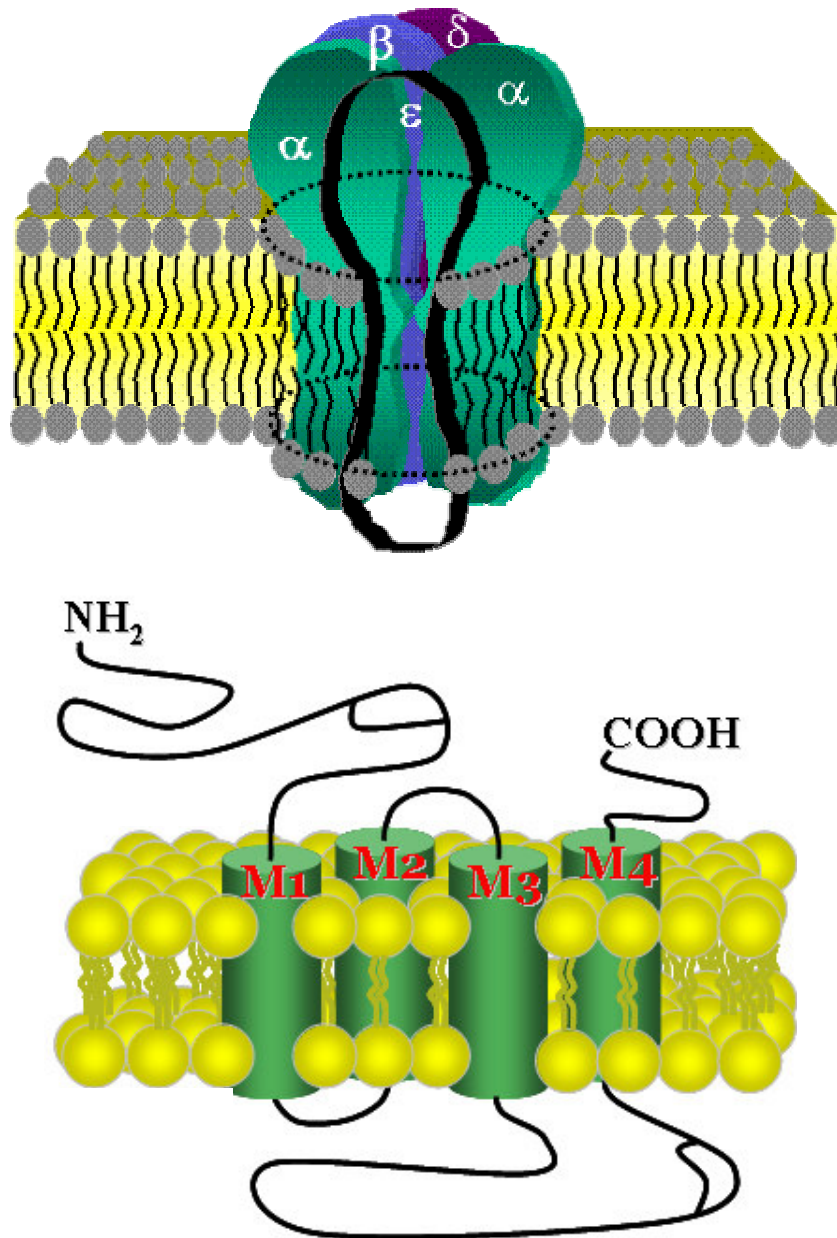
Fig. 1. Schematic arrangement of the adult muscle AChR. *The pentameric receptor is composed of two α and three different non-α subunits (β, ε and δ). Each subunit contains a large extracellular domain and four transmembrane segments. This transmembrane disposition in conserved in all Cys-loop receptors.*

The subunits of this gene superfamily are built on a modular basis. The extracellular domain forms the agonist binding sites. The transmembrane domain contains the pore and the channel gate, the structure that prevents ion flow in the absence of agonist and swings open to allow flow in its presence (Lester et al., 2004).

The extracellular domain: location of the agonist binding sites

Our knowledge of the structure of the extracellular domain of the Cys-loop receptors took a giant step forward with the solution of the high-resolution structure of the acetylcholine binding protein (AChBP) from *Lymnaea stagnalis* (Brejc et al., 2001). This soluble protein is produced and stored in glial cells and is released in an ACh-dependent manner in the synaptic cleft where it regulates synaptic transmission. AChBP lacks the transmembrane region and contains many of the structural cornerstones that give AChRs their unique signature and has therefore become a functional and structural surrogate of the extracellular domain of the Cys-loop receptors (Review in Sine, 2002). It contains 210 amino acids and is 15-24% identical to aligned sequences of the amino-terminal, extracellular halves of AChR, $5HT_3$ and GABA subunits.   Each AChBP monomer consists of an N-terminal α-helix, two short 3_{10} helices, and a core of 10 β-strands that form a sandwich. The N- and C- termini are located at top and bottom of the pentamer, respectively. In the Cys-loop receptors, the end of $\beta10$ connects to the start of M1. Located at the bottom of the subunit, the linker between $\beta6$ and $\beta7$ is the signature Cys-loop found in all members of the superfamily. The binding sites are located at the interfaces between subunits, at about 40 Å above the membrane. One subunit, the α subunit, contributes three loops that span beta strands and harbor key aromatic residues, while the adjacent subunit, called the complementary subunit, contributes three beta strands that harbor aromatic and hydrophobic residues, and a fourth loop that harbors negatively charged residues. AChBP has been used to create homology-based models of the extracellular domains of 5-HT3, AChR and $GABA_A$ (Cromer et al., 2002; Reeves et al., 2003; Niesler al., 2003).

The transmembrane domain: the region that comprises the ion pore and the gate

The transmembrane half of each Cys-loop receptor subunit is composed of 4 transmembrane segments (M1-M4) **(Fig. 1)**. Structural and electrophysiological studies have shown that the ion channel is largely lined by the M2 domains of the five subunits. Using cryo-electron microscopy to a resolution of 4 Å a model of the closed pore of *Torpedo* AChR has been recently reported (Miyazawa et al., 2003; **Fig. 2**). The model shows that the pore is shaped by an inner ring of 5 α-helices (M2 segments) and an outer ring of 15 α-helices, which coil around each other and shield the inner ring from the lipids **(Fig. 2)**. The pore is maximally constricted in the middle of the membrane due to side-to-side interactions between hydrophobic residues of neighboring helices. This tight hydrophobic girdle creates an energetic barrier to ions across the membrane, and thus it has been suggested that it corresponds to the gate (Miyazawa et al., 2003). During gating, twisting of the M2 helices may disrupt the girdle and, in turn, allows ion flux.
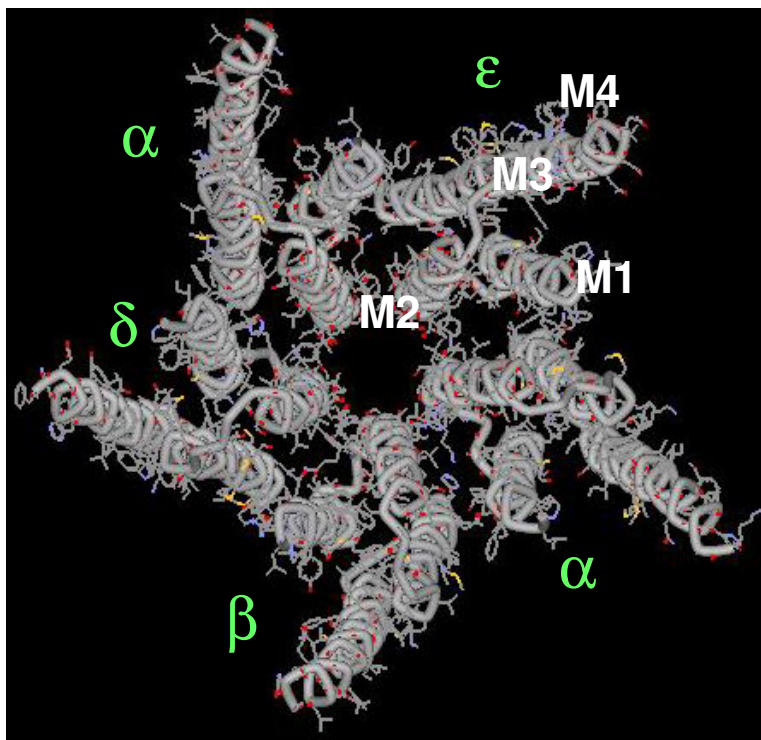
Fig. 2. Molecular model of the closed (resting) AChR pore of Torpedo californica *(modified from Miyazawa et al., 2003). The coordinates were taken from the Protein Data Bank with accession code 10ED (Miyazawa et al., 2003). The models were drawn with ViewerPro 4.2 (Accelrys Inc.).*

The ion pore contains the filter selectivity, which is the structure that determines which type of ions may pass through the channel. Point mutations in the M2 region supported the role of positions –2 to 2′ as main determinants of the selectivity filter of all Cys-loop receptors (Review in Barry and Lynch, 2005).

The long intracellular loop between M3 and M4 contains phosphorylation sites and is thought to be associated with cytoskeletal proteins, such as rapsyin for AChR, gephyrin for GlyR, and GABARAP and MAP1B for $GABA_A$ and $GABA_C$, respectively (Passafaro and Sheng, 1999). These proteins allow the clustering of the receptors at appropriate regions of the membrane. In addition, this loop has been shown to contribute to channel kinetics in muscle AChRs (Bouzat et al., 1994; Wang et al., 2000).

The interface between extracellular and transmembrane domains: a region involved in coupling agonist binding to channel gating

The junction between binding and pore domains has recently emerged as crucial for coupling binding to gating (Bouzat et al., 2004; Kash et al., 2003; Chakrapani et al., 2004). To identify the structural requirements for functionally coupling extracellular and transmembrane domains, we generated a chimeric receptor composed of the AChBP protein, which presumably evolved without the constraint of functional coupling to an ion pore, and the pore domain from the $5HT_3$ receptor (Bouzat et al., 2004). Only when amino-acid sequences of three loops (β1-β2, Cys and β8-β9 loops) in AChBP were changed to

their $5HT_3$ counterparts does ACh bind with low affinity characteristic of activatable receptors and trigger opening of the ion pore. Thus, the functional coupling process is mediated by a network of loops from both domains, including three from the binding domain and one from the pore domain (M2-M3 linker). This region mediates a bi-directional allosteric interaction between the binding sites and the pore domain. Structural modeling shows that the Cys-loop and $\beta1$-$\beta2$ linker straddle opposite sides of the M2-M3 linker from the pore domain **(Fig. 3)**, suggesting a twisting motion during coupling that in turn twists the pore-lining M2 domain to allow flow of cations (Bouzat et al., 2004).
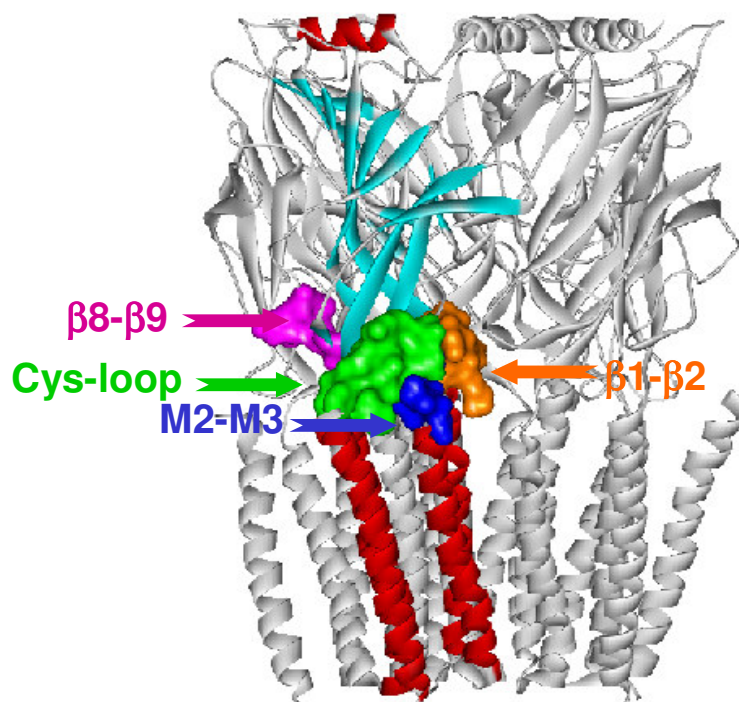
Fig. 3 Homology model of the chimeric AChBP-5HT$_{3A}$ receptor with key regions of one subunit color-coded. *These loops, which are located at the interface between binding and pore domains, are involved in the coupling of agonist binding to channel gating. From Bouzat et al. (2004).*

The complete atomic-resolution structure of any fast synaptic receptor channel has not been resolved yet. However, a refined model of the membrane-associated *Torpedo* AChR in the closed state obtained from electron images at 4 Å resolution has been recently presented (Unwin, 2005). The high level of amino acid sequence conservation imply that all channels of the Cys-loop superfamily are constructed around the same three-dimensional framework and function according to the same global principles (Unwin, 2005).
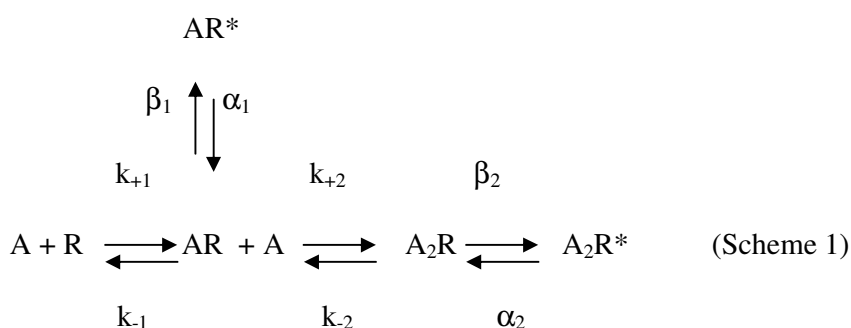
FROM STRUCTURE TO FUNCTION

Cys-loop receptors are allosteric proteins: they are oligomeric, contain multiple agonist-binding sites, non-competitive antagonist sites, and gates that interact at a distance through changes in the quaternary structure of the receptor. They also open and desensitized, albeit with less probability, in the absence of agonist. Receptors can be

therefore found in different conformational states: closed or resting, which is more stable in the absence of agonist; open, which is transient and stabilized by the presence of agonist; and desensitized, which predominates after a prolonged exposure to agonist. In addition, intermediate conformational states between open or closed and desensitized states may exist in the AChR (Spitzmaul et al., 2001; Ryan et al., 2001).

The combination of site-directed mutagenesis and cell expression with single-channel recordings using the patch-clamp technique (Hamill et al., 1981) had a clear impact in the field by disclosing relationships between molecular structure and functional properties for all members of the superfamily. The AChR is the best electrophysiologically characterized receptor. Emerging from the early biophysical studies of the receptor function, the following core mechanism was established and still serves today as a starting point for quantitatively describing muscle AChR activation:

$$
AR^* \\
\beta_1 \Big\uparrow\Big\downarrow \alpha_1 \\
A + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AR + A \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} A_2R \underset{\alpha_2}{\overset{\beta_2}{\rightleftharpoons}} A_2R^* \qquad \text{(Scheme 1)}
$$

where two agonists (A) bind to the receptor (R) in the resting state with association rates k_{+1} and k_{+2}, and dissociate with rates k_{-1} and k_{-2}. Receptors occupied by one agonist open with rate β_1 and close with rate α_1, and AChRs occupied by two agonist molecules open with rate β_2 and close with rate α_2. Further insight into the process of activation has been provided by kinetic analysis of single channels (Cheng et al., 1995; Ohno et al., 1995; Sine et al., 1995; Qin et al., 1996; Wang et al., 2000; Grosman et al., 2000a,b; Bouzat et al., 2000, 2002; Shelley and Colquhoun, 2005). Mutations may produce changes in channel function by affecting one or more of the rate constants in Scheme 1. Kinetic analysis allows the evaluation of the changes in the rate constant(s), thus providing insights into the relationship between structure and channel gating. This approach has been extensively used to associate a specific residue or domain with a kinetic step of the activation process.

FROM FUNCTION TO PATHOLOGY

During the last decade, an ever increasing number of channelopathies have been described. These diseases result from defects in ion channel function. The identification of the molecular mechanisms leading to the disease is necessary for rational therapy.

Neuronal AChRs are involved in a wide variety of diseases affecting the nervous system and non-neuronal tissues, such as Tourette syndrome, attention-deficit hyperactive disorder, schizophrenia, autosomal dominant nocturnal frontal lobe epilepsy, febrile convulsions, depression and anxiety, Alzheimer´s and Parkinson´s disease (Lena and Changeux, 1998; Gotti and Clementi, 2004).

Congenital myasthenic syndromes (CMS) are heterogeneous disorders in which the safety margin of neuromuscular transmission is compromised by one or more presynaptic, synaptic, or postsynaptic mechanisms. The majority of CMS are caused by mutations in the AChR subunits. Some of them lead to kinetic abnormalities which may cause a loss (fast channel syndrome) or a gain of function (slow channel syndrome) (Engel et al., 2003). The slow-channel CMS are dominant mutations that increase activation of the receptor by speeding the rate of opening of the channel, slowing the rate of closing of the channel, or allowing repeated opening during each ACh occupancy. The first study of a CMS revealing how a kinetic abnormality may cause a disease was reported by the groups of Sine and Engel at Mayo Clinic (Ohno et al., 1995). Molecular genetic analysis of a patient suffering from a congenital myasthenic syndrome demonstrated a substitution of proline for threonine at the M2 domain of the muscle ε subunit. Genetically engineered mutant AChRs expressed in HEK cells revealed that the mutation leads to markedly prolonged openings in the presence of agonist and increase openings even in the absence of ACh (Ohno et al., 1995, **Fig. 4**). The result is a staircase summation of endplate potential that cause depolarization block of the muscle action potential. In addition, cationic overloading of the postsynaptic region caused by the cholinergic overactivity results in an endplate myopathy due to destruction of junctional folds. Since then, kinetic analysis of spontaneous mutations in human AChR subunits that cause CMS highlighted functionally significant residues or domain. Mutations causing increase-of-function phenotypes have been shown to occur in the extracellular region, in the M2-M3 linker, and in the transmembrane domains of different subunits (for reviews see Engel et al., 1998, 2002, 2003). Knowledge of the pathological alterations occurring in CMS at the molecular level has important consequences for future therapeutic strategies.
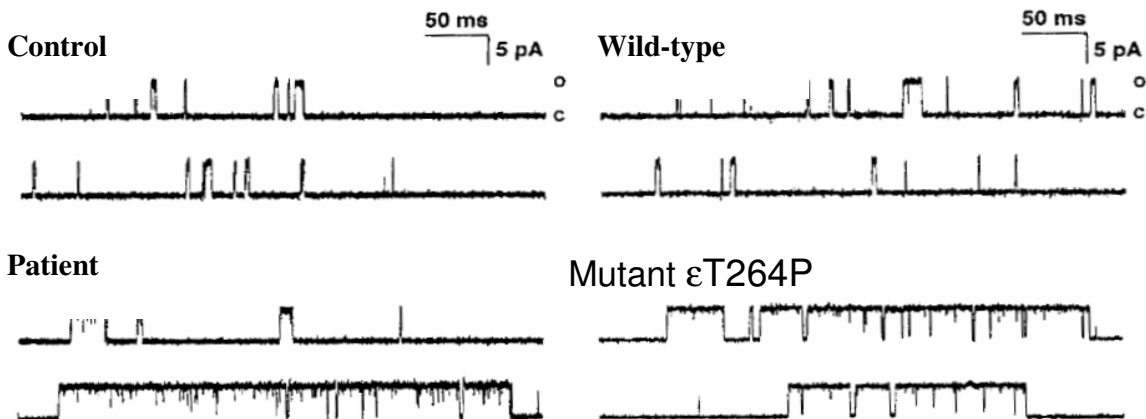
Fig. 4. Slow-channel congenital myasthenic syndromes: The mutation εT264P increases the open duration of the muscle AChR. (Upper panel) Aligned amino acid sequences of the M2 domain of the ε subunit. In the patient, the highly conserved T264 is replaced by a proline. (Lower panel) Comparison of channel events recorded from normal human endplate (control) and a CMS endplate (patient) and from HEK cells expressing wild-type (wild-type) and mutant εT264P (mutant) AChR channels. Markedly prolonged channel events at CMS EP and in HEK cells expressing the εT264P mutation are observed. ACh concentration: 1 μM. Taken from Ohno et al. (1995).

Interestingly, mutations at equivalent regions of different Cys-loop receptors have been shown to lead to diseases, thus confirming a common fundamental mechanism of channel gating. Inherited mutations located in the M2-M3 domain of the GlyR α1-subunit are associated with human startle disease (hyperekplexia) (Shiang et al. 1993) and Startle syndromes in other species (Rajendra and Schofield, 1995). The effect of these mutations is to disrupt signal transduction (Rajendra et al., 1995). An inherited mutation in the M2-M3 loop is associated with a congenital myasthenic syndrome in the muscle AChR (Grosman et al., 2000b), and with a rare form of epilepsy in the GABA$_A$ receptor (Baulac et al., 2001).

CONCLUDING REMARKS

There is still much to learn. Ligand binding and channel gating are dynamic processes that require precise and coordinated movements of the protein, which have recently begun to be unraveled. Desensitization still remains a complex and mysterious process.

If the goal of designing new ligands targeted to specific LGICs subtypes or to mutated receptors at the origin of the disease states is to be fulfilled, we require an intimate knowledge of the structure-function relationship of them. There is hope that the new understanding of the molecular pharmacology of Cys-loop receptors will led to a new generation of more selective drugs with improved safety profiles.

ACKNOWLEDGMENTS

REFERENCES

1. **Barry PH, Lynch JW.** Ligand-gated channels. *IEEE Trans Nanobioscience.* 2005;4, 70-80.

2. **Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme JF, Baulac M, Brice A, Bruzzone R, LeGuern E.** First genetic evidence of

GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nature Genetics.* 2001;28, 46-48.

3. **Beg AA, Jorgensen EM.** EXP-1 is an excitatory GABA-gated cation channel. *Nat Neurosci.* 2003;6, 1145-1152.

4. **Bouzat C, Bren N, Sine SM.** Structural basis of the different gating kinetics of fetal and adult nicotinic acetylcholine receptors. *Neuron.* 1994;13, 1395-1402.

5. **Bouzat C, Gumilar F, Esandi MC, Sine S.** Subunit-selective contribution to channel gating of the fourth transmembrane domain of the nicotinic receptor. *Biophys J.* 2002; 82, 1920-1929.

6. **Bouzat C, Gumilar F, Spitzmaul G, Wang HL, Rayes D, Hansen S, Taylor P, Sine S**. Coupling of agonist binding to channel gating in an ACh-binding protein linked to ion channel. *Nature.* 2004; 430, 896-900.

7. **Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB, Sixma TK**. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature.* 2001; 411, 269-276.

8. **Chakrapani S, Bailey TD, Auerbach A**. Gating dynamics of the acetylcholine receptor extracellular domain. *J Gen Physiol.* 2004; 123, 341-356.

9. **Chen J, Zhang Y, Akk G, Sine S, Auerbach A.** Activation kinetics of recombinant mouse nicotinic acetylcholine receptors: mutations of alpha-subunit tyrosine 190 affect both binding and gating. *Biophys J.* 1995; 69, 849-859.

10. **Connolly CN, Wafford KA.** Molecular structure in ligand-gated ion channel function. *Biochemical Soc. Transactions.* 2004; 32, 529-534.

11. **Cromer BA, Morton CJ, Parker MW.** Anxiety over GABA(A) receptor structure relieved by AChBP. *Trends Biochem Sci.* 2002; 27, 280-287.

12. **Engel A, Ohno K, Milone M, Sine S.** Congenital myasthenic syndromes. *Ann. N. Y. Acad. Sci.* 1998; 841, 140-156.

13. **Engel A, Ohno K, Sine S.** The spectrum of congenital myasthenic syndromes. *Molecular Neurobiology.* 2002; 26, 347-367.

14. **Engel AG, Ohno K, Shen X-M, Sine SM.** Congenital Myasthenic syndromes: multiple targets at the neuromuscular junction. *Ann.N.Y.Acad. Sci.* 2003; 998, 138-160.

15. **Gotti C, Clementi F.** Neuronal nicotinic receptors: from structure to pathology. *Progress in Neurobiology.* 2004; 74, 363-396.

16. **Grosman C, Zhou M, Auerbach A.** Mapping the conformational wave of acetylcholine receptor channel gating. *Nature.* 2000a; 403, 773-776.

17. **Grosman C, Salamone FN, Sine SM, Auerbach A.** The extracellular linker of muscle acetylcholine receptor channels is a gating control element. *J Gen Physiol.* 2000b; 116, 327-40.

18. **Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ.** Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch.* 1981; 391, 85-100.

19. **Hoyer D, Hannon JP, Martin GR.** Molecular, pharmacological and functional diversity of recombinant 5-HT receptors. *Pharmacol. Biochem. Behav.* 2002; 71, 533-554.

20. **Jones AK, Sattelle DB.** Functional genomics of the nicotinic acetylcholine receptor gene family of the nematode, *Caenorhabditis elegans. Bioessays.* 2003; 26, 39-49.

21. **Kash TL, Jenkins A, Kelley JC, Trudell JR, Harrison NL.** Coupling of agonist binding to channel gating in the GABA(A) receptor. *Nature* 2003; 421, 272-475.

22. **Lena C, Changeux JP.** Allosteric nicotinic receptors, human pathologies. *J. Physiol.* 1998; 92, 63-74.

23. **Le Novere N, Changeux JP.** Molecular evolution of the nicotinic acetylcholine receptor subunit family: an example of multigene family in excitable cells. *J. Mol. Evol.* 1995; 40, 155-172.

24. **Le Novere N, Changeux JP.** The Ligand Gated Ion Channel database: an example of a sequence database in neuroscience. *Philos Trans R Soc Lond B Biol Sci.* 2001; 356, 1121-1130.

25. **Lester HA, Dibas MI, Dahan DS, Leite JF, Dougherty DA.** Cys-loop receptors: new twists and turns. *Trends in Neurosci.* 2004; 27, 329-336.

26. **Miyazawa A, Fujiyoshi Y, Unwin N.** Structure and gating mechanism of the acetylcholine receptor pore. *Nature.* 2003; 423, 949-955.

27. **Niesler B, Kapeller FB, Rappold GA.** Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. *Gene.* 2003; 310:101-11.

28. **Ohno K, Hutchinson DO, Milone M, Brengman JM, Bouzat C, Sine SM, Engel AG.** Congenital myasthenic syndrome caused by prolonged acetylcholine receptor channel openings due to a mutation in the M2 domain of the epsilon subunit. *Proc. Nat. Acad Sci U S A.* 1995; 92, 758-762.

29. **Ortells MO, Lunt GG.** Evolutionary history of the ligand-gated ion-channel superfamily of receptors. *Trends Neurosci.* 1995; 18, 121-127.

30. **Passafaro M, Sheng M.** Synaptogenesis: The MAP location of GABA receptors. *Curr Biol.* 1999; 9, 261-263.

31. **Putrenko I, Zakikhani M, Dent JA.** A family of acetylcholine-gated chloride channel subunits in Caenorhabditis elegans. *J Biol Chem.* 2005; 280, 6392-6398.

32. **Qin F, Auerbach A, Sachs F.** Estimating single-channel kinetic parameters from idealized patch-clamp data containing missed events. *Biophys. J.* 1996; 70, 264-280.

33. **Rajendra S, Schofield PR.** Molecular mechanisms of inherited startle syndromes. *Trends Neurosci.* 1995; 18, 80-82.

34. **Reeves DC, Sayed MF, Chau PL, Price KL, Lummis SC.** Prediction of 5-HT3 receptor agonist-binding residues using homology modeling. *Biophys J.* 2003; 84, 2338-44.

35. **Ryan SE, Blanton MP and Baenziger JE.** A conformational intermediate between the resting and desensitized states of the nicotinic acetylcholine receptor. *J. Biol. Chem.* 2001; 276: 4796-4803.

36. **Shiang R, Ryan SG, Zhu YZ, Hahn AF, O'Connell P, Wasmuth JJ.** Mutations in the alpha 1 subunit of the inhibitory glycine receptor cause the dominant neurologic disorder, hyperekplexia. *Nat Genet.* 1993; 5, 351-358.

37. **Sine S, Ohno K, Bouzat C, Auerbach A, Milone M, Pruitt J, and Engel, AG.** Mutation of the Acetylcholine Receptor α-subunit Causes a Slow-Channel Myasthenic Syndrome by Enhancing Agonist Binding Affinity. *Neuron.* 1995; 15, 229-239.

38. **Shelley C, Colquhoun D.** A human congenital myasthenia-causing mutation (epsilonL78P) of the muscle nicotinic acetylcholine receptor with unusual single channel properties. *J Physiol.* 2005; 564, 377-396.

39. **Sine SM.** The nicotinic receptor ligand binding domain. *J. Neurobiol.* 2002; 53, 431-446.

40. **Spitzmaul G, Dilger JP, Bouzat C**,. The noncompetitive inhibitor quinacrine modifies the desensitization kinetics of muscle acetylcholine receptors. *Mol. Pharmacol.* 2001; 60, 235-243.

41. **Unwin N.** Refined structure of the nicotinic acetylcholine receptor at 4A resolution. *J Mol Biol.* 2005; 4;346, 967-989.

42. **Wang HL, Ohno K, Milone M, Brengman JM, Evoli A, Batocchi AP, Middleton LT, Christodoulou K, Engel AG, Sine SM.** Fundamental gating mechanism of nicotinic receptor channel revealed by mutation causing a congenital myasthenic syndrome. *J. Gen. Physiol.* 2000; 116, 449-462.

43. **Yang HS, Yun Kim SY, Choi SJ, Kim KJ, Kim ON, Lee SB, and Sung KW.** Effect of 5-hydroxyindole on etanol potentiation of 5-hydroxytryptamine (5-HT)$_3$ receptor-

activated ion current in NCB-20 neuroblastoma cells. *Neurosci. Lett.* 2003; 338, 72-76.

44. Zheng Y, Hirschberg B, Yuan J, Wang AP, Hunt DC, Ludmerer SW, Schmatz DM, Cully DF Identification of two novel Drosophila melanogaster histamine-gated chloride channel subunits expressed in the eye. *J Biol Chem.* 2002; *277*, 2000-2005.