0026-895X/06/7001-5–7$20.00
MOLECULAR PHARMACOLOGY
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*Mol Pharmacol* 70:5–7, 2006

Vol. 70, No. 1
25767/3124488
*Printed in U.S.A.*



*PERSPECTIVE*

# Potentiation of Acetylcholine Receptors by Divalent Cations

Jon Lindstrom

*Department of Neuroscience, University of Pennsylvania Medical School, Philadelphia, Pennsylvania*

Received April 14, 2006; accepted April 26, 2006

**ABSTRACT**

Divalent cations promote activation of several nicotinic acetylcholine receptor (AChR) subtypes, presumably by lowering the energetic barrier between open and closed conformations. In wild-type $\alpha$7 AChRs, binding of calcium to a particular part of the extracellular domain is required for potentiating activation. McLaughlin et al. (p. 16) tested the hypothesis that movements involved in agonist activation and calcium modulation involve a nearby $\beta$ sheet by linking strands within this sheet through disulfide bonds formed by replacing adjacent amino acids with cysteines to alter its mobility. These studies are helping to reveal how movements initiated by agonist binding to ACh binding sites are propagated through the extracellular domain of AChRs to regulate opening of the cation channel through the membrane.

The Cys-loop ligand-gated ion channel (LGIC) family of neurotransmitter receptors includes nicotinic acetylcholine receptors (AChRs) as well as A type receptors for GABA, glycine receptors, and type 3 serotonin receptors (5HT-3R) (Lindstrom, 2000; Lester et al., 2004; Sine and Engel, 2006). All of these receptors are formed by five homologous or identical subunits arranged to form a central ion channel. The subunits consist of a large N-terminal extracellular domain, three closely spaced transmembrane domains, of which the second (M2) contributes to the lining of the channel, a large cytoplasmic domain, and a fourth transmembrane domain that leads to a short extracellular C-terminal sequence.

A model for the overall structure of these receptors is provided by the low resolution crystal structure of the muscle type $\alpha$1 AChR found in *Torpedo marmorata* electric organs (Unwin, 2005) (Fig. 1). High resolution crystal structures of acetylcholine binding proteins secreted by mollusk glia provide detailed models for the structure of the extracellular domain of AChRs and other related LGICs (Brejc et al., 2001; Celie et al., 2004; Bourne et al., 2005; Hansen et al., 2005) (Fig. 1). Many approaches are being taken using these static structures to understand how the binding of agonists, antagonists, and modulators to the extracellular domain produce movements in the protein. These movements are propagated through the receptor structure to regulate opening of distant ion channel gates, resulting in the rapid dynamics of activation and desensitization that characterize the electrophysiological properties of these receptors. The implications of these structural and functional considerations for human muscle AChRs on neuromuscular transmission are being revealed by analysis of AChR mutations that cause congenital myasthenias (Sine and Engel, 2006).

Movements of the extracellular domain near the ACh binding sites at the subunit interfaces in AChBPs have been revealed by comparing the crystal structures with agonists bound versus those with antagonists bound. By far, the biggest movements are associated with the C loop near the ACh binding site (Fig. 1). This contributes to smaller movements that propagate from the binding site to the channel gate. In the resting unliganded state, the C loop is open. It remains open when a small antagonist is bound and is wedged even more widely open by binding of the large antagonist cobratoxin (Bourne et al., 2005; Hansen et al., 2005). In the active or desensitized conformations caused by binding of small agonists such as nicotine or epibatidine, the C loop slams shut over the ACh binding site (Celie et al., 2004; Hansen et al., 2005).

The conformation changes observed with ligand-bound

J.L. is supported by the National Institutes of Health grants NS11323 and NS052463.

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.

doi:10.1124/mol.106.025767.

Please see the related article on page 16.

**ABBREVIATIONS:** LGIC, Cys-loop ligand-gated ion channel; AChR, nicotinic acetylcholine receptor; 5HT, 5-hydroxytryptamine; AChBP, acetylcholine binding protein.

AChBP reflect those in intact AChRs because a chimera with the extracellular domain of an AChBP and the remainder of a 5HT-3 subunit produces electrophysiologically active AChRs (Bouzat et al., 2004). To insure communication between these two components, three loops in the AChBP at the interface with the 5HT-3 subunit had to be changed to their 5HT-3 counterparts. Two of these loops, the Cys loop (for which the family is named) and the $\beta$1-$\beta$2 loop, straddle opposite sides of the M2-M3 linker of the pore domain in electric organ AChRs, suggesting that movements of the ligand binding domain might produce a twist that is propagated through these three meshing gear teeth to produce a twist in the pore domain to cause the cation channel to open (Bouzat et al., 2004; Unwin, 2005). Electrostatic linkage between peripheral and inner $\beta$ sheets from the extracellular domain positions them to engage with the top of the M2-M3 linker and pivot with respect to M1 (Lee and Sine, 2005).

Interactions between the cholinergic ligand binding to the extracellular domain and the cation channel of AChRs are in delicate, reciprocal balance. $\alpha$7 AChR leucine 247 is conserved in other LGICs. Mutation to threonine (L247T) causes some small antagonists like curare and dihydro-$\beta$-erythroidine to behave as agonists, but the large antagonist $\alpha$-bungarotoxin remains an antagonist (Bertrand et al., 1992). In addition, this mutation prevents the rapid desensitization characteristic of wild-type $\alpha$7 AChRs. This mutation was initially proposed to promote formation of an open channel-desensitized state (Bertrand et al., 1992), but a likely alternate explanation is that this mutation simply alters gating to favor the open state (Filatov and White, 1995; Labarca et al., 1995).

Calcium potentiates the responses of many AChR subtypes, and there is evidence for several categories of calcium binding sites in the extracellular domain of $\alpha$7 AChRs (Galzi et al., 1996). Mutation of $\alpha$7 glutamate 172 or its homolog in the 5HT-3 receptor can prevent potentiation by calcium. Calcium potentiation was proposed to result from promoting the transition from the resting to the active state. $\alpha$7 glutamate 172 was proposed not to play a role in cholinergic ligand binding, but its homolog in AChR $\delta$ subunits was proposed to also contribute to ligand binding (Czajkowski et al., 1993).

McLaughlin et al. (2006) chose to study the $\alpha$7 L247T mutant described by Bertrand et al. (1992) because of its simple homomeric structure, large responses, ease of activation (even by some small antagonists), and because Galzi et al. (1996) had already mapped calcium binding sites on $\alpha$7 AChRs. The detailed structures available for AChBPs allowed them to re-examine potentiation of $\alpha$7 AChR function by divalent cations.

Previously, they reasoned that because the $\beta$9 strand had Glu172 (which is involved in $Ca^{2+}$ modulation) at its N terminus and the ACh binding site at its C terminus, agonist binding might involve movement of $\beta$9 (Lyford et al., 2003). Using the substituted cysteine accessibility method developed by Karlin and Akabas (1998), they replaced single amino acids in $\beta$9, such as Glu172, with cysteines and found that binding of agonists, but not antagonists, inhibited the rate of binding of methanethiosulfonate reagents to these cysteines, indicating that $\beta$9 participates in conformational changes triggered by ligand binding. Glu172, through which calcium increases the potency and efficacy of agonists, is at the N terminus of $\beta$9, which is located in the lumen of the channel vestibule adjacent to the top of the transmembrane domain (Eddins et al., 2001, 2002). It is affected by permeant but not impermeant divalent cations.

In this issue of *Molecular Pharmacology*, McLaughlin et al. (2006) further investigate the movements of $\beta$ strands in the extracellular domain associated with activation by agonists and potentiation by divalent cations. Adjacent positions on $\beta$7 and $\beta$10 strands were stapled together with disulfide bonds to test how this would alter function. These positions were identified using a model of the $\alpha$7 extracellular domain based on the structure of AChBP. One pair, K144C/T198C, permitted divalent cations alone to activate $\alpha$7 L247T AChRs; this was blocked by the antagonist methyllycaconitine. This supports the idea that the $\beta$7/$\beta$9/$\beta$10 sheet moves together similarly under the influence of agonists and/or divalent cations. The L247T mutant, which favors transition to the open state, permits cations alone to act as full agonists, whereas the K144C/T198C link on a wild-type background still required both agonist and divalent cations to act together to cause activation.

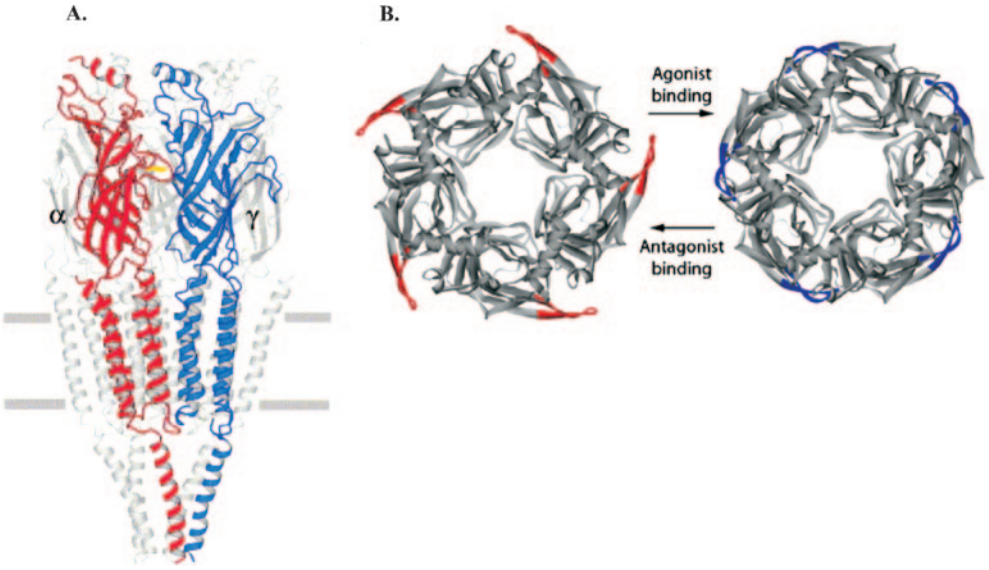


**Fig. 1.** A, a side view of the structure of *T. marmorata* AChR highlighting the $\alpha$1 and $\delta$ subunits at whose interface one of the two ACh binding sites in this heteromeric $(\alpha 1)_{2\beta}1\gamma\delta$ AChR is formed. This part of the figure is from Unwin (2005). B, a top view of the structure of AChBP in its resting and agonist bound conformations. AChBPs, like $\alpha$7 AChRs, are homomeric with five identical ACh binding sites. The C loop is open in the resting state and closed in the agonist-bound active and desensitized states. This part of the figure is from Hansen et al. (2005). Figures reproduced with the permission of the authors.

$Zn^{2+}$ inhibits wild-type $\alpha$7 AChRs, but low concentrations of $Zn^{2+}$ activate the L247T mutant and high concentrations block the mutant channel (Palma et al., 1998). A unique LGIC found in humans and dogs but not rodents has 15% amino acid identity with $\alpha$7, exhibits spontaneous activation, is activated by $Zn^{2+}$, and is inhibited by curare (Davies et al., 2003). $Zn^{2+}$ and curare might act on this LGIC at sites homologous to those proposed for divalent cations and ACh on $\alpha$7 AChRs by McLaughlin et al. (2006).

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**Address correspondence to:** Jon Lindstrom, Department of Neuroscience, University of Pennsylvania Medical School, 217 Stemmler Hall, 36th and Hamilton Walk, Philadelphia, PA 19104-6074. E-mail: jslkk@mail.med.upenn.edu