PROBING THE ROLES OF RECEPTOR STRUCTURE, DRUG-RECEPTOR INTERACTIONS, AND RECEPTOR CROSSTALK IN LIGAND-GATED ION CHANNEL FUNCTION

Thesis by

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To my Angels,

who are always by my side

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Abstract

Ligand-gated ion channels are multi-subunit transmembrane proteins that play crucial roles in synaptic transmission in the nervous system. These include the Cys-loop receptor superfamily, the ionotropic glutamate receptor (iGluR) family, and the purinergic P2X receptor family. Binding of specific neurotransmitters at the ligand-binding site triggers a series of conformational changes that ultimately leads to ion channel opening. This dissertation describes three molecular-scale functional studies on these receptors.

The first project (**Chapter 2**) describes structure-function studies of the conserved Phe-Pro motif in the Cys loop of the nicotinic acetylcholine receptor (nAChR) of the Cys-loop superfamily. Both residues were substituted with natural and unnatural amino acids. A strong interaction between the Phe and Pro residues is evident, as is a preference for aromaticity at the Phe site. Hydrophobicity is preferred at both sites. A correlation between receptor function and the *cis* bias at the proline backbone suggests a significant role for the *cis* proline conformer in receptor function.

The second project (**Chapter 3**) concerns the key binding interaction of memantine, a prescribed drug for Alzheimer's disease, on the *N*-methyl-*D*-aspartate (NMDA) receptor of the iGluR family. The data suggest that the

special property of memantine as an NMDA receptor blocker stems from the presence of the two methyl groups and a proper shape-matching to the binding site. Comparing affinities of memantine and amantadine, a structurally related drug, in response to pore mutations allows an identification of the methyl group binding pockets on the NMDA channel pore.

The final project (**Chapter 4**) involves a study of inhibitory crosstalk between two families of ion channels: α6β4-containing nAChRs and P2X receptors. When these two distinct receptors are co-expressed, their properties are modulated from their normal behavior when expressed alone. The effect is constitutive and does not require channel activation. When they are co-activated by their respective agonists, the observed current is smaller than the sum of the currents evoked by individual application of their agonists. This functional interaction between these nicotinic and purinergic receptors in dorsal root ganglion neurons is proposed to be involved in pain sensation.

Table of Contents