Chemical-Scale Studies of Ligand-Gated Ion Channels

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Dedicated to my parents:

Dennis and Jean McMenimen

And in memory of Fae Eloise Miller

Acknowledgements

Chemistry is all about getting lucky.

-Robert Curl

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Abstract

The studies discussed in this dissertation are aimed at the chemical-scale interactions involved in neuroreceptor structure and function. Unnatural amino acids were incorporated into several ligand-gated ion channels. Two different ionotropic glutamate receptors (iGluRs), the N-methyl-D-aspartate (NMDA) receptor and the α–amino-3-hydroxy-5-methyl- 4-isoxazolepropionic acid (AMPA) receptor were studied, along with an acetylcholine receptor - the nicotinic acetylcholine receptor (nAChR), and all were analyzed with electrophysiology–an assay of receptor function.

In Chapter 2, a highly conserved tryptophan (Trp607) in the ion channel pore of the NMDA receptor was investigated for its role during extracellular Mg^{2+} block. Previous studies hypothesized that a cation-π interaction between NR2BW607 and Mg^{2+} contributed to the receptor blockade. However, our studies suggest that Trp607 is not involved in a cation-π interaction with Mg^{2+}, instead it is a structural component of the pore. NR2B Trp607 acts as a steric "plug," preventing Mg^{2+} permeation through the ion channel. These studies were the first to incorporate unnatural amino acids into a glutamate receptor, extending the scope of nonsense suppression methodology to a new class of neuroreceptors.

Chapter 3 describes the incorporation of unnatural amino acids into the ligand binding domain (LBD) of NMDA and AMPA receptors. Previous structural studies of AMPA receptors established the overall topology of the LBD to be a clamshell, two domains clamp down around a central cleft. Further studies utilizing agonists that induce full receptor activation and partial receptor activation demonstrate a relationship between cleft closure and agonist efficacy, which is the ability to activate a receptor. Full agonists correlate with more cleft closure than partial agonists, which induce less cleft closure. To examine this relationship, we used unnatural amino acid mutagenesis to convert an NR2-conserved tyrosine to homotyrosine and an NR1 glutamine to homoglutamine, residues designed to disrupt clamshell closure by expanding the side chain without altering its functionality**.** The development of our functional probe demonstrates that the clamshell closure mechanism, previously shown for AMPA receptors, likely also applies to NMDA receptors, but to different degrees in the NR1 and NR2 subunits.

Finally, in Chapter 4 we use unnatural amino acids, mutagenesis, and computational simulations to probe the binding interactions that are involved in agonist selectivity at the muscle-type nicotinic acetylcholine receptor. Acetylcholine (ACh) and nicotine, both agonists for nAChRs, have a high potency for neuronal receptors. However, nicotine is a weak agonist for the muscle-type nAChRs, yet the amino acids that contribute to the binding site remain the same between both types of receptors. These studies use mutagenesis and unnatural amino acids to introduce changes in the muscle-type receptor to increase nicotine potency. Although some of the mutations increase nicotine potency, none of the mutations result in a muscle-type receptor with nicotine potency as great as the neuronal receptors. A second set of studies generated a mouse muscle homology model and used molecular dynamics to simulate movements in the receptor with and without agonist bound. These structures demonstrate the importance of a hydrogen-bonding network that contributes to the pre-organization of the aromatic box.

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