Chemical-Scale Studies of the Nicotinic and Muscarinic Acetylcholine Receptors

Thesis by

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In memory of my grandparents:

Margaret and Ronald McCann

Virginia and Carl Torrice

Acknowledgments

The struggle itself towards the heights is enough to fill a man's heart. One must imagine Sisyphus happy.

- Albert Camus

My six years of pushing the stubborn rock of graduate school uphill have come to an end. Thankfully, I've had many sets of hands helping me shove.

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Abstract

This dissertation describes three chemical-scale studies of neuroreceptor structure and function. Incorporation of unnatural amino acids into two acetylcholine receptors—the nicotinic acetylcholine receptor (nAChR) and the M_2 muscarinic acetylcholine receptor (M_2AChR)—and an electrophysiology assay of receptor function were performed in each of the studies. The nAChR is a ligand-gated ion channel (LGIC) and the M_2AChR is a G-protein-coupled receptor (GPCR).

In Chapter 2, a highly conserved aspartate residue (D89) that is near the agonist binding site of the nAChR was probed for its role in agonist binding. We found that the side chain of D89 establishes a redundant network of hydrogen bonds and preorganizes the agonist binding site by positioning a critical agonist-binding residue, tryptophan 149 (W149). Previous studies of a D89N mutant led to the proposal that a negative charge at D89 was essential for receptor function. However, our studies show that neutral side chains at position 89 function well, only if an unfavorable electrostatic clash is avoided.

Chapter 3 describes our attempts to incorporate unnatural amino acids into the M_2AChR, a GPCR. GPCR activity is assayed through second messenger signaling pathways, unlike the direct readout assays of LGICs. These second messenger pathways require significant amounts of optimization to create assays that produce reliable and robust data. In our experiments, variability of dose-response relationship data between batches of cells was the most significant concern. Several factors were investigated to reduce this batch-to-batch variability. After a reliable means to assay M_2AChR function

was found, we performed a preliminary search for tryptophan residues in the agonist binding site that form a cation-π interaction with acetylcholine.

Finally, in Chapter 4, we discuss the use of hydroxy acids to scan the αM1 transmembrane helix of the nAChR for residues that undergo structural rearrangements during gating. Hydroxy acids disrupt hydrogen bonding in protein backbones and thus provide a means to detect backbone interactions that form or break during gating. The hydroxy acid analog of valine, valic acid (Vah), was incorporated at ten positions along the αM1 helix. Backbone mutations at five residues on the intracellular side of a conserved proline (P221) produced shifts in dose-response relationships.

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