

Structure-Function Studies of Nicotinic Acetylcholine Receptors Using Unnatural Amino Acids

Thesis by

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To my loving mother and father:

Cynthia Jean Puskar and James Michael Puskar

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It feels like only yesterday when my mom and grandpa drove me across the United States with everything I owned in the back of my grandpa's pick-up truck. During these past five-and-a-half years I have grown immensely not only as a scientist, but also as a person. And for that, I would like to personally thank the special people who helped me along the way.

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ABSTRACT

This dissertation primarily describes structure-function studies of the nicotinic acetylcholine receptors (nAChRs). These studies use a combination of unnatural amino acid mutagenesis and electrophysiology to determine the specific molecular interactions required for neurotransmitter binding to nAChRs.

Chapter 2 examines the mode of agonist activation for the $\alpha 4\beta 2$ nAChR, the receptor responsible for nicotine addiction. This study investigates the molecular interactions that differentiate the $\alpha 4\beta 2$ receptor from other receptor subtypes and endow it with the ability to mediate nicotine addiction. We report that the high affinity for nicotine at the $\alpha 4\beta 2$ receptor is a result of a strong cation- π interaction and a strengthened backbone hydrogen bond to a conserved tryptophan (TrpB) of this receptor. We also establish that a point mutation just four residues away from TrpB appears to influence the shape of the agonist binding site, such that it can differentiate the agonist binding mode of the $\alpha 4\beta 2$ and muscle-type receptors.

Chapter 3 extends studies of the point mutation near TrpB, termed the “loop B glycine.” We examine the muscle-type, $\alpha 4\beta 2$, and $\alpha 7$ subtypes and show that the identity of this residue strongly correlates with agonist potency. Low-potency receptor subtypes have a glycine at the loop B site, while high-potency receptors have a lysine at this site. We establish that mutation of this residue can convert a low-potency receptor to a high-potency receptor and vice versa.

Chapter 4 investigates the agonist binding mechanism of the $\alpha 4\beta 4$ receptor. We show both ACh and nicotine make a strong cation- π interaction to TrpB, and nicotine makes a strong hydrogen bond to the backbone carbonyl of TrpB. Additionally, chimeric

β subunits are used to examine the influence of the complementary binding component on receptor pharmacology for the $\alpha 4\beta 2$ and $\alpha 4\beta 4$ receptors.

Last, chapter 5 is a methodology-based project focused on optimizing the incorporation of unnatural amino acids into mammalian cells. Using HEK293T cells, we successfully suppressed an amber stop codon using HSAS, an *in vivo* aminoacylated tRNA. Additional studies will pursue the viability of *in vitro* aminoacylated tRNAs for nonsense suppression in mammalian cells.

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