Chemical Scale Investigations of Drug-Receptor Interactions at the Nicotinic Acetylcholine Receptor

Thesis by

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The thing that goes the farthest towards making life worthwhile,
That does the most and costs the least, is just a pleasant smile

- A friend of Gigi’s

I first read this quote in an autograph book of my grandmother Gigi’s and it has stuck with me ever since. After a quick Google search, I now know it was originally written by Wilbur Nesbit. Anyone who works with me knows I usually have a smile on my face. My experiences at Caltech have brought many smiles to my face. For that, I have many people to thank!

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Abstract

Biological signaling pathways employ a vast array of integral membrane proteins that process and interpret the chemical, electrical, and mechanical signals that are delivered to cells. Among these proteins, ligand gated ion channels (LGIC) are therapeutic targets for Alzheimer’s disease, Schizophrenia, drug addiction, and learning and memory. High-resolution structural data on neuroreceptors are only just becoming available, yet the functional importance of particular structural features can be challenging.

The primary focus of the present work is to gain a chemical scale understanding of the ligand-receptor binding determinants of LGICs. In particular, these studies explore drug-receptor interactions at the nicotinic acetylcholine receptor (nAChR), the most extensively studied members of the Cys-loop family of LGICs. The present study utilizes in vivo nonsense suppression methodology to perform chemical scale investigations of nAChR agonist activity.

The binding of three distinct agonists–acetylcholine (ACh), nicotine, and epibatidine–to the nicotinic acetylcholine receptor (nAChR) has been probed using unnatural amino acid mutagenesis. ACh makes a cation-π interaction with Trp α149, while nicotine employs a hydrogen bond to a backbone carbonyl in the same region of the agonist binding site. The nicotine analogue epibatidine achieves its high potency by taking advantage of both the cation-π interaction and the backbone hydrogen bond.

Nonsense suppression was also utilized to probe the importance of residues outside of the binding box in nAChR function. These studies demonstrate a structural role of the highly conserved αD89 residue in stabilizing the agonist binding site near αW149. In addition to outer shell residue, αK145 is shown to be important for proper nAChR
function. In combination with additional evidence from other recent advances, this site is proposed to be important in initiating the nAChR channel gating pathway.

Residues outside the aromatic binding site were also examined through computational protein design studies. Results from these studies identify outer shell mutations 116Q and 57R (AChBP numbering) that enhance nAChR specificity for nicotine, over ACh and epibatidine compared to wild-type receptors.

Finally, a series of cationic polyamides were shown to enhance polyamide affinity while maintaining specificity by varying the number, relative spacing, and linker length of aminoalkyl side chains.
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