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

Thesis by

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In Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

California Institute of Technology
Pasadena, California
2006
(Defended February 23, 2006)

Acknowledgements

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!

I would like to thank my advisor, Dennis Dougherty. Without asking any questions, he accepted me into his group after candidacy my second year. I could not ask for a better mentor than Dennis. He shows a genuine interest in his students both on a professional level and on a personal level. Even without the kind words from his wife, Ellen, it is evident that Dennis truly enjoys working with students and being a teacher. His broad scientific knowledge and his ability to interpret experimental results amaze me. I appreciate his support in giving me the freedom to explore professional growth opportunities both inside and outside of lab.

It has been nice to work with Henry Lester as well. Collaborations with the Lester group have taught me a lot about biology and about the importance of analyzing every bump on an electrophysiology trace. I would also like to thank my thesis committee, Linda Hsieh-Wilson, David Tirrell, and Doug Rees, for their time and commitment.

Daily life at Caltech was also enjoyable because of the members of the Dougherty Lab. From home-made group videos, to chair races, to Strong Bad e-mails, to fantasy sports, to the matching game, there was always welcomed entertainment in lab. When I first joined the lab, the friendly personalities of Niki Zacharias and Sarah May made me feel right at home. Josh Maurer and Gabe Brandt helped me catch up to speed by teaching me lab techniques and showing me the importance of picking good projects. James Petersson and Darren Beene taught me a lot about experiments as well. James's passion for science and his ambitious goals are inspiring. I also enjoyed getting to know Darren and his adorable family. The lab post-doc, David Dahan, was also a great resource for any electrophysiology advice. I always enjoyed working with Don Elmore and I truly miss his music selections in lab. It has been a lot of fun working with Steve Spronk as well. I will always remember his excitement about his courtship of his now wife, Tiffany. Tingwei Mu is a kind and generous person and I know he will continue to do great things in life.

The current Dougherty group members are continuing the lab spirit. Lori Lee, a fellow defector to the lab, makes a great chocolate cake. I wish this California girl the best of luck during the Michigan winters of her post-doc. Erik Rodriguez will undoubtedly continue to improve the lab methodology. It has been a pleasure collaborating with Mike Torrice the past few months. His ability to crack himself up always makes me smile. I have enjoyed getting to know Amy Eastwood. Her commitments to running and to "The Best Team Ever" are motivational. The lab is also lucky to have Katie McMenimen around. Her love of science is evident and I have enjoyed becoming friends with her the past few years. Joanne Xiu is very energetic and fun to be around. Her ability to learn new techniques will undoubtedly advance the lab.

Kiowa Bower has also been fun to get to know. After getting used to his personal style, I have really enjoyed our conversations and his passion for the mountains. I can easily sleep at night knowing that Ariele Hanek is taking good care of the Opus. Kristin Rule's energy, as evidenced by her role as the ultimate Caltech Chemistry Cheerleader, will continue to bring her success during her studies at Caltech. Jinti Wang will surely continue to hold the record for the highest number of mutations studied in the lab. Jai Shanata and Kay Limapichat are welcomed newcomers to the lab. Jai is bravely taking on single cannel studies that will undoubtedly add depth to our research. I would also like to thank members of the Lester Lab. Rigo Pantoja, Purnima Deshpande, Nivalda Pinguet, Stephanie Huang, John Leite, and Fraser Moss have been great resources and a pleasure to work with.

I am also thankful for the time that I spent in Peter Dervan's lab my first two years at Caltech. Peter's enthusiasm and his energy are unlike any other. Clay Wang, Doan Nguyen, and Eric Fechter made my time in the lab more enjoyable and I am thankful for their advice and support. I am thankful for Clay, my first mentor at Caltech, for teaching me molecular biology skills and how to run the perfect gel. Doan, pursuing a career at the interface of science and business, remains a role model for me today. Eric is the ideal classmate and friend. Another classmate, Ben Edelson was also important to me. I will always remember my collaboration with Ben and his joy of sharing his knowledge with others. He is missed.

Outside of lab, I have had the opportunity to make great friends and to experience Caltech beyond my research experience. My friendship with Jessica Mao has extended from TA'ing, to research collaborations, and to a terrific partnership in leading the Caltech Biotech Club. Xin Qi, Christie Morrill, and Anand Vadehra have also become

great friends. The relationships I have built and the exposure that I have gained from my experiences with the Caltech Biotech Club are invaluable. I am grateful to the club founders, Dee Datta and Jessica Mao, for bringing this long-needed club to Caltech.

I save my final thanks for my family. The continued encouragement of my parents and their value of education brought me to where I am today. I thank my grandparents for their support. I thank my mother for her love and support. I thank my big-sister Diane and brother-in-law Marcus for their love and encouragement. I thank Pete Choi whose love and friendship continues to make me smile. I look forward to sharing our futures together.

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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