STRUCTURE AND FUNCTION STUDIES OF THE HUMAN DOPAMINE RECEPTORS

Thesis by

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This thesis is dedicated to my biological and scientific parents:

Mohammad Kalani, LL.M., and Afrouz Mehrazarin, LL.M.

William A. Goddard, III, Ph.D., Kendall N. Houk, Ph.D., and Vaidehi Nagarajan, Ph.D.

Acknowledgments

I hereby would like to thank all those who have played a part in my development as a member of the society and the scientific community.

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The next chapter of my life will take me to medical school at Johns Hopkins University School of Medicine where I will pursue my studies towards a Doctor of Medicine degree. Thank you to all who supported me as I prepared for medical school. Most notably, I would like to thank Drs. G. Jennifer Wilson, Robert N. Schwartz, Dominic Thomas, David Cohen (RIP), Steve Hardinger, and Steven G. Clarke.

This thesis is appropriately dedicated to the individuals whose support, love, and dedication have prepared me for a career as a medical scientist: Mom, Dad, Bill, Ken, and Vaidehi.

Abstract

Dopamine neurotransmitter and its receptors play a critical role in cell signaling process responsible for information transfer in neurons functioning in the nervous system. Development of improved therapeutics for such disorders as Parkinson's and schizophrenia would be significantly enhanced with the availability of the three-dimensional (3-D) structure for the dopamine receptors and of the binding site for dopamine and other agonists and antagonists. In this thesis, I report the 3-D structures of the 5 subtypes of the human dopamine receptors, predicted from primary sequence using first principles theoretical and computational techniques. I use the term "first principles" to mean that we do not use the high resolution crystal structure of rhodopsin as a template, nor do we use homology modeling or threading of any kind to determine the structure. Predicting the binding sites, and the relative binding affinities of endogenous ligands and various pharmaceuticals to the 5 receptors validates the predicted structures. These structures correctly predict the critical residues for binding dopamine and several antagonists, identified by mutation studies and give relative binding affinities that correlate well with experiment. The predicted binding site for dopamine and agonists is located between transmembrane helices (TM) 3, 4, 5, and 6, while the best antagonists bind to a site involving TM helices 2, 3, 4, 6, and 7 with minimal contacts to TM 5. We identify characteristic differences between the binding sites of agonists and antagonists, as well as factors that cause differential binding to the 5 subtypes of the human dopamine receptors.

This thesis consists of five chapters that have, or will shortly result in publications. The first chapter is a brief introduction to the field, the motivation for the project, my scientific contributions, and contribution of others on the team. Chapter two introduces the methods and their successes at reproducing experimentally known results for the human D_2 dopamine receptor; it discusses, in great detail, the active site of pharmaceutical agonists and antagonists to the human D_2 dopamine receptor, and highlights the strengths and shortcomings of homology modeling for membrane bound proteins; this chapter will be submitted for publication to the *Journal of Molecular*

Biology. Chapter three reports the results of a blind study performed in collaboration with Aventis Pharmaceuticals. For this study, we were provided with the two-dimensional structure of 9 antagonists and were asked to predict their binding sites, binding affinities, and to explain the differential binding of the ligands to the human D_2 and D_3 dopamine receptors and the human α1A adrenergic receptor. The results of this study are in preparation for submission to the *Journal of Medicinal Chemistry*. Chapters four and five of the thesis give preliminary results of comparative studies of the agonist and antagonist binding sites of the five subtypes of the human dopamine receptors. Chapter 6 contains results of another blind study on the G2A receptor with Professor Owen Witte.

In addition to the six main chapters, this thesis contains 6 independent appendices that report results of similar studies in other systems. The first 2 appendices are work that has already been published. The remaining 4 appendices will shortly result in publications, but at this time, they are not publication worthy; these appendices represent data that has been analyzed but has not been written in paper format.

In addition, I would like to make note of the studies that I have conducted on the 9 subtypes of the human adrenergic receptors with Mr. Peter Freddolino, the 4 human histamine receptors that were conducted with Mr. Freddolino and Mr. Maziyar Kalani, and the 4 G2A-like lipid receptors conducted with Mr. Rene Trabanino, Dr. Radu, Dr. Yang, and Professor Owen Witte of the Howard Hughes Medical Institute at the David Geffen School of Medicine at the University of California, Los Angeles.

Annotated Résumé of Yashar Kalani

Career Goals and Objectives: I intend to work as a Professor at a large research institution, combining my skills in Physical Chemistry with Biology and Medicine.

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Publications:

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References:

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