

Levandoski Lab, Summer 2008

Modulator Binding Sites on Nicotinic Acetylcholine Receptors

We are interested in the behavior of nicotinic acetylcholine receptors (nAChRs), in particular the interactions of these proteins with their ligands and the conformational changes they undergo. The nicotinic receptors are proteins that mediate chemical communication between excitable cells. As the site of nicotine action in the central nervous system, these receptors are involved in the effects of tobacco smoking, and have been implicated in disease states such as epilepsy, schizophrenia and Parkinson's and Alzheimer's diseases. A related research interest is the use of thermodynamic and kinetic analyses of binding specificity and allostery for these proteins. Our research involves the areas of protein biochemistry, molecular neurobiology, and physical chemistry, and utilizes techniques of electrophysiology, biochemistry and molecular biology.

One nicotinic receptor is a large cell membrane-spanning complex of five homologous protein subunits (e.g., $(\alpha 3)_2(\beta 4)_3$). Each receptor has two binding sites for acetylcholine, the endogenous neurotransmitter (= ligand). The nAChRs are ligand-gated ion channels, meaning that when acetylcholine released from a neighboring nerve cell binds to the receptor, a pore opens within the protein complex, allowing ions to flow through the membrane. We are interested in how various ligands like acetylcholine and nicotine bind to the receptor and cause channel opening, and in how the knowledge of drug-receptor specificity might be utilized to better understand higher-order problems such as the function of nAChRs in the brain.

Projects 1 and 2. Nicotinic Receptors in Zebrafish Brain

The drugs levamisole and morantel are used to treat parasitic infections of worms, and they act at the nicotinic receptors of (at least) the worm muscle. We have discovered some interesting pharmacological effects of these drugs on human and rat neuronal nAChRs: Their primary effect is to act in concert with acetylcholine, causing more opening of the channel than when the receptors are treated with acetylcholine alone; we call this enhanced activity *potentiation*. However, at high enough concentrations, the effect is opposite to that of acetylcholine, leading to a block of channel opening. In very recent work which we have submitted for publication, we were able to show that the underlying mechanism for morantel potentiation is more efficient channel gating once the activating ligands have bound (and we ruled out the alternative explanations). We are now poised to complete another major part of the project which aims to identify the binding sites for morantel on nicotinic receptors. Importantly, levamisole and morantel are but two members of a class of *modulatory* compounds, another of which may even be nicotine.

We approach our questions experimentally using pharmacological analysis of nAChR subtypes following their expression in frog oocytes. Using a simple electrophysiological technique, the current that passes across the oocyte membrane when nAChRs respond to an application of acetylcholine (channel opening) can be measured. Since in the functional assay we measure these modulatory effects indirectly, we can use a second approach – radioligand binding experiments – in order to observe binding of the compounds directly. A third approach combines these techniques but employs a set of chimeric or mutant subunits that should allow for identifying residues of the protein that interact with (bind to) the compounds. The results of these projects will further our understanding of nicotinic receptor pharmacology and the properties of receptor allostery, that is, the various conformational changes the protein complex undergoes upon interacting with ligands.

In these projects, students can learn: 1) preparation of mRNA coding for nAChR subunits and a micro-injection technique, 2) the electrophysiology technique of two-electrode voltage-clamping, 3) biochemical preparation of oocyte membranes and radioligand binding, and 4) pharmacological binding analysis and mathematical modeling.

Project 3. Nicotinic Receptors in Zebrafish Brain

We have been characterizing the nicotinic receptor proteins of the zebrafish brain; the ultimate aim of these studies is to understand the role of nAChRs in the development of the zebrafish nervous system or to use this animal model in studies of nicotine addiction. In recent years the zebrafish has been increasingly used as a

model system because the embryos are transparent up through about two weeks of life, which greatly facilitates both cellular and molecular methods based on visualization techniques, and because powerful genetic methods, not feasible with other vertebrates, can be applied to many problems using zebrafish. In this project we aim to characterize the pharmacology of cloned neuronal zebrafish genes using the oocyte assay. A related effort will be characterization of native zebrafish nicotinic receptors by biochemical and/or microscopy techniques.

In this project, students can learn: 1) the electrophysiology technique of two-electrode voltage-clamping, 2) some basic techniques of protein purification and analysis, including a type of Western blot assay, 3) some techniques of fluorescence microscopy, and 4) methods for breeding zebrafish and analysis of their neuroanatomy.