

## Modulator Binding Sites on Nicotinic Acetylcholine Receptors Levandoski Group, Spring 2009

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. We are also interested in using thermodynamic and kinetic analyses of binding specificity and allostery to study these proteins. Our research draws on 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.,  $(\alpha_4)_2(\beta_2)_3$ ). 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 can measure such currents (ion flow) and thereby deduce the behavior of the receptor. We want to know how various ligands like acetylcholine and nicotine bind to the receptor and cause channel opening, and 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.

The drugs levamisole and morantel are used to treat parasitic worm infections, 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*. We have shown that the underlying mechanism for morantel potentiation is more efficient channel gating once the activating ligands have bound. In very recent work which we have submitted for publication, we identified the morantel binding site on nicotinic receptors: whereas acetylcholine and similar ligands bind at the interface of  $\alpha(+)$  and  $\beta(-)$  subunits (they are unidirectional), morantel binds at  $\beta(+)/\alpha(-)$  interfaces. As of the summer 2008, we have begun three new directions stemming from this earlier work: 1) further probing of subtype specificity, 2) identifying structural components of inter-subunit information transmission, and 3) identifying structural components of intra-subunit information transmission. 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. Because this assay measures these modulatory effects functionally (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. Some emerging areas in the program may involve more protein biochemistry and possibly fluorescence microscopy.