184-Pos

Selectivity of Binding To Estrogen Receptors Alpha and Beta As Determined by Fluorescence Polarization

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Estrogen's role in cell growth and proliferation has long been appreciated both in the normal development of secondary sexual characteristics and in diseased states in cancers of the breast, ovaries and uterus. We are beginning to appreciate estrogen's expanded role in maintaining such diverse functions as the skin's elasticity, the health of the central nervous system, bone density and cardiac health. Estrogen plays out its roles in varied tissues by binding to two major ligand activated nuclear receptors, estrogen receptor alpha (ER-α) and estrogen receptor beta (ER-β). The interrelationship of the two receptors plays a role in the responsiveness of certain breast cancers to drug treatment. Though the ligand binding sites of the two receptors differ by only two amino acids, the overall degree of homology between ER-α and ER-β is low. The body uses the receptor selectivity to its advantage by dispersing the receptors in varying ratios to different tissues. Of these actions ER-α is thought to be responsible for the majority with ER-β playing a minor role in all and having more significance in the cardiovascular and skeletal systems. Small molecules have been identified which bind to one or the other receptors with differing binding affinities. These selective estrogen receptor modulators (SERMs) hold the potential to be pharmacologically effective in treating diseases specific to one type of estrogen receptor while not affecting the other. For example, ER-a and ER-b are both present in breast tissue, and the ratio of beta to alpha is being examined as one indicator in determining the likelihood of successful treatment of breast cancer by certain drugs. Here we use changes in the fluorescence polarization to calculate binding affinities for each of several small molecules to ER-α and ER-β.

185-Pos

Ion Channel Blockade of NMDA Receptors By Argiotoxins: Identification of Structural Determinats For Subtype Selectivity

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The NMDA family of ionotropic glutamate receptors are ligand-gated ion channels that mediate the majority excitatory synaptic transmission in the brain. Dysfunction of NMDA receptor signalling is involved in a range of neurological and psychiatric diseases for which NMDA receptors are considered important drug targets. NMDA receptors are tetrameric assemblies comprised of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits. Four different GluN2 subunits (GluN2A-D) exist with distinct expression and functional profiles. Identification of subunit-selective NMDA receptor agonists, antagonists, or modulators could prove to be both valuable pharmacological tools as well as potential new therapeutic agents.

Polyamine toxins are a group of small molecules found in spiders and wasps that are open-channel blockers of NMDA receptors. Polyamine toxins have found valuable use as pharmacological tools based on their high affinity and selectivity, but have not yet been explored as templates for the development of NMDA receptor drugs. Argiotoxin-636 (ArgTX-636) is a polyamine toxin isolated from the venom of the orb weaver spider Argiope lobata. ArgTX-636 is among the most potent inhibitors of NMDA receptors and blocks these in a use- and voltage-dependent manner, presumably by binding to the ion channel region. We have recently shown that modifications within the polyamine tail of ArgTX-636 can control subtype selectivity. In this study, we explore the rational design of ArgTX-636 analogs to produce NMDA receptor inhibitors with marked improvement in subtype selectivity. Systematic variation of the polyamine tail lead to identification of ArgTX analogs with pronounced subtype selectivity, such as 100-fold differential potency between GluN2A, GluN2B, or GluN2C versus GluN2D containing receptors. To study the structural basis for NMDA subtype selectivity, compounds were docked into models of the NMDA receptor ion channels.

186-Pos

A Survey for Small Multidrug Resistance Protein Multimerization in the Presence of Ligand Using Sds-Page Analysis

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EmrE and SugE are members of the small multidrug resistance protein family that can efflux quaternary cation compounds (QCC) via proton motive force within the *Escherichia coli* plasma membrane. Members of this integral membrane protein family are characterized by their short (~100-140 amino acids) four transmembrane (TM) alpha- helix conformation and highly conserved glutamate residue within the active site. EmrE protein can confer broad multidrug resistance to the host strain unlike SugE protein, which demonstrates limited multidrug resistance. The exact multimeric state or states of both proteins during transport and ligand binding is not well understood and often yield conflicting results that are specific to the conditions of study. To explore SMR multimerization as influenced by QCC ligands, organic solvent extracted EmrE and SugE protein from *E. coli* membranes were characterized in the detergents, sodium dodecyl sulfate (SDS) and dodecyl maltoside (DDM) at varying protein concentrations. SMR proteins solubilized in both detergents demonstrated a predominately monomeric state but upon increasing particular QCC ligand concentrations resulted in multimer formation or enhancement using SDS- tricine polyacrylamide gel electrophoresis (PAGE). The results from this PAGE based assay demonstrate that: i) SMR multimers are induced by particular ligands that may relate to ligand shape and ii) only EmrE multimerization is induced by particular ligands, whereas SugE appears to be insensitive to drug enhanced oligomerization. Therefore, SMR multimer variability may be dependent upon the nature of the transported substrate and SMR subclass; only EmrE can alter its subunit composition in response to particular QCC substrates. This PAGE based assay provides the framework to explore the influence of diverse QCC substrates for its affects on SMR multimerization.

187-Pos

Towards Identifying the Structural Basis For Inhibition By a Newly Discovered Class of CLC Chloride-Channel Inhibitors

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CLC chloride channels and transporters play diverse physiological roles. Small-molecule inhibitors of the CLCs would be powerful tools to understand and potentially treat a variety of CLC-related human diseases. In addition to their potential therapeutic benefits, inhibitors can be used to trap channels or transporters in particular conformational states, potentially advancing our understanding of the structural basis for CLC activity. Despite their usefulness, specific small molecule inhibitors for CLC proteins are scarce. To address this shortfall, we have embarked on a search for novel inhibitors of the CLC proteins. We have discovered a panel of small molecules active against both the human ClC-K and the prokaryotic ClC-ec1 homologs. Because ClC-ec1 is amenable to structural studies by X-ray crystallography, co-crystallization experiments with these novel inhibitors are in progress. As an alternative method to identifying the inhibitor-binding site, we have synthesized photo-reactive diazirine derivatives of the inhibitors and shown that these photo-affinity reagents specifically inhibit ClC-ec1. Experiments to identify the binding site using protease digestion coupled with mass spectrometry are currently underway. Once located, protein/inhibitor interactions gleaned from the labeling of ClC-ec1 could allow us to rationally design more potent inhibitors of mammalian transporters and channels.

188-Pos

Structural Basis For the Allosteric Mechanism of Serine Protease Inhibition By An Antibody

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The widespread use of antibody therapeutics and the emergence of proteases as compelling disease targets contrast sharply with our poor understanding of how antibodies achieve protease inhibition. Recent structural studies have outlined the mechanism of serine protease inhibition by active site-directed antibodies. However, the molecular basis of allosteric inhibition by antibodies has been elusive. The present study, with three crystal structures and a comprehensive kinetic analysis, now provides a detailed view of how an allosteric antibody inhibits protease activity. We determined the structure (2.35 Å resolution) of the trypsin-like serine protease hepatocyte growth factor activator (HGFA) in complex with the allosteric antibody Ab40, a competitive inhibitor of HGFA catalytic activity. The antibody binds at the periphery of the substrate binding cleft and imposes a conformational change on the entire 99-loop (chymotrypsinogen numbering). Remarkably, a single residue deletion (Trp96H) of Ab40 abolished inhibition of HGFA activity commensurate with the reversal of the 99-loop conformation to its 'competent' state as evidenced from the structure of HGFA/ Fab40.ΔTrp96H resolved at 2.9 Å. In order to understand the precise mechanism by which Ab40 interferes with the proteolytic activity of HGFA, we determined the structure of HGFA/Fab40.ΔTrp96H in complex with substrate-mimicking peptidic inhibitor (Ac-KQLR-chloromethlyketone) at 2.7 Å. Comparison of these three structures led us to propose a model wherein the binding of Ab40 to HGFA and the concomitant change in the conformation of the 99-loop may result in the partial collapse of substrate binding subsite S2 and the reorganization of subsite S4, leading to the inhibition of HGFA activity