approach that allows the ''unbiased'' screening for biological activity of compounds *in vivo* against molecular targets on various types of neurons with cholinergic, glutamatergic or electrical synapses and muscles. For this, we use the Giant Fiber System, which is a simple neuronal circuit that mediates the escape response in the fly. The giant fiber cell bodies and dendrites are localized in the brain and each extends a single axon into the second thoracic neuromere, where it makes a mixed electrical (GAP junctions) and chemical (ACh neurotransmitter) synapse on the tergo trochanteral motorneuron, which further innervates the jump muscle. The GF also connects to a peripheral synapsing interneuron (PSI), which makes a cholinergic synapse onto the dorsal longitudinal motorneurons (DLM). Both the TTM and the DLM neuromuscular junctions are using glutamate as the neurotransmitter. Here, we show that we are able to routinely screen components of the venom of cone snails by injecting then into the fly while continuing the recordings from GF circuit allowing us to instantly determine whether a compound has an effect on neurons or muscles of this neuronal circuit. Components of the venom of cone snails have been shown to elicit a wide range of physiological effects and are well-established neuronal probes or drug-lead candidates. The use of the tiny drosophila (a model organism) to evaluate the activity of conotoxins represents an efficacious in vivo assay that can be expanded to evaluate other compounds.

575-Pos

Probing Interactions Within Anthrax Toxin by Electron Paramagnetic Resonance

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Anthrax toxin, besides its role in the pathogenesis of *Bacillus anthracis*, is also an important model system in understanding how proteins cross cellular membranes. Anthrax toxin consists of three proteins: two enzymes, edema factor (EF) and lethal factor (LF), and a pore-forming protein called protective antigen (PA) that acts as a delivery vehicle for the two enzymes. The toxin enters the cell through endocytosis and is trafficked to the endosome where, upon a decrease in pH, PA inserts into the membrane forming a pore through which LF and/or EF are subsequently translocated. Although the details of PA-assisted translocation are still unclear, biochemical studies indicate that LF binds to the surface of PA with its unstructured N-terminal region (residues 1-26) poised above the entryway of the pore, suggesting that these residues may extend into and bind within the lumen of PA, thus initiating translocation. To probe such putative interactions, we attached a nitroxide spin label to the N-terminal, PA-binding domain of LF (LFn) at a number of positions within the N-terminal region. We then used electron paramagnetic resonance to measure the mobility of these spin labels with LFn alone and in complex with PA. We found that for LFn spin labeled at position 2 or 5 the mobility of the label significantly decreases when LFn is in complex with the PA pore, indicating a binding interaction between these N-terminal residues and the pore. Additionally, translocation-compromising mutations within the PA phenylalanine clamp eliminate the observed interaction between the LFn N-terminus and PA. These results suggest that the LFn N-terminus binds within the lumen of the PA pore, likely at or near the phenylalanine clamp, initiating translocation.

576-Pos

A Cytotoxic Peptide from a Marine Sponge, Polytheonamide B: I. Channel Activity and Vectorial-Insertion Into the Membrane

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A peptide from marine sponge *Theonella swinhoei*, polytheonamide B (pTB), shows potent cytotoxic activity. The cytotoxic activity to various types of cells was examined and found that pTB was most effective to eukaryotic cells. We examined mechanisms underlying the cytotoxic activities of pTB. The amino acid sequence of pTB is unprecedented, having alternative D- and L-amino acid residues throughout the 48-mer peptide. The alternative chiral sequence suggests the formation of a β-helix similar to gramicidin channels, and planar bilayer experiments were performed. pTB forms monovalent cation-selective channels (the selectivity sequence: $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$), which is compatible with the inner pore diameter of ~4 Å for a β-helical structure. The single channel current-voltage curve showed slightly outward-rectifying. Single-channel conductance was 18 pS for symmetrical 1 M CsCl solution. Concentration-dependent macroscopic current amplitude exhibited the Hill coefficient of one, suggesting that the channel is formed by monomer. We found a periodic pattern of unusual amino acids which align on one side of the β-helix and may form a hydrogen-bonded chain through those side-chains. This novel motif may reinforce the long pore structure. pTB penetrated vectorially into the membrane, formed a channel by means of a single molecule and was retained in the membrane. A hydrophobic lead of the pTB molecule may drive a wedge into membrane. Retaining pTB in the first membrane prevents further access to the next membrane in cells with outer membranes, suggesting alleviated cytotoxic activity towards cells of this type.

577-Pos

Real-Time Afm Imaging of Surface-Induced Oligomerization of the Non-Amyloidogenic P3 Peptide: Implications for Membrane Insertion and Ion Channel Formation

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The non-amyloidogenic p3 ($A\beta_{17-42}$) peptide is produced by cleavage of amyloid precursor protein (APP) by α and β secretases. The p3 peptide is present in amyloid plaques and is a main component of cerebellar preamyloid lesions in Down's Syndrome (DS). Its pathogenic potential is just beginning to emerge. Like other amyloidogenic peptides, interaction of p3 with cell membrane surfaces will be a critical determinant in its pathogenicity. This study aims to examine biophysical properties and structures of p3 on different surfaces. Using atomic force microscopy (AFM) and molecular dynamics (MD) simulations, we have studied the adsorption properties of p3 peptides on surfaces with varying degree of hydrophobicity. On hydrophobic graphite surfaces, low peptide concentrations produce parallel fibrils of ~ 5 nm diameter and ~ 1 nm height oriented along graphite superstructures over extended periods of time (~ 5 hr). At higher concentrations, peptides reorient on the surface over time and form a more disordered pattern. The observed structures are modeled as hydrophobic C-terminal β-strands in contact with the graphite surface by MD simulations. Mature fibers were not observed in our study. Because of their hydrophobic nature, p3 peptides either did not adsorb on hydrophilic mica surfaces or adsorbed too weakly to be imaged. Preliminary AFM data suggest an adsorption stage where p3 peptides form small agglomerates on lipid bilayers. These results agree with MD simulations that predict peptide adsorption as a preliminary step to subsequent insertion into the lipid bilayer. The insertion of p3 into the lipidic bilayers is a prerequisite for p3 peptide to form toxic ion channels that we have described previously.

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578-Pos

Designer Ligands Specific for Kv1.3 Channels from a Scorpion Neurotoxin-Based Library

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Venomous animals immobilize prey using protein toxins that act on ion channels and other targets of biological importance. Broad use of toxins for biomedical research, diagnosis and therapy has been limited by inadequate target discrimination, for example, among ion channel subtypes. Here, a synthetic toxin is produced by a new strategy to be specific for human Kv1.3 channels, critical regulators of immune T-cells. A phage-display library of 11,200 novel proteins is designed using the α-KTx scaffold found in 31 scorpion toxins that bind to potassium channels and mokatoxin-1 (moka1) isolated by sorting on purified target. Moka1 blocks Kv1.3 at nanomolar levels that do not impact Kv1.1, Kv1.2 or KCa1.1. Thus, moka1 suppresses CD3/28-induced cytokine secretion by T-cells without cross-reactive gastrointestinal hyperactivity. The 3D structure of moka1 rationalizes its specificity and validates the engineering approach revealing a unique interaction surface supported on an α-KTx scaffold. This scaffold-based/target-biased strategy overcomes many obstacles to production of selective toxins. Success with other toxin scaffolds and sorting with cell-surface targets has extended utility of the approach.

579-Pos

Gramicidin Pores Report the Activity of Membrane-Active Enzymes

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Phospholipases constitute a ubiquitous class of membrane-active enzymes that play a key role in cellular signaling, proliferation, and membrane trafficking. Aberrant phospholipase activity is implicated in a range of diseases including cancer, inflammation, and myocardial disease. Characterization of these enzymes is therefore important, both for improving the understanding of phospholipase catalysis, and for accelerating pharmaceutical and biotechnological