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

applications. This work describes a novel approach to monitor, in-situ and in real-time, the activity of phospholipase D (PLD) and phospholipase C (PLC) on planar lipid bilayers. This method is based on enzyme-induced changes in the electrical charge of lipid bilayers and on the concomitant change in ion concentration near lipid membranes. The approach reports these changes in local ion concentration by a measurable change in the ion conductance through pores of the ion channel-forming peptide gramicidin A. This enzyme assay hence takes advantage of the amplification characteristics of gramicidin pores to sense the activity of picomolar to nanomolar concentrations of membrane-active enzymes without requiring labeled substrates or products. The resulting method proceeds on lipid bilayers without the need for detergents, quantifies enzyme activity on native lipid substrates within minutes, and provides unique access to both leaflets of well-defined lipid bilayers; this method also makes it possible to generate planar lipid bilayers with transverse lipid asymmetry.

Voltage-gated Na Channels I

580-Pos

pH Modulation of the Cardiac Voltage Gated Sodium Channel, Nav1.5

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Alterations in the function of the cardiac voltage-gated sodium channel ($Na_V1.5$) are a known cause of cardiac disease and arrhythmia. Elevated concentrations of protons decrease conductance and depolarize the voltage dependence of activation and steady-state fast inactivation (SSFI) of $Na_V1.5$ channels (Zhang & Siegelbaum, 1991, Khan *et al.*, 2006). A complete analysis of the effects of low pH on $Na_V1.5$ channel kinetics has not previously been reported. We sought to characterize the effects of low pH on $Na_V1.5$ kinetics. $Na_V1.5$ was co-expressed in *Xenopus laevis* oocytes with the β_1 subunit, and currents were recorded at 20 °C using the cut-open voltage clamp technique with the extracellular solution titrated to either pH 7.4 (control) or pH 6.0. Application of solution at pH 6.0 significantly depolarized the voltage dependence of activation and SSFI; -34.4 ± 0.3mV to -25.2 ± 0.2mV and -76.4 ± 0.1mV to -72.7 ± 0.2mV, respectively. The apparent valences of activation and SSFI were significantly decreased; from $3.4 \pm 0.12e$ to $2.5 \pm 0.04e$, and from $-4.6 \pm 0.07e$ to $-4.1 \pm 0.09e$, respectively. At pH 6.0, the fast time constant of use-dependent inactivation was significantly increased and the use dependent current reduction was decreased from $40.6 \pm 0.12\%$ to $34.8 \pm 0.05\%$. The rates of open-state fast inactivation onset were significantly decreased at potentials between −30mV and +30mV, and the rates of recovery at −90mV and −80mV were significantly increased. There was also a visible increase in window current. All effects were reversible upon reperfusion of solution at pH 7.4. Taken together, these data suggest that lowering extracellular pH from 7.4 to 6.0 destabilizes the fast-inactivated state of $Na_V1.5$ channels, an effect that could act as an arrhythmogenic trigger during ischemic events.

581-Pos

Differential pH-Dependent Regulation of NaV Channels

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Brain and skeletal muscle NaV channels play a crucial role in neuronal and muscle excitability. Using whole-cell recordings we studied effects of low extracellular pH on the biophysical properties of rNaV1.2 and hNaV1.4, stably expressed in CHO cells. Activation in both channel isoforms was unaffected at low pH. In hNaV1.4, low pH slightly increased the apparent valence of steady-state fast inactivation and accelerated recovery from the fast-inactivated state, although voltage dependence of fast inactivation was not shifted. Time course of cumulative inactivation in hNaV1.4 was unchanged at pH 6.0. In contrast, both fast and slow inactivation in rNaV1.2 were susceptible to acidification. Consistent with our previous studies, the fast-inactivated state in rNaV1.2 was destabilized at pH 6.0, as suggested first-order two-state Eyring model. Slow inactivation at pH 6.0 was more complete than at pH 7.0 and cumulative inactivation was enhanced at low pH. Thus, our data suggest that pH differentially regulates brain and skeletal muscle NaV channels. This differential regulation might reflect unique physiological roles of these isoforms and tissue-specific distributions of NaV1.2 and NaV1.4 channels.

582-Pos

Recovery of Voltage-Gated $Na_v1.4$ Channels from Slow Inactivation Reflects Memory of Prior Stimulation in Multiple Molecular Processes

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Slow inactivation (SI) regulates availability of voltage-gated Na^+ (Na_v) channels in neurons, cardiac myocytes and skeletal muscle cells thereby determining excitability. In native cells, inactive Na_v channels accumulate when the time between action potentials is inadequate to allow recovery from SI. SI can be simulated with cloned channels by repetitive application of seconds-long depolarizing pulses to cells expressing the channels. Others have shown that, in contrast to voltage-gated K^+ channels, the rate of recovery from SI for $Na_v1.2$ channels (a neuronal isoform) depends on the duration of the previous depolarizing pulse - the channels show memory (Toib et al. 1998. J. Neurosci. 18:1893-903). To investigate this phenomenon in $Na_v1.4$ channels (a muscle isoform), we measure ionic and gating currents in cut-open oocyte mode and employ voltage clamp fluorimetry to correlate motion of each of the four S4 voltage sensing domains with currents. Like $Na_v1.2$, $Na_v1.4$ recovery from SI is found to depend on prepulse duration. A model for memory of prepulse duration is proposed based on the kinetics of processes associated with specific S4 domains and others independent of gating charge movement.

583-Pos

Role of the S4 Charges on Activation Gating of the Sodium Channel

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The conformational changes in the S4 voltage-sensors of the sodium channel on depolarization of the membrane result in gating of the voltage-gated sodium channel. The movement of the positively charged residues of the voltage-sensors in the membrane electric field generates a measurable transient current referred to as the gating current. The four S4 voltage-sensors in the sodium channel are homologous, but non-identical and prior work supports the hypothesis that each of the voltage-sensors may have a different role in the processes of activation and inactivation. In an attempt to characterize the role of each voltage-sensor in the process of activation, we generated a series of mutants in which the first three extracellular charges of each voltage-sensor were concurrently mutated to the neutral amino acid glutamine (Q3 mutants). These mutants show a tetrodotoxin (TTX) insensitive current component at hyperpolarized potentials presumably due to current flow through the voltage-sensors. Charge neutralization of DII resulted in a reduced Cole-Moore shift compared to the wild type channels. Gating current measurements at 15 degrees Celsius show that the rate of charge movement is the most rapid for the DII-Q3 mutant compared to the WT, and other Q3 mutants. These experiments may provide insights into the mechanisms underlying activation gating of the sodium channel.

584-Pos

Arginine Mutations in the S4 VSD of NaV1.4 Associated with Hypokalemic Periodic Paralysis, But Not with Paramyotonia, Create a Gating Pore Conductance

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Hypokalemic periodic paralysis (HypoPP) is a dominantly inherited disorder of skeletal muscle in which attacks of weakness occur as a result of inexcitability from persistent depolarization. Thirteen missense mutations have been identified in HypoPP, and remarkably all occur at arginines in S4 voltage sensor domains (VSD) of sodium channels (NaV1.4, 8 mutations) or calcium channels (CaV1.1, 5 mutations). Prior studies have shown that all five HypoPP mutations in the outermost two arginines of DIIS4 in NaV1.4 cause an accessory permeation pathway or ''gating pore''. Recently, an R1132Q mutation has been identified in DIIIS4 of NaV 1.4 in a family with HypoPP. We used the cut-open oocyte voltage-clamp to demonstrate that rat NaV1.4 R1125Q (homologous to human R1132Q) has a hyperpolarization activated cation current consistent with an accessory gating pore. The amplitude of the current, normalized to total gating charge displacement, was 150 nA/nC at −140 mV in 115 mM $[K^+]_o$. Selectivity for cations was $K^+ > Na^+ >> NMDG^+$. Paramyotonia congenita (PMC), a disorder with prominent myotonia and intermittent weakness with cooling or high $[K^+]_o$, is associated with mutations in NaV1.4 which include VSD mutations at R1448 in DIVS4. Rat NaV1.4 R1441C (homologue of human R1448C PMC mutation) expressed robustly in oocytes but failed to demonstrate any gating pore current. Gating pore currents have been detected for all six HypoPP mutations tested to date, now including the first example in DIIIS4. Importantly, we have also shown that an R→C VSD mutation in DIVS4 associated with paramyotonia, but not HypoPP, does not have a gating pore current. This supports the hypothesis that gating pore currents underlie the abnormal depolarization and paralysis observed in HypoPP.

585-Pos

Nav1.4 Voltage Sensor Residues Immobilized During Fast Inactivation

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We investigated the locus of the charge immobilization in S4 voltage sensing segments of skeletal muscle sodium channels. To do this we compared the