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