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 $+30$mV, 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 $R \rightarrow C$ 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

effects of charge reversing mutations of outer and central arginine or lysine residues using the cut open oocyte configuration. While reversal of charge at R1448 (R1 in DIV S4) produced a depolarizing effect on IV and QV curves, and limited immobilization of the gating charge, the analogous mutation at K1126 (K1 in DIII S4) had no effect. Reversal of charge at R4 and R5 in both DIII S4 and DIV S4 also limited immobilization of the gating charge. Dependence on charge for the effects of mutations at these loci was examined by substituting the native arginine residue with lysine. Charge substitution at R1 in DIV S4 and at R5 in DIII S4 or DIV S4 partially restored QV and gating charge immobilization parameters to the wild type phenotype, whereas charge substitution at R4 in either DIII S4 or DIV S4 did not. Effects of R1457E and R1457K (R4 in DIV S4) on gating charge remobilization suggested that this residue plays a pivotal role in gating charge movement associated with accessibility of the IFMT motif during fast inactivation. This work was supported by NIH R15NS064556-01 to JRG and NIH P20RR16454 to ISU from the INBRE program of the National Center for Research Resources.

586-Pos
Disulfide Locking Reveals a Closed State Interaction Within the Voltage Sensor of NaChBac

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S4 transmembrane segments of voltage-gated ion channels move outward upon depolarization initiating a conformational change that opens the pore. Formation of ion pairs between gating-charge-carrying arginine residues (R1-4) in S4 and negatively charged amino acid residues in neighboring transmembrane segments are thought to catalyze movement of S4. We have demonstrated three open-state interactions of gating charges R3 and R4 with negative charges D60 and E70 in NaChBac using the disulfide-locking method (DeCaen et al. PNAS 2008, 2009). Here, we studied cysteine pairs hypothesized to stabilize the resting state of the voltage sensor. Single mutations D60C or R113C (R1C) yield viable channels and I_{Na}, but the double mutant (D60C:R1C) is not functional when transfected into tsA201 cells. Confocal microscopy of cells transfected with D60C:R1C-eGFP revealed lack of membrane trafficking and sequestration to intracellular inclusions, whereas trafficking of singly mutated channels was comparable to WT. We also studied D60C:L112C which does not impair membrane trafficking but disulfide-locks in the closed state as assessed by whole-cell voltage clamp. This disulfide interaction is abolished by extracellular perfusion with reducing agents BME and TCEP. Evidently, the position of the S4 segment at negative potentials allows disulfide-locking of D60C and L112C, and S4 immobilization at this position maintains the voltage sensor in a resting conformation and keeps the central pore closed. These data suggest that the first gating charge forms an ion pair with D60 in a resting state and their side chains approach within ~2 Å, as required for formation of disulfide bonds. These new molecular interactions allow further refinement of the ROSETTA sliding helix model of NaChBac gating (see poster by Yarov-Yarovoy).
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587-Pos
Fast Real-Time Computation of Na Channel Kinetic Models for Dynamic Clamp

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Voltage-gated Na channels play a critical role in action potential generation and control of the spiking pattern in mammalian neurons. Understanding how Na channels regulate the firing pattern of a particular type of neuron requires not only a good understanding of the sodium channel gating kinetics, but also of the complex interactions between Na currents and all other currents, some of which may be unknown or incompletely characterized. A powerful tool for studying the function of voltage gated ion channels in their cellular context is dynamic clamp, by which individual conductances can be functionally replaced with computational models on a background of otherwise native conductances. We present new computational techniques for both deterministic and stochastic integration by which Markov models with as many as 20-30 states can be solved at rates as high as 150 kHz, while allowing complex data visualization, recording and stimulation in a standard Windows environment. Furthermore, ensembles of as many as 5000 channels can be integrated stochastically at the same rates. The speed relies on efficient use of all available processors, deterministic integration using pre-computed transition matrices, and parallelized, optimized stochastic integration using Gillespie's algorithm. We illustrate the power of the technique with applications of realistic Markov models for sodium channels in central neurons.

588-Pos
Automated Two-Electrode Voltage-Clamp Recording with Additional Compensation Electrode

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Two-electrode voltage-clamp (TEV) of Xenopus laevis oocytes is easily applied for the rapid screening of ion channel function, in particular in pharmacological experiments. However, conventional TEV hardware is not straightforwardly operated by technical personnel because adjustment of the electronics requires considerable practical experience. Moreover, the faithful interpretation of experimental data is often compromised by an incomplete control of all physical parameters determining the voltage-clamp performance. We therefore designed and implemented a hardware/software combination with a built-in 16-bit DA/AD USB interface board providing complete software control of a TEV amplifier featuring full digital calibration and tuning as well as automatic operation via electrophysiological data acquisition software. By means of automated features, such as offset compensation, filter setting, software adjustable amplifier controls (type of controller, gain, response time), and electrode resistance measurements, TEV experiments can be performed in a highly reproducible manner while monitoring the complete set of amplifier control settings. Direct software access to stimulation bandwidth, clamp mode, gain, and response time allows for the objective and automated optimization of voltage-clamp parameters. Two methods for obtaining optimized clamp control parameters will be discussed. An automatic hardware transient compensation increases the dynamic range, particularly important when assaying voltage-gated ion channels. The method is applied for the recording of currents mediated by voltage-gated potassium and sodium channels. In addition to the voltage recording electrode and the current injecting electrode, we implemented a third electrode that injects current in parallel to the second electrode. According to Baumgartner et al. (Biophys. J. 77:1980-1991, 1999), this additional compensation electrode placed in the extracellular space corrects for local current flows and helps improving the voltage clamp performance in big cells such as Xenopus oocytes. Additional operation modes of a third electrode will be presented.

589-Pos
Automated Patch Clamp Electrophysiology Enables the Differentiation of Compound Mode of Action at Na_v Channels

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Differentiation of a compounds mechanism of action and selectivity at different sodium channels is a key goal towards the discovery of novel therapeutics. It remains unclear whether therapeutic targeting of pain blockade should focus on a single Na_V channel type or a combination of subtypes. The interaction between Na_V1.8 and Na_V1.7 in nociceptive sensory neurones is crucial for the transduction of nociceptive signals. Na_V1.8 generates a slow inactivating current with a high activation threshold underlying action potential generation. Na_V1.7 channel has faster kinetics and is involved in amplification of generator potentials in the sensory neurones.
Recombinant hNa_V1.7 cell lines have shown high channel expression and upon activation currents typically >1nA. This current magnitude enables hNa_V1.7 compound profiling on the IonWorks platform. However a hNa_V1.8+β3 sodium channel cell line failed to resolve currents on IonWorks of similar magnitudes, typically <0.2nA.
Using the PatchXpress 7000 automated electrophysiology platform, we were able to resolve hNa_V1.8 currents to enable biophysical characterisation of this receptor. Analysis of current voltage relationships indicated a receptor activation threshold typically −40mV, with a typical peak current of -1.64 ± 0.83nA. $V_{1/2}$ of activation was determined to be -3.37 ± 0.83mV (n=23), and the steady state inactivation $V_{1/2}$ of this channel was -43.7 ± 2.4mV (n=6). Na_V1.7 shows a similar activation threshold typically −50mV, with a mean peak current -2.05 ± 0.18nA (n=24), currents >4nA were excluded from analysis. The Na_V1.7 $V_{1/2}$ of activation was -26.32 ± 3.4mV (n=12) and the steady state inactivation of this channel equalled -62.5 ± 1.6mV (n=8). Biophysical pharmacological profiles for known sodium channel blockers, Amitriptaline, Tetracaine, were determined, as well as the degrees of tonic and use dependent block at both sodium channels. PatchXpress 7000 electrophysiology enables the determination of both biophysical properties and complex mode of action pharmacology at hNa_V1.8.