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

Paul G. DeCaen[1], Chen Yuan Pan[2], Vladmir Yarov-Yarovoy[1], Todd Scheuer[1], William A. Catterall[1].
[1]University of Washington, Seattle, WA, USA, [2]University of Taiwan, Taipai, Taiwan.

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

Lorin S. Milescu.
Harvard Medical School, Boston, MA, USA.

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

Stephan G. Bode[1], Hubert Affolter[1], Christian N. Heinemann[1], Stefan H. Heinemann[2].
[1]HEKA Elektronik Dr. Schulze GmbH, Lambrecht, Germany, [2]Center for Molecular Biomedicine, Department of Biophysics, Friedrich Schiller University Jena, Jena, Germany.

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

Andrew W. Baxter, David Downie, Andrew J. Powell.
GlaxoSmithKline, Harlow, United Kingdom.

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 $hNa_V1.8+\beta3$ 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.