occupancy of the intrapore receptor. The fraction of long-lived states was tightly linked with the degree of receptor occupancy. The findings provide strong support for the *m* gate trapping of LIDO.

1268-Pos Board B112

Molecular Determinants of μ-Conotoxin KIIIA Block of Voltage-Gated Sodium Channels

Jeff R. McArthur[1], Min-min Zhang[2], Layla Azam[2], Songjiang Luo[3], Baldomero M. Olivera[2], Doju Yoshikami[2], Grzegorz Bulaj[2], S. Rock Levinson[3], Robert J. French[1].
[1]University of Calgary, Calgary, AB, Canada, [2]University of Utah, Salt Lake City, UT, USA, [3]University of Colorado, Denver, CO, USA.

mu-Conotoxin (muCTX) KIIIA is of special interest both functionally and structurally because (1) it blocks neuronal voltage-gated sodium (Na_v) channels involved in pain signalling (Zhang et al., 2007, J. Biol. Chem.) and (2) unlike previously discovered muCTXs (most >22 amino acids), KIIIA has only 16 amino acids, missing amino acids in the N-terminal section. We have performed preliminary molecular dynamics simulations of muCTX KIIIA docking to a model of the $Na_v1.4$ outer vestibule (Choudhary et al, 2007, Channels). The results are consistent with a possible binding orientation in $Na_v1.4$ with K7 facing down into the pore, interacting with the outer ring charges (E403 & E758) in domains I and II. To exam this possible orientation, single-channel bilayer recordings from rat brain (preparation includes $Na_v1.1$, 1.2, 1.3 and 1.6) and rat skeletal muscle (muscle, predominantly $Na_v1.4$) preparations demonstrated that when lysine-7 (K7) is neutralized, channels show an increase in fractional residual current (f_{res}) upon KIIIA[K7A] addition (brain, $48 \pm 3\%$ & muscle, $45 \pm 9\%$) compared to wild type KIIIA (brain, $19 \pm 3\%$, muscle $19 \pm 3\%$). The wild-type non-zero f_{res} hints that the lack of N-terminal residues or the use of a lysine residue (instead of arginine) to occlude the pore in KIIIA leads to incomplete toxin block, suggesting KIIIA has a ''looser'' interaction with the channel, with the key basic residue, K7, playing a smaller role in toxin block than in GIIIA and PIIIA. This data is supported by whole-cell experiments looking at KIIIA and KIIIA[K7A] interactions with multiple Na_v isoforms. The single-channel and whole-cell data suggest KIIIA binds to the outer vestibule with the lysine at position 7 blocking current through the pore, similar to R13 in GIIIA (skeletal muscle specific) and R14 in PIIIA (blocks both skeletal and neuronal channels).

1269-Pos Board B113

ProTxII Interacts Specifically with the Domain II Voltage Sensor of $Na_V1.4$ Modifying Gating Without Immobilization

Gabrielle B. Edgerton[1], **Fabiana V. Campos**[1], Kenneth M. Blumenthal[2], Francisco Bezanilla[1], Dorothy A. Hanck[1].
[1]University of Chicago, Chicago, IL, USA, [2]SUNY Buffalo, Buffalo, NY, USA.

ProTxII, a peptide extracted from the venom of the tarantula *Thrixopelma pruriens*, binds to multiple voltage-gated sodium channel isoforms. In $Na_V1.2$ ProTxII reduces the sodium conductance and decreases total gating charge (Sokolov et al., Mol. Pharm. 73:1020). In this isoform the toxin's effect could be partially reversed with strong depolarization indicating interactions with one or more of the voltage sensors. Mutagenesis data further implicated the domain II voltage sensor in the toxin-channel interaction in $Na_V1.2$. Here we have shown that, as was seen for $Na_V1.2$, ProTxII (2-5μM) produced a decrease in maximum conductance (~60%) and a decrease in total gating charge (~20%) in the $Na_V1.4$ isoform expressed in Xenopus oocytes. Unlike $Na_V1.2$, however, these effects on $Na_V1.4$ could not be reversed with strong depolarization. We also discovered a single residue, S660, located at the S3-S4 linker/S4 boundary of DII, which when mutated, renders this channel insensitive to toxin. We used site-specific fluorescent measurements to determine the effect of ProTxII on the movement of individual voltage sensors. In the presence of ProTxII the voltage dependence of the fluorescent signal of DIIS4 was modified, but not eliminated, indicating that this voltage sensor is not completely immobilized upon toxin binding. The fluorescent signals measured from domains I and III were not significantly affected by ProTxII implicating a specific interaction with DII in producing the effect of ProTxII on $Na_V1.4$. Supported by GM30376 (FB) and NS061535-01(GBE).

1270-Pos Board B114

Tryptophan Scanning Mutagenesis to Identify the Residues Involved in Coupling between the Pore and DIII Voltage-Sensor of a Sodium Channel

Yukiko Muroi, Manoel Arcisio-Miranda, Baron Chanda.
University of Wisconsin-Madison, Madison, WI, USA.

In response to membrane depolarization, voltage-gated ion channels undergo a structural rearrangement that moves the voltage sensing segments in the electric field and initiates a series of conformational transitions that ultimately opens the channel pore. The mechanism of coupling between the voltage-sensing domain and pore domain remains poorly understood. To characterize the molecular basis of this coupling, we have systematically substituted tryptophan residues in the S4-S5 region (from C-terminus of S4 to N-terminus of S5) and C-terminus of S6 of domain III in the skeletal muscle sodium channel. The effects of these perturbations on the movement of the voltage-sensor were monitored by using a site-specific fluorescent reporter on S4 of domain III using voltage-clamp fluorometry. Conformational changes in the pore were tracked by measuring the inward sodium currents. Our study identifies a number of mutants, which stabilize the voltage-sensors in the activated conformation while destabilizing the open pore conformation relative to the wild type. We suggest that the residues at these positions play an important role in coupling the voltage-sensor of domain III to the pore of the sodium channel.
Support: National Institutes of Health, American heart Association and Shaw Scientist Award.

1271-Pos Board B115

Double Mutant Perturbation Analysis Reveals High Conformational Stability Of The Domain IV S6 Segment Of The Voltage-gated Na Channel

Rene Cervenka[1], Touran Zarrabi[1], Xaver Koenig[1], Harry A. Fozzard[2], Karlheinz Hilber[1], **Hannes Todt**[1].
[1]Medical University of Vienna, Vienna, Austria, [2]The University of Chicago, Chicago, IL, USA.

The S6 segment of domain IV (DIV-S6) of the voltage-gated Na channel is considered to be a key player in gating and local anesthetic drug block. Thus, some mutations in DIV-S6 substantially alter the channel's inactivation properties. In order to get a comprehensive picture of the kinetic role of DIV-S6 in fast inactivation we performed a cysteine scanning analysis of sites 1575-1591 in the DIV-S6 of the rNav1.4 channel. In addition, we produced the same cysteine replacements in the background of the mutation K1237E. K1237 is located in the P-loop of domain III and mutations at this site have dramatic effects both on permeation and gating properties. Hence, K1237E most likely causes a complex conformational change of the channel. We sought to explore whether K1237E changes the pattern of gating perturbations by the serial cysteine replacements in DIV-S6. The constructs were expressed in *Xenopus laevis* oocytes and studied by means of two electrode voltage-clamp. The half-point of availability following a 50 ms conditioning prepulse (V05) was -44 ± 1 mV and -51 ± 1 mV in wild-type and K1237E, respectively ($P < 0.001$) . Most serial amino acid replacements in DIV-S6 produced shifts in V05, both in wild-type and in K1237E background, ranging from +17 ± 1 mV to -9 ± 2 mV. A plot of the shifts in V05 by single DIV-S6 mutants relative to wild-type versus the shifts in V05 by double mutants relative to K1237E showed a significant positive correlation ($R = 0.72$, $P = 0.002$). This indicates that the general pattern of gating perturbations in DIV-S6 is not affected by K1237E, suggesting a high conformational stability of the DIV-S6 segment during the fast inactivated state. Support: FWF P21006-B11

1272-Pos Board B116

Charge Immobilization From The Open And Closed States Of Voltage-Gated Sodium Channels

James R. Groome.
Idaho State University, Pocatello, ID, USA.

We compared the immobilization of voltage sensor gating charge during open- and closed-state fast inactivation in skeletal muscle sodium channels. To do this we compared charge movement and its immobilization using ionic and gating current recordings in the cut open oocyte configuration. Charge movement and its immobilization were steeply voltage dependent at subthreshold voltages. Kinetics of charge immobilization during open- or closed-state fast inactivation were more rapid than the concomitant decay of ionic current. The extent of charge immobilized was correlated with the completion of closed-state fast inactivation at the most negative pulse commands, and reached a maximum of 2/3 of the ON gating charge. Anthopleurin-A decreased the votlage dependence of charge immobilization compared to wild type channels. Whereas anthopleurin slowed open-state fast inactivation, the toxin accelerated currrent decrement at voltages for which only closed-state fast inactivation was possible. Anthopleurin selectively accelerated remobilzation of the gating charge in fast inactivated channels without slowing the onset or decreasing the extent of charge immobilized. This work was supported by NIH P20RR016454 to ISU.

1273-Pos Board B117

Structure-activity Relationship of Primaquine and Sodium Channel rNav 1.4

Eduardo M. Salinas[1], Evelyn Martinez[1], Alfredo Sanchez[1], Lourdes Millan[2], Thomas F. Scior[3].
[1]Instituto de Fisiologia-BUAP, Puebla, Mexico, [2]Centro de Quimica-BUAP, Puebla, Mexico, [3]Facultad de Ciencias Quimicas-BUAP, Puebla, Mexico.

Mutations in the putative selectivity filter region of the voltage-gated Na^+ channel, the so-called DEKA-motif not only affects selectivity and the