Voltage-gated potassium (Kv) channels undergo a process termed slow inactivation in response to prolonged depolarizations. This process is thought to occur in response to structural rearrangements near the selectivity filter, which ultimately result in nonconducting (inactivated) channels. Although substantial efforts have been made to understand this process, the details of slow inactivation at the atomic level have yet to be unraveled in eukaryotic Kv channels. Based on information gleaned from crystal structures, the indole nitrogen of a highly conserved Trp, Trp435 in Shaker potassium channels, had been proposed to form a hydrogen bond with Tyr445 in the GYGD selectivity filter signature sequence, thus contributing to open pore stability and likely slow inactivation. Functionally, however, we find that the indole nitrogen of Trp435 does not contribute to slow inactivation as mutations to Phe or Tyr at this site do not affect slow inactivation. In contrast, removal of the hydroxyl group from Tyr445 by Phe substitution results in a rapidly inactivating phenotype, and this could be slowed by application of external TEA, thus suggesting a role for Tyr445 in slow inactivation. A closer examination of the available structural information of side chains in close physical proximity of Tyr445 pointed towards a possible role of Thr439, a highly conserved side chain in the pore helix. Indeed, Thr439Val channels were non-conducting and behaved similar to the permanently inactivated Trp434Phe phenotype. Re-introduction of a hydroxyl moiety in position 439 via the Thr439Ser mutation led to a WT-like conductance-voltage relationship and only a modestly increased inactivation rate compared to WT. Together, these results suggest a novel and energetically significant hydroxyl-hydroxyl interaction between Tyr445 and Thr439 that regulates slow inactivation in Kv channels.

631-Pos Board B400

Three Novel Pathways to Stabilize the Conductive Conformation of a Voltage-Gated K^+ Channel

Jose S. Santos, **Ruhma Syeda**, Mauricio Montal.
University of California San Diego, La Jolla, CA, USA.

Voltage-gated K^+ channels (Kv) are tetrameric assemblies in which 4 voltage sensors open a shared K^+ selective pore located at the center of the array at activating membrane potentials. A feature of many Kv channels- indispensable for the repolarization of the membrane potential following depolarization of neuronal cells- is to shut off their flux after prolonged activation. Here we show that in KvLm, a well characterized bacterial voltage-gated channel, the premature closing of the pore is bypassed by the association of the filter gate of the pore with three distinct and novel open-conformation stabilizers: A positively charged lipid, a small-molecule anti-depressant, and a protein toxin. This analysis is based on an extensive set of single channel current measurements of purified KvLm and its sensorless pore reconstituted in symmetric and asymmetric lipid bilayer membranes. Comparative assessment of the consequences produced by the three modulators in relieving the ''inactivation'' establishes that stabilization of the open conformation proceeds in the absence or presence of the sensors. We propose two plausible mechanisms by which all three modulators exert their demands on the pore.

This work was supported by NIH grant GM 49711.

632-Pos Board B401

Locked-Open Activation Gate Prevents the Recovery of Shaker K^+ Channels from Slow Inactivation

Tibor Szanto G, Florina Zakany, Ferenc Papp, Zoltan Varga, **Gyorgy Panyi**.
University of Debrecen, Debrecen, Hungary.

In the absence of N-type inactivation Shaker potassium channels display slow (C-type) inactivation. It has been shown earlier that Cd^{2+} traps the V476C *Shaker* channels in the open state, even at very negative voltages, by forming a metal bridge between a cysteine in one subunit and a native histidine (H486) in a neighboring subunit (Webster et al., Nature, 2004). However, none of the previous studies determined the relationship between inactivation/recovery from inactivation and the locked-open activation gate. The current experiments tested the hypothesis that locking the activation gate in the open configuration prevents recovery from inactivation. To address this hypothesis we compared the extent of recovery from inactivation for control conditions and in the presence of 20 μM Cd^{2+}. V476C/IR channels contained an alanine in position 449 to facilitate the entry of the channels into the slow-inactivated state. All ionic current experiments were performed with excised inside-out patches. A fast-step perfusion system was used for rapid solution exchange. 2.0-s-long depolarizing pulses from a holding potential of −120 mV to +50 mV were applied and when applicable, 20 μM Cd^{2+} was added to the fully inactivated channels. The duration of the Cd^{2+} pulse was 1.0 s long which was started 800 ms after the start of the depolarization. Under control conditions 100% of the channels recovered from inactivation within 60 s at −120 mV, whereas upon Cd^{2+} application less than 10 % of the current recovered under identical conditions (n=3). The lack of recovery from inactivation of the locked-open channels suggests that closure of the activation gate is essential for the recovery from slow inactivation.

633-Pos Board B402

Tuning the Single Channel Conductance of Shaker K-Channels

Ignacio Diaz-Franulic, Gaspar Herrera, Nieves Navarro, David Naranjo.
Centro de Neurociencias de Valparaiso/Universidad de Valparaiso, Valparaiso, Chile.

Potassium channels are membrane proteins that allow passage of K^+ ions across the hydrophobic core of the membrane. The extremely conserved signature sequence in the residues lining the pore (TTVGYGD) allows discrimination between ions having similar radii (Hegimbotham et al 1994). Despite of this conservation, closely related potassium channels display differences of up to two orders of magnitude in their K^+ ion transport rates. Searching for the molecular determinants accounting for such a large difference in transport rates, we made several charge substitutions at the internal entrance of the pore of the low conductance Shaker K-channel. We expressed these variants in Xenopus oocytes and measured their single channel conductance in inside-out membrane patches in 100 and 1000 mM of symmetrical [K^+]. The substitution of Pro475 by Asp or Gln increases Shaker's unitary conductance 8 and 4 fold in 100 mM K^+, respectively, as shown earlier (Sukhareva, et al., 2003). But at 1000 mM K^+ the increases are 4 and 3 fold, respectively. In these high conductance backgrounds (475D or P475Q), we also introduced either negative or positively charged side chains (Asp or Arg) to positions 476 and 479. These positions align with the negatively charged rings that electrostatically control conductance in BK channels. Our results shows that, as in BK and KcsA channels, electrostatics at the inner mouth of the pore plays an important role on increasing the conductance of this Shaker variant, suggesting similar architecture in the pore of low and high conductance K-channels.

Financed by Fondecyt 1120819 and the Millennium Initiative (P09-022-F), IDF is a Mecesup Doctoral Fellow.

634-Pos Board B403

Dependency of Ion Permeation Pattern on K^+ Concentration through the Kv1.2 Channel

Takashi Sumikama[1], Shinji Saito[2], Shigetoshi Oiki[1].
[1]University of Fukui, eiheiji-cho, Yoshida-gun, Fukui, Japan, [2]Institute for Molecular Science, Okazaki, Aichi, Japan.

It is widely known that ions and water molecules alternately permeate (Pattern A) through the selectivity filter of the K^+ channels. However, microscopic mechanisms underlying the exclusive use of the Pattern A remain unsolved. Here we performed the molecular dynamics simulation of ion permeation through the Kv1.2 channel at several different K^+ concentrations. We found that the main permeation pattern is, as is expected, Pattern A at all concentrations, while the other kinds of pattern also emerge. At low concentrations, Pattern B, where an ion is transported accompanied with two water molecules, is observed. This behavior is consistent with the recent experiment measuring the streaming potential. On the other hand, Pattern C, where ions are transported without intervening water molecules, is also seen at high concentrations. Pattern C has been reported by some theoretical studies. At the physiological concentration, Pattern B and C, as well as Pattern A, are nearly equally used. In order to clarify why Pattern B and C emerge, the positions of the ions in the selectivity filter were analyzed. In the case of Pattern B, when a next incoming ion approaches the channel, ions are located at the downstream of the selectivity filter, thus two water molecules, having been occupied the selectivity filter, are franked by ions. Once an incoming ion enters into the selectivity filter that bears ions at the upper stream, no water molecule is inserted between the ions, and Pattern C emerges. Thus, the macroscopic observation such as the water-ion coupling ratio reflects differences in the microscopic ion distributions in the selectivity filter at the instance of an upcoming ion enters.

635-Pos Board B404

Defining the Interactive Surfaces of Tarantula Toxins for Voltage Sensors in Kv Channels

Maryam Zamanian[1], Chan Hyung Bae[2], Jae Il Kim[2], Kenton Swartz[1].
[1]National Institutes of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA, [2]Department of Life Science, Gwangju Institute of Science and Technology, Gwangju, Korea, Republic of.