sensing domain, and depends on the presence of K^+, leading to an *Oscillating Gate Hypothesis*: the open gate swings between a configuration in which it complexes a K^+, driving the central K^+ upward to the selectivity filter, and a relatively open state in which the K^+ moves up to the cavity center, while the gate awaits another K^+. The closed gate has protons on the H418, repelling K^+ and keeping the gate shut. This also accounts for the connection of the voltage dependent gate to the selectivity filter, and thus inactivation, a phenomenon noted by several groups, and for the dependence of the conductivity on intracellular K^+ concentration. With certain sets of ions, a proton pathway with water forms, while it does not with others.

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An Oscillating Gate Hypothesis for the Potassium Channel: Quantum Calculations on the Vestibule

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Quantum calculations (DFT) on the vestibule, or cavity, region of the potassium channel, with 12, 14, 16, and 18 water molecules, plus a K^+ ion, give the free energy profile for the ion at five positions: the center of the cavity, and 2, 4, 5 and 6 Å above this position. This is sufficient to show that 16 or 18 water molecules are approximately the correct number, and that the ion must move up a free energy gradient to reach the selectivity filter. The conductivity of the channel depends on the intracellular K^+ concentration (LeMasurier et al, JGP,**118**, 303, 2001); their data, replotted, shows a linear dependence of log σ on log(K^+ activity); log(K^+ activity) is proportional to the ion free energy, implying a free energy barrier to K^+ conductivity, as calculated in this work. We show that this is most easily understood with an *oscillating gate* that alternates complexing K^+ and releasing it (see Abstract, Kariev and Green: The Switch at the Potassium Channel Gate: Quantum Calculations Comparing Open and Closed States.) The amount of water in the cavity largely controls the energy barrier, which is approximately 20 kcal. The calculations show substantial charge transfer to the K^+, which at the center has a charge of only 0.8 - 0.85 e. However, this would suffice to repel an ion entering the gate, leading to a reverse ''knock-on'' effect, hence no conductivity.
However, a complexed ion at the gate could push the central K^+ up to the selectivity filter, leaving space for the ion at the gate to follow to the cavity center, repeating the cycle. This is also a part of the oscillating gate hypothesis.

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Modulation of Kv1 Voltage-Gated Potassium Channels by Sodium Channel Beta Subunits

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Sodium channel beta subunits (SCN1b-SCN4b) are integral members of voltage-gated sodium channel (VGSC)-complexes at nodes of Ranvier, axon initial segments, and cardiac intercalated disks, where they modulate the function of VGSCs. Mutations of these genes results in neurological (e.g. epilepsy) and cardiovascular (e.g. Brugada syndrome) diseases. Here we report that SCN1b modulates the Kv1-subfamily of K^+ channels, each in a unique fashion, when co-expressed in *Xenopus* oocytes or mammalian cells (Table). SCN2b, but not SCN3b, has similar modulatory properties. Pull-down experiments show that SCN1b is physically coupled to Kv1 channels. Using chimeras of SCN1b and the myelin Po protein, we demonstrate that the external domain of SCN1b is essential for channel modulation. Two known epilepsy-causing mutations in the Ig-domain of SCN1b, R85C and C121W, disrupt Kv1 channel-modulation. Thus, sodium channel beta subunits may regulate action potential firing and propagation in normal and diseased conditions by modulating the function of both VGSCs and Kv channels.

Ca-activated Channels

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State-Dependent FRET Reports Large Gating-Ring Motions in BK Channels

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Large conductance voltage- and calcium-dependent potassium channels (BK channels) are key regulators of many important physiological processes and a key feature to their physiological role is that the channel's open probability is regulated both by changes in transmembrane voltage and by intracellular calcium concentration. The voltage sensor resides within the transmembrane region of the channel, while Ca2+ binding is sensed by a large C-terminal intracellular region, where eight Regulator of Conductance for K+ (RCK) domains form a ''gating ring''. Calcium binding to this region reduces the energy required to open the channel, but the exact mechanism underlying this process is still uncertain. Structural studies using isolated gating rings from prokaryotic channels and a biochemical study of the isolated gating ring from the human channel suggest that Ca2+ binding expands the gating ring. The large movement of the gating ring would physically pull and open the gate located at the pore domain. In the present study we investigate the calcium and voltage-dependence of conformational changes in the intact human BK channel by patch-clamp recordings and simultaneous measurements of fluorescence energy transfer between CFP and YFP variants of the green fluorescent protein, inserted into three sites in the BK gating ring. Depending of the site studied, different movements are detected that differ in their Ca- and V-dependence. Here we show that Ca2+ binding produces surprisingly large structural changes that, contrary to current theories, are not obligatorily coupled to the opening of the pore and are not strictly cooperative. Instead, a mechanism such as the ''flip'' transitions that have been identified in pentameric neurotransmitter receptor-channels is operative.

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BK and Ca_V Channel Interactions in the Plasma Membrane

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Activation of the human voltage- and Ca^{2+}-gated (BK, Slo1) K^+ channel is triggered by both depolarization and increases in intracellular Ca^{2+} concentration. BK channels and different types of voltage gated (Ca_V) channels associate in the cell membrane such that Ca^{2+} entering via Ca_V channels activates BK channels. Protein co-localization and distribution at the plasma membrane level are typically addressed by fluorescence microscopy. We seek to gain insight into the relative proximity and positioning of BK channels and different types of Ca_V channels ($Ca_V1.2$ or $Ca_V2.2$) in the membrane by fitting to a mathematical model ionic current recordings obtained from *Xenopus* oocytes co-expressing BK and Ca_V channels. Prior to voltage clamp, the oocytes were injected with known concentrations of EGTA or BAPTA. Using the cut-open oocyte voltage-clamp technique, oocytes were subjected to a protocol consisting of two 80 mV pulses, separated by a −70 mV or 0 mV intermediate pulse of variable duration. The magnitude of the BK channel currents, during the second 80 mV pulse, depended on the amplitude and duration of Ca^{2+} entry during the intermediate pulse, as well as intracellular Ca^{2+} buffering conditions. Under the same experimental conditions, $Ca_V2.2$ channels activated BK channels more efficiently than $Ca_V1.2$ types, as suggested by the longer-lasting potentiation of the BK current when $Ca_V2.2$ and BK channels were co-expressed. We are interpreting the degree of physical association in view of a mathematical model that computes the activation of Ca_V and BK channels, as well as intracellular Ca^{2+} dynamics, and allows for varying density and relative distance between these channels. This model may be useful for predicting the relative distribution of Ca_V and BK channels in native cells.

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Calcium and Heme Induce Distinct Conformational States of the Human BK (Slo1) Channel Gating Ring

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The human BK channel gating ring (GR) is the site of action of numerous signaling molecules such as Ca^{2+} and heme, which ultimately modulate pore