Our previous studies revealed a key role for the Ca^{2+}-activated K^+ (BK) channel in determining the spontaneous contractions of detrusor smooth muscle (DSM) in mammals. Using tissue baths and isometric smooth muscle tension recordings, we examined the contribution of BK channels in the neurogenic contractions of DSM strips isolated from rats and Guinea pigs. Neurogenic contractions were induced by increasing electrical field stimulation (EFS) frequencies (0.5-50 Hz) under stimuli with constant amplitude (20 V), pulse width (0.75 ms), and duration (3 s). These EFS-induced contractions were abolished by the Na^+ channel inhibitor, tetrodotoxin (1 ∈ 1/4M), indicating their neurogenic origin. Blocking the BK channel with its specific inhibitor, iberiotoxin (200 nM), caused a statistically significant increase in the EFS-induced contraction amplitude at all stimulation frequencies in both species. We further dissected the BK channel contribution to the cholinergic and purinergic neurogenic contractions using specific neurotransmitter receptor inhibitors. Atropine (1 ∈ 1/4M) was used to block the cholinergic component and a combination of suramin (10 ∈ 1/4M), and α,β-meth-ATP (10 ∈ 1/4M), was used to block the purinergic component of the EFS-induced neurogenic contractions. In both species, blocking the BK channel caused a statistically significant increase in both cholinergic and purinergic components of the EFS-induced neurogenic contraction amplitude. However, the BK channel contributed more to the purinergic component. With a blocked BK channel, the maximum response of the purinergic component was doubled and achieved at lower frequencies of stimulation. Time controls were performed for each experimental series to confirm the stability of the preparations. The data indicate that the BK channels function to oppose neurogenic contractions in rat and Guinea pig DSM. Supported by NIH DK084284 & DK070909.

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The Putative Ca2+-Acting Site in ANO1

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Ca^{2+}-activated Cl- channels (CaCC) mediate numerous physiological functions including vectorial Cl- movements across epithelia. Anoctamin1/TMEM16A (Ano1) confers Ca^{2+}-activated Cl^- currents. ANO1 having TMs with a putative pore region between TM5 and TM6 shows similar biophysical as well as pharmacological properties as those known for endogenous CaCCs. More importantly, ANO1 is activated by intracellular Ca2+ with EC50 of 2.6 microM at −60 mV. Furthermore, ANO1 activation is also voltage dependent as known for endogenous CaCCs. Because functional importance lies on its activation by intracellular Ca2+, the Ca2+ action site in ANO1 was determined with mutagesis. However, unlike other Ca2+-activated channels such as BK channels, there is no consensus sites for Ca2+ binding except one region that shows weak sequence homology with the Ca2+ action site in BK channel. This region contains many negatively charged amino acids. When we deleted 14 amino acids including the highly negatively charged region, the mutant ANO1 was rarely activated by intracellular Ca2+ with right shift of G-V curves, indicating that this region is important for Ca2+ action. With various mutants in this region, we can localize a sensitive site for Ca2+ response. However, when negatively charged amino acids were replaced by alanine, this mutant showed a comparable sensitivity to Ca2+. Judging from the experimental results of chimera studies with other ANOs, we can conclude that Ca2+ action on this site is essential for its activation.

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Heme-Driven Conformational Changes in the Human Slo1 BK_{Ca} Channel Gating Ring

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Large-conductance voltage- and Ca^{2+}-activated K^+ channels (BK_{Ca}) are regulated by signaling molecules acting on two intracellular Regulator of K^+ Conductance (RCK) domains which assemble into a gating ring superstructure. The discovery of heme as a BK_{Ca} channel modulator has unveiled novel aspects of its physiological function (Tang et al., *Nature* 2003). A recent study (Horrigan et al., *JGP* 2005) suggests that heme induces conformational changes within the BK_{Ca} gating ring altering its interaction with the voltage sensor.

Here, we report direct evidence of heme-induced conformational transitions in the purified human BK_{Ca} gating ring, expressed and purified from *E. coli* as reported previously. Its assembly into physiologically-relevant homotetramers (~310kDa) was confirmed by size-exclusion chromatography. Heme-binding properties of the gating ring were studied under physiologically-relevant conditions using steady-state and time-resolved fluorescence spectroscopy in combination with biochemical methods. Heme binding to BK_{Ca} gating ring was detected using UV-visible absorption spectroscopy, which revealed a strong peak at 420nm (Soret band) and a peak at 550nm (α/β-band), characteristic of heme-protein complex formation. The gating ring's Tryptophan fluorescence decreased by up to $44 \pm 1.5\%$ with the addition of heme in a dose-dependent manner ($K_{0.5}$=211 ± 24.0nM, n=2.7 ± 0.10). In contrast, addition of protoporphyrin IX (900 nM), a heme analog lacking Fe, did not affect the Tryptophan fluorescence intensity. Time-correlated single-photon counting (TCSPC) spectroscopy resolved heme-induced structural alterations of the gating ring. Heme binding reduced the average excited-state Tryptophan lifetime from ∉″$_{avg}$=2.6ns (no heme) to ∉″$_{avg}$=1.6ns ([heme]=687nM), revealing an altered conformational state. Effects of heme and Ca^{2+} on fluorescence lifetime were not additive as, after saturating Ca^{2+}-induced effect were reached (35∈ 1/4M), addition of saturating [heme]=687nM produced minimal effect (∉″$_{avg}$=1.5ns). In summary, we demonstrate heme-induced conformational transitions in the human BK_{Ca} gating ring, likely correlated to the modulation of channel activity.

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MthK Gating Explored with a High Affinity Quaternary-Ammonium Blocker

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The bacterial potassium channel MthK is activated by Ca^{2+} binding to intracellular RCK-domains. The channel is homologous to the K^+ conductive pore and C-terminal Ca^{2+} binding domains of the eukaryotic BK channel but lacks the N-terminal voltage-sensing domain. A low resolution x-ray crystal structure showed the MthK pore in a proposed open conformation, consistent with the presence of Ca^{2+} in the crystals (Jiang et al. Nature, 2002). It is widely assumed that the closed state of the channel involves an intracellular constriction of pore-helices as seen in the KcsA crystal structure (Doyle et al. Science, 1998; Jiang et al. Nature, 2002). However, several ligand-gated K^+ channels, including the BK channel, have been proposed to use the selectivity-filter near the extracellular side and not an intracellular constriction as the conduction gate (Wilkens and Aldrich, JGP, 2006). We tested the presence of an intracellular gate in MthK by using a quaternary-ammonium (QA) blocker, bbTBA. QA blockers bind within the aqueous vestibulethat lies between the proposed intracellular gate and the selectivity-filter in KcsA and other voltage-gated potassium channels (Armstrong and Hille, JGP, 1972; Holmgren et al. JGP, 1997; Zhou et al. Nature, 2001; Lenaeus et al. NSMB, 2005; Yohannan et al. JMB, 2007). We measured the bbTBA binding affinity to the open channel and have studied the state-dependence of channel block using single-channel recording in artificial bilayers. Preliminary data indicate the channel can close while the blocker remains bound. Further experiments involving macroscopic recordings may distinguish whether the blocker is trapped inside the closed channel or has state-independent binding.

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Mechanism Underlying pH-Modulation of Ca^{2+}-Dependent Gating in the MthK Channel

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MthK is a Ca^{2+}-gated K^+ channel whose activity is modulated by cytoplasmic pH. To determine possible mechanisms underlying the channel's pH sensitivity, we recorded current through MthK channels, which were purified from E.coli membranes, reconstituted into liposomes and then incorporated into planar lipid bilayers. Each bilayer recording was obtained at up to six different [Ca^{2+}] (ranging from nominally 0 to 30 mM) at a given pH, in which the solution bathing the cytoplasmic side of the channels was replaced via a perfusion system to ensure complete solution exchanges. We observed a steep relation between [Ca^{2+}] and open probability (Po), with a mean Hill coefficient (n_H) of 9.9 ± 0.9. Neither the maximal Po (0.93 ± 0.005) nor n_H changed