Ca-activated Channels

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Using Ca_V-Type Dependent BK Channel Behavior to Probe BK-Ca_V Channel Coupling and Submembrane [Ca^{2+}] Dynamics

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Voltage- and Ca^{2+}-gated K^+ (BK) channels and voltage-gated Ca^{2+} (Ca_V) channels assemble into macromolecular complexes such that Ca^{2+} entering through Ca_V channels during membrane depolarization activates BK channels. To understand the [Ca^{2+}] dynamics underlying BK channel activation, we used the cut-open *Xenopus* oocyte preparation to record BK channel currents activated by Ca^{2+} entering the cell through co-expressed $Ca_V1.2$ or $Ca_V2.2$ channels at an imposed buffering condition of 5 mM EGTA. We found that $Ca_V1.2$ and $Ca_V2.2$ channels exhibit distinctly different functional coupling to BK channels: $Ca_V2.2$ activated BK channels more potently than $Ca_V1.2$, as evidenced by a larger magnitude and faster activating I_{BK} (τ_{act} = 0.20 ±0.02 ms and τ_{act} = 0.29 ±0.04 ms, respectively). Moreover, when I_{BK} amplitude was reduced by reducing the number of available Ca_V channels (by imposing different degrees of Ca_V channel inactivation), the I_{BK} activation kinetics in the presence of $Ca_V2.2$ remained unchanged but slowed in the presence of $Ca_V1.2$ channels. These results strongly suggest that each BK channel is functionally coupled to one $Ca_V2.2$ channel but to several $Ca_V1.2$ channels. We have also determined the P_O and activation kinetics of BK channels using inside-out patch clamp recordings in different known fixed [Ca^{2+}]. Based on these properties and measured Ca^{2+} currents for each Ca_V type, we are fitting a 3-dimensional diffusion-reaction model combined with a kinetic BK channel model to the I_{BK} data. Ultimately, we will adjust the parameters of this model to fit comparable recordings from the more physiologically-relevant synapses formed in *Xenopus* nerve-muscle cell co-cultures.

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BK_{ca} Channel Alpha Subunit Variants are Differentially Modulated by the Beta-1 Subunit

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Uterine myometrial cells are maintained in a quiescent state during pregnancy, in part due to hyperpolarization of the membrane by the large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel. During labor, uterine contractility is increased and BK_{Ca} current is suppressed, however, expression of BK_{Ca} is increased, suggesting that multiple mechanisms regulate BK_{Ca} channel function. We have focused on the roles of an auxiliary β1-subunit and two N-terminal α-subunit BK_{Ca} variants, whose peptide sequences start at different methionines, MANG and MDAL. We found that whereas MDAL expression was equivalent in all samples, MANG was expressed at a higher level in myometrium from term laboring women than from term non-laboring or non-pregnant women. To examine the possibility that differential expression of these variants reduces BK_{Ca} channel activity at the onset of labor, we expressed the MANG and MDAL variants, with or without the β1-subunit, in a heterologous system and recorded currents using patch-clamp. Although whole-cell voltage-gated currents were similar between variants, the β1-subunit enhanced Ca^{2+} activation of MDAL but not MANG. Inside-out single-channel recordings confirmed that MANG currents were not modulated by β1. Our finding that β1 co-immunoprecipitated with both MDAL and MANG variants suggests that the longer N-terminus of MANG does not affect the interaction between BK_{Ca} α- and β1-subunits, but instead inhibits the functional modulation of the channel by β1. These observations suggest that BK_{Ca} variants are differentially regulated by auxiliary subunits to regulate myometrial contractility during pregnancy and labor.

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BK Gene Disruption Enhances Acute Renal Vascular Response to Angiotensin II

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Renal vascular resistance is involved in the regulation of blood pressure through its contribution to the overall peripheral resistance and role in salt/fluid balance. Renal vascular tone is determined by the equilibrium between vasoconstrictive and vasodilative forces in renal arterial smooth muscle cells, where Angiotensin II type 1 receptor (AT1R) and BK channels are functionally coupled. In the present study, we investigated the role of BK in the maintenance of arterial blood flow and in its response to Angiotensin II (ANG II) stimulation. To this end, we used BK knockout (BK-/-) mice and ultrasound imaging to measure renal and aortic blood flow. In right renal arteries, peak systolic blood flow velocities (PSV) were similar in wild type, wt (502 ± 122 mm/s, n=5) and BK-/- (482 ± 64 mm/s, n=4) mice. End-diastolic velocities (EDV) also showed no differences (EDV_{wt}=180 ± 125 mm/s vs. $EDV_{BK-/-}$=181 ± 135 mm/s). Renal arterial resistive indexes (RI=[PSV-EDV]/PSV) appeared to be at the same level (RI_{wt}=0.63 ± 0.11, n=5 vs. $RI_{BK-/-}$=0.63 ± 0.05, n=4). Likewise, in abdominal aorta there were no significant differences in PSV, EDV, and RI. After administration of ANG II to wt mice (s.c., 500 ng/g body weight), right renal arterial PSV was reduced to 30.9 ± 5.5% (n=6) of its initial value and recovered to 77.7 ± 7.9% after 1 h. Remarkably, in BK-/- (n=3) PSV was reduced to 15.9 ± 0.4%, and only recovered to 37.6 ± 0.4% within the same time frame. Our findings show that loss of BK function does not cause alterations in basal arterial blood flow but critically potentiates the vasoconstrictor action of ANG II in renal arteries. Supported by NIH.

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Omega-3 Fatty Acids Activate Slo1 BK Channels and Lower Blood Pressure

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Long-chain omega-3 fatty acids such as docosahexaenoic acid (DHA) found in oily fish may offer various health benefits but the underling mechanisms are only poorly understood. In vascular smooth muscle cells, large-conductance Ca^{2+}- and voltage-dependent K^+ (Slo1 BK) channels provide a vasodilatory influence. We found that DHA with EC_{50} of ~500 nM directly and reversibly activates BK channels composed of the pore-forming Slo1 subunit and the auxiliary subunit β1 in excised-patches, increasing currents by up to ~20-fold. The DHA action does not require voltage-sensor activation or Ca^{2+} binding but depends on an electrostatic interaction within 1 or 4. DHA acutely lowers blood pressure in anesthetized wild-type but not in Slo1 knockout mice. DHA ethyl ester (DHA EE), found in dietary supplements, fails to activate BK channels and antagonizes the stimulatory effect of DHA. On an equimolar basis, the stimulatory effect DHA on Slo+1 channels was greater than that of eicosapentaenoic acid, alpha-linolenic acid, arachidonic acid, or linoleic acid. Slo1 BK channels are thus receptors for long-chain omega-3 fatty acids that, unlike their ethyl ester derivatives, activate the channels and lower blood pressure. Supported in part through the NIH, DFG FOR 1738, and Shanghai Science and Technology Commission.

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Global Fit to Model Modulation of Voltage and Calcium Activated K-Channels by Auxiliary ß Subunits

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Calcium and voltage activated potassium (BK) channels are regulated by a multiplicity of stimuli including voltage,intracellular calcium, and phosphorylation; they may even act as hormonal receptors. The prevailing view is that the different gating mechanisms are allosterically coupled in the sense that the processes involved in channel opening influence each other but are not coupled obligatorily. In most instances the pore forming subunit of these channels is associated to one of four alternative ß subunits that appear to target specific gating mechanisms for regulating the channel activity. To identify the relative impact on the different sensors, gates and/or allosteric factor coupling these gates, a detailed and comprehensive kinetic model becomes necessary. Furthermore, to quantify the different transition rates and hence the energy landscape connecting the different conformational states is necessary to include data from very different types of experiments such as direct recording of the movement of the voltage sensor (gating currents), macroscopic ionic currents and single channel activity. Each one of these types of signals provides information on a reduced number of states. Here, by developing a global fit strategy of complex models combining gating and ionic currents recording, we were able to indentify an additional step in the path to channel opening that become relevant only when the pore forming subunit of the channel co-exist with ß1 or ß2 but not the ß3 subunit.