Voltage- and Ca^{2+}-gated, large conductance K^+ (BK) channels are ubiquitously expressed and play a key role in linking membrane excitability to Ca^{2+} signaling. In most tissues, BK channel complexes consist of channel-forming α and regulatory β subunits. Each β contains short intracellular N- and C-termini, and two transmembrane domains (TM1, TM2) joined by an extracellular loop. The smooth muscle-abundant BK β1 increases the channel's apparent Ca^{2+} sensitivity and is required for channel activation by cholane steroids (*Brenner et al., 2000; Bukiya et al., 2009*). The participation of different β1 regions in regulating channel function has not been fully settled. Here, we used BK channel-forming cbv1 and β1 subunits cloned from rat cerebral artery myocytes, neuron-abundant β4, and chimeras constructed by swapping regions between β1 and β4 to determine the contribution of specific β1 regions to the β1-containing BK phenotype. After co-expressing cbv1 with *wt* βs or chimeric βs in *Xenopus* oocytes, macroscopic currents were evoked by 200 ms-long, 10 mV depolarizing steps from −150 to +150 mV (V_H=-80 mV). At Ca^{2+} levels found near the BK channel during smooth muscle cell contraction (10 μM), channel complexes that included chimeras combining both TMs from β1 with remaining regions (EC loop, IC ends) from β4 showed a phenotype (V_{half}, τ_{act}, τ_{deact}) identical to that of channel complexes containing *wt* β1. However, this phenotype could not be evoked by channel complexes that included chimeras combining either β1 TM1 or β1 TM2 with the remaining β4 regions (TM2-EC loop-IC ends and TM1-EC loop-IC ends, respectively). We conclude that at physiological voltages and Ca^{2+}, both TMs from β1 are necessary to confer the characteristic current phenotype of β1-containing BK channels. Support: R37-AA11560, R01-HL10463 (AMD).

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Phosphorylation of a Constitutive Serine (S642) in BK Channel Variants Containing the Alternate Exon 'SRKR' Alters Current Properties

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Kcnma1, the gene encoding the pore forming subunit of the BK Ca^{2+}-activated K^+ channel, undergoes extensive alternative splicing across tissues, tuning BK current properties. Phosphorylation and β subunit association further contribute to diverse BK current properties, but the interplay of these mechanisms have not been well-investigated in BK variants cloned from native tissues. We used RT-PCR to identify two *Kcnma1* splice variants in the mouse suprachiasmatic nucleus (BK_0 and BK_{SRKR}). Both variants contain an alternate translation start (MANG) compared to the canonical start (MDAL), no insert at splice site 2 (STREX), an insert at site 3 (near the Ca^{2+} bowl), as well as an alternate C-terminus (VYR). However, they differ at splice site 1, containing a four amino acid SRKR alternate exon located near the end of the RCK1 domain. Voltage-clamp recordings from the variants expressed in HEK293 cells revealed that BK_{SRKR} channels produced BK currents with distinct properties from the insert-less BK_0 variant, with significantly right-shifted G-Vs, slower activation, and faster deactivation kinetics at 0, 1, and 100 μM Ca^{2+}. Co-expression of the β4 subunit enhanced these differences in a Ca^{2+}-dependent manner. Inclusion of SRKR resulted in two predicted phosphorylation sites in BK_{SRKR}, S642 (a constitutive residue present in both variants) and S644 (within the SRKR exon). Alkaline phosphatase treatment and mutations at both positions revealed that phosphorylation of S642 is both necessary and sufficient to produce the observed differences between BK_0 and BK_{SRKR} current properties. These data demonstrate that phosphorylation of S642 underlies the differences in current properties between BK_{SRKR} and BK_0, and suggests the alternate exon SRKR serves to enhance S642 phosphorylation in native tissues.

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Uncovering Mitobk$_{Ca}$ Channel Mitochondrial Translocation Mechanisms

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The mitochondrial large-conductance Ca^{2+}-activated K^+ channel (mitoBK$_{Ca}$) of adult cardiomyocytes is encoded by the Kcnma1 gene and contains a C-terminal 50 amino acid insert (DEC) essential for mitoBK$_{Ca}$ targeting. We tested the hypothesis that DEC may bind to the mitochondrial protein import system. To this end, we used recombinant GST-DEC and GST as baits to immunoprecipitate proteins using mitochondrial lysates from ventriculocytes (8 rats/experiment, n=3). Mitochondrial proteins bound to GST-DEC and GST (control) were analyzed using 1-D in gel electrophoresis and LC-MS/MS. After subtracting GST-bound proteins from those binding to GST-DEC, we found that DEC peptide interacts with more than 200 different mitochondrial proteins with scores >21. These include components of the outer membrane TOM complex, TOM70 (2 peptides, n=1), TOM22 (1 peptide, n=2), and TOM40 (2 peptides, n=1); of the inner membrane TIM23-PAM complex, GrpE (4 peptides, n=1); and matrix proteins involved in folding, Hsp60 (6 peptides, n=1) and "import signal" cleavage, MPP (mitochondria processing peptidase) subunits A (6 peptides, n=1) and B (6 peptides, n=1). Interestingly, DEC also interacted with SAM50 (10 peptides, n=2), a component of the outer membrane insertion machinery. The results suggest that BK$_{Ca}$-DEC may use a novel import pathway as DEC associated with TOM70 and TIM23-PAM, which differs from the established TOM70→TIM22 and TOM20→TIM23-PAM pathways. In the new TOM70→TIM23-PAM pathway, BK$_{Ca}$-DEC would be translocated across the outer and inner membranes reaching the matrix where BK$_{Ca}$-DEC would be processed by MMP and correctly folded by Hsp60 for its final insertion to the inner membrane. Since MMP is known to be a cleavage signal peptidase, the results also indicate that DEC might serve as a cleavable C-terminal targeting signal. The unexpected finding of SAM50 association with DEC, suggests that BK$_{Ca}$-DEC could also be targeted to the mitochondrial outer membrane.

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Proteomic Analysis Identifies MaxiK Channel Intracellular Partners from Human Coronary Artery

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The large conductance voltage and calcium-activated potassium (MaxiK) channel, a key modulator of vascular tone, interacts with several types of proteins (e.g. regulatory subunits, G-protein coupled receptors, signaling kinases). In this study, we searched for MaxiK channel partners in human coronary artery using a proteomic approach. A MaxiK polyclonal antibody and 3 coronary arteries from explanted hearts were used to immunoprecipitate MaxiK. IgG was used as negative control. Immunoprecipitated proteins were separated by 1-D SDS-PAGE; subsequently, gel bands were excised for trypsin digestion and LC/MS/MS analysis. As positive control, we searched for MaxiK peptides and found 7 peptides (93 amino acids) from MaxiK C-terminus. No peptides of MaxiK were found in IgG negative controls (n=3). Various groups of intracellular proteins that form complex with MaxiK channels were identified. A group of proteins are cytoskeleton proteins, which include coronin-1A, PACN2, tubulin beta-3 chain and beta-centractin. PACN2 plays a role in the formation of flask-shaped caveolae at the plasma membrane and coronin-1A is involved in cell locomotion. Another group of proteins are linked to transcription and mRNA-splicing. These proteins are TFIID subunit 9B, DDX1, and pre-mRNA-splicing factor SYF1. Mitochondria metabolic proteins were also identified as MaxiK partners including ADP/ATP translocase and PCCB (Propionyl-CoA carboxylase beta chain). In addition, we found that Hsp90 co-chaperone CDC37 (CDC37), WASL and WASL-interacting protein family member 1 (WIPF1) associate with MaxiK. These three proteins also form complex with Hsp90. WASL and WIPF1 are involved in the formation of actin filament and cell motility. Hsp90 binds to WASL regulating actin polymerization. CDC37 and Hsp90 assemble to stabilize protein kinases. In summary, proteomic analysis identified intracellular proteins forming complex with MaxiK channel and the underlying functions for these interactions need further investigation. Supported by NIH.

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All-or-None Effect of γ_1 Auxiliary Subunit on BK Channel Gating

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Ca^{2+}-and-voltage-gated K^+-channels (BK) are symmetrical complexes whose minimal functional unit is a tetramer composed of four identical α-subunits. BK α-subunits can coassemble with auxiliary subunits which drastically influence channel gating. Two families of BK auxiliary subunits have been described, β and γ subunits. Up to four β-subunits can assemble with the α-subunits in a BK channel complex, where each individual β-subunit contributes an identical additive increment to the total β-induced gating shift. At present, the stoichiometry of the assembly of α and γ subunits is unknown. Here we show that, in contrast to gating shifts produced by β-subunits, the γ_1-effect on BK channel is an 'all-or-none' type of functional regulation. The presence of a single γ_1 subunit in BK channels is enough to produce the full γ_1-induced gating shift, although probably more than one γ_1 can assemble in a BK channel complex. Our results describe an uncommon example of asymmetric functional regulation of a symmetric oligomeric protein. (Supported by NRSA F32 GM103138 to VGP and GM081748 to CJL).

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Cytosolic Activation Dynamics in the KV Channel Probed by a Fluorescent Unnatural Amino Acid

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