Myristic and/or palmitic acid incorporation to proteins is a mean by which cells tether proteins to the intracellular leaflet of plasma membranes. Two types of protein myristoylation have been reported; one occurs co-translationally at the N-terminus (e.g. c-Src) and the other post-translationally at an internal amino-acid residue. Here, we tested whether Slo1 might undergo post-translational myristoylation as it lacks an N-terminal consensus site for myristoylation. HEK-293T cells expressing Slo1 or c-Src (positive control) were metabolically radiolabeled with [^{3}H]-myristic acid and subjected to immunoprecipitation; radiolabeled proteins were detected by autoradiography. Our data show that Slo1 incorporates [^{3}H]-myristic acid (n=5) via a post-translational mechanism as assessed by the lack of effect upon inhibition of protein synthesis with cyclohexamide. As control, cyclohexamide treatment reduced c-Src myristoylation confirming its co-translational incorporation (n=3). Next, we sought to determine what type of chemical bond is involved in Slo1 protein myristoylation. Hydroxylamine (NH_2OH) at pH10 but not Tris-HCl at pH10 (negative control) or NH_2OH at pH 7, cleaves hydroxyester bonds. Treatment of **[^{3}H]-**myristoyl-Slo1 with NH_2OH, pH10 but neither treatment with Tris-HCl at pH10 nor NH_2OH at pH7, completely removed incorporated myristic acid from Slo1 (n=3). Possible palmitoylation of Slo1 via a thioester bond was excluded because treatment of labeled Slo1 with NH_2OH at pH7 which cleaves thioester bonds or 1.4 M β-mercaptoethanol, a reducing agent, did not alter the signal. Further, we did not observe Slo1 labeling using [^{3}H]-palmitate (n=2). These data strongly support an involvement of a hydroxyester chemical bond between myristic acid and Slo1 S/T/Y residue(s). In conclusion, we show for the first time that Slo1 protein is post-translationally myristoylated at an internal site. This myristoylation might play a role in controlling Slo1 channel structure, function or trafficking. Supported by NIH.

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Palmitoylation Controls BK Channel Regulation By Phosphorylation

Lijun Tian, Owen Jeffries, Heather McClafferty, Adam Molyvdas, Iain Rowe, Fozia Saleem, Lie Chen, **Michael J. Shipston**.
University of Edinburgh, Edinburgh, United Kingdom.

Large conductance calcium- and voltage- gated potassium (BK) channels are important regulators of physiological homeostasis and their function is potently modulated by protein kinase A (PKA) phosphorylation. PKA regulates the channel through phosphorylation of residues within the intracellular C-terminus of the pore-forming α-subunits. However, how PKA phosphorylation of the α-subunit effects changes in channel activity are unknown. The STREX variant of BK channels is inhibited by PKA as a result of phosphorylation of a serine residue within the evolutionary conserved STREX insert. As this inhibition is dependent upon phosphorylation of only a single α-subunit in the channel tetramer we hypothesised that phosphorylation results in major conformational rearrangements of the C-terminus. Using a combined imaging, biochemical and electrophysiological strategy we have defined the mechanism of PKA-inhibition of BK channels. We demonstrate that the cytosolic C-terminus of the STREX BK channel uniquely interacts with the plasma membrane via palmitoylation of evolutionary conserved cysteine residues. PKA-phosphorylation of STREX dissociates the C-terminus from the plasma membrane resulting in channel inhibition. Abolition of channel palmitoylation by site-directed mutagenesis or pharmacological inhibition of palmitoyl-transferases prevents PKA-mediated inhibition. Thus PKA inhibition of BK channels is conditional upon the palmitoylation status of the channel. Palmitoylation and phosphorylation are both dynamically regulated thus cross-talk between these two major post-translational signalling cascades provides a novel mechanism for conditional regulation of BK channels. Interplay of these distinct signalling cascades has important implications for the dynamic regulation of BK channels and the control of physiological homeostasis.

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Bovine and Mouse SLO3 K^+ Channels: Many Functional Differences Map to the Same Region

Celia M. Santi, Alice Butler, Aguan D. Wei, Lawrence Salkoff.
Washington University School of Medicine, Saint Louis, MO, USA.

Genes pertaining to male reproduction, especially those involved in sperm production, morphologically and functionally evolve much faster than their non-sexual counterparts. *SLO3* is an especially intriguing example of such a rapidly evolving gene. The *SLO3* gene encodes a K^+ channel which is expressed only in mammalian sperm and is evolving much faster than its close paralogue *SLO1* which is expressed in brain and other organs. We cloned the bovine orthologue of *SLO3* (*bSLO3*) and compared its primary sequence and functional properties to its mouse orthologue (*mSLO3*) which we previously cloned. A comparison of bSLO3 and mSLO3 primary sequences showed far less conservation than for SLO1 proteins in mouse and bovine species. Functionally, bSLO3 and mSLO3 also differ markedly with respect to their voltage range of activation, their ion selectivity, and their activation kinetics. Remarkably, although there are many regions of low conservation between bSLO3 and mSLO3 proteins, we found that all of the different functional properties that we measured map to a small region of low conservation in the RCK1 domain. One or more of these different functional properties may reflect differences in the resting membrane potentials of sperm in bovine and mouse species. This work was supported by National Institute of Health grants 1R21HD056444-01A1 to C.M.S. and R24 RR017342-01 and R01 GM067154-01A1 to L.S.

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Multiple Components of Ca-activated K currents in mouse pancreatic beta cells

Khaled M. Houamed, Leslie S. Satin.
University of Michigan, Ann Arbor, MI, USA.

Multiple Components of Ca-activated K currents in mouse pancreatic beta cells
In beta cells, two types of Ca activated K channels have been described. One component, K_{slow}, is believed to be mediated by small-conductance, voltage-independent (SK) channels; it is thought to regulate the duration of intervals between successive action-potential bursts observed when beta cells are exposed to moderately elevated glucose. In contrast, the functional role of the second type of Ca activated K channels, the large conductance, Ca- and voltage-activated, BK channels, is not well understood. BK channel subunit genes have been detected in insulin-secreting cell lines, and BK channels have been observed functionally in rodent beta cells; however, early studies with BK channel blocking drugs have failed to identify a role for these channels in the electrical excitability or the stimulus-secretion coupling of beta cells.
Using patch clamp recording under quasi-physiological conditions, we show that the BK channel current can contribute up to a half of the outward current activated by depolarizing pulses whose amplitude resembles the voltage excursion of beta cell action potentials. Kinetic and pharmacological experiments reveal that the beta cell BK current consists of several pharmacologically, kinetically, and possibly spatially, distinct components. Our results suggest that the BK current could play a significant role in regulating beta cell electrical excitability of stimulus secretion coupling.

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Calcium Binding Causes A Conformational Change in The RCK1 Domain of The BK(Ca) Channel

Akansha Saxena, Jianmin Cui, David Sept.
Washington University, St Louis, MO, USA.

Calcium plays a major role in controlling the opening and closing of the large conductance BK_{Ca} channels. Two high affinity binding sites have been identified in the channel structure and one of these sites is the DRDD loop in the N-terminus of the RCK1 domain. Mutation of the first aspartate in this conserved DRDD motif significantly reduces Ca^{2+} sensitivity and hence this residue has been implicated as a coordinating group in the binding site. Here we present results on the prediction of the Ca^{2+} binding site based on a series of detailed computational studies. The basic protocol involves multiple iterations of random ion placement, implicit solvent molecular dynamics simulations and statistical analysis. Our resulting model matches very well with existing mutagenesis data, and subsequent explicit solvent molecular dynamics simulations have been performed using this Ca^{2+} bound structure. Comparison of the dynamics and conformations of the Ca^{2+} bound and unbound simulations reveal a concerted conformational change in the structure and suggest a potential mechanism for calcium dependent activation of these channels.

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Comparative Mechanisms Of Activation Of The Slo1 BK Channel By Ca2+ And H+ Mediated By The RCK1 Domain

Shangwei Hou[1], Frank T. Horrigan[2], Stefan H. Heinemann[3], Toshinori Hoshi[1].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Baylor College of Medicine, Houston, TX, USA, [3]Friedrich Schiller University Jena, Jena, Germany.

Large-conductance Ca2 ± and voltage-gated K+ (Slo1 BK) channels are allosterically activated by depolarization and intracellular Ca2+. High-affinity activation of the channel by Ca2+ involves two sites, the Ca2+ bowl sensor and the RCK1 sensor, the latter of which is also required for the stimulatory action of intracellular H+ (Hou et al., Nat Struct Mol Biol. 15, 403, 2008). We investigated the comparative effects of Ca2+ and H+ on activation of the Slo1 BK channel mediated by the RCK1 sensor using a Ca2+ bowl-defective mutant expressed in HEK cells. Decreasing pHi from 7.5 to 6.2 shifted the voltage-conductance (GV) curve to the left by ~50 mV. The shift in GV by H+ was, however, only ~40% of that caused by a saturating concentration of Ca2+ in the mutant. Single-channel measurements at negative voltages where voltage sensor activation is negligible verified that 200 uM Ca2+ drastically increased open probability, corresponding to the allosteric coupling factor C = ~4 in