interactions in the ''bundle-crossing'' gate of a closed channel. Our observations with BK channels are unlikely to result from disruption of specific interactions, as multiple positions in the BK deep-pore, some of them adjacent along the S6 helix, showed aspartate mediated constitutive activities.

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Effects of Palmitoylation on the Movement of BK_{CA} Channels In Live Cells using Site-Specific Labeling with Quantum Dots

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It was shown that the large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels are palmioylated at a cluster of cysteine residues within the cytosolic linker connecting the 1st and 2nd transmembrane domains,and the lipid modification affects the surface expression of the channel proteins. Since the lipid modification is known to influence the dynamics of the membrane proteins, we investigated the effect of palmitoylation on the lateral movement of BK_{Ca} channels within the live cell membrane. The wild-type channel and a triple mutant (C53:54:56A) in which three cysteine residues were substituted to alanine residues were tagged with an acceptor peptide sequence at their N-terminus and expressed in COS-7 cells for metabolic modification by endogenous biotin. The biotin-modified BK_{Ca} channels presented on the cell surface were specifically labeled with streptavidin-conjugated quantum dots (QDs). The QD-labeled BK_{Ca} channels were visualized in live cells and tracked at real-time. Unlike the wild-type channels exhibiting a confined diffusion, the movement of the mutant channels was much less confined and close to be random. The diffusion of the mutant channel was also much faster than that of the wild type. Thus, the lateral movement of BK_{Ca} channelsin live cell membrane is greatly influenced by lipid modification.

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Apical Ca^{2+} activated K^+ Channels in Mouse Lacrimal Acinar Cells

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Fluid secretion of salivary glands relies on intracellular calcium transients activating calcium dependent Cl^- conductances in the luminal membrane of acinar cells. Older parotid acinar cell models place Ca^{2+}-activated K^+ channels into the basolateral membrane because early studies found Ca^{2+}-activated K^+ channel activity in cell-attached patches targeting this membrane domain. Recently we have used the combination of whole cell patch clamp electrophysiology, spatially localized flash photolysis of caged Ca^{2+} and temporally resolved digital Ca^{2+} imaging to show that Ca^{2+} -activated K^+ channels colocalize with Cl^- channels and occur in much higher density in the luminal membrane of parotid acinar cells compared to the basal. This finding provided evidence that supported a new mathematical model in which optimal fluid secretion requires significant K^+ conductance inserted into the luminal membrane. The aim of the present study was to test the localization of Ca^{2+} -dependent K^+ channels in acinar cells from a different tissue, the lacrimal gland. Our initial experiments have shown both intermediate (IK) and big conductance (BK) Ca^{2+}-activated K^+ currents in these cells, although the abundance of IK conductance is less when compared to parotid. Flash-induced localized Ca^{2+} elevations at the luminal membrane resulted in a robust increase in K^+ and Cl^- currents. The K^+ current evoked by apical photolysis was abolished by 10 μM paxilline, a specific inhibitor of BK channels. Our failure to activate IK in these experiments suggests that the role of IK in lacrimal acinar cells is smaller compared to parotid. In contrast, when the $[Ca^{2+}]$ was increased at the basal plasma membrane, no increase in either K^+ or Cl^- currents was evoked. These data indicate that the density of large conductance Ca^{2+} activated K^+ channels appears higher in the apical versus basal plasma membrane.

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Phosphatidylinositol-3-Kinase C2B and TRIM27 Function to Positively and Negatively Regulate IGE Receptor Activation of Mast Cells

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Cross linking of the IgE receptor (FceRI) on mast cells plays a critical role in IgE-dependent allergy including allergic rhinitis, asthma, anaphylaxis, and immediate type hypersensitivity reactions. Previous studies have demonstrated that the K^+ channel, KCa3.1, plays a critical role in IgE-stimulated Ca^{2+} entry and degranulation in both human and mouse mast cells. We now show that the class II phosphatidylinositol 3 kinase C2b (PI3KC2b) is necessary for FceRI stimulated activation of KCa3.1, Ca^{2+} influx, cytokine production and degranulation of bone marrow derived mast cells (BMMC). In addition, we found that the E3 ubiquitin ligase, tripartite motif containing protein 27 (TRIM27), negatively regulates FceRI activation of KCa3.1, and downstream signaling by ubiquitinating and inhibiting PI3KC2b.*TRIM27*$^{-/-}$ mice are also more susceptible in vivo to acute anaphylaxis. These findings identify TRIM27 as an important negative regulator of mast cells in vivo, and suggests that PI3KC2b is a potential new pharmacologic target to treat IgE mediated disease.

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The Stoichiometry of an SK Channel Peptide and Calmodulin

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SK potassium channels modulate the cellular membrane potential in response to intracellular calcium. SK requires that calcium binds to the calcium sensor, calmodulin (CaM), to open. Comprised of four identical subunits, SK has at least four putative CaM binding sites; at least one site is encoded per carboxyl tail. Understanding how SK channels operate requires knowing how many CaMs bind to SK. Previous work has shown that in a crystal the SK peptide (SKp) forms a '2:2 complex' with CaM in saturating calcium. We have used a recombinant CaM binding SK peptide (SKp) without affinity tags to measure stoichiometry. Orthogonal approaches were used to measure the molecular mass of particles in solution and in the presence of 1 mM calcium. Light scattering results show that SKp and CaM form a complex with a molecular mass close to 29 kDa, which is consistent with a 1:1 SKp:CaM complex. Sedimentation equilibrium studies show an intermediate molecular weight consistent with alternate stoichiometries, but not likely present as a 2:2 complex. In sedimentation velocity measurements, the sedimentation rate of the complex that forms varies with the molar ratio of SKp:CaM . For example, when SKp and CaM are equimolar, van Holde-Weischet analyses yields a sedimentation constant of 2.50 s (s=1x10^{-13}s). If CaM is in two fold molar excess over SKp, the sedimentation constant increases to 2.96 s. If SKp is in two fold molar excess over CaM, a distinct species forms with a sedimentation constant of 2.75 s. Finally, 2D spectrum analyses combined with Monte Carlo statistics support alternative stoichiometric ratios, but also exclude the formation of a '2:2 complex'. The data suggest these proteins have different modes of interaction in a dilute solution than occurs in a crystal.

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ANO1 Activation does not require Calmodulin

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The Ca^{2+}-activated Cl channel anoctamin-1 (ANO1, also called TMEM16A), a member of the Anoctamin superfamily, plays a variety of important physiological roles including epithelial fluid secretion. ANO1 is activated and gated by intracellular Ca^{2+}, but there is uncertainty whether Ca^{2+} binds directly to ANO1 or whether an additional Ca^{2+}-binding subunit such as calmodulin (CaM) is required. Here, we report that CaM is not a necessary component for ANO1 activation for the following reasons: 1) ANO1 activation by photolysis of caged Ca^{2+} is very rapid (<1ms), suggesting that Ca^{2+} binds directly to the channel. 2) Exogenous CaM has no effect on the currents generated by either the ac or abc isoforms of ANO1 in inside-out excised patches. 3) Over-expression of functionally defective CaM has no effect on ANO1 (abc isoform) currents while it eliminates the small conductance Ca-activated K (SK2) current. 4) CaM does not physically interact with ANO1 as determined by co-immunoprecipitation. 5) ANO1 is activated in excised patches by Ba, which is a very poor CaM activator. We also re-examined the effect of the CaM inhibitor trifluoperazine (TFP). TFP blocks ANO1 in a voltage-dependent manner, suggesting that TFP blocks the current by lodging in the ANO1 pore. These results show CaM is not required for ANO1 activation and supports the hypothesis that Ca binds directly to ANO1.

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Modeling SK-Channels and Electrical Activity in Human Beta-Cells

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Electrical activity in pancreatic beta-cells plays a pivotal role in glucose-stimulated insulin secretion by coupling metabolism to calcium-triggered exocytosis. Mathematical models based on rodent data have helped in understanding the mechanisms underlying the electrophysiological patterns observed in laboratory animals. However, human beta-cells differ in several aspects, and in particular in their electrophysiological characteristics, from