Channel Regulation & Modulation I

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Regulation of Kv1.3 Channels by a Matrix Metalloproteinase
Hai M. Nguyen

Matrix metalloproteinase 23 (MMP23) contains a functional K+ channel-blocking toxin domain (TxD) with structural similarity to the sea anemone toxins BKgK and ShK (J. Biol. Chem. 2010.285:9124-9136). MMP23 co-localizes with and traps TxD-sensitive Kv1.3 channels intracellularly without affecting TxD-resistant Kv1.2 channels (J. Biol. Chem. 2010.285:9124-9136). Here we provide time- and concentration-dependent evidence for the generation of MMP23 required for regulation of human Kv1.3. Confocal microscopy showed that eGFP-Kv1.3 co-localized with dsRed-tagged MMP23 and with three deletion constructs of MMP23 (lack the IgCAM domain, IgCAM + TxD, IgCAM + TxD + Catalytic Domain), but not with dsRed (vector control), in COS-7 cells. Patch-clamp experiments revealed suppression of Kv1.3 currents by full-length dsRed-MMP23 and by the deletion construct lacking all three external domains (IgCAM+TxD+Catalytic domain), but not by dsRed. These results indicate that the N-terminal segment of MMP23 (stretching from the N-terminus, through the single transmembrane segment, and ending at the external furin-cleavage site) is sufficient for both current suppression and co-localization with Kv1.3 channels. Western blot analysis demonstrated cleavage of external loops of Kv1.3 by full-length MMP23. Thus, MMP23’s N-terminal segment associates with and retains Kv1.3 intracellularly, while the TxD binds to the channel pore and positions the catalytic domain to cleave external loops of Kv1.3.

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Differential Influence of DOPG and DOPA Anionic Phospholipids on Single Channel Conductance of KcsA
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Binding of anionic phospholipids to non-annular binding site of KcsA has been shown with crystallographic and biochemical methods. To gain insight into the functional importance of these specific protein-lipid interactions we studied the single channel properties of KcsA in planar lipid bilayer using anionic phospholipid content of the membrane. Phospholipids with identical acyl chains and equivalent headgroup charges have been chosen for this purpose. Single channel properties of KcsA were unchanged, when the content of anionic phospholipids was kept below 30% in planar lipid bilayers formed from DOPC neutral phospholipid as remainder component. Upon gradual increase of membrane DOPG content up to 100%, a significant increase in single channel conductance was observed only at positive potentials (from 82.2 ± 1.4 pS to 116,3 ± 0.7 pS at +100mV in symmetrical 150 mM KC1 solution). Surprisingly, equimolar increase of DOPA content of the membrane had no influence on single channel conductance of KcsA. Differential influence of DOPG and DOPA phospholipids was preserved at varying K+ concentrations of recording solutions ranging from 50 to 250 mM. Using KcsA-Kv1.3 chimera we could show that the stretch of amino acids between E51 and A65 in KcsA was responsible for differential sensitivity of KcsA for DOPA and DOPG. Taken together, our data show that the structure of the phospholipid headgroup is important for phospholipid-specific modulation of single channel conductance in KcsA.

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Dual Regulation of Kv1.2 Activation by PIP2

Phosphatidylinositol (4,5)-bisphosphate is now recognized as a ubiquitous regulator of ion channels and transporters. We report here that PIP2 regulates activation of Kv1.2, a Shaker family voltage-gated potassium channel. In inside-out macroscopic patches of Xenopus laevis oocytes, loss of PIP2 induced a leftward shift in the voltage-dependence of activation and a reduction in maximal current, which could be restored by exogenous PIP2 application. These findings were reproduced using the voltage-dependent lipid phosphatase Ci-VSP, which preferentially dephosphorylates PIP2(4,5)P2 to PIP2(4)P when activated by depolarization. The dual effect of PIP2 on the voltage-dependence of activation and current level exhibited distinct kinetics. The time course of PIP2 depletion (e.g. during rundown of activity) or recovery (e.g. upon re-phosphorylation of PIP2(4)P) indicated that the effect on the voltage-dependent shift proceeded with faster kinetics than the current level. Furthermore, high concentrations of diC8-PIP2, a soluble analog of PIP2, partially restored the shift in the voltage dependence of activation caused by PIP2 antibody but failed to do the same for the current level. Taken together, the two effects of PIP2 on the voltage-dependence of activation and the current level of Kv1.2 channels proceeded with distinct kinetics and sensitivity to PIP2, suggesting distinct underlying molecular determinants.

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The Cytoplasmic N-Terminal Domain of DPP6K Disrupts KChIP Modulation of Kv4 Channels

Kv4 channel modulatory subunits, including the cytoplasmic Kv channel interacting proteins (KChIPs) and transmembrane dipeptidyl peptidase-like proteins (DPLPs), have variable N-terminal domains that can dramatically influence Kv4 channel function. These modulatory effects probably underlie the distinctiveness of subthreshold A-type potassium currents (I_A) in different neuron types. In this study we have characterized unique modulatory effects encoded in the N-terminal domain of DPP6K, a brain DPP6 isoform named for a distinctive N-terminal lysine cluster. In co-expression studies of Kv4.2+KChIP3a+DPP6 ternary channel complexes in Xenopus oocytes, DPP6K produces dramatically slower recovery from inactivation (at -100 mV: t+ ~ 84 ms (58%), t- = 324 ms (42%)) when compared to DPP6S (t+ ~ 25 ms for DPP6S (t+ ~ 10 ms). The slower recovery kinetics produced by DPP6K are accompanied by a hyperpolarizing shift (~ 8 mV) in steady-state inactivation. To explore the functional determinant for these DPP6K effects, we conducted deletion analysis and point mutations on the DPP6K N-terminal domain. The results show that the unique functional effects of DPP6K are not attributable to the distinctive lysine residues but instead depend on hydrophobic residues 12-16. Alanine scanning of residues 12-16 shows that Met-12 and Val-16 are the critical residues for these modulatory effects, with substitution of both residues completely eliminating the effects of DPP6K on inactivation. To examine the mechanism for DPP6K modulation of channel inactivation, we compared the properties of Kv4.2+KChIP3a+DPP6K to Kv4.2/D2-40+DPP6K. Surprisingly, Kv4.2/D2-40+DPP6K and Kv4.2+KChIP3a+DPP6K channels recover from inactivation similarly and have similar midpoints of steady-state inactivation (~ -70 mV), as if DPP6K blocks the normal modulatory effects of KChIP3a.

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The Two-Pore-Domain Potassium Channels, TASK-1 and TASK-3, Regulate Pancreatic Beta-Cell Membrane Potential in Response to pH and Anesthetics
Prasanna Dadi, Louis H. Philipson, David A. Jacobson.

Glucose stimulation of the pancreatic β-cell depolarizes the membrane potential to allow activation of voltage dependent calcium channels resulting in action potential (AP) firing, calcium influx, and insulin secretion. Two-pore-domain potassium (K2P) channels regulate the membrane potential from where AP firing occurs in many neurons. However, a role of K2P channels in regulating the pancreatic β-cell membrane potential is unknown and thus we addressed expression and function of the TASK K2P channels in the mouse β-cell. We find that TASK-1 and TASK-3 channels are expressed in mouse β-cells. Furthermore, β-cell TASK-like currents are sensitive to external pH, showing inhibition with acidic pH and stimulation with alkaline pH. Interestingly, glucose regulates β-cell intracellular pH causing acidic conditions in high glucose, which may serve to regulate β-cell TASK channel activity. Increasing glucose from 2 to 14 mM caused polarization of the β-cell membrane potential whereas reducing glucose from 14 to 2 mM caused membrane depolarization by 6.1 +/- 2.2 mV when KATP was inhibited with tolbutamide. Similarly extracellular alkalization (pH 8) resulted in polarization of the β-cell membrane potential by 9.19 +/- 2.0 mV, whereas extracellular acidification (pH 6) caused membrane depolarization by 9.84 +/- 2.9 mV. Anesthetic compounds that activate (halothane) or inhibit (lidocaine) TASK channels were evaluated for their ability to regulate β-cell membrane potential. Treatment of islets with lidocaine depolarized the β-cell membrane potential by 6.2 +/- 1.5 mV and halothane treatment resulted in β-cell membrane polarization. Both lidocaine and halothane have been shown to cause perturbations in human glucose homeostasis. Thus, this data implicate important roles for TASK channels in regulating the β-cell membrane potential, which may influence anesthetic and pH/glucose induced modulation of insulin secretion.

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Membrane Modulation of KcsA Structural Dynamics and Ion Channel Function
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The chemical environment presented by the lipid bilayer is hypothesized to have potentially dramatic effects on the structure and function of integral membrane proteins, especially ion channels. Previously, Valiyaveetil, et al. reported that although a variety of phospholipids provide sufficient structural stability to refold KcsA, loss of membrane structure, negatively charged lipids are required to re-establish K-channel
functionality [1]. To provide a more detailed understanding of the dynamic details of membrane-mediated perturbations of KcsA structure and function, we performed bulk and single-molecule fluorescence and electrophysiological assays of reassembled KcsA tetramers in neutrally charged DOPC and negatively charged DOPE/DOPG bilayers. Labeling of KcsA monomers was performed using the GS56C KcsA mutant [2] which provides a soluble accessible reactive thiol for covalent coupling of fluorophores for FRET assays between neighboring monomers and fluorescently labeled membranes. The results suggest that structural rearrangement is predicated by KcsA-phospholipid headgroup binding which excludes non-negative phospholipids at the cytoplasmic TM2-membrane interface. Additionally, a limited number of inter-monomer structural perturbations are consistent with the data in the presence of negatively charged phospholipids, including a decrease in the angular relationship of KcsA monomers normal to the bilayer or decreased toroidal packing. The results of these assays shed light on the downstream structural effects of interactions of phospholipid headgroups on transmembrane proteins and how these correlate to function in a highly stable, model potassium channel.


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Semi-Simultaneous Imaging of Ca²⁺ and Ca²⁺-Dependent Calsmodulin Interactions by FRET

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Calcium dynamics and its linked molecular interactions contribute to a variety of biological responses, thus it is essential to exploit techniques for detecting both efficiently. Our new describe a new method to image Ca²⁺ and protein-protein interaction on semi-simultaneous base through combined application of Fura-2 fluorescence imaging and Fluorescence or FRET and proteins' in HEK cells, we could obtain quantitative and synchronous dose-response relations for [Ca²⁺], versus protein-protein interaction between Ca²⁺ binding protein 'calmodulin' (CaM) and its binding domain from an enzyme, which were fused with fluorescenes CFP and YFP, respectively. The same approach applied to voltage-gated Na⁺, Ca²⁺ and TRP channels revealed their distinct sensitivities to basal Ca²⁺ through CaM binding. This semi-simultaneous imaging system could be applicable to the identification of Ca²⁺-dependent protein interactions with further potential application to elucidating the interrelations between Ca²⁺ signals and cellular functions such as enzyme activities, signal transductions, and membrane protein regulations in various living cells.

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KCNK3 (Task-1) Potassium Leak Channels Internalize in a PKC- and 14-3-3-beta-Derpend Manner


Ion channels encoded by the human KCNK3 gene give rise to the pH-sensitive outwardly rectifying potassium leak current in a variety of cell types, where they play a decisive role in determination of the hyperpolarized resting membrane potential, controlling excitability and hastening repolarization. These tandem-pore domain channels are regulated by various agents and stimuli, including neurotransmitters, hormones, volatile anesthetics, oxidases and protein kinases. Several studies have shown that activation of protein kinase C (PKC) inhibits KCNK3 (Task-1) currents; however, the cellular mechanism underlying this inhibition has not been elucidated. Using a combination of electrophysiological, biochemical and imaging approaches, we have determined that PKC-activation induces KCNK3 channel internalization. Moreover, we have identified specific residues in the KCNK3 C-terminus that contribute, but are not solely required, for PKC-mediated internalization. Since it was previously shown that the interaction with phosphoserine binding protein 14-3-3-beta promotes ER exit of KCNK3, we examined the role of 14-3-3-beta on KCNK3 internalization. Using overexpression and shRNA knockdown experiments of 14-3-3beta, we show that PKC-dependent KCNK3 internalization is also crucially dependent on the cellular levels of 14-3-3-beta protein. Our results reveal a novel mechanism of KCNK3 current regulation by channel internalization in a PKC- and 14-3-3-beta-dependent manner.