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DPP6A Confers Redox Sensitivity to Kv4 Channel Inactivation

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Reactive oxygen species (ROS) are important modulators of excitability and may play a critical role in the etiology of many neurodegenerative disorders. Previous studies have shown that the fast N-type inactivation of Kv1.4 channels is suppressed by ROS-mediated cysteine oxidation whereas Kv4 channel N-type inactivation is not. However, in native neurons Kv4 channel subunits form large macromolecular complexes with KChIP and DPL proteins, and fast N-type inactivation is conferred to Kv4 channel complexes by a specific isoform of DPL proteins, either DPP10a or DPP6a. To investigate whether the DPP6a-mediated fast inactivation is regulated by ROS, tert- butyl hydroperoxide (tBHP) was applied to oocytes expressing Kv4.2+KChIP3a+DPP6a channels. tBHP (1 mM) application dramatically increases the peak current amplitude by ~ 44% and slowed inactivation kinetics. The effects of tBHP are reversed by the reducing agent dithiothreitol (DTT, 10 mM). In contrast, ternary complex channels containing another DPP6 isoform (DPP6K) are not affected by tBHP, indicating the importance of the DPP6a variable N-terminal domain for the tBHP effect. Alignment of N-terminal sequences from DPP6a and DPP10a with Kv1.4 reveals a common cysteine residue in position 13 (Cys-13), which in Kv1.4 is critical for the redox-regulation of N-type inactivation. Substituting Cys-13 of DPP6a with serine (DPP6a/C13S) results in a loss of regulation by tBHP, consistent with a similar critical role for DPP6a Cys-13. To test if other cysteines in the channel are also required for this regulation, we switched to mutant Kv4.1 (C11xA) and KChIP3a (KChIP3a/del2-59) constructs that remove most intracellular cysteine residues. Channels composed of C11xA, KChIP3a/del2-59, and normal DPP6a show disrupted regulation by tBHP, suggesting that ROS likely induces an intersubunit disulfide linkage to regulate DPP6a-mediated N-type inactivation. This work was supported by a grant from the National Institute of Health (R01 GM090029).

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Ci-KCNQ1, an Ortholog of Vertebrate KCNQ1 from Ciona Intestinalis, has Revealed that KCNE1 and KCNE3 Utilize Different Domains of KCNQ1 for the Modulation of Gating

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KCNQ1 channel is a voltage-gated potassium channel expressed in various tissues such as heart, intestine, inner ear, kidney and pancreas. The gating property of KCNQ1 is largely determined by the type of coexpressed KCNE proteins. For example, coexpression of KCNQ1 with KCNE1 produces slowly-activating potassium current which is known as I_{Ks} in heart, while KCNE3 makes KCNQ1 voltage-independent and constitutively-active. We previously isolated Ci-KCNQ1, a vertebrate KCNQ1 ortholog from marine invertebrate *Ciona intestinalis*. As KCNE genes are not present in the *Ciona* genome, we hypothesized that Ci-KCNQ1 might lack important amino acid residues for the gating modulation by mammalian KCNE proteins. Ci-KCNQ1, by itself, produced large voltage-gated potassium current (about 10 μA at + 40mV) in *Xenopus* oocyte. As we hypothesized, KCNE proteins did not properly modulate Ci-KCNQ1 as they did with human KCNQ1. KCNE1 shifted the *G-V* curve to the negative direction and failed to transform Ci-KCNQ1 into a slowly-activating potassium channels. Ci-KCNQ1 remained voltage-dependent in the presence of KCNE3. By making series of chimeras of human KCNQ1 and Ci-KCNQ1, we identified that some amino acid residues in the pore region of Ci-KCNQ1 are responsible for the improper modulation with KCNE1. On the other hand, the difference of S1 segment seemed to be responsible for the lack of modulation by KCNE3. Point mutation revealed that Phe127 on the S1 segment of human KCNQ1, which is substituted with valine in Ci-KCNQ1, plays an important role in the KCNE3 modulation. Taken together, we showed that KCNE1 and KCNE3 utilize different domains of KCNQ1 channels, and that may be the reason why different KCNE proteins exert extremely different modulation effects on KCNQ1.

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Competitive Interactions of Kv7 Channel Carboxy-Termini with PIP_2 and Calmodulin

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Calmodulin (CaM) is a ubiquitous calcium sensor which binds to the carboxyl terminus of Kv7 channels, thus regulating channel function. Ca^{2+}-dependent interactions of Kv7 with CaM mediate channel inhibition by specific G protein coupled receptors (GPCR). Yet other GPCR's inhibit Kv7 channels via the depletion of membrane phosphatidylinositol 4,5-bisphosphate (PIP_2), which is required for Kv7 activity. Putative binding sites for PIP_2 and CaM are in close proximity or overlap within the proximal C-termini of Kv7 channels. We investigated whether calmodulin and PIP_2 binding to Kv7 channels is competitive, a phenomenon that would predict an increased affinity for Kv7-CaM interaction upon PIP_2 depletion, and, conversely a decrease of Kv7 channel PIP_2 affinity upon CaM binding. We performed co-immunoprecipitation between CaM and Kv7.4 overexpressed in HEKMSR cells under conditions of tonic PIP_2, chronic PIP_2 depletion (overexpression of PIP_2 sequestering construct) and chronic PIP_2 elevation (overexpression of phosphatidylinositol 5-kinase, PI5K). Sequestering PIP_2 increased the co-immunoprecipitation of CaM with KCNQ4, while overproduction of PIP_2 decreased CaM-binding. Next, we evaluated fluorescence recovery after photobleaching using TIRF illumination (TIRF-FRAP) between KCNQ4 and eYFP-CaM under conditions of normal, low and high membrane PIP_2. When only eYFP-CaM was expressed in HEKMSR cells, TIRF-FRAP had a time constant of 43 ± 9 s (n=24), co-expression of CaM with Kv7.4 showed an increase in the recovery time constant to 93 ± 21 s (n=24, $p \leq 0.05$), indicating a fraction of CaM molecules are tethered to the plasma membrane by the interaction with Kv7.4. Depletion of PIP_2 with wortmannin (10 μM) resulted in a further increase in recovery time to 453 ± 165 s (n=27, $p \leq 0.001$); overexpression of PI5K resulted in the recovery time of 118 ± 19 s (n=20, $p \leq 0.001$ vs. wortmannin). Collectively, these data strongly suggest a competitive nature for CaM and PIP_2 interactions at the Kv7 C-terminus.

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The CRAC Motif Nearest to the N-End of Slo1 Cytosolic C-Tail is the Main Determinant of BK Channel Sensitivity to Membrane Cholesterol

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Increased membrane cholesterol (CLR) decreases BK channel activity (Po) (reviewed by Levitan et al., 2010). Electrophysiological data from a recent structure-activity study of CLR and several analogs on BK channel-forming slo1 proteins (cbv1) reconstituted into phospholipid bilayers led us to hypothesize that CLR action resulted from CLR selective and direct recognition by a site(s) in cbv1 (Bukiya et al., 2011).

We identified ten Cholesterol Recognition/Interacting Amino acid Consensus (CRAC) domains in cbv1: CRACs 1-3 in the transmembrane core (S0-S6), and CRACs 4-10 in the cytosolic C tail (CTD). Bilayer electrophysiology demonstrated that construct trcbv1S6, which lacked CTD, failed to respond to CLR. However, an extended construct that included CRAC4 (trcbv1-CRAC4) was CLR-sensitive, indicating that a major determinant of CLR sensitivity lies in CRAC4 and/or the linker between this motif and S6. We next removed two CRACs at a time: immediately distal to CRAC6 (trcbv1-CRAC6) and to CRAC8 (trcbv1-CRAC8). Trcbv1-CRAC6 and trcbv1-CRAC8 were CLR-sensitive, further supporting the hypothesis that the S6-CRAC4 linker and/or CRAC4 itself is essential for CLR action. However, elimination of CRACs 7-10 or CRACs 9-10 somewhat decreased CLR action. To determine whether CTD CRACs themselves contributed to CLR responses, we introduced progressive Phe substitutions of signature tyrosines in CTD CRACs. The most drastic reduction of CLR action occurred with Y450F in CRAC4. However, cumulative Tyr to Phe substitutions in CRACs 5-10 gradually reduced CLR action. In conclusion: 1) CRAC4 in BK CTD is the main region contributing to CLR action; 2) the signature CRAC residue Y450 is essential for CLR sensitivity; 3) tyrosines in CRACs distal to CRAC4 also contribute to overall CLR sensitivity of BK channels. Support: R01-HL104631;R37-AA011560 (AMD); UTHSC NI Fellowship (AKS).

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Sodium 3-Hydroxyolean-12-en-30-Oate is a Novel and Selective Activator of β1 Subunit-Containing BK Channels and thus Cerebral Artery Dilator

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Lithocholic acid (LCA) is a cholane steroid that causes cerebrovascular dilation *via* activation of β1 subunit-containing BK channels in vascular smooth muscle (Bukiya et al., 2007; 2009). In search of selective β1-containing BK activators devoid of a steroidal nucleus, we used LCA as a template and performed structure similarity search with the threshold of 70% in the Hit2lead.com database. We identified methyl 3-hydroxyolean-12-en-30-oate, which after hydrolysis, rendered sodium 3-hydroxyolean-12-en-30-oate (HENA). Patch-clamp results demonstrated that HENA activated BK channels (cbv1+β1) cloned from rat cerebral artery myocytes. While EC_{50} (~50 μM), E_{max} (~300 μM) and apparent