this increase in the Ca$^{2+}$ spark rate was associated with a decrease in the SR Ca$^{2+}$ content. This is consistent with the increase in the SR Ca$^{2+}$ leak (as evidenced by the increase in the Ca$^{2+}$ spark rate) that followed the H$_2$O$_2$ application. Since ROS has been shown to activate other signaling systems in heart (e.g., CaMKII), the interactions between H$_2$O$_2$ dependent ROS elevations and both CaMKII and PKA were examined. While significant interactions between rapid, transient ROS elevation and CaMKII and PKA were observed, it was also determined that the actions of these ROS elevations on Ca$^{2+}$ sparks was not mediated by either CaMKII or PKA. How ROS may affect EC coupling under these conditions is also examined and discussed.

**Voltage-gated K Channels II**

**2709-Pos Board B401**

The Cytochrome C-Like Domain of the Human BK Channel

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The voltage- and calcium-activated BK channel (Slo1) is modulated by small ligands that bind to its intracellular gating ring (GR) formed by four pairs of tandem homologous RCK1 and RCK2 domains. Heme binds reversibly to the GR at site 612C-KACH$^{616*}$, a conserved heme regulatory motif (CXXCH) in the Cytochrome C (CytC) protein family, and it is located in the ~120-residue linker connecting RCK1 and RCK2 domains. Most of this linker has thus far evaded structural definition. To gain structural insight on this functionally-significant region, we performed a sequence alignment of BK with CytC and CytC-like domains from different hemoproteins. We found that CytC-$\delta$ domain is conserved in the BK RCK1- RCK2 linker: firstly, the portion of the BK region resolved in the available atomic structures shares secondary structure elements with CytC proteins; secondly, CytC positively-charged residues critical for Apaf-1 and cardiolipin interaction align with BK residues K606, K623, R648, K684, K685 and K698; finally, CytC methionine-80, the second axial ligand to the heme iron, aligns with BK M691. These similarities support the premise that a CytC-like domain exists in the BK GR. To experimentally test this hypothesis, we expressed and purified this region (598ASL...L5G1) and probed its structural composition with Circular Dichroism spectroscopy. The z-helical composition of this protein increased following addition of heme (150 mM) from ~35% to ~51%, approaching the z-helical content of CytC (~53%). Moreover, in the full GR, mutation M691A significantly attenuated heme-binding properties as shown by reduced Soret band formation compared to WT, suggesting that M691 is important for heme binding. These results demonstrate that BK channels possess four intracellular CytC-like domains, which may confer novel physiological functions to these ubiquitous ion channels.

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Enzymatic Activity of the Human BK Channel: A Function Beyond Electrical Signaling

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BK (Slo1) channels open probability is acutely regulated by heme, which associates with their intracellular multi-ligand sensor, the gating ring. We found that the gating ring region encompassing the heme-binding site shares structural homology with CytC, a protein known for gating ring catalytic activity, i.e. the oxidation of suitable substrates using peroxides. To probe for peroxidase activity of the Cytochrome C-like domain in a purified BK channel gating ring, we used the chromatophore 2,2’-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the oxidizable substrate. We found that the BK gating ring complexed with ABTS catalyzes the formation of ABTS$^2-$ cation radical, monitored by absorption at 415nm. The initial rate of ABTS$^2-$ formation was linearly correlated with [gating ring] between 0.05-3.0μM. Disruption of the heme regulatory motif (C615H616R) significantly decreased the initial rate of ABTS$^2-$ formation. The kinetic parameters of the enzymatic reaction were determined by performing two-substrate Michaelis-Menten analysis, which yielded for gating ring: $k_{cat}/K_{M1}$ = 12 ± 2 s$^{-1}$mM$^{-1}$ and $k_{cat}/K_{M2}$ = 0.5 ± 0.2 s$^{-1}$mM$^{-1}$. For CytC, we estimated $k_{cat}/K_{M1} = 3.5$ s$^{-1}$mM$^{-1}$ and $k_{cat}/K_{M2} = 0.35$ s$^{-1}$mM$^{-1}$. These results suggest that, under our experimental conditions, the gating ring catalytic efficiency is ~10 times higher than CytC. Finally, we found that HEK cells expressing BK channels (blocked with 100 nM Iberiotoxin) are significantly more resistant to oxidative insult (200 μM H$_2$O$_2$) than cells expressing BK channels with impaired heme binding (C615H616R) (p<0.05) as revealed by the increased cell viability (MTT assay). Thus, the BK channel exhibits peroxidase activity and confers a protective effect against oxidative cell damage. These results redefine the role of BK channels, assigning a catalytic property, in addition to their established K$^+$ conducting properties.

**2711-Pos Board B403**

BK B1 Transmembrane Regions Crucially Control the Characteristic Phenotype of B1-Containing Bk Channel Currents

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