inactivation observed in these neurons is not due to the presence of associated inactivating subunits such as β2 or β3. Interestingly, the $V_{1/2}$ activation values obtained are shifted toward more hyperpolarized potential compared to what has been reported for the heterologous coexpression of the BKα and β4 subunits. Thus, biophysical properties of native BK channels from intracardiac cholinergic neurons cannot be completely explained by the sole interaction between BKα and β4 subunits.

Supported by AHA.

535-Pos Estrogen Alters Expression Levels and Splicing Pattern of Human BK Channel Alpha Subunit

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Board B383

Studies in animal models have shown that estrogen has a protective role on cardiovascular function. However, in humans the long-term protective role of estrogen on vascular function is less clear. Estrogen regulation of vascular smooth muscle tone may be mediated via BK (MaxiK, Slo) channels through a non-genomic pathway of estrogen action. However, this mechanism may not completely explain the long-term effects of estrogen observed on cardiovascular physiology. Here, we present evidence of a genomic regulation of human BK channel alpha subunit (hSlo) expression and splicing by estrogen. In isolated primary culture of human coronary and aortic smooth muscle cells estrogen treatment increased transcript levels of hSlo. Sequence analysis of the promoter-regulatory regions of the gene revealed the presence of multiple half sites of estrogen response element (ERE) that can bind estrogen receptor alpha. In addition, multiple Sp1 transcription factor binding sites that can assist or mediate estrogen action were detected. In a heterologous system hSlo promoter could be stimulated with estrogen in a saturating dose-dependent manner. We also monitored changes in splicing patterns after estrogen stimulation of coronary smooth muscle cells. Two splice inserts namely, STREX and SV29 (contained in hBr5) were upregulated when compared to the constitutive mRNA expression levels. Thus, estrogen via a genomic mechanism modulates hSlo expression that may contribute to effects observed in the vascular system after long-term estrogen treatments

(Supported by NIH).

536-Pos Statistical Mechanical Evaluation of an Allosteric Three-Gate Four-subunit Model of the BK Channel

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Board B384

BK channels are opened by two main stimuli: membrane depolarization and elevation in cytoplasmic [Ca^{2+}]. It appears that two high affinity Ca^{2+} binding sites exist within the cytoplasmic C-terminus. It has been recently proposed that each binding site is located within functional domains known as RCK1 and RCK2 (Yusifov et al Bioph Meet 2007, 1312-pos). Voltage and Ca^{2+} sensors are separate domains that operate independently in inducing channel opening. However, experimental and theoretical evidence suggests that some degree of cooperativity exists between voltage and Ca^{2+} sensors of the channel. To date, the most comprehensively studied model of calcium and voltage activation of the BK channel possesses a single Ca-binding site (Horrigan and Aldrich, 2002). We propose a physiologically meaningful allosteric model of BK activation that contains three regulatory gating structures: the S4 voltage-sensitive gate and two Ca^{2+} sensors represented by the RCK1 and RCK2 domains. As in the Horrigan and Aldrich model, the channel opens and closes in concerted fashion involving all four subunits analogous to the Monod-Wyman-Changeux allosteric model, but the complete set of allosteric interactions among the three gates evokes the Koshland-Nemethy-Filmer sequential model. We used an energetics approach to derive the partition function of this rather large gating scheme. The model was used to perform a global, simultaneous fitting of equilibrium conductance and S4-labeled fluorescence vs. voltage for three BK channels (WT, neutralized RCK1 Ca^{2+} sensor (D362A/D367A) and neutralized RCK2 Ca^{2+} sensor (Ca bowl, D894-898N). The results indicate that mutation of RCK1, which is closely located downstream the pore gate, greatly affects the interaction energy with the pore while mutation of RCK2 seems to affect mainly its interaction with the S4 voltage sensor.

Voltage-gated K Channels - II

540-Pos Comparing the Functional Expression of BK Channels Using Heterologous Expression in Different Cell Backgrounds

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Board B385

Modified insect baculoviruses containing mammalian gene expression cassettes (BacMam viruses) were used to transiently express BKα, BKαβ1 and BKαβ4 ion channels in modified CHO and human osteosacroma (U-2 os) cell backgrounds. We compared the transient BacMam expression of the BK channel complexes with those same channels expressed in stable CHO-K1 cell lines. Functional expression was determined by direct measurement of ionic currents using an Ionworks platform or a fluorescent readout assay measuring thallium influx using FLIPR. There was a positive correlation between the magnitude of outward current and the amount of BKα BacMam virus added to the culture medium with apparent saturation reached at a multiplicity of ~100 viral particles per cell. Resistance to iberiotoxin served as a marker of BKαβ4 expression while slowing of the activation time course was a marker for BKαβ1 expression. We co-transduced BKα with either BK β1 or BKβ4

subunits and observed an increase in the number of cells with the functional characteristics of BKαβ1 or BKαβ4. This increase correlated with increasing amounts of the β1 or β4 baculovirus (in the presence of a fixed amount of BKα). All BK channel types produced robust currents when expressed in either CHO or U-2 os cells, but the magnitude of current was greater in U-2 os cells. Likewise, currents activated at lower voltages in U-2 os cells than in CHO cells. Comparison of G-V curves for BK currents expressed in CHO and U-2 os cells showed a marked left-shift in $V_{0.5}$ for the U-2 os cells compared to CHO.

541-Pos Inhibition Of So1 BK Channels By Possible Cerebral Vasospasmogens

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Board B386

Cerebral hemorrhage is often followed by delayed cerebral vasospasm but the mechanism of delayed cerebral vasospasm is yet to be elucidated. Recent evidence shows that bilirubin-oxidation end products (BOXes) present in cerebrospinal fluid of patients with subarachnoid hemorrhage constrict cerebral vessels in both in vitro and in vivo animal models, suggesting that BOXes are important spasmogens (Clark JF et al., J Cereb Blood Flow Metab 2002, 472). However, how BOXes promote vasospasm is not known. Because large-conductance Ca2+- and voltage-gated K+ (BK) channels play an important role in regulation of vascular smooth tone, we examined whether BOXes alter the functional properties of BK channels. We report here that BOXes potently inhibit Slo1 BK channels in native vascular smooth muscle cells and also recombinant human Slo1 BK channels heterologously expressed in HEK cells. Treatment of the Slo1 channel with BOXes decreased open probability by altering a specific gating transition of the channel without altering the single-channel conductance. The effect of BOXes on the channel gating was irreversible and did not require the presence of auxiliary Î² subunits. The So1 channel after treatment with BOXes retained the Ca2+ and voltage sensitivity as well as the sensitivity to the BK channel activator NS1619. Our results show that the inhibitory effect of BOXes on vascular Slo1 BK channels contributes to delayed cerebral vasospasm following cerebral hemorrhage and suggest a possible new therapeutic direction.

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542-Pos Structural Analysis of the Calcium Sensors in BK_{Ca} Channel

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Board B387

The C-terminus of the large-conductance Ca^{2+}-activated K^+ channel (hSlo) comprises two RCK (Regulators of K^+ Conductance) domains, which constitute the vast majority (85%) of this intercellular region. Since an atomic structure for BK channels is not available, information about the structure and function of RCK modules is limited. Here, we report a study of the structure and Ca^{2+}-dependent conformational changes in the BK channel C-terminus. The C-terminus of hSlo channels was purified in two separate parts corresponding to the aa sequences 322*IIE*. . .*DPL*667 (hSloRCK1) and 668*LLI*. . .*KAL*1004 (hSloRCK2). hsloRCK1 and hSloRCK2 were expressed in E. coli and purified. Protein identity was confirmed by mass spectrometry. CD spectroscopy revealed that hSloRCK1 adopts α-helix and β-strand content similar to hSloRCK2. We found that β-strand content increased by ~10% as the free [Ca^{2+}] was increased from 0.015μM to 31.2 μM. This Ca^{2+} induced increase in the β strand fraction was paralleled by a similar decrease in α-helix content, while the turns and unordered fractions remained practically unchanged. Structure and Ca^{2+} dependent properties of hSloRCK1 are similar to our previously reported hSloRCK2 study (Yusifov et al Bioph. Meet. 2007). The structural similarities between the hSloRCK domains and the MthK RCK domain allowed us to create a 3D model of heteromeric hSloRCK1-hSloRCK2 complex using the crystal structure of MthK (PDB ID 1LNQ) (Jiang et al., *Nature* 2002,). The structural models of hSloRCK1 and hSloRCK2 contain the high affinity Ca^{2+} sites D362/D367, M513 (hSloRCK1) and Ca^{2+} bowl (hSloRCK2). These sites are located near the base of the αG helix-turn-αF helix regions and may influence the interactions of RCK domains across this interface in a Ca^{2+} -dependent manner. We predict that interactions through the αG-turn-αF helices of RCK1 and RCK2 create a gating ring similar to that observed in MthK.

543-Pos Bimane Fluorescence Scanning Suggests Secondary Structure near the S3–S4 linker of the BK Channel

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Board B388

Large conductance Ca^{2+}-activated K^+ channel (BK or Maxi-K channel) gating is controlled by a Ca^{2+}-sensor, formed by the channel's cytoplasmic carboxy-terminal domain, and a voltage sensor, formed by its S0–S4 transmembrane helices. Here we analyze structural properties of a portion of the BK channel voltage sensing domain, the S3–S4 linker (or voltage sensor "paddle"), using fluorescence lifetime spectroscopy. Single residues in the S3–S4 linker region were substituted with cysteine, and cysteine-substituted mutants were expressed in CHO cells and covalently labeled with the sulfhydryl-reactive fluorophore monobromo trimethylammoniobimane (qBBr). QBBr fluorescence is quenched by tryptophan and, to a lesser extent, tyrosine sidechains. We found that qBBr fluorescence in several of the labeled cysteine-substituted channels is quenched, as indicated by an increase in the fractional amplitude of a brief lifetime component of the qBBr fluorescence decay from ~0.6 at the position of Leu-199, to ~0.9 at the position of

Ser-202. Quenching can be reduced with the mutation W203F, suggesting that Trp-203 may provide the primary quenching group, though other native aromatic sidechains may act as minor quenching groups. Our results suggest a working hypothesis for secondary structure in the BK channel S3–S4 region, and places residues Leu-204, Gly-205, and Leu-206 within the extracellular end of the S4 helix.

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544-Pos The Voltage Dependent Dye $DiBAC_4(3)$ Activates Native Arterial Smooth Muscle BK Channels with No Essential Requirement for the β1 Subunit

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Board B389

Modulation of Ca^{2+} activated potassium (BK) channels is central to arterial smooth muscle physiology. It has recently been shown that the slow-response voltage-dependent dye $DiBAC_4(3)$ is a potent activator of BK channels, only when the α subunit is coexpressed with the β1 or β4 subunits. Since BK channels from arterial smooth muscle are known to be formed by α and β1 subunits, we used wild type (WT) and transgenic β1 knockout (KO) mice to test the effect of $DiBAC_4(3)$ on single BK channels from cerebral artery myocytes. Bath application of $DiBAC_4(3)$ on inside-out patches from WT animals promotes a dose-dependent activation of BK channels with EC_{50}= 14 ± 0.6 μM and a Hill coefficient n=1.4 (N=9). Similarly, $DiBAC_4(3)$ also promotes activation of KO channels (EC_{50} =15 ± 0.5 μM; n= 2.94; N=16). DiBAC-induced activation is mainly due to abbreviation of the mean closed time. We also found that high concentrations of DiBAC (30 μM) induce a high open probability mode characterized by a ~25% reduction in slope conductance, and an increase in the open channel noise. At this concentration, $DiBAC_4(3)$ shifts the voltage activation curves by ~150 mV in the hyperpolarizing direction in both WT and KO channels. 30 μM $DiBAC_4(3)$ was less effective when applied from the extracellular side , suggesting that DiBAC promotes the high Po mode acting from the intracellular side of the channel. Our data indicates that $DiBAC_4(3)$, in the micromolar range, can activate native arterial smooth muscle BK channels with or without their β1 subunits. However, in the absence of the β1 subunit, the sigmoidicity (Hill coefficient) of dose response curves is increased without a change in the apparent affinity (EC_{50}~15 μM).

Supported by NIH.

545-Pos Maxi-K Single-Ion Channel Activity And Pharmacology Studied In Tethered Bilayer Lipid Membranes - Role In Biosensor Development

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Board B390

Advances in ion channel study have revealed these membrane proteins as viable candidates for the development of biosensors for detection of biological analytes. The ideal biosensor should both be chemically and structurally stable and additionally be highly sensitive to the specific agents it is designed to target; a significantly high signal-to-noise ratio would be desirable. High conductance calcium-activated (maxi-K, BK) potassium channels stand out as an excellent choice for these applications due to their inherently high conductance, ease of genetic manipulation and expression in *Xenopus laevis* oocyte membranes, as well as their well-known pharmacological profile. We study maxi-K single-channel activity by the interfacing of the ion channel to a gold microelectrode array device in a tethered bilayer system that converts biological events into measurable electronic signals. The modulation of single ion channel activity is probed by stochastic sensing in the picoampere range. A number of broadly studied peptidyl agents and quaternary ammonium compounds known to influence the gating properties of the maxi-K ion channels are used to analyze the pharmacological responses. Charybdotoxin (ChTX), a 37 amino acid peptide purified from the venom of the *Leiurus quinquestriatus* scorpion is known to potently inhibit the maxi-k channel, however it is also known to inhibit voltage-gated potassium channels. Iberiotoxin, which bears significant structural homology to ChTX, is specific to maxi-K channels and also shows blockade of the channel at micromolar concentrations. Further, pharmacological responses of the channel are observed and studied under exposure to tetraethylammonium. The inhibition of the flow of ionic currents associated with gating events as a result of exposure to these compounds reflects on the analyte sensing capacity of the channel and potential use for biosensor development.

546-Pos Distinct Activity Of BK Channels In Cerebral (systemic) And Pulmonary Artery Smooth Muscle Cells

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Board B391

It is well established that big-conductance, Ca^{2+}-activated K^+ (BK) channels play an important role in the regulation of contraction in cerebral (systemic) artery smooth muscle cells (CASMCs) by providing a negative feedback mechanism to inhibit membrane depolarization, voltage-dependent Ca^{2+} channel opening and Ca^{2+} influx; and pulmonary artery SMCs (PASMCs) differ in many aspects from systemic arterial SMCs. In this study, thus, we sought to examine and compare the activity of BK channels in CASMCs and PASMCs. Our data indicate that the frequency and amplitude of

STOCs are significantly higher in CASMCs than PASMCs under perforated whole-cell patch clamp conditions; and the frequency and amplitude of spontaneous Ca^{2+} sparks are higher as well in CASMCs than PASMCs. Using the inside-out patch clamp recording technique, we have found that BK channels show a higher sensitivity to voltage in CAMSCs than PASMCs; however, the maximum current amplitude, number of active channels and channel conductance are all similar in both cell types. Real-time qualitative RT-PCR reveals that the BK α and β1 subunit mRNA expression are similar in CASMCs and PASMCs, whereas Western blot analysis shows that the α and β1 subunit protein expression are lower in cerebral than pulmonary arteries. Taken together, we for the first time provide evidence that the differential activity of Ca^{2+} sparks (ryanodine receptors) and BK channel regulatory β1 subunit may confer the distinct functions of BK channels in cerebral (systemic) and pulmonary artery SMCs, contributing to the diversity of cellular responses in these two different vascular systems.

547-Pos The Mechanism of Regulation of the IK1/maxi-K Interaction: Suggested Roles of Maxi-K Beta and Cholesterol

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Board B392

Recently we demonstrated a novel interaction between large conductance (maxi-K) and intermediate conductance (IK1) Ca^{2+}-activated K channels: maxi-K activity is strongly inhibited by activation of IK1. To understand the molecular mechanism of the interaction we used iberiotoxin (IBX). It has been shown that co-expressing of beta subunit of maxi-K dramatically reduces the inhibitor sensitivity of the channel. In parotid acinar cells IBX partially inhibited maxi-K current and the remaining current showed very low sensitivity to activation of IK1. This suggests that maxi-K beta subunit negatively regulates the IK1/maxi-K interaction. The IK1/maxi-K interaction is also regulated by another, cholesterol-dependent mechanism: cholesterol depletion attenuates the channel interaction. Similar attenuation was observed when endogenous cholesterol was partially substituted with its optical isomer, epicholesterol. This is consistent with the role of specific sterol-protein binding in regulation of the IK1/maxi-K interaction. To investigate further this mechanism we used cholesterol substitution with coprostanol and epicoprostanol, which has been shown to have strong effect on membrane physical properties and stability of cholesterol-rich microdomains - a similar effect is produced by cholesterol depletion. While substitution with coprostanol did not affect the IK1/maxi-K interaction, epicoprostanol weakened the interaction. Therefore, change in membrane physical properties and cholesterol-dependent microdomains are not required for cholesterol-dependent regulation of the IK1/maxi-K interaction. This conclusion is consistent with the previously found independence of the channel interaction on caveolin, the scaffolding protein of caveolae. Finally, cholesterol depletion did not affect IBX sensitivity of native maxi-K current but reduced IK1-dependent inhibition of both IBX-sensitive and IBX-insensitive fractions of maxi-K. This suggests that

(i) association of maxi-K with its beta subunit is cholesterol-independent and
(ii) regulation of the IK1/maxi-K interaction by cholesterol is downstream of maxi-K alpha/beta association or is an independent mechanism.

548-Pos The Accessory Beta4 Subunit Regulates The Response Of Bk Channels To Dephosphorylation

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Board B393

Large conductance calcium-activated potassium (BK) channels regulate membrane excitability in diverse cell types. BK channels are composed of a pore-forming alpha subunit and tissue-specific accessory beta subunits that tailor BK channel properties to different cells types. Since BK channel function is affected by phosphorylation status, we wished to test if the neural-specific beta4 subunit alters the response to dephosphorylation. Our approach was to transfect BK alpha channels in HEK293 cells with or without beta4, and test channels' response to a variety of Thr/Ser protein phosphatase inhibitors. BK currents were assayed using the in the inside-out configuration of the patch clamp technique. Application of 500 nM okadaic acid (OA, which blocks PP1 and PP2A) in 4 micromolar calcium shifted the conductance-voltage (G/V) relation to more positive potentials when BK channels were co-expressed with beta4 but did not change G/V relations for alpha subunit alone. In 130 micromolar calcium, application of 500 nM caused a positive G/V shift of alpha subunit alone but did not affected G/V relation of alpha+beta4 BK channels. Selective blockers of PP2A (Calyculin A and 10 nM OA) failed to alter G/V relations of both alpha alone or alpha+beta4 BK channels in both 130 and 4 micromolar calcium. Surprisingly, we found that a selective blocker of PP1, Fostriecin did not reproduce the OA effects. Thus, neither specific blocker of protein phosphatases reproduces the effect of OA. By default, these results indicate that OA-sensitive phosphatases, PP1 and PP2A, both have overlapping roles in modulating beta4-dependent effects on BK channels.

549-Pos Probing Molecular Interactions Between hSloRCK1 and hSloRCK2 Domains in BK_{Ca} Channels

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Board B394

We are investigating the interaction between RCK1 and RCK2 domains in the human BK channels (hsloRCK). We took advantage

of the fluorescent properties of the N-(1-pyrene)maleimide dye (Py). Py is a thiol-reactive fluorophore that reports changes in its surrounding environment. hSloRCK1 (322*IIE*. . .*DPL*667) and hSloRCK2 (668*LLI*. . .*KAL*1004) were expressed in E. coli and purified. Endogenous cysteines of hSloRCK1 and hSloRCK2 were labeled with Py (10μM) and Py emission spectra ($\lambda_{EM=}$360–600nm) were recorded for different free [Ca^{2+}], ranging from to 0.01 to 32 μM ($\lambda_{EX=}$320nm). Increasing [Ca^{2+}] caused a progressive quenching of fluorescence intensity in hSloRCK1 or hSloRCK2, suggesting Ca^{2+}-induced conformational changes. On the contrary, the equimolar mixture of Py-labeled hSloRCK1 and non-labeled hSloRCK2 (or vice versa) elicited a fluorescent signal that increased with [Ca^{2+}] and was associated with a relative decrease of excimer fluorescence (485nm) suggesting an interaction between hSloRCK1 and hSloRCK2. To investigate possible regions of interaction between hSloRCK domains, we performed covalent cross-linking of equimolar amounts of purified hSloRCK1 and hSloRCK2 using disuccinimidyl suberate followed by digestion with chymotrypsin to generate cross-linked peptides. Digestion products were analyzed by reverse phase liquid chromatography and tandem mass spectrometry on a Thermo Orbitrap. Database searching of peptide spectra identified multiple unmodified peptides from both domains; we focused our manual analyses on peptides modified by the cross-linker. The analyses revealed a cross-linked peptide pair corresponding to the additive mass of a peptide fragment each from hSloRCK1 and hSloRCK2 derived from their αG-helix regions (Yusifov et al., BJ 2007, 278A). A structural model of the putative hSloRCK1-RCK2 heterodimeric complex (based on MthK channel, Jiang et al., 2002) predicts close physical proximity of the two crosslinked peptides. Thus, several lines of evidence indicate that hSloRCK1 and hSloRCK2 co-assemble, possibly forming a BK gating ring structure.

550-Pos BK Channel α and β Subunits in Complex with the Thromboxane A2 Receptor: Protein-Protein Interactions and Functional Consequences

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Board B395

Thromboxane A2 (TxA2), a potent constrictor of smooth muscles, mediates the pathogenesis of vascular diseases like stroke and infarction. U46619, a TxA2 analogue, inhibits the activity of coronary BK channels reconstituted in lipid bilayers. We hypothesized that TXA2 receptors (TP) and BK may form a tight complex in native arteries. BK α subunit was immunoprecipitated (IPed) with TP from aorta and coronary arteries. To elucidate the epitopes of this association, human TPα and human BK α (hSlo) +/− β subunit (tagged with c-Myc or Flag sequences) were co-transfected in HEK 293T cells and different intracellular regions of TPα were produced as GST-fusions. Reverse co-IPs and fluorescence confocal microscopy showed that hSlo and TPα are closely associated. This association was independent of β subunit coexpression as the robust hSlo-TPα co-IP signal was unaltered. Also, β-subunit can by itself associate with TPα independently of hSlo. GST-pull down assays demonstrated an intracellular loop of the TP receptor as the interaction site with hSlo. Inside-out patches of freshly dissociated human coronary artery myocytes showed that U46619 inhibits BK channel activity. This inhibition was not changed by a phospholipase C inhibitor, but can be reversed by SQ 29548, a specific TP receptor antagonist. Moreover, mechanical experiments showed that BK blocker, Iberiotoxin, modified the ability of U46619 to induce contraction by decreasing the agonist EC_{50} from 17 to 5 nM. We conclude that TP receptors and BK α and β subunits form a unique complex facilitating the signaling between TP receptor activation and BK channel inhibition.

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551-Pos Inter-domain Formation of A Metal-Ion Binding Site For BK Channel Activation

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Board B396

BK-type K^+ channels, activated by voltage, intracellular Ca^{2+} and Mg^{2+}, are essential for regulating muscle contraction and various neuronal activities such as synaptic transmission and hearing. Our studies have shown that E374/E399 in the cytosolic RCK domain form part of the Mg^{2+} binding site and the bound Mg^{2+} activates the voltage sensor via an electrostatic interaction with R213 in the S4 transmembrane segment [1–2]. The Mg^{2+}-coordination requirements and the spatial proximity of Mg^{2+} to S4 imply that the membrane-spanning domain may also contribute to Mg^{2+} coordination. Here we show that D99, located at the linker between the transmembrane S0 and S1 helices, provides another Mg^{2+} coordinate. Among individual Ala mutations of all oxygen-containing residues in the membrane-spanning domain, only D99A completely abolishes Mg^{2+} effects on channel activation. A positive charge covalently added to the vicinity of the Mg^{2+} binding site, but not Mg^{2+}, still affects the gating current of D99A, indicating that this mutation prevents Mg^{2+} binding. To verify that D99 forms a ligand for Mg^{2+}, we mutated D99 to various amino acids and found that the carboxylate or carbonyl group on its side chain is required for Mg^{2+} sensing. Further, a disulfide bond between C99 and C397, which is in the vicinity of the E374/E399 site, was detected, indicating the spatial closeness between D99 and the cytosolic part of the Mg^{2+} binding site. These results indicate that the Mg^{2+} binding site is formed by residues from both the membrane-spanning and the cytosolic domains. Thus, the two structural domains with distinct origins have evolved to engage in intimate interactions and control the activation of BK channels synergistically.

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552-Pos Stimulation of β3-adrenoceptors with BRL 37344 Relaxes Rat Urinary Bladder Smooth Muscle via Activation of Large Conductance Ca^{2+}-activated K^+ (BK) Channels

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Board B397

Here, we investigated the role of large conductance Ca^{2+}-activated K^+ (BK) channels in the β3-adrenoceptor (β3-AR)-induced relaxation in rat urinary bladder smooth muscle (UBSM). BRL 37344 (0.1–100 μM), a specific β3-AR agonist, inhibits the spontaneous contractions of isolated UBSM strips. Iberiotoxin (200 nM), a specific BK channel inhibitor, shifts the BRL 37344 concentration-response curves for contraction amplitude (n=7; $P<0.01$), force (n=7; $P<0.05$), and tone (n=7; $P<0.01$) toward higher BRL 37344 concentrations. To further determine the role of BK channels in β3-AR-induced relaxation, UBSM cells were freshly isolated for perforated patch-clamp experiments. 100 μM BRL 37344 increased transient BK current frequency to 146.0±20.1% (n=5; $P<0.05$) but had no significant effect on transient BK current amplitude. This effect was due to an increase in Ca^{2+}-sparks activity because 100 μM BRL 37344 did not change single BK channel activity. In cell-attached mode at 0 mV holding potential the single BK channel amplitude was 5.17±0.28 pA, while in the presence of BRL 37344 was 5.55±0.41 pA (n=5; $P>0.05$). The BK channel open probability was also unchanged, 0.0022±0.0018 NP_o/pF (control) versus 0.0019±0.0012 NP_o/pF (BRL 37344) (n=5; $P>0.05$). In the presence of ryanodine and nifedipine, the steady state BK current-voltage relationship before and after BRL 37344 application showed no difference (n=8; $P>0.05$). In current-clamp mode, application of 100 μM BRL 37344 caused membrane potential hyperpolarization from −22.02±3.76 mV (control) to −25.47±3.91 mV (n=11; $P<0.0005$). The hyperpolarization induced by BRL 37344 was abolished by application of the non-specific BK channel inhibitor-tetraethylammonium (1 mM) (n=8; $P>0.05$). The data indicate that stimulation of β3-AR with BRL 37344 relaxes rat UBSM by increasing the transient BK current frequency, which leads to membrane hyperpolarization and relaxation.

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553-Pos Is a Large Entrance to the Inner Vestibule of BK Channels Required for their Large Conductance?

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Board B398

BK channels have the largest conductance of all K^+ selective channels. The structure of BK channels is unknown, but may be homologous to the large conductance MthK channel, which has a large entrance to the inner vestibule. We hypothesize that a large entrance to the inner vestibule of BK channels is required for their large conductance. Our study tests this hypothesis by examining whether changing the size of the entrance to the inner vestibule changes the single-channel conductance. The size of the entrance was changed by substituting amino acids with different sized side chains at positions E321/E324 located at the entrance. Two classes of substitutions were made using the hydrophobic amino acids alanine and valine or the polar uncharged amino acids serine, asparagine, and glutamine. Comparisons of the effect of side chain volume on single-channel current amplitude were made only within each functional group. For each group, increasing the side chain volume decreased the outward single-channel current, while having little effect on inward single-channel current. Increasing $[K^+]_i$ from 150 mM to 2.5 M removed the difference in single-channel current associated with different side chain volumes. These observations suggest that a large entrance to the inner vestibule of BK channels contributes to their large conductance.

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554-Pos Studying the Calcium Induced Movement of a Proposed BK(Ca) Gating Ring

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Board B399

The large conductance calcium activated potassium BK(Ca) channel activity is modulated both by voltage across the membrane and by intracellular calcium concentrations. Evidence suggests that the calcium sensitivity of this channel is conferred by a large, intracellular domain. When calcium binds to this domain, opening of the channel (gating) requires less energy. The exact mechanism of this process is not clear. Recently, structures of a tetrameric, prokaryotic, calcium activated, potassium channel (MthK) suggest that its intracellular calcium sensor domain (RCK) forms a ring structure (called the gating ring). In response to calcium binding, the "gating ring" expands, and presumably could apply a force on the channel gate to make its opening more energetically favorable. Since BK (Ca) is also tetrameric, and its calcium binding domain shares some homology with the binding domain of MthK, it has been proposed that the two channels share a similar calcium sensing mechanism. In this study we measure movements within the calcium-binding domain using FRET. By labeling the calcium, binding domain with either CFP or YFP, it is possible to form hetero-tetramers that contain both CFP and YFP tagged domains. Through the use of Confocal Fluorescence Microscopy it is possible to image cell membranes that contain these hetero-tetramers, and to obtain the fluorescence spectra required to measure FRET. In the absence of calcium, we measure a high FRET signal indicating that the CFP and YFP are close to each other in space. In the presence of calcium, the FRET signal decreases, suggesting that the CFP and YFP have moved further apart. These results are consistent with the model where a "gating ring" expands in response to increased calcium

concentrations. The practical methods and results of this study will be discussed.

555-Pos Single-channel Kinetics of BK Channels with Each Subunit Containing One Functional Ca Sensor

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Board B400

Each subunit of BK channels contains a voltage sensor, two high affinity Ca^{2+} sensors (identified by the Ca^{2+} bowl and D362/D367), and one low affinity Ca^{2+}/Mg^{2+} sensor identified by E399A. With four sensors per subunit tetrameric BK channels would require a multi-tiered allosteric model with a minimum of 1250 states to describe the single-channel kinetics. To work towards developing such a model we are studying the single-channel kinetics of BK channels with a single voltage sensor and a single Ca^{2+} sensor per subunit to reduce the model to a minimum of 50 states. Initial experiments are for the Ca^{2+} bowl with 100 micromolar intracellular Ca^{2+} to further reduce the model towards 10 states by saturating the Ca^{2+} binding site. The parameters for the model were obtained by simultaneously fitting 2-D dwell time distributions obtained over a wide range of voltages to maximize the likelihood that the experimental data were drawn from distributions predicted by the model. The ranking of models took into account the number of free parameters. A two tiered 10 state model with cooperative coupling of each voltage sensor to opening and closing transitions could approximate the single-channel kinetics and Po over a range of voltages, but some of the rate constants using systematic allosteric models were physically unrealistic. The inclusion of a third tier of closed 'flicker' states did not correct the problem, but the inclusion of cooperativity among voltage sensors, such that activation of each voltage sensor increased the activation rates of subsequent voltage sensors, did give realistic rate constants.

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556-Pos Prediction Of The High Affinity Ca^{2+}-binding Site In The N-terminus Of The Cytoplasmic Region Of The BK_{Ca} Channel

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Board B401

The Ca^{2+} ion plays a major role in controlling the opening and closing of the large conductance BK_{Ca} channels thereby regulating the K^+ potassium currents passing through the membrane. Ca^{2+} activation in these channels is achieved by binding of Ca^{2+} to the channel structure which triggers the opening of the gate. Two high affinity binding sites have been identified in the channel structure and one of the sites is located in the conserved DRDD loop in the N-terminus of the cytoplasmic domain. The first aspartate in this conserved DRDD loop, residue D367, reduces Ca^{2+} sensitivity by a significant amount when mutated to alanine and hence is thought to be a putative binding site, however the mutations R368A, H365A and D362A in and around the loop also reduce Ca^{2+} sensitivity by a significant amount. Here we present results on the prediction of the Ca^{2+} binding site based on a series of computational studies. The basic protocol involves multiple iterations of random ion placement, implicit solvent molecular dynamics calculations to allow rearrangement of coordinating side chains, and then explicit solvent MD simulations to allow the system to equilibrate. The correspondence between the mutagenesis results and our finding is presented.

557-Pos Activation Of Rat Glioma Bk Channels Induces Paraptosis That Stimulates Immunity: A Novel Cancer Vaccine?

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Board B402

High grade gliomas kill thousands every year and are resistant to typical cancer treatments. Work by our labs has implicated large-conductance Ca^{2+}-activated K^+ channels (BK or Maxi-K Channels) in the mechanism by which human glioma cancer cells are killed by macrophages expressing membrane macrophage colony stimulating factor (mM-CSF) (Hoa et. al., *Laboratory Investigation*, *87*, 115–129, 2007). We show that paraptosis can be initiated through a Maxi-K channel dependent process initiated by reactive oxygen species (ROS). Electrophysiological recordings from cell attached patches revealed the presence of low expression of voltage dependent large conductance channels (>180 pS). By a combination of confocal and electron microscopy, flow cytometry, electrophysiology, pharmacology and genetic knock-down approaches, we show that these ion channels control cellular swelling and vacuolization of rat T9 glioma cells. Our data suggests that when macrophages encounter the mM-CSF cell, ROS are produced that cause hemoxygenase and P450 reductase to enzymatically produce carbon monoxide (CO). CO mediates the opening of Maxi-K channels on the cell membrane, as well as in the ER and the mitochondria thereby allowing pooled stores of K+ to be expelled. In whole body animal experiments, these cells vaccinated the rats towards the parental cells, which implicate an immune response. These results are the first to show that glioma cellular death induced by prolonged Maxi-K channel activation naturally improves tumor immunogenicity. Strategies that induce paraptosis may prove quite valuable in clinical immunotherapy against cancer.

558-Pos Determining the Affinities of the BKCa Channel's High-Affinity Ca2+ Binding Sites with High Resolution Ca2+ Dose-Response Curves

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Board B403

It has been established that the large conductance Ca2+-activated K+ channel contains two high-affinity Ca2+ binding sites. The affinities of these sites and how they change as the channel opens, is still a subject of some debate. Previous estimates of these affinities have relied on fitting a series of conductance voltage relations determined over a series of Ca2+ concentrations with models of channel gating that include both voltage sensing and Ca2+ binding. This approach requires that some model of voltage sensing be chosen, and differences in the choice of voltage-sensing model may underlie the different estimates that have been produced. Here, to better determine these affinities we have measured Ca2+ dose-response curves at constant voltage for the wild-type mSlo channel (minus its low-affinity Ca2+ -binding site) and for channels that have had one or the other Ca2+ binding site disabled via mutation. To accurately determine these dose-response curves we have used a series of 22 Ca2+ concentrations, and we have used unitary current recordings, coupled with changes in channel expression level, to measure open probability over 6 orders of magnitude. Our results indicate that at −80 mV the Ca2+-bowl related Ca2+ binding site has an affinity of 3.28 uM when the channel is closed and 0.94 uM when the channel is open, while the RCK1-related site has lower affinities, 23.3 uM when the channel is closed and 5.44 uM when the channel is open.

559-Pos Ca^{2+}- and Thromboxane-Dependent Distribution of MaxiK Channels in Cultured Astrocytes: From Microtubules to the Plasma Membrane

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Board B404

Large-conductance, voltage- and Ca^{2+}-activated K^+ channels (MaxiK, BK) are broadly expressed ion channels comprised of pore-forming α-subunits arranged in a tetrameric conformation in close association with regulatory β-subunits. MaxiKα is typically observed as a plasma membrane protein in various cell types such as neurons. However, the physiological role of MaxiKα in astrocytes remains to be elucidated. Our previous results using high-resolution confocal microscopy have revealed the novel finding that MaxiKα in cultured murine astrocytes are distributed intracellularly, colocalized along the microtubule network. This MaxiKα association with microtubules was further confirmed by his-tag pulldown assays and microtubule depolymerization experiments (100μM colchicine, 20μM nocodazole). In order to gain a better understanding of the physiological relevance behind the intracellular deposition of astrocytic MaxiKα, we applied a variety of stimuli to elicit a change in MaxiKα distribution. Our current findings indicate that changes in intracellular Ca^{2+} elicited by general pharmacological agents, caffeine (20mM) or thapsigargin (1μM), result in the increased distribution of MaxiKα at the plasma membrane of cultured astrocytes. In an additional result supporting the hypothesis that intracellular Ca^{2+} may influence MaxiKα in a physiological system, application of thromboxane A2 receptor (TPR) agonist U46619 (100nM), which activates Ca^{2+} release pathways, also elicits a similar increase in surface expression of MaxiKα. These changes in MaxiKα plasma membrane distribution are effectively blocked by preincubating astrocytes with a cell permeable Ca^{2+}-chelator, BAPTA-AM. The results of this study provide novel insight that suggests Ca^{2+} released from intracellular stores may play a key role in regulating the traffic of intracellular, microtubule-associated MaxiKα stores to the plasma membrane of cultured murine astrocytes.

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560-Pos The BK Channel β_1 (*KCNMB1*) Subunit TM2 Is A Discrete Sensor For Lithocholate

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Board B405

Lithocholate (LC) and other bile acids increase the activity (NPo) of large conductance, calcium- and voltage-gated potassium (BK) channels in cerebral artery myocytes and, thus, cause cerebrovascular dilation (Bukiya et al., 2007). Native cerebral artery myocyte BK channels consist of pore-forming (cbv1) and accessory (β_1) subunits. Interestingly, LC-induced cerebrovascular dilation is blunted in vessels from *KCNMB1* knockout mice. Moreover, after channel subunit expression in *Xenopus* oocytes, LC potentiates cbv1± β_1 NPo (≈ × 2.5 times) but fails to modify cbv1, cbv1+β_2, cbv1+β_3, or cbv1+β_4 channel activity (Bukiya et al., 2007). These results point to BK β_1 as the LC-sensor in the BK protein complex. Beta subunits consist of N- and C- intracellular termini, two transmembrane domains (TM1, TM2) and an extracellular loop (L). To determine the β_1 region that senses LC we constructed chimeras by swapping subunit regions between β_1 (which provides LC-sensitivity to cbv1) and β_4 (which does not), and investigated LC action using patch-clamp methods following channel expression in *X.* oocytes. Vehicle- and LC-containing solutions were applied to the intracellular side of I/O patches ($[Ca^{2+}]$=10 μM; V=+20 or −20mV). LC (150 μM) failed to activate cbv1+β_4L_1 but caused a reversible increase in cbv1+β_1L_4 NPo (≈ ×2.2 times). Thus, transmembrane or intracellular regions in β_1 are involved in LC sensing. Moreover, LC failed to activate cbv1+β_1TMs_4 while reversibly increasing (≈ ×2.4 times) cbv1+β_4TMs_1 NPo. Finally, cbv1+β_4TM1_1 channels remained insensitive to LC; whereas, cbv1+β_4TM2_1 were LC-sensitive, with NPo in LC increasing ≈

×2 times. This effect is similar to that observed with LC on cbv1+β_1 (*P>0.05;* n=6). We conclude that TM2 acts as the sensing region in the β_1 subunit for LC activation of BK channels.

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561-Pos BK Channel Alpha S0 Location

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Board B406

The large-conductance potassium channel (BK) alpha subunit contains a seventh transmembrane helix, S0, N-terminal to the conserved S1–S6. We mutated to Cys the first four residues flanking the extracellular ends (tops) of S0, S1, S2, S5, and S6. The extracellular S3-S4 loop contains only 4 residues, two of which we mutated to Cys. We generated ~50 double-Cys mutants, in which one Cys was in the S0-flanking region and one was in the region flanking one of S1-S6. These were expressed in HEK-293 cells, which were surface-labeled with an impermeant biotinylation reagent before their solubilization. BK channels trafficked to the cell surface were selected with avidin beads, and the extent of direct disulfide crosslinking was determined by SDS-PAGE and immunoblotting. Several of the Cys pairs involving the S0 flank and the S3–S4 loop formed intrasubunit crosslinks to an extent >90%. In three such pairs, there was almost no effect of this crosslinking on the G-V curve or the kinetics of opening and closing. This implies that the top of S0 contacts the S3–S4 loop and, to the extent that the S3-S4 loop moves during gating, the top of S0 moves with it. Furthermore, these disulfides can be reduced by dithiothreitol applied outside of outside-out patches and, with the voltage held at −80 mV and the channel closed, can be reformed by a doubly charged, presumably membrane-impermeant oxidizing agent. Cys flanking S0 formed intrasubunit crosslinks with Cys flanking S1 and S2 to an extent of ~60%, and some of these crosslinks caused 50–100 mV rightward or leftward shifts in V50 and slowing of the gating kinetics, consistent with relative movement among S0, S1 and S2 during gating. S0 did not crosslink to S5 and S6. The tops of S1–S4 surround the top of S0.

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562-Pos Subunit-specific Effect of the Voltage Sensor Domain on Ca2+ Sensitivity of BK Channels

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Board B407

Large conductance, Ca2+- and voltage-activated K+ (BK) channels, composed of pore-forming alpha subunits and auxiliary beta subunits, play important roles in diverse physiological processes. The differences in BK channel phenotypes are primarily due to the tissue-specific expression of beta subunits (beta1-4) that modulate channel function differently. Yet, the molecular basis of the subunit-specific modulation is not clear. Here we report that the perturbation of voltage sensor movements by mutations of the putative voltage sensing residues in BK channels selectively disrupted the ability of the beta1 subunit, but not that of the beta2 subunit, to enhance apparent Ca2+ sensitivity. Among these mutations, D153C, R167A and R213C abolished the beta1-induced, Ca2+-dependent shift of the conductance-voltage (G-V) relationship, while D186A results in a partial elimination. In contrast, none of these mutations reduced the beta2-induced G-V shift. Furthermore, mutations of non-voltage sensing residues R207 and Y163 did not change the effect of the beta1 subunit although both mutations altered the voltage dependence of activation. These results suggest that mutations of the voltage sensing residues in BK channels influence a specific aspect of voltage sensor movements that is also crucial for the beta1-induced G-V shift in various [Ca2+]i, while the similar function of the beta2 subunit is underscored by a distinct mechanism.

563-Pos Acute Alcohol Tolerance Is Intrinsic To The Bk_{Ca} Protein, But Is Modulated By The Lipid Environment

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Board B408

Ethanol tolerance, in which exposure leads to reduced sensitivity, is an important component of alcohol abuse and addiction. However, the molecular mechanisms underlying this phenomenon remain poorly understood. The BK_{Ca} channel plays a central role in the behavioral response to ethanol in *C. elegans* (1), and in *Drosophila* (2). In neurons, ethanol tolerance observed in BK_{Ca} channels has two components: a reduced number of membrane channels, and a decreased potentiation in the remaining channels (3). Here, we use heterologous expression, coupled with planar bilayer techniques to examine two additional aspects of alcohol tolerance in human BK_{Ca} channels:

1. What is the minimal unit capable of showing acute tolerance; i. e. can we observe tolerance in a single channel protein complex within a lipid environment reduced to only two lipids?
2. Does lipid bilayer composition affect the appearance of acute tolerance in Bk_{Ca} channels? We found that acute ethanol tolerance was observable in BK_{Ca} channels in membrane patches pulled from transfected HEK cells, and in the same channels placed into reconstituted PE/PS membranes.

Further, altering bilayer thickness by incorporating the channel into bilayers of lipid mixtures of DOPE with phosphatidylcholines (PCs) of C (14:1), C (18:1), C (20:1) and C (24:1), or with brain sphingomyelin (SPM)), strongly affected the sensitivity of the channel to ethanol, as well as the time course of the acute ethanol response. Ethanol sensitivity changed from a strong potentiation in thin PC (14:1)/DOPE bilayers, to only inhibition in thick SPM/DOPE bilayers. Our data suggest that tolerance can be an intrinsic property of the channel protein complex, and that lipid thickness plays an important role in shaping the pattern of response to ethanol.

564-Pos Butyl Nitrite (poppers) Activate BK Channels With Beta 1 Subunits Differentially In The Presence Or Absence Of Ca^{2+}

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Board B409

Poppers are inhaled recreational drugs that cause a rapid fall in blood pressure, flushing of the face, smooth muscle relaxation, and feelings of euphoria, Previously we demonstrated that the popper compound butyl nitrite activates the alpha subunit of large conductance calcium-activated potassium, (BK) channels from the intracellular side of the membrane. Here we extend those studies. Macrocurrent recordings demonstrated that neither butanol nor nitrite ions, the breakdowns products of butyl nitrite, activated BK channels, suggesting a direct action of butyl nitrite. We find that butyl nitrite also increases channel activity for BK channels coexpressed with the BK channel beta 1 subunit found in smooth muscle and involved in regulating vasodilation via smooth muscle tone. Single-channel recordings from inside-out patches showed that in 0 Ca^{2+}, 1 mM butyl nitrite increased open probability, Po, and that the increase arose almost entirely by shortening the long shut times between bursts (i.e. by increasing the frequency of bursts), with little effect on open times, burst duration, or number of openings per burst. Interestingly, in the presence of Ca^{2+}, butyl nitrite acted to increase Po, not only by decreasing long shut times but also by increasing burst duration through an increase in the number of openings per burst. In addition we find that butyl nitrite activates BK channels when applied in the extracellular solution. These data suggest that butyl nitrite modulates the BK channel via the transmembrane region and that BK channels may mediate some of the physiological effects of butyl nitrite, such as rapid vasodilation and other smooth muscle relaxation that occurs upon inhalation.

565-Pos Two hERG Activators Work by Different Mechanisms

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Board B410

HERG activators may provide therapeutic actions against acquired or congenital long QT syndrome. Several experimental hERG activators are available; however, their binding sites and mechanisms of action are not clear. We address these issues for 2 such drugs, PD(307243) & NS(1643), using oocyte & COS-7 expression systems. Both PD and NS work from the extracellular side of cell membrane & increase currents (@ 0 mV) by as much as 100 & 200% with apparent K_d 3 & 20 uM, respectively. Their agonist effects on the hERG channel are additive, suggesting distinct binding sites and mechanisms of action. Indeed, although both cause a modest slowing of hERG inactivation, they have distinctly different effects on hERG activation: while PD does not alter voltage-dependence or kinetics of activation or deactivation, NS appears to accelerate the forward transitions and/or slow the backward transitions among closed states in the activation pathway. Furthermore, BeKm-1 (a peptide toxin that binds to hERG outer vestibule to reduce currents without occluding the pore) does not prevent PD's agonist effect but suppresses NS' agonist effect. On the other hand, hERG pore occlusion by extracellular tetrapentylammonium or intracellular dofetilide prevents PD's agonist effect. Cysteine substitution in hERG outer vestibule region can disrupt the inactivation process by different mechanisms: mutating high-impact positions, forming disulfide bonds or MTS modification of side chain. These inactivation-disrupted mutants are not responsive to NS' agonist effect, but they are all responsive to PD's agonist effect. Based on these data, we propose that NS is a hERG gating-modifier: it binds to the outer vestibule (overlapping with the BeKm-1 binding site) and increases currents by interfering with channel inactivation. PD may be a hERG pore-modifier: it increases currents by directly increasing the channel pore conductance with little effects on channel gating.

566-Pos Modulation Of HERG (Human Ether-a-go-go Related Gene) Potassium Channel By Ceramide

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Board B411

HERG (Human ether-a-go-go related gene) potassium channels play an important role in repolarization of cardiac action potential. Ceramide, a sphingolipid-derived second messenger, has been implicated in the regulation of ion channels. However, ceramide mechanisms of HERG regulation are not well understood. Using whole cell patch-clamp, we investigated the effect of C6-ceramide on HERG activity in HEK293 cells stably expressing HERG. C6-ceramide (10 μM) inhibited HERG current with an onset occurring around 40 seconds after the application. The average inhibition of peak tail current was 35 ± 12 % (SD; $n = 5$), whereas the bio-inactive dihydro-C6-ceramide (10 μM) did not significantly affect HERG current (6 ± 5 % SD; $n = 3$). C6-ceramide treatment resulted in negative shift in the activation voltage in a reversible and dose dependent manner. The shift in V1/2 was -4 ± 2 mV at 1 μM and -8 ± 2 mV at 10 μM C6-ceramide (SD, $n = 7$–18). C6-ceramide also significantly increased the speed of deactivation with the fast deactivation tau increased by 34 ± 6 % and the slow tau increased by 48 ± 5 % (SD, $n = 5$) (both $p < 0.01$). Again dihydro-C6-ceramide was without any significant effect on activation voltage and deactivation. The inhibition of HERG by ceramide combined with the shift in voltage of activation can affect the duration of cardiac action potential resulting in arrhythmias. The physiological and pathophysiological significance of these rapid and specific effects of ceramide on HERGs and the mechanisms involved are being investigated using agents that increase the endogenous ceramide levels.

567-Pos Functional Interactions between HERG and KvLQT1 Downregulate I_{kr} Currents

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Board B412

KvLQT1 and HERG code for the α-subunits of ***IKs*** and ***IKr***, the two main repolarizing K^+ currents of most mammalian cardiomyocytes. Whether KvLQT1 and HERG interact with each other is what we are currently concerned. We have created two transgenic rabbit models for long QT syndrome 1 (LQT1) and long QT syndrome 2 (LQT2) by over-expression of human a loss-of-function pore mutant of KvLQT1 (KvLQT1Y315S) and HERG (HERGG628S), respectively in the heart. The result showed that as expected KvLQT1Y315S abolished the endogenous IKs currents, however, it also down-regulated the IKr currents. Similarly, over-expression of HERGG628S transgene abolished the endogenous IKr currents and reduced the IKs currents.

To further investigate the possible interaction between HERG and KvLQT1, we have created a CHO cell line stably expressing HERG with a FLAG epitope on its N-terminus. Transient transfection of either WT KvLQT1 or KvLQT1Y315S resulted in the down-regulation of HERG currents. Immunocytochemistry experiments confirmed that the surface HERG was down-regulated with the transfection of either WT KvLQT1 or KvLQT1Y315S. Coimmunoprecipitation experiment shows that HERG polypeptides could be co-precipitated with either WT KvLQT1 or KvLQT1Y315S. Moreover, KvLQT1 polypeptides could be co-precipitated with either WT HERG or HERGG628S polypeptides. The down regulation observed *in vitro* correlates with our observation in LQT1 and LQT2 rabbits. Collectively, these results suggest that HERG and KvLQT1 polypeptides might specifically and significantly interact with each other (directly or indirectly) and that these interactions could modify the level of expression of the currents encoded by these α subunits.

568-Pos Biochemical and Spectroscopic Studies of Heteromeric Interactions of HERG1a and HERG1b Potassium Channel Subunits

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Board B413

Human Ether-a-go-go Related Gene (HERG) encodes a voltage-gated potassium (K^+) channel that is abundantly expressed in the heart and in nervous tissue. Two isoforms of HERG, 1a and 1b, have been described in heart and neurons. In the heart, 1a and 1b have been shown to form heteromeric channels and underlie the native rapidly activating delayed rectifier K^+ current (I_{Kr}). Here, we examined the direct interaction of 1a and 1b subunits. Single cysteine mutations were introduced to 1a and 1b. Cysteines spontaneously formed disulfide bonds and in the absence of reducing agents were stable through biochemical purification. Western blots of homomeric single cysteine mutants showed two bands corresponding to subunit monomers and dimers. Mixing 1a and 1b cysteine-containing subunits showed a band consistent with 1a-1b dimers, indicating direct 1a–1b subunit interactions. Homomeric double cysteine mutants showed two additional bands which were consistent with trimeric and tetrameric complexes. Coexpression of 1a and 1b subunits with double cysteine mutations showed formation of a 1a-1b dimer as well as multiple bands at high molecular weights. The sizes of these bands were consistent with the formation of multiple combinations of heteromeric complexes. To investigate subunit interactions at the cell surface, HERG1a and HERG1b were fused to the Enhanced Cyan Fluorescent Protein (eCFP) and Citrine fluorescent proteins. We used Forster Resonance Energy Transfer (FRET) to show direct interactions between 1a and 1b subunits at the membrane surface. We used Fluorescence Intensity Ratio studies to measure relative 1a and 1b subunit expression levels at the membrane surface and electrophysiology to measure channel function. Taken together, the results indicate that that the interaction of HERG1a and HERG1b subunits is random in membranes and at the membrane surface.

Anion Channels

569-Pos GaTx1: A New Tool For The Study of ATP-dependent Gating In The CFTR Chloride Channel

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Board B414

We recently reported the isolation and initial characterization of a novel 3.7 kDa peptide toxin, GaTx1, which is a potent and reversible inhibitor of CFTR, acting from the cytoplasmic side of the membrane. While many peptide inhibitors of cation-permeable channels have been described, and used as highly specific probes of those channel targets, peptide toxin inhibitors of chloride channels of known molecular identity have not been available. Thus, GaTx1 is the first peptide toxin identified which inhibits a chloride channel of known molecular identity. GaTx1 was prepared in synthetic form using solid-phase chemistry, and was then folded under oxidizing conditions and purified by HPLC. In excised, inside-out patches pulled from oocytes expressing wildtype CFTR, GaTx1 inhibited CFTR channel activity by prolonging the interburst closed duration. GaTx1 exhibited high specificity, showing no effect on a panel of nine transport proteins including Cl^- channels, K^+ channels, and ABC Transporters. To study dose-response relations for activity of synthetic toxin, we used multichannel patch recordings from oocytes expressing Flag-cut-ΔR-CFTR, taking advantage of the insensitivity of this CFTR variant to dephosphorylation-mediated rundown. GaTx1-mediated inhibition of CFTR channel activity is strongly state-dependent; both potency and efficacy are reduced in presence of high [ATP]. When CFTR channels were pre-incubated