paralleled by impaired K^+ coordination in the selectivity filter (Choi et al., 1991; Hoshi et al., 1990; Lopez-Barneo et al., 1993). The mechanism of KCNQ1 inactivation and its modulation by external K^+ are dissimilar to the mechanism described for C-type inactivation in *Shaker*-like K^+ channels (Gibor et al., 2007). Further, inactivation of wild-type (WT) KCNQ1 channels becomes evident only in the characteristic hooked tail currents which reflect recovery from inactivation (Abitbol et al., 1999; Pusch et al., 1998; Tristani-Firouzi and Sanguinetti, 1998). We use a combination of functional-structural analysis combined with mathematical and 3D-structural modeling to gain insights into the structural rearrangements during KCNQ1-inactivation. We show that the Kv7.1 α-subunits act in a concerted way to initiate KCNQ1-inactivation.

640-Pos

Non-Obligatory Gating of Kv7.1 Potassium Channel

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The response of voltage-dependent K+ (Kv) channels to a change in membrane voltage involves a molecular device, which couples voltage-driven conformational changes to gate opening and closing within the channel's conduction pathway. To further our understanding of the coupling choreography we have studied how changes in coupling strength instigate the Kv channel to open before and after voltage-sensor activation. We used single and double mutations in a Kv channel pore domain to analyze coupling sensitive sites. We observed in the mutational effects a correlation between coupling strength and non-obligatory Kv channel gating that is well described with a four-state allosteric gating model. Mapping the data onto known Kv channel structures showed that coupling-sensitive amino acid residues are strategically clustered to a small area between pore gate and the interface of pore and voltage sensors. We propose that the physical contact at the interface between voltage sensor and pore domain is an important determinant of altered coupling strengths leading to obligatory and non-obligatory Kv channel gating.

641-Pos

Diclofenac Activates Kv7.4 and Inhibits Kv7.5 Potassium Channels Heterologously Expressed in A7r5 Vascular Smooth Muscle Cells

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Members of the KCNQ (Kv7) voltage-gated potassium channel gene family are differentially expressed through the body. Five Kv7 subtypes play major roles in regulation of membrane potential and cell excitability within different tissues. Well known functions of Kv7.1 in cardiac action potential duration, Kv7.2/7.3 in neuronal excitability, Kv7.4 in hearing and an emerging role of Kv7.5 in vascular tone, increases the demand for channel modulating drugs that exhibit selectivity among Kv7 subtypes. Diclofenac, an anti-inflammatory drug, was found to be a novel Kv7.2/7.3 channel opener and was used as a template to synthesize new activators and inhibitors of neuronal KCNQ channels (Kv7.2/7.3 and Kv7.4). We compare affects of diclofenac on human Kv7.4 and Kv7.5 currents using A7r5 rat aortic smooth muscle cells as an expression system and perforated patch-clamp techniques. Diclofenac, at 100∈ 1/4M, increased maximal conductance of Kv7.4 channels by 1.5-fold and induced a negative shift in the activation curve (by 9mV). Surprisingly, the same concentration of diclofenac (100∈ 1/4M) reduced maximal conductance of Kv7.5 channels by 2-fold, but also induced a robust negative shift in the activation curve (by 30mV). For the both Kv7.4 and Kv7.5, application of diclofenac (100∈ 1/4M) reduced the deactivation rate of the current. For Kv7.4, the deactivation rate was 1.7-fold slower in the presence of diclofenac, independent of the voltage in the range from −120mV to −90mV. In contrast, for Kv7.5 the reduced deactivation rate in the presence of diclofenac was voltage-dependent, changing linearly from 2-fold at −120mV to 4-fold at −90mV. These differences in diclofenac action on two members of the Kv7 channel family may reflect structural differences between Kv7.4 and Kv7.5 and make diclofenac a useful tool to distinguish between Kv7.4 and Kv7.5 currents in native tissues.

Ca-Activated Channels

642-Pos

Cholesterol Depletion Alters Amplitude and Pharmacology of Vascular Calcium-Activated Chloride Channels

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Calcium-activated chloride channels (CACCs) share common pharmacological properties with SLO (KCNMA1)-encoded large conductance K^+ channels ($K_{Ca}1.1$) and it has been suggested they may co-exist in a macromolecular complex (Greenwood, I. A., and Leblanc, N. *Trends Pharmacol Sci*28: 1-5, 2007; Saleh *et al.* J Pharmacol Exp Ther 321: 1075-1084, 2007; Sones *et al. Br J Pharmacol* 158: 521-531, 2009). As $K_{Ca}1.1$ channels are known to localise to cholesterol and caveolin-rich lipid rafts (caveolae) the present study investigated whether Ca^{2+}-sensitive Cl^- currents in vascular myocytes were affected by the cholesterol depleting agent Beta-methyl cyclodextrin (Beta-MCD). Calcium-activated chloride and potassium currents were recorded from single murine portal vein myocytes in whole cell voltage clamp. Western blot was undertaken following sucrose gradient ultracentrifugation using protein lysates from whole portal veins. Ca^{2+}-activated Cl^- currents were augmented by Beta-MCD with a rapid time-course ($t_{0.5}$ = 1.8 min). Beta-MCD had no effect on the bi-modal response to niflumic acid or anthracene-9-carboxylate but completely removed the inhibitory effects of the $K_{Ca}1.1$ blocker paxilline and the stimulatory effect of the $K_{Ca}1.1$ activator NS1619. Discontinuous sucrose density gradients followed by Western blot analysis revealed that $K_{Ca}1.1$ was present in lipid fractions, co-localising with lipid raft markers caveolin and flotillin-2. The newly identified candidate for calcium-activated chloride channels TMEM16A, co-localised to the same fractions as $K_{Ca}1.1$. These data reveal that CACC properties are influenced by lipid raft integrity. The results also provide a structural basis explaining the intimate functional interaction that exist between $K_{Ca}1.1$ and CACCs in generating STOCs and STICs and how they regulate resting membrane potential and tone in vascular myocytes.

643-Pos

Ca2$^+$-Activated Cl- Currents of Pulmonary Artery Smooth Muscle Cells are Enhanced in Monocrotaline-Induced Pulmonary Arterial Hypertension in the Rat

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Pulmonary arterial hypertension (PAH) in humans is defined by a pulmonary artery pressure (PAP) exceeding 25 mm Hg at rest, and 30 mm Hg during physical activity. Three major factors contribute to elevating PAP in PAH patients: 1) enhanced vasoconstriction; 2) reduction of lumen diameter due to remodeling of the arterial wall; and 3) enhanced clot formation. It has been recently suggested that Cl^- currents can regulate proliferation of cultured rat pulmonary artery (PA) smooth muscle cells (Liang et al., *Hypertension* 54: 286-293, 2009). The purpose of the present study was to determine if Ca^{2+}-activated Cl^- currents ($I_{Cl(Ca)}$) are altered in PA smooth muscle cells from monocrotaline(MCT)-treated rats. Aged-matched male rats were either injected with saline or a single dose of MCT (50 mg/kg) to induce PAH, and the animals from both groups were sacrificed after 3 weeks. Rats treated with MCT displayed an increase in right ventricular weight with no change in left ventricular and septum weights, consistent with PAH. Patch clamp experiments revealed that the cell capacitance, an index of cell surface, of PA cells from MCT-injected rats was 40% greater than that of cells from saline-injected rats. Time- and voltage-dependent $I_{Cl(Ca)}$ elicited by 500 nM internal free Ca^{2+} (buffered with 10 mM BAPTA) displayed outward rectification in both groups but was more than ~ 2-fold larger in the MCT *vs.* saline group. In both groups, the current ran down over time but significantly more on a percentage basis in MCT than control cells. In conclusion, the properties and regulation of $I_{Cl(Ca)}$ appear to be altered in a validated animal model of PAH and these results suggest that this anion current may represent a new therapeutic target.

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The Interaction of Antrhacene-9-Carboxylic Acid with Calcium-Activated Chloride Channels is Influenced by the State of Global Phosphorylation in Pulmonarty Artery Smooth Muscle Cells

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Ca^{2+}-dependent Cl^- currents (I_{ClCa}) are inhibited by phosphorylation in arterial smooth muscle cells. We recently reported that niflumic acid (NFA), an inhibitor of I_{ClCa}, is less efficacious at blocking these currents in conditions promoting phosphorylation (Wiwchar *et al.*, *Br J Pharmacol*, in press, 2009). This

study aimed to assess whether another Cl^- channel blocker, anthracene-9-carboxylic acid (A9C), is also affected by channel phosphorylation. A9C blocks I_{ClCa} at positive potentials but paradoxically stimulates the inward I_{ClCa} tail after repolarization to negative potentials (Piper & Greenwood, *Br J Pharmacol* 138: 31-38, 2003). I_{ClCa} was evoked by pipette solutions containing 500 nM free Ca^{2+} with or without 5 mM ATP to alter the state of phosphorylation. Although A9C (1-500 uM) dose-dependently blocked steady state I_{ClCa} at potentials positive to 0 mV in all cell groups, its maximal effect and sensitivity to voltage were enhanced in cells dialyzed with 0 vs. 5 mM ATP. For example, maximal block by 100 uM A9C was 35 and 73%, and $V_{0.5}$ was 110 and 67 mV, in cells with 5 vs 0 ATP, respectively. A9C enhanced I_{ClCa} tail at −80 mV by causing a negative shift in voltage-dependence in both cell groups, with a larger shift occurring in cells dialyzed with 5 mM ATP. Interestingly, 100 but not 500 uM A9C stimulated steady-state $I_{Cl(Ca)}$ at potentials < 0 mV in cells dialyzed with 0 ATP, a potential range where $I_{Cl(Ca)}$ was unaffected in myocytes dialyzed with 5 mM ATP. As with NFA, the complex actions of A9C on I_{ClCa} are influenced by the state of channel phosphorylation and we propose the existence of at least two binding sites with different affinities for A9C.

645-Pos

F233A Mutation in AT_1R Interrupted Caveolae Targeting and Abolished Regulation of hSlo Channel by Angiotensin II

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The large conductance Ca^{2+}-activated K^+ (BK) channels play an important role in the regulation of vascular tone in response to changes in intracellular metabolic status and Ca^{2+} homeostasis. Angiotensin II (Ang II)-mediated c-Src activation is known to inhibit the activities of BK channels. Recently, trafficking of the Ang II type I receptor (AT_1R) into caveolae was shown to be essential for Ang II signaling and activation of c-Src. We found that BK channels and the AT_1R signaling complex are colocalized in the caveolae of vascular smooth muscle cells (VSMC). In this study, we examined the role of caveolae in the Ang II-mediated modulation of BK channels by co-expressing hSlo channels, AT_1R, caveolin-1, and c-Src in HEK293 cells. Immunoblot analysis confirmed that hSlo and AT_1R were co-precipitated by anti-caveolin-1 antibody only in cells co-transfected with caveolin-1, but not in those without caveolin-1, suggesting that hSlo, AT_1R, and caveolin-1 were physically associated. Exposure to Ang II (2 μM) inhibited the hSlo current density by 32.7 ± 2.8 %, and the Ang II effect was blocked by Losartan (2 μM) with only 1.2 ± 7.8 % current inhibition. However, in cells coexpressing hSlo, c-Src, caveolin-1, and the AT_1R F233A mutant, which abolished its interaction with the caveolin-1 scaffolding domain, exposure to Ang II produced only 6.0 ± 3.7% hSlo current inhibition suggesting that caveolae targeting of AT_1R is crucial for Ang II-mediated BK channel regulation. These results were confirmed by experiments using mouse VSMC. Ang II produced 40% inhibition of BK currents in cells from wt mouse but had no effect on those from cav-1(-/-) animals. Hence, translocation of AT_1R into caveolae upon agonist activation represents a critical step in Ang II regulation of BK channels and vascular function.

646-Pos

Angiotensin II Effects on BK Channel in Mesenteric Arterial Smooth Muscle Cells of SS, BN, and Congenic Renin+ and Renin- Rats

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Cardiovascular sensitivity to anesthetics has been linked to the renin-angiotensin system (RAS), and it is thought to be related to the differences in cardiovascular collapse observed between SS and BN rats. In a previous study we found that congenics carrying the BN renin allele (renin+) in the SS background had the same cardiovascular sensitivity and low BK channel activity as BN rats. On the other hand, congenics carrying the SS renin allele (renin-) displayed high BK activity and behaved like SS rats, suggesting that RSA could be involved in differential responses. To test this hypothesis, the inhibition of BK channel by angiotensin II (AngII, 100 nM) was evaluated in four strains. Activity of BK was monitored from isolated mesenteric arterial smooth muscle cells of SS and BN rats, and renin+ and renin- congenic rats in the cell-attached mode at +80 mV Em and in symmetrical 150 mM K+. Blockade by paxilline (1 μM) confirmed identity of BK channel. Similar to findings from our previous study, in the cell-attached mode the probability of BK channel opening (Po) was different between SS (high Po) and BN (lower Po). The BK activity of renin2- resembled that of SS, whereas BK activity of renin+ matched BN. AngII had a greater inhibitory effect on channel Po in BN (−55+7%) and renin+ (−94+2%) strains than in SS (−9+6%) and renin- (−7+2%) strains. Impaired renin expression and impaired RAS are associated with lower sensitivity of BK to inhibition by AngII in SS than in BN rats. The fact that renin+ and renin- strains follow a similar pattern appears to support this conclusion.

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Maxik Channel Beta1 Subunit Interacts and Regulates Thromboxane A2 Receptor Function

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MaxiK channel, composed of the pore-forming α (MaxiKα) and regulatory β1 subunits, controls vascular tone via the activation or inhibition of its pore conducting activity. Thromboxane A2 receptor (TPR), a G-protein coupled receptor, induces potent vasoconstriction mediated by its agonist, thromboxane A2. Previously, we demonstrated that U46619, the stable analogue of thromboxane A2 inhibits MaxiK channel in vascular smooth muscle cell contributing to U46619-induced vasoconstriction. In this study, we report that this inhibition is reversed by nanomolar dehydrosoyasaponin I (DHS-I), a pharmacological tool that indicates MaxiKα and β1 association and functional coupling. The reversing effect of DHS-I indicated that the MaxiK channels inhibited by U46619 could be coupled with regulatory β1 subunits. Co-immunoprecipitation and double immunolabeling in co-expressing cells showed that TPR form a complex with β1 on the plasma membrane. To identify the interacting sites in β1 responsible for TPR-β1 complex formation, we prepared serial carboxyl-terminal deletions of β1 and analyzed their interaction properties with TPR in co-immunoprecipitation experiments. β1 lacking amino acids 103-191 reduces the TPR-β1 association by 44 ± 14% ($p<0.01$), while deletion of residues 73-191 completely reduces the TPR-β1 interaction, suggesting that amino acids 73 to 191 predominantly contribute to the TPR-β1 interaction. To further investigate how β1 regulates TPR-MaxiKα functional coupling, inside-out patch clamp experiments were performed in HEK293T cells expressing TPR + MaxiKα +/- β1 subunit. We found that the β1 subunit reduces U46619-induced MaxiKα inhibition in a dose-dependent manner. In summary, β1 interacts with TPR forming a tripartite complex with MaxiKα and opposes to TPR agonist-induced MaxiK channel inhibition serving as a buffer to vasoconstriction. Thus, in pathological situations like hypertension or aging where β1 expression is compromised the TPR-MaxiK complex would induce severe vasoconstriction. Supported by NIH and AHA.

648-Pos

Kca3.1 Blockers as Potential New Drugs for the Prevention of Renal Fibrosis and Chronic Allograft Rejection

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The calcium-activated potassium channel $K_{Ca}3.1$ is critically involved in the proliferation and migration of T cells, macrophages, dedifferentiated vascular smooth muscle cells and fibroblasts by regulating membrane potential and calcium influx. $K_{Ca}3.1$ has therefore been suggested as a potential therapeutic target for various diseases where activation and excessive proliferation of one or more of these cell types is involved in the pathology. Using the selective small molecule $K_{Ca}3.1$ blocker TRAM-34 as a pharmacological tool compound we previously demonstrated that $K_{Ca}3.1$ blockade prevents restenosis in both rats and pigs and reduces atherosclerosis development in $ApoE^{-/-}$ mice. We now used two models of chronic allograft rejection and one model of kidney fibrosis to evaluate whether $K_{Ca}3.1$ blockers might also be useful for the prevention of transplant rejection and fibrotic kidney changes. In a murine model of obliterative airway disease, where tracheas from CBA mice were heterotopically transplanted into the greater omentum of C57Bl6 mice, both genetic deficiency or pharmacological blockade of $K_{Ca}3.1$ with TRAM-34 reduced luminal obliteration from 92 ± 7% to 60 ± 29% or 61 ± 28% ($n = 6$ per group). We further performed orthotopic aortic transplantations in the PVG-to-ACI rat model and evaluated chronic allograft vasculopathy after 120 days. TRAM-34 at 10 mg/kg (−35%) and 40 mg/kg (−60%) dose-dependently reduced chronic aortic luminal obliteration. Genetic disruption of $K_{Ca}3.1$ and pharmacological blockade also reduced fibrotic marker expression, chronic tubulointerstitial damage, collagen deposition and alphaSMA(+) cells in kidneys following unilateral ureteral obstruction in mice. Taken together, our findings suggest that $K_{Ca}3.1$ channels are involved in the pathology of obliterative airway disease, chronic allograft vasculopathy and fibrotic kidney disease.
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