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.

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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.

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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.

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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