Administration of 10 and 30 mg/kg SKA-31 lowered mean arterial blood pressure by 4 and 6 mmHg in normotensive mice and by 12 mmHg in angiotensin-II-induced hypertension. These effects were absent in KCa3.1-deficient mice. In conclusion, with SKA-31 we have designed a new pharmacological tool to define the functional role of KCa2/3 channel activation *in vivo*. The blood pressure lowering effect of SKA-31 suggests KCa3.1 channel activation as a new therapeutic principle for the treatment of hypertension.

2454-Pos Board B424

Molecular Action Of CFTR Potentiators On The Kca3.1 Channel

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Airway epithelial cells are the site of Cl- secretion through the cystic fibrosis transmembrane regulator (CFTR). Cystic fibrosis (CF) is a fatal genetic disease caused by mutations in CFTR. The most frequent mutation consists of a deletion of the phenylalanine at position 508 (ΔF508-CFTR) that impairs protein maturation and alters channel gating. In the last years, several small molecules were identified by high throughput screening that could restore ΔF508-CFTR function. Compounds addressing ΔF508-CFTR gating defects are referred to as potentiators and have been documented to increase the activity of ΔF508-CFTR to a level similar to wild-type CFTR. The basolateral K+ channel KCa3.1 has been documented to play a prominent role in establishing a suitable driving force for CFTR-mediated Cl- secretion in airway epithelial cells. Thus, in a global approach of transepithelial transport, the research for physiologically relevant ΔF508-CFTR potentiators should also consider their effects on the KCa3.1 channel. A characterization of the effect of different ΔF508-CFTR potentiators on the KCa3.1 channel was undertaken using inside-out patch clamp measurements on cDNA injected *xenopus* oocytes and on transformed HEK-293 cells that express the KCa3.1 channel. In this work we present preliminary results on the effects of different ΔF508-CFTR potentiators on KCa3.1. Our inside-out patch-clamp measurements show that VRT-532 has a state independent inhibitory effect on KCa3.1, but very little action on the V282G mutant of KCa3.1, which is constitutively active. In contrast, CBIQ succeded to activate KCa3.1, through a mechanism likely to involve an action on the channel gate. These effects were observed at concentrations known to activate ΔF508-CFTR. Supported by CCFF.

2455-Pos Board B425

Mechanism of Benzofuroindole-induced Potentiation of BK_{Ca} channel

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In our previous studies, we reported that the activity of the large-conductance calcium-activated potassium channels (BK_{Ca} channel) could be strongly potentiated by certain derivatives of benzofuroindole scaffold (Gormemis *et al.*, 2005; Ha *et al.*, 2006). Here, we characterized the mechanism of action of these compounds. Benzofuroindoles potentiated the channel by shifting its conductance-voltage relations toward the more negative direction without affecting its voltage sensitivity. This drug was proven to act on the alpha-subunit of the channel (Slo1) from the extracellular side. The dose-response curve of the drug could be well fitted with the Hill coefficient close to 1. While the apparent affinity of the drugs was not affect by tetraethyl ammonium, a channel-blocking quaternary ammonium, the co-treatment of charybdotoxin significantly decreased the potency of the compounds, suggesting the potential competition between the drug and the peptide blocker. Guided by these results, we performed the mutagenesis studies on the outer vestibule of the BK_{Ca} channel in order to localize the drug binding site. Among one deletion and 19 alanine substitutions, four mutant channels showed significantly smaller shifts in their conductance-voltage curves by the drug treatment compared to the wild-type. Since these mutations were clustered at the 'turret' region of the channel, benzofuroindole derivatives may stabilize the open conformation of BK_{Ca} channel by binding to this area.

2456-Pos Board B426

Energetic Performance is Improved by Specific Activation of K+ Fluxes through KCa Channels in Heart Mitochondria

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K^+ movement across mitochondrial membranes is involved in volume regulation and may play a role in cardioprotection. The Ca^{2+}-dependent K^+ (KCa) channel has been proposed as a contributor to mitochondrial K^+ uniport activity, but its functional role is not well understood. To investigate the impact of KCa channels on mitochondrial energetics, we measured K^+ fluxes in parallel with $\Delta\Psi_m$ and light scattering in isolated mitochondria from guinea pig hearts. We first analyzed the role of different anions on K^+ fluxes. Mitochondria loaded with the K^+-sensitive fluorescent probe PBFI were incubated with 5mM glutamate-Na^+/malate-Na^+ in isotonic sucrose medium and subjected to pulses containing different concentrations of KCl, KAc or KH_2PO_4. K^+ fluxes saturated at $\approx$10mM regardless of the anion, attaining maximal rates (nmol K^+/min/mg) of 172 ± 17 (KCl), 84 ± 2.4 (KAc), and 74 ± 3.8 (KH_2PO_4), with similar $K_{0.5}$ in all three cases. We then analyzed the effect of NS11021, a novel activator of KCa channels, on the maximal K^+ uptake rate. In the presence of KH_2PO_4 or KAc, 20-50nM of NS11021 increased mitochondrial volume and K^+ flux by ~2.5-fold whereas KCl increased K^+ uptake by 30% with little change in volume. $\Delta\Psi_m$ was minimally affected in this concentration range. The NS11021 effect was blocked by 200nM charybdotoxin, a KCa channel blocker. At 50nM NS11021, the respiratory control ratio of the mitochondria increased 2.5-fold in the presence of KH_2PO_4, but not KCl, indicating that a regulatory volume increase is required to improve oxidative phosphorylation. At higher concentrations of the compound ($\geq 1\mu M$) substantial effects on $\Delta\Psi_m$ and state 4 respiration were observed, which were not inhibited by Chtx. The findings indicate that activation of K^+ fluxes through KCa channels, coupled with swelling without loss of $\Delta\Psi_m$, improves mitochondrial energetic performance.

Cyclic Nucelotide-gated Channels

2457-Pos Board B427

Structural and Energetic Analysis of the Cyclic nucleotide binding domain from the MlotiK1 Potassium Channel

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MlotiK1 is a cyclic nucleotide-dependent ion channel which contains an intracellular C-terminal cyclic nucleotide binding domain (CNB domain). We have used X-ray crystallography to determine several different structures of the MlotiK1 CNB domain structures in the bound and unbound state. In combination, the five MlotiK1 CNB domain structures provide a unique opportunity for analyzing, within a single protein, the structural differences between the *apo* and bound states and the structural variability within each state. With this analysis as a guide, we have probed the nucleotide selectivity and importance of specific residue side chains in ligand binding and channel activation. These data help to identify ligand-protein interactions that are important for ligand-dependence in this channel and more globally in the class of nucleotide-dependent proteins.

2458-Pos Board B428

Enhancement of Voltage Sensitivity of a cGMP-gated Channel

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Activity of cyclic nucleotide-gated (CNG) cation channels underlies signal transduction in vertebrate visual receptors. These channels must be primarily activated by the binding of cGMP so that the activity of these highly specialized receptor channels be controlled by ligands in a finely graded manner required for transducing sensory stimuli of varying intensity. Significant voltage sensitivity of the channels would generate voltage-driven positive feedback and thus reduce the signal-transduction sensitivity. Indeed, the CNGA1 channel is only modestly voltage sensitive in low cGMP concentrations, and the voltage sensitivity vanishes with increasing cGMP concentration. We have found that loosening the attachment of the selectivity filter to the surrounding ''pore shell'' dramatically increases the channel's voltage sensitivity, which is independent of the positively charged residues in S4. Thus, proper attachment of the selectivity filter is essential to avoid significant, adverse voltage sensitivity in these channels.

2459-Pos Board B429

Lidocaine Inhibition of HCN1 Channels is Fast, Voltage-dependent and Reversible

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Hyperpolarization-activated Cyclic Nucleotide-modulated (HCN) channels underlie the funny current or the hyperpolarization-activated current (If or Ih), which is important in regulating excitability in the neurons of the central nervous system and in the conduction tissue of the heart. There are four mammalian isoforms of HCN channels (HCN1-4), each exhibiting different kinetics, voltage dependence, and amounts of inward, time-dependent current (If). Lidocaine, a local anesthetic and antiarrhythmic drug, has been shown to inhibit HCN-mediated currents in the rabbit sinoatrial (SA) node, which expresses various HCN isoforms. Previously, we showed that lidocaine, at concentrations ranging from 20 to 200 μM, inhibits mouse HCN1 channels, but the rate and extent of both