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

Ariane Longpré-Lauzon, Line Garneau, Helene Klein, Rémy Sauvé.
GÉPROM, Université de Montréal, Montréal, QC, Canada.

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

Byoung-Cheol Lee[1], Hyun-Ho Lim[2], Yong-Chul Kim[1], Chul-Seung Park[1].
[1]Gwangju institute of science and technology, Gwangju, Republic of Korea, [2]Department of Biochemistry, Howard Hughes Medical Institute, Brandeis University, Waltham, MA, USA.

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

Miguel Aon[1], Sonia Cortassa[1], Morten Grunnet[2], Brian O'Rourke[1].
[1]Johns Hopkins University, Baltimore, MD, USA, [2]NeuroSearch A/S, Ballerup, Denmark.

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

Joao Morais Cabral[1], Stephen Altieri[2], Gina Clayton[2], William Silverman[2], Adrian Olivares[2], Enrique De La Cruz[2], Lise R. Thomas[3].
[1]IBMC, Porto, Portugal, [2]Yale University, New Haven, CT, USA, [3]Quinnipiac University, New Haven, CT, USA.

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

Juan R. Martinez-Francois[1], Yanping Xu[1], Zhe Lu[2].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia, PA, USA.

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

Raymond Yip, Damiano Angoli, Christopher A. Ahern, Eric A. Accili.
University of British Columbia, Vancouver, BC, Canada.

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

inhibition and drug washout, were not determined. Here, these issues are examined using Chinese hamster ovary (CHO) cells, transiently transfected with mouse HCN1 DNA, measurement of resulting currents using the whole-cell patch clamp technique, and a fast perfusion system for the application of lidocaine. We found that the onset of lidocaine action is fast (~6 seconds) with maximum inhibition occurring at approximately 20 seconds, and that its effects are partially reversible. With 200 μM lidocaine perfused directly onto the cell under study, If elicited in response to -85mV and -100mV test pulses was reduced by 18.4 ± 3.6 % (n= 6 cells) and 8.3 ± 1.0 % (n= 7 cells), respectively, with average current return of 49.1 ± 10.9 % and 73.9 ± 9.4 % to the level prior to lidocaine addition. The fact that currents did not return completely may be attributed to a phenomenon called current rundown, or to incomplete washout of lidocaine. The significant difference in current reduction between -85mV and -100mV suggests that the effect of lidocaine on HCN1 channels depends on voltage, with greater If inhibition at less negative voltages.

2460-Pos Board B430

Ligand Binding and Gating in HCN2 Channels

Jana Kusch[1], Christoph Biskup[1], Susanne Thon[1], Eckhard Schulz[2], Klaus Benndorf[1].

[1]Universitétsklinikum Jena, Jena, Germany, [2]Fachhochschule Schmalkalden, Schmalkalden, Germany.

HCN channels are nonselective tetrameric cation channels that are activated by hyperpolarizing voltages and modulated by the ligand cAMP. They generate spontaneous rhythmic activity in heart and brain. Ligand binding to the intracellular cyclic nucleotide-binding site accelerates activation kinetics, shifts the steady-state activation to more positive voltages and increases the open probability. Though it is relatively simple to determine an apparent affinity for the ligand action, it is not so simple to determine the true ligand affinity during channel activation because, according to the principle of reciprocity, ligand binding and efficacy depend on each other, i.e. the affinity of the binding sites must increase when the channel opens. It is therefore important to determine the binding of the ligands to the channels and channel activation simultaneously.

Activation of homotetrameric HCN2 channels was studied in inside-out macropatches simultaneously with ligand binding by means of patch-clamp fluorometry, using a fluorescent cAMP (fcAMP) that activates the channels in a similar manner as cAMP. With 1 μM fcAMP the binding of the ligand to the open channels exceeded that to closed channels. The slowness of the activation time course of HCN2 channels allowed us to monitor the ligand binding during the activation process. As predicted, the slow activation time course was accompanied by an increase of ligand binding. Moreover, the increase of binding was exponential whereas activation obeyed the typical sigmoidal time course. Hence, in the superimposed normalized time courses, the initial binding preceded activation whereas at later times activation preceeded binding. These results show that activation gating indeed increases the binding affinity for the ligands, quite as predicted by the principle of reciprocity, and that the gating of the first of the four subunits, which does not lead to channel opening, is associated with ligand binding.

2461-Pos Board B431

Electrophysiological Evaluation of Novel Blockers of If Current

Martina Del Lungo[1], Michele Melchiorre[2], **Laura Sartiani**[1], Martin Biel[3], Andras Varro[4], Maria N. Romanelli[2], Elisabetta Cerbai[1].

[1]Center of Molecular Medicine University of Florence, Florence, Italy, [2]Department of Pharmaceutical Sciences, University of Florence, Florence, Italy, [3]Department of Pharmacy and Pharmacology, University of Munich, Munich, Germany, [4]Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary.

In the sino-atrial node (SAN) a major role in rhythm generation is played by f-channels. They mediate a sodium-potassium inward current (If) activated upon hyperpolarization and encoded by HCN genes (HCN1-4). If is overexpressed in cardiac diseases at ventricular level, where it may contribute to the increased propensity for arrhythmias. Selective f-channel blockers have a potential therapeutic use as bradycardic and antiarrhythmic agents. Zatebradine and ivabradine act as f-channel blockers but they lack cardiac selectivity, blocking the neuronal HCN isoforms. As a consequence an unmet need exists to develop new blockers selective for the mammalian SAN channel isoform, HCN4. Zatebradine analogues (C1-C5) were synthesized and the effect on If was measured on patch-clamped HEK293 cells expressing mHCN1, mHCN2 and hHCN4 and native guinea-pig and rabbit SAN cells. At 10 μM concentration all compounds reduced maximal If amplitude; however, potencies (defined by EC50) differed considerably. Ivabradine, taken as reference compound, showed no isoform selectivity; C1 and C4 were more potent on HCN1, the ratio being 4.3 (HCN2/HCN1) and 7.5 (HCN4/HCN1). C2 was more potent on HCN4, the ratio being 6 and 17 vs. HCN1 and HCN2, respectively. C3 was equipotent on HCN1 and HCN4 and C5 had low activity on all isoforms. Blockade was concentration-dependent, did not reverse upon drug removal and did not change current activation properties. Finally, data obtained in SAN cells show that effects on native If resemble those obtained on HCN4 isoform, in accordance with the hypothesis that HCN4 has a major contribution in SAN cells. Present results indicate that drug interaction with different HCN isoforms has diverse structural requirements. Current investigations are aimed to characterize the pharmacological profile of the new f-channel blockers and improve their isoform selectivity (Supported by EU - LSH M/CT/2006/018676, Normacor).

2462-Pos Board B432

Functional Consequences Of Disease-associated Mutations In The Pore Region Of Human Cone Photoreceptor CNG Channels

Katja Koeppen, Peggy Reuter, Thomas Ladewig, Bernd Wissinger.

Institute for Ophthalmic Research, Tuebingen, Germany.

CNGA3 encodes the A-subunit of the cone photoreceptor cyclic nucleotide-gated (CNG) channel. Mutations in the *CNGA3* gene have been associated with achromatopsia, a congenital, autosomal-recessively inherited retinal disorder characterized by lack of color vision, severely reduced visual acuity, photophobia and nystagmus. The aim of this study was the functional characterization of five mutant CNGA3 channels with amino acid substitutions in the pore region (S341P, L363P, G367V, P372S and E376K), which had been identified in achromatopsia patients. Mutant channels were heterologously expressed in HEK293 cells and their functional properties were assessed by calcium imaging and patch-clamp measurements. For patch-clamp recordings mutant CNGA3 was co-expressed with the wild-type B3 subunit present in native channels and transfected cells were incubated at 27°C in order to enhance folding and trafficking of the channel mutants. Furthermore, immunocytochemical experiments were performed after incubation at either 27°C or 37°C to determine the extent of co-localization of mutant channels with the cell membrane.

All five pore mutations rendered the channel non-functional in calcium imaging experiments, indicating severely reduced calcium influx through the mutant channel pore. Interestingly, cGMP-induced potassium currents could be recorded from patches containing channels with the mutations S341P, G367V and E376K. Even though macroscopic currents were small compared to wild type channels, these three pore mutants have been shown to possess residual potassium conductivity. While channels with the mutation G367V, P372S or E376K showed normal co-localization with the plasma membrane after incubation at 37°C, reduced surface expression was observed for channel mutants S341P and L363P, suggesting impaired folding and/or trafficking of the mutant proteins.

2463-Pos Board B433

Bimodal Agonism In A Cyclic Nucleotide-Gated Channel Is Coordinated By Two Adjacent Binding Domains

Kerry Chan, Edgar Young.

Simon Fraser University, Burnaby, BC, Canada.

Bimodal agonism is a cGMP-dependent desensitization found in the catfish CNGA2 subtype of the cyclic nucleotide-gated channel. In bimodal agonism, initial cGMP binding events at lower concentrations (<3 mM) increase channel open probability (P_o); however, additional cGMP binding events at higher concentrations (>3 mM) decrease P_o. Understanding bimodal agonism could lead to building cyclic nucleotide analogues that can specifically open or close the channel. The C-terminal region (β7-C helix) of the ligand-binding domain (BD) was previously shown to determine ligand selectivity and efficacy, so we tested its role in bimodal agonism. The corresponding region from the normal (non-bimodal) CNGA4 BD was substituted into the bimodal CNGA2 BD to form a chimeric channel subunit. We expressed the chimera as homomers and tested its activation in the excised patch-clamp. Steady-state currents were measured at high and low concentrations of cGMP. The chimera is bimodal ($I_{10mMcGMP}/I_{3mMcGMP}$ is 0.72) suggesting that the C-terminal region of the BD is not essential in bimodal agonism. The roles of intersubunit interactions in bimodal agonism were studied through fusing together two bimodal and two normal pseudo-subunits into tandem tetramers. Tetramers with the two bimodal pseudo-subunits arranged either adjacent (*cis*) or diagonally opposite (*trans*) to each other were tested. The *cis* tetramer is bimodal ($I_{30mMcGMP}/I_{3mMcGMP}$ is 0.87) but the *trans* tetramer is not ($I_{30mMcGMP}/I_{3mMcGMP}$ is 1.05). The *cis* and *trans* tetramers have an identical number of bimodal subunits yet their capability for bimodal agonism (and by extension their P_o at high cGMP concentration) is different. This suggests that the bimodal subunits could be coupled in a dimer within a ''dimer-of-dimers'' mechanism. This also suggests a putative additional cGMP binding pocket may be located in the regions between two adjacent bimodal BDs.

2464-Pos Board B434

The Voltage Sensor Of Cnga1 Channels Becomes Functional When The Hydrophobic Bond Between Phe380 And Leu356 Is Impaired

Monica Mazzolini.

SISSA, Trieste, Italy.