To test the hypothesis that the arginine mutation affects permeation by inhibiting conduction through the pore due to its size and charge, we generated double mutant G156R/N160D channels. Double mutant channels were functional; in addition, G156R/N160D did not show strong rectification in contrast to N160D, suggesting electrostatic interaction between the two residues. Single channel activity of double mutant channels exhibit altered intraburst gating kinetics compared to WT, suggesting the mutations or their interaction affects the selectivity filter. However, double mutant channels were sensitive to inhibitor ATP and activators MgADP and long-chain acyl Coenzyme A similar to WT channels. Collectively, our results demonstrate functional rescue of the putative glycine hinge position caused by a disease mutation in KATP channels.

2404-Pos Board B374

Atrophy and Phenotype Transition Signaling Exert Opposite Actions on the KATP Channels of Disused Rat Soleus Muscle

Domenico Tricarico[1], Antonietta Mele[1], Giulia Maria Camerino[1], Lorenza Brocca[2], Alfred L. George Jr.[3], Roberto Bottinelli[2], Diana Conte Camerino[1].
[1]Dept. of Pharmacobiology, Fac. of Pharmacy, Univ. of Bari, Bari, Italy, [2]Human Physiology Unit, Dept. of Experimental Medicine, Univ. of Pavia, Pavia, Italy, [3]Div. of Genetic Medicine, Dept. of Medicine, Vanderbilt Univ., Nashville, TN, USA.

ATP-sensitive-K^+channel(KATP) is involved in several pathophysiological conditions; whether this channel is affected by atrophy and/or skeletal muscle phenotype transition characterizing muscle disuse is unknown. Here, we combined patch-clamp with MHC expression experiments and measurements of the diameter on the same fibers of slow-twitching soleus muscle(SOL) from controls and 14-days-unloading(HU) rats, an animal model of disuse characterized by atrophy and slow-to-fast phenotype transition. Evaluation of gene expression of KATP channel subunits have been performed in the same muscles. Single fibers analysis showed that 47% of the sampled fibers of SOL from 14-HU rats were atrophic showing a reduced diameter of 45 ± 8 μm and KATP current of -14 ± 3 pA; in contrast not atrophic fibers showed an high KATP current of -120 ± 12 pA and a fiber diameter of 72 ± 7 μm. The atrophic fibers were mostly labeled by MHC1 antibodies(Freq.=41%), had a reduced diameter of 48 ± 8 μm and KATP current of -16 ± 3 pA; with the exception of 1 fiber of MHC2A-type showing a reduced KATP current and diameter. For not atrophic fibers, 29% were of MHC1 showing KATP current of -85 ± 11 pA and diameter of 65 ± 8 μm resembling those of controls; while a significant number of fibers(Freq.=23.5%) were labelled by MHC2A antibodies and showed an enhanced KATP current of -150 ± 12 pA and diameter of 78 ± 0.3μm. RT-PCR experiments showed a reduced expression levels of Kir6.2, SUR1 and SUR2B with no change in the SUR2A subunits in SOL from 14-HU rats. KATP channel is therefore up-regulated in the MHC2A-type fibers in the absence of atrophy, while it is down-regulated in the atrophic MHC1-type fibers indicating that atrophy and slow-to fast phenotype transitions exert opposite actions of this channel type affecting its subunits composition. Supported by ASI-OSMA.

2405-Pos Board B375

Loss Of Regulation Of Primary Afferent Neuronal KATP Channels By Calcium-Calmodulin- CaMKII Mediates Hyperalgesia After SNL

Takashi Kawano, Vicky Zoga, Geza Gemes, Wai Meng Kwok, Mei Ying Liang, Quinn Hogan, Constantine Sarantopoulos.
Medical College of Wisconsin, Milwaukee, WI, USA.

Painful nerve injury decreases IKATP (1) and intracellular calcium (2) in axotomized DRG neurons. Therefore, we hypothesized that: 1) Calcium-Calmodulin-CamKII regulates IKATP in DRG neurons; and 2) painful axotomy attenuates IKATP opening via altering the Calcium-CaM-CamKII signaling. Male rats were subjected to either L5 SNL axotomy (3) or sham skin (SS) operation, and subsequently to sensory testing looking for hyperalgesia or normal response (4). We then compared L5 DRG neurons from: 1) hyperalgesic rats after SNL (SNL-H); 2) rats without hyperalgesia after SNL (SNL-NH); or 3) control neurons from SS rats. Single-channel recordings were obtained from cell-attached (CA) or inside-out (IO) patches.

Neurons exhibited spontaneous single channel opening consistent with IKATP. Channel properties in IO patches did not differ between groups. However, NPo in CA patches was decreased in SNL-H compared to controls ($p<0.01$) or SNL-NH ($p<0.02$). Ionomycin activated IKATP in control ($p<0.01$) or SNL-NH ($p<0.01$), but not in SNL-H DRG neurons. In IO patches, physiological calcium concentration, without or with CaM, did not activate IKATP. However, addition of CaMKII enhanced NPo equally between control and SNL. Finally, in CA patches, CaMKII inhibitors AIP and KN93 blocked ionomycin-induced IKATP activation in control ($p<0.01$), or SNL-NH ($p \leq 0.01$) DRG neurons. In contrast, CaMKII inhibitors did not have any effect in neurons from SNL-H DRG.

Conclusions: Calcium-CaM-CamKII regulates IKATP in DRG neurons. This pathway is attenuated after painful nerve injury, and by less KATP channel opening may explain increased excitability leading to hyperalgesia and neuropathic pain.

References
1 Neurosci Lett 2003; 343: 185-9.
2 Anesthesiology 2007; 107: 117-27.
3 Pain 1992; 50: 355-63.
4 Anesthesiology 2004; 101: 476-8.

Support was provided by the NIH K08 NS049420-01 grant

2406-Pos Board B376

Regulation of Neuronal K_{ATP} Channels by Signaling Elicited by cGMP-Dependent Protein Kinase Activation

Yu-Fung Lin, Yong-ping Chai.
University of California, Davis, CA, USA.

The ATP-sensitive potassium (K_{ATP}) channel couples intracellular metabolic state to cell excitability. Recently, we have demonstrated that activation of the nitric oxide (NO)/cGMP/cGMP-dependent protein kinase (PKG) signaling cascade results in stimulation of Kir6.2/SUR1 (i.e. the neuronal-type K_{ATP}) channels. To understand how PKG activation induces plasma-membrane K_{ATP} channel stimulation, in the present study we investigated the potential involvement of the mitochondrial K_{ATP} (mitoK_{ATP}) channel and reactive oxygen species (ROS) in signal transduction. By performing single-channel recordings in transfected human embryonic kidney (HEK) 293 cells and neuroblastoma SH-SY5Y cells, we found that the enhancement of Kir6.2/SUR1 channel currents by PKG activation observed in cell-attached patches was diminished by the selective mitoK_{ATP} channel inhibitor 5-hydroxydecanoic acid (5-HD), ROS scavengers, and catalase, an enzyme that decomposes hydrogen peroxide (H_2O_2). 5-HD, ROS scavengers and catalase also significantly attenuated Kir6.2/SUR1 channel stimulation induced by NO donors. Moreover, bath application of H_2O_2 increased the activity of Kir6.2/SUR1 channels in cell-attached but not inside-out patches, and the stimulatory effect was not affected by 5-HD, excluding ROS as a signal upstream of the mitoK_{ATP} channel to mediate Kir6.2/SUR1 channel stimulation. In addition, H_2O_2 failed to stimulate tetrameric Kir6.2LRKR368/369/370/371AAAA channels expressed without the SUR subunit in intact cells. Altogether, these novel findings suggest that PKG stimulates neuronal K_{ATP} channels via opening of mitoK_{ATP} channels and ROS generation in a SUR1 subunit-dependent manner, implicating functional coupling between mitoK_{ATP} and plasma-membrane K_{ATP} channels upon PKG activation. The NO/cGMP/PKG/mitoK_{ATP}/ROS signaling cascade may contribute to neuroprotection under ischemic conditions by enhancing the function of plasma-membrane K_{ATP} channels whose activation reduces cell excitability.

2407-Pos Board B377

Glucose Deprivation Regulates K_{ATP} Channel Trafficking via AMPK in Pancreatic Beta-Cells

Ajin Lim, Sun-Hyun Park, Jong-Woo Sohn, Ju-Hong Jeon, Suk-Ho Lee, Won-Kyung Ho.
Seoul Natl Univ Col Med, Seoul, Republic of Korea.

AMP-activated protein kinase (AMPK) and ATP-sensitive K^+ channel (K_{ATP} channel) are metabolic sensors that are activated during metabolic stress. The importance of AMPK has been appreciated by its role as a regulator of metabolism, whereas K_{ATP} channel is known as a regulator of cellular excitability. Cross-talks between two systems are not well understood. In pancreatic β-cells or INS-1 cells, we measured K_{ATP} currents by the patch clamp technique and examined distributions of K_{ATP} channel proteins (Kir6.2 and SUR1) using immunofluorescence imaging and surface biotinylation studies. When K_{ATP} channels were activated by washout of intracellular ATP using a ATP- and Mg^{2+}-free internal solution, the increase in whole cell conductance was surprisingly small in cells incubated in 11.1 mM glucose medium, but the increase was significantly higher in cells pre-incubated in glucose-free medium for 2 hrs. We confirmed that K_{ATP} channel proteins were mostly internalized in 11.1 mM glucose, but recruited to the plasma membrane by glucose deprivation without changes in total levels. The effects of glucose deprivation on K_{ATP} channels were abolished by an AMPK inhibitor or a knockdown of AMPK using siRNA, but mimicked by an AMPK activator. These results suggest that regulation of K_{ATP} channel trafficking by AMPK is a prerequisite for K_{ATP} channel activation in pancreatic β-cells in response to glucose deprivation. The interplay between AMPK and K_{ATP} channels may play a key role in inhibiting cellular excitability and insulin secretion under low energy status.

2408-Pos Board B378

Artificial Ligand-Gated Channels Engineered by Assembly of Potassium Channels and G-Protein Coupled Receptors

Christophe J. Moreau, Jean Revilloud, Julien P. Dupuis, **Michel Vivaudou**.
Institut de Biologie Structurale, Grenoble, France.

Inspired by the natural example of the K-ATP channel in which an ion channel (Kir6.2) is regulated by an associated, unrelated membrane protein, the ABC protein SUR, we have engineered Ion-Channel Coupled Receptors (ICCRs) by physical coupling of G-protein coupled receptors (GPCRs) with Kir6.2.

A first ICCR was constructed using the muscarinic M2 receptor and Kir6.2. Our strategy consisted of creating various fusion proteins by linking the receptor C-terminus to the channel N-terminus and progressively removing residues from either termini until functional coupling was achieved, i.e., agonist binding to the receptor modified channel activity. The fusion proteins were heterologously expressed in Xenopus oocytes and characterized with the two-electrode voltage-clamp and patch-clamp techniques.

Successful coupling was achieved with limited deletions of the channel N-terminal with an optimum of 25 residues. The optimal construct was reversibly upregulated by the M2 agonist, acetylcholine. To further establish proof-of-concept, a second ICCR was obtained by using the dopaminergic D2 receptor. This ICCR was also regulated by D2 agonists and antagonists although, unexpectedly, the D2 ICCR responses were the opposite of those of the M2 ICCR, i.e., agonists caused channel downregulation.

We observed that, i) agonist modulation of Kir6.2 was concentration-dependent and saturatable, ii) agonist effects were abolished by receptor antagonists, iii) the GPCRs within the fusion remained functional as verified by their capacity to activate coexpressed G-protein-activated Kir3 channels. iv) receptor-mediated responses were independent of G-protein activation because they persisted in the presence of pertussis toxin, and v) ICCRs remained functional in cell-free, outside-out patch conditions.

ICCRs could be useful tools for the study of GPCR activation and K+ channel gating and could also serve as biosensors for drug screening and diagnostics.

Ref :

Moreau et al, Nature Nanotechnology. 2008, in press.

2409-Pos Board B379

KirBac1.1: It's An Inward Rectifying Potassium Channel

Wayland W.L. Cheng[1], Decha Enkvetchakul[2], Colin G. Nichols[1].
[1]Washington University School of Medicine, Saint Louis, MO, USA, [2]Saint Louis University School of Medicine, Saint Louis, MO, USA.

The structures of KirBac1.1 and KirBac3.1 have been used extensively to generate in silico homology models of eukaryotic Kir channels and to explore ion permeation, gating and drug-channel interactions by computational approaches. Functional studies of KirBac1.1 have been limited to 86Rb+ flux assays, but we have now succeeded in measuring voltage-clamp currents of KirBac1.1 reconstituted in giant liposomes. Using the patch-clamp technique, we recapitulate the results of 86Rb+ flux experiments, showing that KirBac1.1 currents are potassium-selective, blocked by barium, and inhibited by PIP2. These findings suggest that KirBac1.1 channels are functionally similar to eukaryotic Kir channels. Like the weak inward rectifiers Kir1.1 and Kir6.2, introduction of a negative charge at the ''rectification controller'' residue in the inner cavity of KirBac1.1 (I138D) confers strong inward rectification (i.e. steep voltage-dependent block by spermine). Steady state single channel currents of KirBac1.1 show multiple subconductance levels and gating modes in 150 mM symmetrical K+. However, similar to eukaryotic Kir channels, single channel amplitudes exhibit mild intrinsic inward rectification, with a maximum conductance at ~56 pS (-100 mV), and open probability is higher at positive potentials. Multiple conductance states are still present in single channel currents of other permeant ions such as Rb+ and Tl+. However, similar to many K+ channels, including KcsA, Rb+ and Tl+ single channel currents show increased mean open time and decreased conductance. We find that KirBac1.1(T142C), equivalent to the Kir6.2 high P(o) mutant L164C, also has a high open probability and is effectively blocked by Cd+. These electrophysiological results confirm that KirBac1.1 is a bona fide inward rectifying K+ channel and a tractable model for study of the molecular basis of inward rectification, permeation and gating in eukaryotic Kir channels.

2410-Pos Board B380

Stabilization of KirBac1.1 Tetramer by Blocking Ions

Shizhen Wang[1,2], Yewande Alimi[1], Ailing Tong[2], Colin G. Nichols[2], Decha Enkvetchakul[1].
[1]Saint Louis University, Saint Louis, MO, USA, [2]Washington University, Saint Louis, MO, USA.

Potassium channels are tetrameric proteins that mediate K^+ selective transmembrane diffusion. For the prototypic potassium channel KcsA, interactions between ions and the channel pore can modulate the stability of the tetrameric structure of KcsA, where permeant and strongly blocking ions increase stability, and impermeant or weakly blocking ions tend to decrease stability. Because of the structural similarity between the transmembrane regions of KirBac1.1 and KcsA, we examined the role of blocking ions on KirBac1.1 tetramer stability. In 150 mM KCl, purified KirBac1.1 protein migrates as a monomer (~40kD) on SDS-PAGE. Addition of Ba^{2+} ($K_{1/2}$ ~ 50 μM) prior to loading results in an additional tetrameric band (~160 kD). Mutation A109C, at a residue located near the expected Ba^{2+} binding site, decreased tetramer stabilization by Ba^{2+} ($K_{1/2}$ ~300 μM), while I131C, located nearby, stabilized tetramers in the absence of Ba^{2+}. Neither mutation affected Ba^{2+} block of channel activity (using $^{86}Rb^+$ flux assay). In contrast to Ba^{2+}, Mg^{2+} had no effect on tetramer stability (even though Mg^{2+} was a potent blocker). Many studies have shown Cd^{2+} block of K channels as a result of introduced cysteines in the cavity-lining M2 (S6) residues, with the implicit interpretation that coordination of a single ion by cysteine side-chains along the central axis effectively blocks the pore. We examined blocking and tetramer stabilizing effect of Cd^{2+} on KirBac1.1 with cysteine substitutions in M2. Cd^{2+} block potency followed an alpha-helical pattern consistent with the crystal structure. Significantly, Cd^{2+} strongly stabilized tetramers of I138C, located in the center of the inner cavity. This stabilization was additive with the effect of Ba^{2+}, consistent with both ions simultaneously occupying the channel; Ba^{2+} at the selectivity filter entrance and Cd^{2+} coordinated by I38C side-chains in the inner cavity.

2411-Pos Board B381

Kirbac 1.1 activity in liposomes is suppressed by cholesterol

Dev K. Singh[1], Decha Enkvetchakul[2], Colin G. Nichols[2], Irena Levitan[1].
[1]University of ILLINOIS, Chicago, IL, USA, [2]Washington University, Saint Louis, MO, USA.

Inwardly rectifying potassium channels (Kir) are responsible for regulating diverse processes including: cellular excitability, vascular tone, heart rate, renal salt flow, and insulin release. Our earlier studies have shown that inwardly-rectifying K channels from Kir2 family are strongly suppressed by the elevation of cellular cholesterol. Little is known, however, about the mechanism responsible for cholesterol modulation of Kir channels. The goal of this study is to test whether cholesterol-induced suppression of Kir channels is observed when purified channels are incorporated into liposomes. To achieve this goal, we reconstituted a bacterial channel Kirbac 1.1, a structural homolog of mammalian Kir channels, into liposomes of defined composition consisting of 3:1 phosphatidyl ethanolamine: phosphatidyl glycerol ratio and variable concentrations of cholesterol. The activity of the channels was assayed using $^{86}Rb^+$ uptake. Our results show that $^{86}Rb^+$ flux through the Kirbac 1.1 is strongly inhibited by incorporating cholesterol. Incorporation of 5% (mass Cho/PL) cholesterol into the liposome suppresses more then 50% $^{86}Rb^+$ flux, and the activity is completely inhibited at 12-15% (mass Cho/PL). No effect was observed at cholesterol levels below 1% (mass Cho/PL). Furthermore, epicholesterol, a stereo isomer of cholesterol that has physical properties similar to those of cholesterol, also suppresses $^{86}Rb^+$ flux but its effect is significantly less pronounced. Purified KcsA, structurally similar K^+ ion channel from *Streptomyces lividans* was not at all inhibited by cholesterol when incorporated into the liposomes instead of Kirbac 1.1.These observations demonstrate that cholesterol suppresses Kir channels in a non-cellular environment and suggest that it may interact with the Kirbac 1.1 channels directly.

2412-Pos Board B382

Physical Determinants of Strong Voltage Dependence of K^+ Channel Block

Yanping Xu[1], Hyeon-Gyu Shin[1], **Zhe Lu**[2].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Howard Hughes Medical Institute; University of Pennsylvania, Philadelphia, PA, USA.

Many pharmacological agents, as well as some endogenous biological molecules, act by blocking ion channels in a strongly voltage-dependent manner. In retrospect, the first and most dramatic example of such action is the ''anomalous'' voltage dependence of inwardly rectifying K^+ (Kir) conductance discovered by Bernard Katz six decades ago. He observed that, contrary to the classic voltage-gated K^+ conductance, the Kir conductance tends to zero with membrane depolarization but increases with hyperpolarization. In intact cells and within physiological voltage ranges, certain Kir channels are as steeply voltage dependent as Kv channels yet, unlike Kv channels, they have no inherent voltage sensors: the observed voltage sensitivity instead reflects voltage-dependent block of their ion pore by intracellular cations such as the polyamine spermine. Our group has proposed that the high valence associated with block of strong rectifiers primarily reflects the movement, not of tetravalent spermine itself, but of five K^+ ions displaced by spermine across the steep electric field in the narrow K^+ selectivity filter. We will present experimental evidence for key requirements of this blocker-K^+ displacement model, and will discuss the essential features that render a pore-blocking process strongly voltage dependent.