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.

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

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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.

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

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

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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.

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

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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.

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

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