K Channels, Other

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Nerve Growth Factor Sensitizes Superior Cervical Ganglion Neurons to Bradykinin
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The sympathetic nervous system plays a role during chronic inflammation. Since levels of nerve growth factor (NGF) are high in inflamed tissues and, in sensory neurons, NGF controls nociception by regulating neuronal sensitivity to noxious stimuli, we hypothesize that NGF also sensitizes sympathetic neurons to bradykinin (BK), a proinflammatory peptide. We cultured rat superior cervical ganglion (SCG) neurons in the presence or absence of NGF. To assess the response of SCG neurons to Bk, we measured cytoplasmic Ca^{2+} elevations induced by an acute application of Bk. We found Ca^{2+} elevation to be increased 3-fold in the presence of NGF. These Ca^{2+} elevations in the presence of NGF depended on external Ca^{2+} and depolarization of the plasma membrane. Next, we assessed the effect of Bk on the membrane potential by electrophysiological recordings. NGF-treated neurons were significantly depolarized after Bk application, while neurons cultured in the absence of NGF showed no change in membrane potential with Bk. How does NGF render SCG neurons more responsive to a Bk stimulus? We first tested whether NGF application alters BK-induced M current inhibition. Interestingly, we observed full inhibition of M current upon application of BK with and without culture in NGF. Next, we asked whether incubation of SCG neurons with NGF alters activity of other ion channels. One of the ion channels tested, KCa1.1 (Maxi K^+ channel), was reduced in current density by 50% in neurons cultured in the presence of NGF. In addition, inhibition of KCa1.1 channels with iberiotoxin in SCG neurons cultured in the absence of NGF depolarized the plasma membrane potential after Bk application. In conclusion, NGF increased Bk-induced electrical excitability of rat SCG neurons by reducing a repolarizing current mediated by KCa1.1 channels. Supported by NIH grant NS008174.

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Postnatal Development of K_V Currents in Cultured Small Mouse Dorsal Root Ganglion (DRG) Neurons
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Voltage-gated potassium (Kv) channels play an important role in the regulation of the electrophysiological properties of neurons. Neuronal excitability depends on the spatial and temporal expression of many different Kv subunits. Small dorsal root ganglion (DRG) neurons exhibit four different voltage-gated K^+ currents: the M current (I_M), the hyperpolarization-activated current (I_H), the transient outward current (I_A), and the delayed rectifier current (I_K). It has been demonstrated that ~60% of I_K is carried by both homotetrameric Kv2.1 channels and heterotetrameric channels consisting of Kv2.1 and silent Kv subunits (i.e. Kv5-Kv6 and Kv8-Kv9) while Kv1.4, Kv3.4 and members of the Kv4 subfamily underlie I_A. Little is known about the postnatal development of I_K and I_A and how these developmental changes influence the electrophysiological properties of small DRG neurons. Here we report a decrease of the stromatoxin (ScTx)-sensitive component of the whole-cell outward DRG current during postnatal development: the fraction of ScTx-sensitive current decreased from 64% to 51% between 1 month and 3 months old mice. ScTx is a gating modifier of both Kv4.2- and Kv2-containing channels and therefore this decrease could reflect a decrease in expression of one or more of these Kv subunits during development. However, the fraction of the anti-Kv2.1 antibody-sensitive current - which only reflects Kv2-containing channels - increased between 2 weeks and 2 months old mice from 32% to 42% of the whole-cell DRG current. These results together suggest that the decrease in the ScTx-sensitive component is due to a decrease in Kv4.2 expression. Furthermore, these results suggest that the balance between I_A and I_K and consequently the electrophysiological properties change during the postnatal development of small DRG neurons.
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Characterization of the Slo1 Channel as a Principal Potassium Channel of Human Sperm
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Mammalian spermatozoa gain their competence to fertilize an oocyte as they travel through the female genital tract. This process - termed capacitation - goes along with an elevation of sperm intracellular calcium and a membrane hyperpolarization. The latter is evoked by K^+ efflux. However, the molecular identity of the potassium channel of human spermatozoa (hKSper) is unknown. Here, we characterize hKSper and show that this chanel is regulated by intracellular calcium but is insensitive to intracellular alkalinization. We also demonstrate that human KSper is inhibited by charybdotoxin, iberiotoxin and paxilline, while mouse KSper is insensitive to these compounds. These properties suggest that the Slo1 ion channel is the molecular identity for hKSper. We show that Slo1 is localized to the sperm flagellum and is inhibited by progesterone. Inhibition of hKSper by progesterone, in turn, may depolarize the spermatozoon to open the calcium channel CatSper, thus raising $[Ca^{2+}]$ to produce hyperactivation and allowing sperm to fertilize an oocyte.

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Modulation of Sarcolemmal ATP-Sensitive Potassium Channels by Atrial Natriuretic Peptide in Ventricular Cardiomyocytes
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ATP-sensitive potassium (K_{ATP}) channels couple cell metabolic status to membrane excitability and are crucial for stress adaptation in the heart. Natriuretic peptides (NPs) produced by the heart, the vasculature and the kidney exert physiologically important actions such as natriuresis and inhibition of sympathetic tone and cardiomyocyte hypertrophy. Our recent study reveals that the gaseous messenger nitric oxide evokes cardiac K_{ATP} channel stimulation via activation of soluble guanylyl cyclase and subsequently a cGMP-dependent signaling pathway. Binding of NPs like atrial natriuretic peptide (ANP) to the type 1 NP receptor (NPR-A) also increases intracellular cGMP level, through activating guanylyl cyclase intrinsic to the receptor; however, how ANP modulates the function of cardiac K_{ATP} channels remains to be established. By performing cell-attached patch recordings in ventricular cardiomyocytes acutely isolated from adult rabbits, we found that the single-channel activity of sarcolemmal K_{ATP} ($sarcK_{ATP}$) channels induced by sodium azide through metabolic inhibition was potentiated by addition of ANP (100 nM) (n=16), whereas the potentiation by ANP was abolished when the NPR-A antagonist anantin (1 μM) was coapplied (n=5); these findings suggest that the stimulatory action of ANP is mediated by NPR-A activation, which may depend on generation of diffusible cGMP. Indeed, $sarcK_{ATP}$ channel stimulation by ANP in intact ventricular myocytes was significantly abated by inhibition of cGMP-dependent protein kinase (PKG) with KT5823 (1 μM) (n=5). Moreover, the ANP effect was prevented by selective suppression of extracellular signal-regulated protein kinase (ERK)1/2 (n=6) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) (n=6). Collectively, here we demonstrate that the polypeptide hormone ANP positively modulates ventricular K_{ATP} channel function via activation of NPR-A, PKG, ERK1/2 and CaMKII. By opening myocardial K_{ATP} channels, this signaling pathway may regulate cardiac excitability and contribute to cytoprotection against ischemia-reperfusion injury.

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An Intramolecular Interaction Controls a Rate-Limiting Step in ATP-Dependent Gating of Kir6.2 Channels
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ATP-sensitive potassium (KATP) channels are complexes of inwardly-rectifying Kir channels (Kir6.x) and sulfonylurea receptors (SUR). These two subunits work together to encode sensitivity to intracellular adenine nucleotides (ATP and ADP) and other regulatory ligands including PIP2. We investigated the kinetics of ATP-dependent regulation of KATP (Kir6.2 + SUR1) channels using rapid concentration jumps. WT Kir6.2 channels rapidly reopen after washout of ATP, with a time constant of ~60 ms. Extending similar kinetic measurements to mutants around the bundle crossing revealed modest effects on gating kinetics despite significant changes in ATP sensitivity. However, we identified a pair of highly conserved neighboring amino acids (Trp68, Lys170) that controls the rate of channel recovery and inhibition by ATP. Mutations of Trp68 or Lys170 have the paradoxical effect of dramatically slowing the kinetics of channel opening, while increasing channel open probability. Formalization of the transition state effects of these residues using phi-value analysis revealed a consistent steep negative slope. This finding implies that