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Ankyrin regulates K_{ATP} channel membrane trafficking and gating in excitable cells

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Abstract

K(ATP) channels play critical roles in many cellular functions by coupling cell metabolic status to electrical activity. First discovered in cardiomyocytes,[1] K_{ATP} channels (comprised of Kir6.x and SUR subunits) have since been found in many other tissues, including pancreatic beta cells, skeletal muscle, smooth muscle, brain, pituitary and kidney. By linking cellular metabolic state with membrane potential, K_{ATP} channels are able to regulate a number of cellular functions such as hormone secretion, vascular tone and excitability. Specifically, a reduction in metabolism causes a decrease in the ATP:ADP ratio, opening of K_{ATP} channels, K^+ efflux, membrane hyperpolarization, and suppression of electrical activity. Conversely, increased cellular metabolism causes an increase in the ATP:ADP ratio that leads to closure of the K_{ATP} channel, membrane depolarization, and stimulation of cell electrical activity.

Keywords

ankyrin; spectrin; trafficking; targeting; cytoskeleton; diabetes

Numerous studies in isolated cells and tissues, as well as genetically-modified mice or patients with mutations in K_{ATP} channel genes, have demonstrated that K_{ATP} channels play important roles in a wide range of physiological processes.[2] Their contribution to glucose homeostasis is well-documented with regard to K_{ATP}-dependent regulation of insulin secretion by beta cells,[3] glucagon secretion from pancreatic alpha cells,[4] somatostatin secretion from pancreatic delta cells,[5] and glucagon-like peptide 1 (GLP-1) secretion from L-cells.[6] Moreover, K_{ATP} channels in ventromedial hypothalamic neurons mediate the counter-regulatory response to glucose,[7] while K_{ATP} channels in the arcuate nucleus are hypothesized to play a role in appetite regulation.[8] Interestingly, these glucose-sensing cell types express K_{ATP} channels that are open under resting conditions. In other tissues, however, K_{ATP} channels are closed under physiological conditions and open only in response to ischemia, neurotransmitters, or hormonal stimulation. For example, in cardiac muscle and central neurons, opening of K_{ATP} channels reduces electrical activity to protect against cardiac stress and seizures.[9–12] Moreover, K_{ATP} channels are involved in the phenomenon of ischemic preconditioning in the heart[13] and in the regulation of vascular smooth muscle tone.[14,15] Additionally, K_{ATP} channels modulate electrical activity and

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synaptic neurotransmitter release in the hippocampus, substantia nigra and the hypothalamus.[8,16–19]

Macroscopic K_{ATP} channel current is the highly-coordinated product of open probability, single channel conductance, and the total number of membrane channels. K_{ATP} channel open probability in vivo is tightly regulated by ATP levels ([ATP]:[ADP] ratios). In fact, even minor changes in cellular [ATP]:[ADP] ratios may result in dramatic alterations in K_{ATP} channel opening, cellular excitability, and tissue function.[20] The importance of normal K_{ATP} channel ATP sensitivity for normal metazoan physiology is illustrated by human mutations in Kir6.2 or SUR genes that affect K_{ATP} channel ATP sensitivity and result in neonatal diabetes.[21] Likewise, human gene variants that affect K_{ATP} channel biosynthesis, targeting, or membrane expression may result in abnormal beta cell excitability and human disease.[22,23]

In 2006, Shyng and colleagues identified human K_{ATP} channel gene variants that resulted in neonatal diabetes due to defects in both gating and membrane targeting. For example, Lin et al. specifically demonstrated that Kir6.2 mutants R201C and R201H showed both decreased membrane expression (conductance) as well as abnormal gating phenotypes resulting in diabetes.[24] In a recent publication, our group linked a human disease mutant, Kir6.2 E322K, with a dual mechanistic phenotype (alterations in both gating and membrane targeting).[25] Specifically, our findings identified a critical motif in the C-terminal domain of Kir6.2 that is essential not only for normal Kir6.2 membrane targeting, but also metabolic regulation by ATP.[25] The Kir6.2/SUR K_{ATP} channel complex directly interacts with the membrane adaptor protein ankyrin-B[26,27] and Kir6.2 targeting is significantly reduced when the Kir6.2 motif is disrupted or when ankyrin is depleted from the cell. Interestingly, ankyrin plays a key role in regulating ATP sensitivity, as Kir6.2 ATP sensitivity is decreased in the presence of peptides that disrupt the ankyrin-B:Kir6.2 interaction. Finally, we demonstrated that a previously identified human neonatal diabetes mutation in Kir6.2 (E322K) blocks the association of ankyrin-B with Kir6.2, resulting in a complex beta cell phenotype.

While K_{ATP} channel function (and therefore cell excitability) is rather predictably affected by gene mutations that alter ATP sensitivity or channel membrane expression alone, it is more difficult to predict how K_{ATP} channel will be affected by a mutation that alters several channel properties simultaneously.[24,25] In our study, we used mathematical modeling of beta cell excitability (Fig. 1) to help gain important insight into how complex changes in K_{ATP} channel properties result in the ultimate cellular phenotype and disease.[25] Interestingly, computational modeling predicts that beta cell excitability (measured as inter-burst period in Fig. 1) is much more sensitive to changes in K_{ATP} channel ATP sensitivity than it is to changes in channel conductance (slope of linear regression = 1.01 and 0.28, respectively). In fact, the most severe trafficking defect associated with the E322K mutant channel resulted in only about a 14% decrease in inter-burst period (arrow in Fig. 1B), while decreased ATP sensitivity due to the mutation (arrow in Fig. 1C) completely eliminated spontaneous electrical activity. Thus, even though fewer mutant channels traffic to the membrane (loss-of-function), the decreased ATP sensitivity of those channels that do localize properly is enough to produce a net gain-of-function. Together, these findings demonstrate a key role of the cytoskeleton in K_{ATP} channel function as well as illustrate the complex cellular phenotypes that have evolved in metazoans to modulate channel function and cellular excitability.

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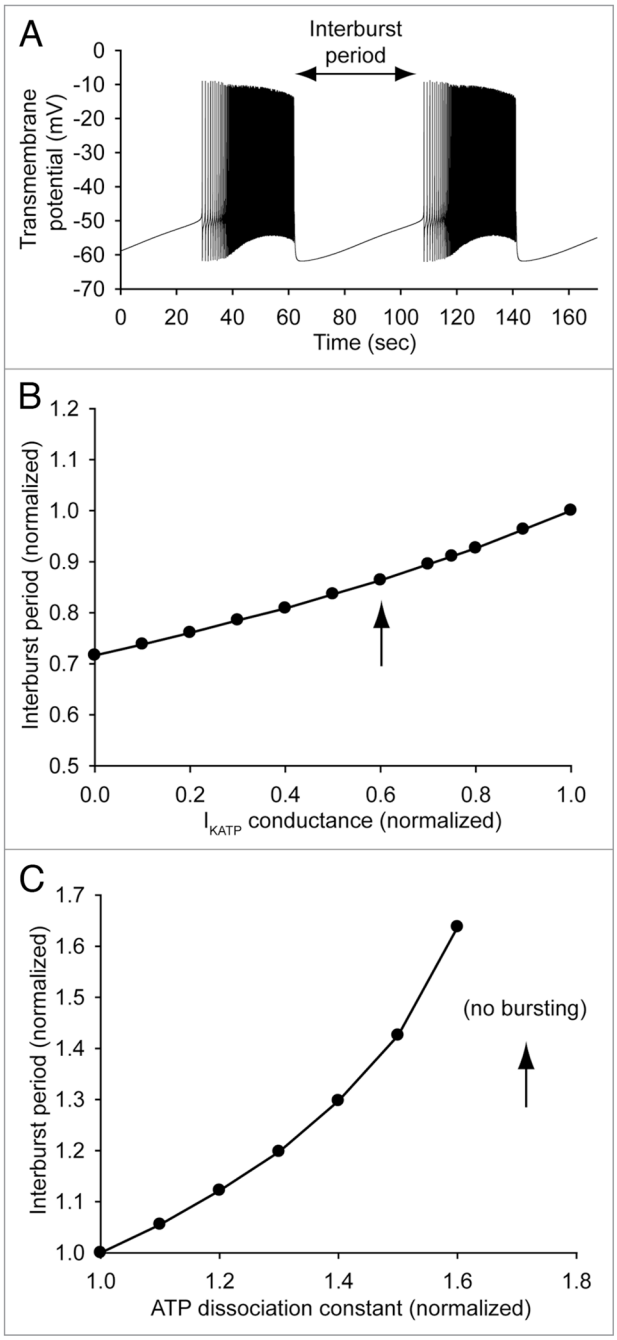

Figure 1.

Simulated beta cell electrical activity as a function of K_{ATP} channel conductance and ATP sensitivity. (A) Pancreatic beta cell electrical activity is simulated using the mathematical model developed by Fridyland et al.[28] (B) Pancreatic beta cell inter-burst period decreases (excitability increases) as I_{KATP} conductance is reduced. However, the relationship between inter-burst period and I_{KATP} conductance is relatively flat. In fact, a 40% decrease in I_{KATP} conductance (corresponding to the more severe trafficking defect associated with ankyrin-B loss in HEK cells[25]) results in only a 14% decrease in inter-burst period (*arrow*). (C) In contrast, beta cell inter-burst period is steeply dependent on the ATP sensitivity of the K_{ATP} channel. A 70% increase in the ATP dissociation constant of the K_{ATP} channel

(corresponding to the E322K heterozygous mutant[25]) results in a complete loss of spontaneous beta cell firing (*arrow*).