Differential roles for SUR subunits in K\textsubscript{ATP} channel membrane targeting and regulation

Thomas J. Hund\textsuperscript{1,2} and Peter J. Mohler\textsuperscript{1,3}

\textsuperscript{1}Division of Cardiovascular Medicine, Department of Internal Medicine; \textsuperscript{2}Department of Biomedical Engineering; and \textsuperscript{3}Department of Molecular Physiology and Biophysics, University of Iowa Carver College of Medicine, Iowa City, Iowa

SINCE THEIR DISCOVERY OVER 25 years ago, ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP}) channels have been studied extensively in many tissues, including heart, pancreas, brain, skeletal and smooth muscle, pituitary, and kidney (6, 10, 18, 19). K\textsubscript{ATP} channels are critical sensors that couple cellular metabolic state to membrane excitability and organ function and regulate a wide range of cellular functions including hormone secretion, vascular tone, cardiomyocyte contractility, and synaptic transmission (6, 18, 19). In heart, the activation of K\textsubscript{ATP} channels during ischemia shortens action potential duration to help preserve energy in the face of a decreased supply (6, 10). In pancreatic \( \beta \)-cells, K\textsubscript{ATP} channels couple membrane potential (and therefore insulin release) to a metabolic state and are the target of the sulfonylurea class of antidiabetic drugs (6, 10). In vascular smooth muscle, K\textsubscript{ATP} channels regulate basal tone and promote vaso-dilation in response to severe hypoxia. Importantly, variants in K\textsubscript{ATP} channel genes have been linked to human disease, including congenital hyperinsulinemia, neonatal diabetes, and atrial and ventricular arrhythmias (2, 18, 19). Despite the importance of K\textsubscript{ATP} channels in many critical cellular functions and their clinical importance as drug targets, little is known about the cellular pathways that regulate the membrane expression of these critical channels. In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Bao and colleagues (1) add to our understanding of this critical channel by studying the roles for K\textsubscript{ATP} channel sulfon-nylurea receptor (SUR) subunits in differential localization of K\textsubscript{ATP} channels in submembrane compartments.

The K\textsubscript{ATP} channel is a hetero-octameric complex comprised of four pore-forming inward rectifier \( \text{K}^+ \) channels subunits (Kir6.1 or Kir6.2, encoded by \textit{KCNJ8} and \textit{KCNJ11}, respectively) and four regulatory SUR subunits (SUR1, SUR2A/SUR2B, encoded by \textit{ABCC8} and \textit{ABCC9}, respectively). Kir6.1 and Kir6.2 are comprised of a cytoplasmic NH\textsubscript{2}-terminus, two transmembrane domains (M1 and M2) with high homology to pore-forming S5–S6 segments of voltage-gated \( \text{K}^+ \) channels, and a cytoplasmic COOH-terminus (10, 18). Whereas Kir6.1 is the dominant pore-forming subunit found in K\textsubscript{ATP} channels from vascular smooth muscle, Kir6.2 is important in pancreatic \( \beta \)-cell, cardiac myocyte, and nonvascular smooth muscle K\textsubscript{ATP} channels (19). Thus Kir6.1 plays important roles in regulating vascular tone, whereas Kir6.2 is critical for glucose homeostasis and ischemic preconditioning (19). SUR subunits are members of the ATP-binding cassette (ABC) membrane protein family. These subunits contain an NH\textsubscript{2}-terminal transmembrane domain (TM0) and cytoplasmic linker (L0) that interact with Kir6, two additional transmembrane domains (although no transport function has been identified for SUR), and two cytoplasmic nucleotide-binding folds (NBF1 and NBF2) that contain binding sites for Mg\textsuperscript{2+}-adenosine nucleotides. While Kir6.2 and SUR2 (SUR2A in particular) are predominantly expressed in heart, Kir6.1 and SUR1 are also found in heart. While knockout mice have generated important insight into the role of different subunits in heart, the story is far from complete. Kir6.2\textsuperscript{-/-} mice have virtually no cardiac K\textsubscript{ATP} channel activity consistent with Kir6.2 being the dominant pore-forming subunit in heart (24). Isolated ventricular myocytes from Kir6.2\textsuperscript{-/-} mice show normal action potential duration and contractility at baseline but diminished ischemic cardioprotection similar to the effect of K\textsubscript{ATP} channel blockers (24, 25, 29). Ventricular myocytes from SUR2\textsuperscript{-/-} mice show a dramatic loss of K\textsubscript{ATP} channel activity; however, SUR2\textsuperscript{-/-} animals are protected from global ischemia likely because of the effects in other tissues (23). Interestingly, data from SUR1\textsuperscript{-/-} mice show normal ventricular K\textsubscript{ATP} currents but a loss of atrial K\textsubscript{ATP} current (7), suggesting that within the heart there is a heterogeneous K\textsubscript{ATP} channel subunit composition.

A hallmark property of the K\textsubscript{ATP} channel is its regulation by intracellular nucleotides. In response to a decrease in the ATP-to-ADP ratio, K\textsubscript{ATP} channels activate, leading to K\textsuperscript{+} efflux from the cell, membrane hyperpolarization, and suppression of electrical activity. At the molecular level, ATP (in the absence of Mg\textsuperscript{2+}) inhibits the channel by binding directly to Kir6 in a binding pocket located at the interface of the NH\textsubscript{2}- and COOH-termini to inhibit the channel. Thus the channel stoichiometry provides each K\textsubscript{ATP} channel with four ATP binding sites. Several mutations in Kir6.2 linked to neonatal diabetes alter ATP sensitivity and/or channel gating (9, 13, 14, 16, 28). In contrast, Mg\textsuperscript{2+}-ADP and Mg\textsuperscript{2+}-ATP both activate the channel by binding to SUR, which effectively decreases the IC\textsubscript{50} of ATP binding to Kir6. Most K\textsubscript{ATP} channel mutations linked with congenital hyperinsulinemia reside in SUR1 and reduce Mg\textsuperscript{2+}-ADP activation, resulting in a loss of channel function (18). In addition to intracellular nucleotides, K\textsubscript{ATP} channel activity is regulated by a wide range of factors including phospholipids [e.g., phosphatidylinositol 4,5-bisphosphate (PIP\textsubscript{2})], acyl-CoA, pH, pharmacological agents, the cytoskeleton, and phosphorylation (e.g., by PKA and PKC) (3, 8, 26). For example, PIP\textsubscript{3} binding to Kir6.2 activates the K\textsubscript{ATP} channel, whereas PIP\textsubscript{2} hydrolysis via phospholipase C inhibits the channel (5, 11).

K\textsubscript{ATP} channels reside within dynamic macromolecular complexes comprised of kinases, cytoskeletal and adapter proteins in addition to Kir6 and SUR subunits (6, 10). Enzymes (i.e., creatine kinase, adenylate kinase, and lactate dehydrogenase) are integral components of this complex and regulate channel function (21). Syntaxin 1A, a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein in-
volved in exocytosis, and the cAMP-binding protein cAMP-guanine nucleotide exchange factor (GEFII) have been shown to bind SUR1 to regulate K$_{ATP}$ channel activity and insulin secretion in pancreatic $\beta$-cells (20, 22). It is well established that the disruption of the actin cytoskeleton activates the channel by altering ATP sensitivity (3, 8, 26). Recently, a potential link between the cytoskeleton and K$_{ATP}$ channel was identified in the cytoskeletal adapter ankyrin-B, which binds directly to Kir6.2 and regulates ATP sensitivity as well as channel trafficking (13, 15). In fact, a human mutation in the ankyrin-B binding motif on Kir6.2 disrupts the association of ankyrin-B with Kir6.2, resulting in abnormal K$_{ATP}$ channel trafficking and function and permanent neonatal diabetes mellitus (13, 28).

Important roles have been identified for SUR in the regulation of channel function. In addition to the binding of Mg$^{2+}$-adenosine nucleotides, SUR regulates the drug sensitivity of K$_{ATP}$ channel (6, 10, 19). Specifically, the sulfonfylurea class of K$_{ATP}$ channel blockers used to treat type II diabetes binds to SUR as do K$_{ATP}$ channel openers such as pinacidil and diazoxide. A role for SUR has also been identified in the trafficking of the octameric K$_{ATP}$ channel complex to the membrane. Kir6 and SUR both contain putative ER retention motifs (R-K-R) that prevent the surface expression of either subunit alone (30), although other regions (i.e., M2 and COOH-terminus) may also play a role in channel trafficking (12). Consistent with this hypothesis, truncated Kir6.2 channels lacking the ER retention motif have been found to traffic to the membrane in the absence of SUR, although at relatively low levels (27). However, data showing full-length Kir6.2 membrane trafficking in the absence of SUR raise questions about whether SUR acts as a chaperone for Kir6.2 through the endoplasmic reticulum/Golgi or stabilizes the expression at the membrane (17).

Bao and colleagues (1) provide compelling evidence for a role in SUR2 as a differential in membrane trafficking of K$_{ATP}$ channels to endosomal compartments within the cell. The authors coexpress Kir6 with SUR1 or SUR2 in a variety of cell lines (including h9c2 and coronary artery smooth muscle cells) and show by immunofluorescence that Kir6/SUR1 targets to the cell membrane, whereas Kir6/SUR2A/B is localized primarily in an endosome/lysosome compartment, indicated by the colocalization with lysosomal-associated membrane protein 2. Interestingly, hemagglutinin-tagged Kir6.2 was rapidly internalized when coexpressed with SUR2 but not SUR1. Cell fractionation on rat ventricular tissue revealed the expression of Kir6.2 in sarcosome and endosome-enriched fractions with a shift to the sarcosomal compartment with global ischemia, consistent with previous studies (4). These data suggest that SUR2-containing K$_{ATP}$ channels in endosomal compartments represent an available pool that may cycle dynamically to the sarcosome and regulate heart function. While the findings of Bao et al. (1) support the view of SUR as a critical player in channel trafficking, the study raises a number of important questions. Although the authors demonstrate their findings in a variety of cell expression systems (including h9c2 cells derived from embryonic rat heart), it will be important to know what happens in the physiological environment of the cardiomyocyte, where the heteromeric channel likely resides in a dynamic multimeric protein complex comprised of signaling, cytoskeletal, and accessory proteins. SUR1 has been found to associate with several proteins involved in exocytosis (20, 22), consistent with the model described by Bao et al. (1). We would expect SUR2 to associate with select endosomal proteins, although such interactions have yet to be identified. Thus it remains to be determined the molecular mechanism for the differential sorting of SUR1 and SUR2 channels and whether this pathway may be exploited to increase/decrease the number of functional K$_{ATP}$ channels at the sarcolemma.

**REFERENCES**


