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Inward rectifying potassium channels

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Inward rectifying potassium channels

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18.1 INTRODUCTION

Empirically, rectification means change of conductance with voltage, and most, if not all, ion channels show some degree of rectification. For potassium channels, inward rectification means that, given an equal but opposite electrochemical driving force, the inward flow of K+ ions exceeds the outward flow. Strongly inwardly rectifying K+ currents were first observed in frog skeletal muscle (Katz 1949) and termed anomalous to distinguish them from the normal or delayed outward rectification that is characteristic of K+ currents in the squid axon, and expected for an electrodiffusive pathway in normal physiological [K+] gradients (Hodgkin et al. 1949). We now know that inward rectification is a consequence of voltage-dependent, asymmetric open channel pore block by cytoplasmic divalent cations, especially Mg2+, and polyamines (Guo et al. 2003, Kurata et al. 2007, Lopatin et al. 1994, Lu and MacKinnon 1994, Matsuda et al. 1987, Tao et al. 2009, Vandenberg 1987, Yang et al. 1995). At hyperpolarizing membrane potentials, the blocking ions are cleared from the channel and K+ currents flow, whereas at depolarizing voltages, the blockers are driven into the pore and the K+ current is blocked (Figure 18.1a). Consequently, strongly rectifying Kir channels are conductive when excitable cells are at rest and nonconductive during excitation, and this property grants Kir channels a key role in the maintenance and modulation of cell membrane potential.

The first Kir channel genes were isolated by expression cloning in 1993 (Ho et al. 1993, Kubo et al. 1993), and there are now at least seventeen eukaryotic Kir channel genes distributed in seven subfamilies (Kir1–7; Figure 18.1b), and multiple prokaryotic isoforms (KirBac1.1–9). Since their discovery, Kir channels have been found in many cell types, with widely varying rectification properties. For example, classical, strong inward rectifying K+ currents are very prominent in cardiac myocytes, and in glial cells and neurons in the central nervous system (Brismar and Collins 1989, Hestrin 1987, Nakajima et al. 1988, Newman 1993, Vandenberg 1994). Rectification of these channels is sufficiently strong that very little current flows through them at potentials positive to about −40 mV; high conductance at negative voltages allows cells to maintain a stable resting potential, but the greatly reduced conductance at positive voltages avoids short-circuiting the action potential. ATP-sensitive K+ (K_ATP) channels are present in all muscle cell types, in the brain, and in pancreatic cells (Ashcroft 1988). In contrast to classical inward rectifiers, K_ATP channels display only weak rectification and allow substantial outward current to flow at positive potentials (Nichols and Lederer 1991, Noma 1983). Between these extremes, the
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Brain is particularly well endowed with K⁺ channels that show intermediate rectification properties, many of these channels being strongly dependent on ligand activation, often through G-proteins or other second messenger systems (Inanobe et al. 1999b, Lesage et al. 1995, Liao et al. 1996). Crystallization of the bacterial KcsA channel revolutionised our understanding of channel structure (Doyle et al. 1998), as it unveiled a basic architectural scaffold that is common to all major cation channel family members, including Kir channels, namely, two transmembrane helices bridged by an extracellular loop that generates the narrow portion of the pore and controls ion selectivity. Solving of the structure of full-length prokaryotic Kir channel homologs KirBac1.1 and KirBac3.1 (Kuo et al. 2003, 2005) revealed an extended cytoplasmic pore and the likely location of ligand binding sites, and the unique properties of eukaryotic Kir channels, as anticipated by decades of biochemical and biophysical experiments, were given a solid structural context with the crystallization of linked N- and C-terminal domains of Kir3.1 and Kir2.1 (Nishida and MacKinnon 2002, Pegan et al. 2005b) and, more recently, of the full-length Kir2.2 (Hansen et al. 2011, Tao et al. 2009) and Kir3.2 (Whorton and MacKinnon 2011).

Figure 18.1 (a) Inward rectification. Under physiological conditions, conductance declines upon depolarization, due primarily to channel block by polyamines and Mg²⁺ ions. In weak inward rectifiers, this decline is very weakly voltage dependent. In strong inward rectifiers, the decline in conductance results in a marked negative slope region, which may be followed by a second region of positive conductance due to punchthrough of the blocking ions. (b) Phylogeny of Kir channels. There are seven major subfamilies of eukaryotic Kir genes, encoding subunits with ≈60% amino acid identity within and ≈40% identity between subfamilies, and generating various native inward rectifying channels as indicated.

18.2 KIR CHANNEL STRUCTURE AND GATING

Functional Kir channels are formed by the homomeric or heteromeric assembly of four subunits, each Kir subunit containing two membrane-spanning domains (TM1 and TM2), an extracellular pore-forming region (H5) that includes the selectivity filter, and cytosolic NH₂- and COOH-termini that associate with each other to form a cytoplasmic domain that controls gating (Figure 18.2) (Nishida et al. 2007, Whorton and MacKinnon 2011).

The Kir channel transmembrane domain is formed by outer (TM1) and inner (TM2) helices, the slide helix, and the pore helix (Figure 18.2). The selectivity filter, containing the signature sequence TXGY(F)G (where X is an aliphatic amino acid), is shared with other K⁺-selective ion channels (Bichet et al. 2003) and forms a constriction in the conduction pathway that separates the central cavity from the extracellular solution. In eukaryotic Kir channels, the pore region is structurally constrained through covalent linkage by an absolutely conserved pair of Cys residues between the pore

Figure 18.2. Kir channel structure. Ribbon diagram of two opposing Kir subunits. The channel is divided into two distinct domains, the transmembrane domain formed by the M1 and M2 helices and the pore loop (generating the inner cavity and selectivity filter, respectively), and the cytoplasmic domain formed by the N- and C-termini, lining the cytoplasmic extension of the pore. Conserved locations of pore-lining negatively charged residues (corresponding to D172, E224 and E299 in Kir2.1) are shown in ball-and-stick format. Also shown is a spermine molecule within the channel pore, at a location envisioned by the fixed-tail model of polyamine block, in which a polyamine is stabilized by interactions with charged residues in the inner cavity and the selectivity filter.

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helix and the selectivity filter (e.g., C123 and C155 in Kir2.2). The central cavity is lined by the TM2 helix from each of the four Kir subunits, in an arrangement very similar to that of other K+ channel families, including KCa and bacterial and mammalian Kv channels (Doyle et al. 1998, Jiang et al. 2003, Long et al. 2005). Beneath the central cavity, large hydrophobic residues (such as I177 and M181 in Kir2.2) form two tight seals that close off the pore to the cytoplasm (Tao et al. 2009). Both rearrangements of the selectivity filter and motion of the TM2 helices have been proposed to occur, and may coexist, in Kir channel gating; mutations introduced in or around the selectivity filter modify the fast gating kinetics (Choe et al. 2001, Enkvetchakul et al. 2000, Proks et al. 2001, Yang et al. 1995), whereas mutations in the region where the bundle of inner TM2 helices cross at the bottom of the inner cavity have been shown to alter slow gating (Enkvetchakul and Nichols 2003, Enkvetchakul et al. 2000, Trapp et al. 1998, Yi et al. 2001); it has been hypothesized that bending of each TM2 around a hinge Gly residue halfway through the membrane would allow for the inner part of the helix to swing away from the permeation pathway (Jin et al. 2002). The TM1 domains are also implicated in the gating machinery of Kir channels: hydrogen bond linking between TM1 and TM2 at the bundle-crossing region modulates gating by intracellular protons and PIP2 (Rapedius et al. 2007), and the slide helix, a transverse amphiphilic domain at the cytoplasmic end of TM1, serves as a mediator of coupling between the ligand-sensing, cytoplasmic interface, and the gating transmembrane domains (Doyle et al. 1998, Kuo et al. 2003).

The cytoplasmic domain of Kir channels, formed by the association of the NH2- and COOH-termini of the four channel subunits (Figure 18.2), lines an extension of the channel pore into the cytoplasm and effectively doubles the conduction pathway length to over 60 Å. This domain also allows for the control of Kir channel gating by ATP, Na+, nucleotides, G-proteins, and phosphatidylinositol-4,5-bisphosphate (PIP2) (Nishida and MacKinnon 2002, Pegan et al. 2005a). For example, all Kir channels require PIP2 for activity, and recent crystallographic and biochemical data suggest that the binding site for PIP2 is formed predominantly by positively charged side chains at the interface between the transmembrane helices and the cytoplasmic domain (D’Avanzo et al. 2013, Hansen et al. 2011, Whorton and MacKinnon 2011). PIP2 interactions determine the sensitivity of Kir channels to regulation by cytoplasmic factors such as ATP, phosphorylation, and pH, and differences among Kir channels in their specific regulation by a given modulator may reflect differences in their apparent affinity for PIP2 (Du et al. 2004). In KATP channels, the ATP-binding pocket is formed at the cytoplasmic domain interfaces in Kir6 subunits (Antcliff et al. 2005), and in Kir1.1 and Kir4.1 channels, intracellular acidification disrupts an intersubunit cytoplasmic salt bridge (E302-R311 in Kir1.1; E288-R297 in Kir4.1), which contributes to channel closing (Rapedius et al. 2006, Sala-Rabanal et al. 2010).

### 18.3 MECHANISMS OF INWARD RECTIFICATION

Block by intracellular Mg2+ and polyamines underlies the Kir channel key functional property of preferential conduction of inward K+ currents (Fakler et al. 1995, Ficker et al. 1994, Lopatin et al. 1994, 1995). As a rapid, voltage-dependent process, polyamine-mediated inward rectification provides a mechanism for K+ current regulation in excitable tissues, which contributes to shaping both the action potential and the resting membrane potential in tissues such as the myocardium (Bianchi et al. 1996, Lopatin et al. 2000, Priori et al. 2005). Polyamine block entails an initial weakly voltage-dependent binding, probably in the cytoplasmic domain of the channel, followed by a steeply voltage-dependent step in which the blocker migrates to a stable binding site in the inner cavity (Guo and Lu 2000, Kurata et al. 2007, Shin and Lu 2005, Xie et al. 2002). Inward rectification is strongly voltage dependent, and the voltage at which Kir channels transition from a conductive state to a blocked state becomes more positive as the extracellular K+ concentration is increased, reflecting competition between conducting ions, polyamines, and Mg2+ for sites in the pore (Guo et al. 2003, Pearson and Nichols 1998, Shin and Lu 2005, Tao et al. 2009). Residues critically involved in each step have been identified: mutations that affect the shallow binding step (e.g., E224 and E299 in the strong rectifier Kir2.1) cluster in the cytoplasmic domain, while the rectification controller residue (D172 in Kir2.1), critical for steep voltage-dependent block, lies at a pore-lining position in the channel inner cavity (Guo and Lu 2003, Kubo and Murata 2001, Kurata et al. 2007, Wible et al. 1994, Xie et al. 2003, Yang et al. 1995), and there is now substantial evidence that the polyamines bind between the rectification controller residue and the selectivity filter (Kurata et al. 2004, 2006, 2008, 2009, 2010).

Kir channel subtypes show differing strengths of inward rectification, and this is critically dependent on the presence or absence of a negatively charged residue at the rectification controller position (equivalent to D172 in Kir2.1) in the inner cavity: the Asp is conserved in strongly rectifying Kir3.x channels and only replaced by Glu in intermediate rectifiers such as Kir4.x, whereas in the weakly rectifying Kir1.x and Kir6.2, this position is occupied by uncharged Asn residues (Kucheryavykh et al. 2007b, Kurata et al. 2004, Stanfield et al. 1994, Wible et al. 1994).

### 18.4 Kir CHANNEL FAMILIES

To date, seven subfamilies of Kir channel subunits have been identified, each sharing ~40% amino acid identity between one another, and ~60% identity between individual members within each subfamily (Figure 18.1b). They can be classified into four groups according to their functional and physiological properties: classical Kir channels (Kir2 subfamily members) are strong rectifiers highly expressed in the heart, where they are the key players in the cardiac inward rectifier current, Iki; G-protein-gated Kir channels, or Kc, channels (Kir3.x), underlie G-protein-coupled receptor-activated currents in the heart, brain, and endocrine tissues; ATP-sensitive K+ channels, or KATP channels, are formed by the assembly of four pore-forming Kir6.x subunits and four regulatory sulfonylurea subunits (SURx), and are tightly coupled to cellular metabolism, and K+ transport channels (Kir1.x, Kir4.x, Kir5.x, and Kir7.x) are intermediate or weak rectifiers predominantly expressed in barrier epithelia and in glial cells, where they contribute to K+ reuptake and membrane repolarization.
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18.4.1 CLASSIC INWARD RECTIFIERS: Kir2.x

Kir2 subunits generate channels that are constitutively active, strong inward rectifiers, and are robustly expressed in the heart and nervous system, where they are essential for the modulation of cell excitability. Five distinct mammalian Kir2 subfamily members have been identified: Kir2.1 (encoded by gene KCNJ2) was the first member of the subfamily to be cloned (Kubo et al. 1993), followed by Kir2.2 (KCNJ12) (Takahashi et al. 1994), Kir2.3 (KCNJ4) (Morishige et al. 1994), Kir2.4 (KCNJ4) (Toper et al. 1998), and, more recently, Kir2.6 (KCNJ16) (Ryan et al. 2010). Kir2.1 is the predominant isoform in skeletal muscle, and in atrial and ventricular myocytes where it associates with Kir2.2 or Kir2.3 to underlie the strong inwardly rectifying IK1 currents (Hibino et al. 2010a); the central role of IK1 currents in cardiac electrophysiology is manifested by severe arrhythmias linked to Kir2.x channel gain- or loss-of-function mutations, such as the Andersen–Tawil syndrome, certain forms of short QT syndrome (SQTS), tachycardia, and atrial fibrillation (AF) (Anumowono and Lopatin 2010). In vascular endothelial cells, classical Kir channels, mostly made up of Kir2.2 subunits, have a prominent role in modulation of vascular tone (Adams and Hill 2004, Fang et al. 2005), and in arterial smooth muscle cells in the brain, K+ reuptake by Kir2.1 contributes to vasodilation and the control of local blood flow (Bradley et al. 1999, Filosa et al. 2006). Kir2.x subunits are differentially expressed in the central nervous system, where classical Kir currents are critically involved in the regulation of neuronal excitability: Kir2.1 is expressed diffusely throughout the brain; Kir2.2 strongly in the cerebellum; Kir2.3 in spinal cord, forebrain, and olfactory bulb; and Kir2.4 in the midbrain, pons, and medulla (de Boer et al. 2010). Kir2.6 is transcriptionally regulated by thyroid hormone and controls skeletal muscle cell excitability; loss-of-function mutations in Kir2.6 channels have been associated with thyrotropic hypokalemic periodic paralysis (Ryan et al. 2010).

18.4.1.1 IK1 channel assembly and regulation

IK1 currents dominate the background conductance and establish the negative resting potential of ventricular cardiomyocytes, including Purkinje fibers, and play a critical role in shaping the cardiac action potential; the lack of outward conductance at positive potentials prevents K+ efflux and helps maintain the depolarization during the characteristic elongated plateau phase. Once repolarization is initiated by the activation of K+ channels, large outward currents pass through IK1 channels, which accelerate the final stages of repolarization (Anumowono and Lopatin 2010, Hibino et al. 2010a).

While it is well established that members of the Kir2 subfamily underlie IK1 currents, subunit composition varies among species, cell types, and membrane domains. In the human heart, for example, Kir2.1 and Kir2.3 dominate over Kir2.2 in Purkinje cells; in the right ventricle, Kir2.1 is the most abundant subunit, whereas Kir2.3 is prominent in the right atrium; and Kir2.4 is not expressed (Gaborit et al. 2007). Kir2.2 appears to be absent in guinea pig and sheep cardiomyocytes (Dhamoon et al. 2004), and very little Kir2.3 is found in the mouse heart (Zaritsky et al. 2001). The molecular makeup of IK1 channels has been assessed in knock-out animal models, native cells, and heterologous expression systems based on the differential biophysical properties of individual Kir2 channel assemblies, but due to the tissue- and species-specific distribution, in many cases, the exact contribution of Kir2.x subunits to the conductance of heteromeric channels is still unknown. There is solid evidence, however, that in all species, Kir2.1 is the core subunit of cardiac IK1 channels; in mouse and rabbit heart, IK1 is generated by heteromeric Kir2.1/Kir2.2 channels (Zaritsky et al. 2001; Zobel et al. 2003); and in human cardiomyocytes, IK1 currents likely result from heteromultimerization of Kir2.1, Kir2.2, and Kir2.3 subunits (Schramp et al. 2003).

IK1 currents are modulated by α and β adrenergic stimulation (Scherer et al. 2007, Zitron et al. 2008), intracellular Ca2+ (Zaza et al. 1998), and phosphorylation (Karle et al. 2002, Zitron et al. 2004). More notably, Kir2.x channels are selectively activated by PIP2, and can be inhibited by other phosphoinositides (Cheng et al. 2011, D’Avanzo et al. 2010). Multiple positively charged residues in the cytoplasmic side of Kir2.1 have been found to control sensitivity to PIP2 activation (Soom et al. 2001, Xie et al. 2008); recently, biochemical data and the atomic structures of Kir2.2 and PIP2-bound Kir3.2 have revealed that some of these key residues cluster in one specific site just beyond the transmembrane segments, at the interface of the cytoplasmic NH2- and COOH-termini (D’Avanzo et al. 2013, Tao et al. 2009, Whorton and Mackinnon 2011). Kir2 channels are constitutively closed in the absence of PIP2; PIP2 strengthens N- and C-termini interactions by tethering them to the cytoplasmic side of the membrane, thus mechanically opening the pore (D’Avanzo et al. 2013, Enkvetchakul and Nichols 2003, Xie et al. 2008). The cholesterol content of cell membranes has been shown to affect human Kir2.1 channel function, which could have pathophysiological implications (D’Avanzo et al. 2011).

18.4.1.2 Pathophysiology of IK1 channels

Atrial IK1 currents are upregulated in patients with chronic AF (Dobrev et al. 2002), and in congestive heart failure, atrial and ventricular IK1 current density may be moderately reduced (Anumowono and Lopatin 2010). IK1 appears to be implicated in protective ischemic preconditioning after acute hypoxia or cyanide poisoning, by action potential shortening and early repolarization (Piao et al. 2007). More notably, four channelopathies with severe cardiovascular and musculoskeletal manifestations that originate from loss- or gain-of-function mutations in Kir2.1 have been identified: type 1 Andersen–Tawil syndrome (ATS1), catecholaminergic polymorphic ventricular tachycardia (CPVT), short QT3 syndrome, and hereditary AF. ATS1 is characterized by cardiac electrophysiological abnormalities, including prolongation of the QT interval that can result in biventricular tachycardia and arrhythmias, and may lead to AF and sudden cardiac death; additionally, ATS1 patients are dysmorphic, with short stature, scoliosis, and cleft palate, as well as skeletal muscle weakness with episodes of paralysis (Sansone and Tawil 2007). Since the causal relationship between mutant human Kir2.1 channels and ATS1 was first established (Plaster et al. 2001), over 30 KCNJ2 dominant-negative mutations associated to the syndrome have been described, most located on the N-terminal slide helix and the C-terminal cytoplasmic domain, and all resulting in disruption of Kir2.1 channel function through
mechanisms that include abnormal trafficking, altered interaction with second messengers or gating, and incorrect folding of the Kir2.1 protein (Tristani-Firouzi and Etheridge 2010). Several of these mutations are located within the cluster of residues involved in PIP2 sensitivity (Lopes et al. 2002). CPTV, on the other hand, presents with ventricular arrhythmias and sudden cardiac death associated with physical activity, but no dysmorphic features (Lehnart et al. 2007); in most cases, the symptoms have been linked to mutations in the ryanodine receptors (CPTV1 or CPTV2), but in a subset of CPTV patients, several novel mutations in the KCNJ2 gene, leading to loss of Kir2.1 function by mechanisms distinct from those of ATS1 mutations, have been identified (Eckhardt et al. 2007, Vega et al. 2009).

SQT3 is an inherited condition that predisposes to atrial and ventricular fibrillation and sudden death; the SQT1 and SQT2 variants of the syndrome are a result of gain-of-function mutations in KCN channels KCNH2 (HERG channel) and KCNQ (Iκ, channel), respectively, but a third form of the syndrome, SQT3, arises from overactive Kir2.1 channels (Priori et al. 2005). Finally, enhanced Iκ, current density in the atrial myocardium, by overexpression of Kir2.1 protein or due to a gain-of-function mutation in the KCNJ gene (Girmatsion et al. 2009, Li et al. 2004, Xia et al. 2005), has been linked to familial AF, a condition that often becomes chronic due to sustained action potential shortening and appearance of reentry circuits (Ravens and Cerbai 2008).

18.4.2 G-PROTEIN-GATED KIR CHANNELS: Kir3.x

Activation of G-protein-coupled receptors (GPCRs) by hormones or neurotransmitter ligands results in the release of two intracellular molecules, Gα and Gβγ, which modulate multiple cellular processes. Kir3 channels, or Kκ channels, are targets of GPCRs (Pfaffinger et al. 1985): in atrial cardiac myocytes, for example, muscarinic K+ channels (KAC3), generated by Kir3.1/3.4 subunits, are activated by GPCRs in response to acetylcholine and adenosine, resulting in slowing of the heart rate (Anumonwo and Lopatin 2010). Kκ channels are also prominent in the brain, where they have been implicated in synaptic plasticity and in mechanisms underlying drug addiction (Chung et al. 2009, Robbins and Everitt 1999), and in endocrine pancreas, where they help to regulate hormone secretion (Sharp 1996).

18.4.2.1 Kκ channel assembly, regulation, and pharmacology

Functional Kκ channels are homomeric or heteromeric tetramers of Kir3.x channel subunits. Presently, four Kir3 subfamily members are known, namely, Kir3.1, Kir3.2, Kir3.3, and Kir3.4, and various combinations of Kir3 subunits form Kκ channels of unique properties, finely tuned by their localization, protein–protein interactions, and modulation by intracellular factors. Kir3.1/GIRK1 (encoded by gene KCNJ3) was the first Kir subunit shown to participate in the formation of Kκ channels, and interestingly, Kir3.1 channel subunits do not generate Kκ currents on their own (Corey and Clapham 1998, Wischmeyer et al. 1997) but are generally incorporated into heteromers with other Kir3.x subunits to form Kκ channels in native cells and tissues (Duprat et al. 1995, Velimirovic et al. 1996); most notably, cardiac KAC3 channels are formed by the heteromeric assembly of Kir3.1 and Kir3.4 (GIRK4/KCNJ5) subunits (Krapivinsky et al. 1995, Wickman et al. 1998). There are four different isoforms of Kir3.2/GIRK2, generated by alternative splicing of the KCNJ6 gene (Wickman et al. 2002), which exhibit differential expression patterns in various tissues, such as brain and pancreas, where they form functional homomeric channels or heteromerize with Kir3.1 or Kir3.3 subunits (Inanobe et al. 1999a,b, Jelacic et al. 2000); on the other hand, two isoforms of Kir3.3/GIRK3 (KCNJ9) have been cloned, but their expression profile and functional differences have not been fully established (Jelacic et al. 1999, Lesage et al. 1994).

Kκ channel opening is triggered by membrane-bound G-proteins (Kurachi et al. 1986), and after a long controversy, it has been established that Kκ channels are activated only by the Gβγ subunits (Kofuji et al. 1995, Wickman and Clapham 1995), although the Gα subunits may play important regulatory roles (Yamada et al. 1994). In the absence of agonists, Kκ channels associate with GDP-bound Gα and Gβγ to make the preformed complex, which effectively reduces their basal activity; when the GPCR is activated, the Gα is released and Gβγ activates the associated Kκ channel (Nobles et al. 2005, Riven et al. 2006). A recent Kir3.2-Gβγ cocystal structure (Whorton and MacKinnon 2013) confirms an extensive interaction surface between Gβγ and the cytoplasmic domain of Kκ channel subunits, and the key roles of specific residues identified functionally including L344 and G347 in Kir3.2 (Finley et al. 2004) and H64 and L262 in Kir3.4 (Ivanina et al. 2003). Several G-protein-mediated signal pathways are negatively modulated by regulator of G-protein signaling (RGS) proteins by accelerating intrinsic GTP hydrolysis in the Gα subunit (Ross and Wilkie 2000), and this has been implicated in the control of Kκ channel activity (Fujita et al. 2000, Inanobe et al. 2001, Saijoh et al. 1997). Like other Kir channels, Kκ channels require PIP2 to maintain their activity (Huang et al. 1998), but unlike in most Kir channels, PIP2–dependent activation in Kκ channels is strengthened by interactions with Gβγ subunits and intracellular Na+ (Ho and Murrell-Lagnado 1999, Rosenhouse-Dantsker et al. 2008, Zhang et al. 1999). Additionally, Kκ channels are modulated by redox signaling, which may contribute to protecting cells from hypoxic or ischemic insults (Zeidner et al. 2001), and are inhibited by extracellular acidification, which may be involved in the control of cellular respiration and CO2 chemoreception in tissues such as the brain (Mao et al. 2003). Ethanol, methanol, and other n-alcohols can activate Kκ channels containing Kir3.1, Kir3.2, and Kir3.4 subunits (Lewohl et al. 1999), and Kκ channels made up of various combinations of Kir3.x subunits can be modulated by antipsychotic drugs (Kobayashi et al. 2000), antidepressants (Kobayashi et al. 2004), and volatile and local anesthetics (Weigel and Schreibmayer 2001, Zhou et al. 2001), in many cases, by interaction with specific locations in the cytoplasmic domain.

18.4.2.2 Physiology and pathophysiology of Kκ channels

Kir3 proteins are expressed throughout the brain (Koyrakh et al. 2005, Wickman et al. 2000) in various homomeric or heteromorphic complexes. At least Kir3.1/Kir3.2, Kir3.2/Kir3.3, and Kir3.2/Kir3.4 assemblies have been identified in various regions of the brain (Jelacic et al. 2000, Liao et al. 1996). Kir3.1 and Kir3.2 are found in presynaptic and postsynaptic termini,
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whereas Kir3.3 and Kir3.4 localize to axons and dendrites (Hibino et al. 2010a). Postsynaptic Kir3.2 is critically involved in the generation of slow inhibitory postsynaptic potentials that suppress neuronal excitability, and Kir3.2-deficient mice develop spontaneous seizures and are more susceptible to seizure-inducing drugs (Signorini et al. 1997). A mutation in the signature sequence of the selectivity filter of Kir3.2 in weaver mice results in nonselective cation permeation, which leads to a severe phenotype including locomotor defects, male sterility, deficiencies in the midbrain dopaminergic system, and neuronal death (Navarro et al. 1996, Patil et al. 1995). Heteromeric Kir3.1/Kir3.4 channels have been identified in the rat pituitary gland, where the action of thyrotropin-releasing hormone enhances dopamine- and somatostatin-induced inhibitory K⁺ currents, and this constitutes an effective mechanism for control of plasma hormone levels (Morishige et al. 1999). In the heart, the acetylcholine released from vagal nerve endings activates Kₑ Channels composed by Kir3.1 and Kir3.4 subunits (Krapivinskaya et al. 1995), and this causes an increase in K⁺ efflux and membrane hyperpolarization that results in a deceleration of the heartbeat. Iₖᵋ(Cₐ₃) has been found to play a critical role in AF, as electrical remodeling in AF patients causes Kₑ CHANNELS to be constitutively active, and this leads to persistent action potential shortening (Dobrev et al. 2005). Conversely, Kₑ CHANNELS could be a target of for AF treatment and prevention, as Iₖᵋ(Cₐ₃) can be inhibited by classical antiarrhythmic agents, such as quinidine (Kurachi et al. 1986).

18.4.3 ATP-SENSITIVE Kir CHANNELS: Kir6.x/SURx

ATP-sensitive potassium (Kₐ₃) channels are expressed in most excitable tissues and serve to couple intracellular energetics to electrical activity (Nichols 2006). Kₐ₃ CHANNELS are uniquely generated by complexes of four pore-forming Kir6 channel subunits with four auxiliary sulfonylurea receptors (SURs). Kₐ₃ CHANNELS are activated by Mg²⁺-bound nucleotides in states of low metabolism and inhibited by cytosolic ATP in states of high metabolism. Since being first described in ventricular myocytes (Noma 1983, Trube and Hescheler 1984), Kₐ₃ CHANNELS have been found in tissues throughout the body, including pancreatic ß-cells (Cook and Hales 1984), skeletal muscle (Spruce et al. 1985), visceral and vascular smooth muscle (Staden et al. 1989), and brain (Bernardi et al. 1988, Gehlert et al. 1990).

The roles of Kₐ₃ CHANNELS in glucose homeostasis and ischemic protection are well established (Flagg et al. 2010, Koster et al. 2005a, Rorsman et al. 2008), and novel functions continue to emerge: recognized as protective against neural apoptosis following a stroke (Wind et al. 1997), brain Kₐ₃ CHANNELS have been implicated in memory (Betourne et al. 2009) and in the regulation of male reproductive behavior (McDevitt et al. 2009). Mutations leading to aberrant Kₐ₃ CHANNEL function have been linked to a variety of diseases, from neonatal diabetes to congenital hyperinsulinism (Denton and Jacobson 2012, Flanagan et al. 2009) and, more recently, to the Cantu syndrome (Nichols et al. 2013). Classic Kₐ₃ CHANNEL openers (KCOs), such as diazoxide and pinacidil, have been used to treat hypertension, angina, and hyperinsulinism of infancy, while antagonists such as sulfonylureas are established antidiabetic agents (Doyle and Egan 2003, Wickenden 2002). The drug industry continues to exploit the tissue-specific pharmacology of Kₐ₃ CHANNELS in the design of novel therapeutic agents aimed at endocrine, vascular, neurological, urological, and even dermatological disorders (Jahangir and Terzic 2005).

18.4.3.1 Kₐ₃ channel architecture and assembly

Kₐ₃ CHANNEL activity in heterologous expression systems requires both Kir6 and SUR subunits to be coexpressed in a 4:4 stoichiometry to generate the functional Kₐ₃ CHANNEL (Clement et al. 1997, Inagaki et al. 1995a, 1997, Shyng and Nichols 1997). Similarly, biochemical studies demonstrate that the SUR2 protein variants, SUR2A and SUR2B, can also coassemble with Kir6 subunits (Babenko et al. 1998, Inagaki et al. 1996, Okuyama et al. 1998, Yamada et al. 1997), presumably in a similar octameric arrangement. How the Kir6 and SUR subunits are physically connected is still unknown, but electron microscopy and intersubunit FRET studies of complete Kₐ₃ CHANNEL assemblies suggest the tight packing of four SUR and four Kir6.x subunits (Mikhaylov et al. 2005, Wang et al. 2013). The genes for Kir6.2 and SUR1 lie sequentially on human chromosome 11p15.1 (Inagaki et al. 1995b), suggesting an as yet unconsidered coregulation at the gene level. In addition, the genes for Kir6.1 and SUR2 are also adjacent on chromosome 12p12.1 (Chutkow et al. 1996, Inagaki et al. 1995b), which implicates an evolutionary duplication event. Classic pancreatic Kₐ₃ CHANNELS are generated by Kir6.2 and SUR1 subunits (Inagaki et al. 1995b), and in vascular smooth muscle (VSM), the predominant Kₐ₃ CURRENT is represented by Kir6.1/SUR2B channels (Beech et al. 1993, Farzaneh and Tinker 2008, Zhang and Bolton 1996). However, the combination of subunits extends beyond the pairing of Kir6.2 with SUR1 and Kir6.1 with SUR2; for example, Kir6.2 associates with SUR2A in cardiac and skeletal muscle (Inagaki et al. 1996). Although it is possible to construct functional channels with more than one SUR isoform or Kir6 isoform in recombinant expression (Chan et al. 2008, Cheng et al. 2008, Cui et al. 2001, Kono et al. 2000, Wheeler et al. 2008), the presence of heteromultimeric Kₐ₃ CHANNELS in native tissues remains controversial (Pountney et al. 2001, Seharaseyon et al. 2000). In addition, alternative splicing of the ABCC9 gene gives rise to SUR2A or SUR2B, which confer distinct physiological and pharmacological properties on the channel complex (Chutkow et al. 1999, Shi et al. 2005).

18.4.3.2 Regulation and pharmacology of Kₐ₃ CHANNELS

The key regulatory features of Kₐ₃ CHANNELS are rapid and reversible closure by cytoplasmic ATP, and activation by nucleotide tri- and diphosphates (Nichols 2006). In the absence of Mg²⁺, nucleotides inhibit Kₐ₃ CHANNEL activity through interaction with the Kir6 subunit (Drain et al. 1998, Tucker et al. 1998), but in the presence of Mg²⁺, both ATP and ADP stimulate channel activity (Dunne and Petersen 1986, Hopkins et al. 1992, Kakei et al. 1986, Lederer and Nichols 1989), through interaction with the SUR subunit (Gribble et al. 1997, Nichols et al. 1996, Shyng et al. 1997). Membrane phospholipids, in particular PIP₃, potently stimulate Kₐ₃ activity by binding the Kir6.2 subunit (Baukrowitz et al. 1998, Fan and Makielski 1997, Ribalet et al. 2005, Shyng and Nichols 1998, Xie et al. 2008), and phospholipase C, which hydrolyzes PIP₃, reduces Kₐ₃ CHANNEL activity (Baukrowitz et al. 1998, Fan and Makielski 1997, Hilgemann and Ball 1996,
Xie et al. 1999). Importantly, there exists a negative coupling between PIP₂ activation and ATP sensitivity of Kₐ₅P channels such that as PIP₂ increases, channel open probability increases and ATP sensitivity decreases (Enkvetchakul and Nichols 2003). Residues involved in PIP₂ binding and activation overlap with the ATP-binding site on Kir6.2, consistent with their competitive effects observed in binding assays (Enkvetchakul and Nichols 2003, MacGregor et al. 2002, Ribalet et al. 2005). Collectively, these observations demonstrate that ATP sensitivity is not a fixed parameter and may change dynamically with changes in membrane composition. Positively charged amino acid residues in the slide helix region directly interact with PIP₂ in the membrane (Cukras et al. 2002, Schulze et al. 2003, Shyng et al. 2000), as in Kir2 channels. While less extensively studied, Kir6.1 channels are also activated by PIP₂ (Quinn et al. 2003), suggesting conservation of the fundamental determinants of phospholipid binding and gating. Long chain acyl-coA molecules (LC-CoA), intermediates of β-oxidation of fatty acids, have also been shown to modulate Kₐ₅P channel activity in an analogous manner to membrane phosphoinositides (Liu et al. 2001a).

The same residues on Kir6.2 that mediate PIP₂ activation also contribute to the stimulatory effect of LC-CoA (Manning Fox et al. 2004, Schulze et al. 2003), suggesting that Kir6.2 is the major site of LC-CoA action. Acidic intracellular pH stimulates Kₐ₅P channel activity (Davies 1990, Davies et al. 1992, Xu et al. 2001), and intracellular acidification concomitant with anaerobic metabolism provides another potential physiological stimulus of Kₐ₅P activity. Although the mechanistic basis of pH regulation is still not completely established, there is a general consensus that protons act to decrease sensitivity to inhibitory ATP. Agonist-dependent protein kinase A (PKA) phosphorylation regulates smooth muscle and pancreatic Kₐ₅P channels: the Kir6.2 subunit has two consensus PKA phosphorylation sites which when phosphorylated increase channel open probability, and in human SUR1, a unique, constitutively phosphorylated PKA site acts to both increase the surface expression of the channel and decrease channel open probability (Beguin et al. 1999, Lin et al. 2000). Finally, protein kinase C (PKC) has mixed actions on native ventricular Kₐ₅P channels, inhibiting at low micromolar ATP concentrations (Light et al. 1995), but activating at high ATP concentrations (Light et al. 1996), through phosphorylation of the highly conserved T180 residue in the Kir6.2 subunit (Light et al. 2000). Kir6.1/SUR2B channels are inhibited by acute PKC treatment due to phosphorylation of residues in Kir6.1 (Quinn et al. 2003), whereas Kir6.2/SUR2B channel activity is reportedly unaffected by PKC (Shi et al. 2008, Thorneoe et al. 2002), highlighting the specificity of Kₐ₅P channel subunit combinations in physiological regulation.

The SUR subunit determines the sensitivity of the channel to a wide range of pharmacological KCOs and blockers (Babenko et al. 2000, D’Hahan et al. 1999, Hambrock et al. 2004). The sulfonurea glibenclamide inhibits all SUR isoforms, with a potency that depends on the intracellular nucleotide concentration, and hence on the metabolic state (Koster et al. 1999, Reimann et al. 1999). The SUR subunits also confer sensitivity to KCOs such as diazoxide, cromakalim, and pinacidil (Kurat et al. 2006). Diazoxide is an effective activator of SUR1 and SUR2B, but not SUR2A, whereas pinacidil and cromakalim are effective activators of SUR2A and SUR2B, but not SUR1 (Flagg et al. 2008, Quayle et al. 1995), and this potential for tissue specificity remains an important pharmaceutical strategy (Ashcroft and Gribble 2000). These openers all require the presence of hydrolyzable ATP (Dickinson et al. 1997, Hambrock et al. 1998, Schwantech et al. 1998), which suggests that they act to stabilize or enhance ATP hydrolysis at the nucleotide-binding folds (NBFs).

### 18.4.3.3 Kₐ₅P channel pathologies

In pancreatic β-cells, Kₐ₅P channels are made up of Kir6.2 and SUR1, and link blood sugar levels to insulin secretion by modulating membrane excitability. Pancreatic Kₐ₅P channels are constitutively active, due to the enhanced stimulatory effects conferred by SUR1 (vs. SUR2A) (Masia et al. 2005) as well as to the relatively low [ATP]/[ADP] ratio in β-cells during fasting, and help set a negative membrane potential. As blood glucose levels increase after a meal, glucose is taken up and metabolized by the β-cells, the [ATP]/[ADP] ratio is increased, and Kₐ₅P channel activity is reduced. This results in cell membrane depolarization and activation of voltage-gated Ca²⁺ channels, and Ca²⁺-dependent secretion of insulin granules (Ashcroft 2005). Loss-of-function mutations in pancreatic Kₐ₅P channel subunits underlie congenital hyperinsulinism (Dunne et al. 2004), and gain-of-function Kₐ₅P channel mutations lead to transient or permanent neonatal diabetes mellitus (Koster et al. 2005b). The most severe permanent form of the disease extends beyond the pancreas to neuronal or other tissues, such that patients experience motor and intellectual developmental delay, epilepsy, and neonatal diabetes, collectively known as the DEND syndrome (Hattersley and Ashcroft 2005); the extrapancreatic symptoms of DEND have been attributed to Kₐ₅P overactivity in muscle and/or nerves and brain, which highlights the role of Kir6.2 and SUR1 in tissues outside the pancreas. Mouse models expressing overactive or inducible Kₐ₅P channel subunits established the Kₐ₅P-dependent basis of neonatal diabetes (Girard et al. 2009, Koster et al. 2000, Remedi et al. 2009), and led to the realization that sulfonureas could be used to treat the disease. Most patients with activating Kₐ₅P mutations have now been successfully treated with oral sulfonureas rather than insulin (Koster et al. 2008, Pearson et al. 2006, Wambach et al. 2010), and these have since become a preferred treatment option for neonatal diabetes (Karges et al. 2012).

Under normal metabolic conditions, cardiac sarcolemmal Kₐ₅P channels are predominantly closed, and do not significantly contribute to cell excitability. However, these channels can open when exposed to a severe metabolic stress such as anoxia, metabolic inhibition, or ischemia, and this has been hypothesized to protect the cells against damage of Ca²⁺ overload (Flagg and Nichols 2005). In cardiac myocytes, Kₐ₅P current activation leads to action potential shortening, which reduces Ca²⁺ entry and inhibits contractility, thereby reducing energy consumption and accelerating recovery after an ischemic event (Lederer et al. 1989). Kir6.2-null mice are intolerant of vigorous exercise, and show a predisposition to cardiac remodeling, heart failure, and death when exposed to hypertension, pressure overload, or ischemia (Kane et al. 2006, Liu et al. 2004, Suzuki et al. 2001, 2002, Yamada et al. 2006, Zingman et al. 2002). The function...
of cardiac K\textsubscript{ATP} channels in less challenging physiological conditions is a matter of debate; for example, baseline cardiac action potential and contractility are unaffected in Kir6.2-null mice (Li et al. 2000, Suzuki et al. 2001, 2002), and no cardiovascular symptoms have been reported for individuals with gain-of-function or loss-of-function mutations in Kir6.2 or SUR1 (Flanagan et al. 2009, Nichols et al. 2007); also, Kir6.2 gain-of-function transgenic mice recapitulate the human condition of neonatal diabetes with no cardiac phenotype (Koster et al. 2001). Studies to ascertain the role of sarcolemmal K\textsubscript{ATP} channels in cardiac rhythm have yielded conflicting results: treatment with KCOs has been shown to stabilize the resting membrane potential and reduce the frequency of arrhythmias in some studies (Grover and Garlid 2000), but has proven pro-arrhythmic in others (Wolleben et al. 1989). Similarly, both increased (Shigematsu et al. 1995) and decreased (Kantor et al. 1990, Wolleben et al. 1989) incidence of tachycardia and ventricular fibrillation following treatment with glibenclamide have been reported.

There have been reports of AF and heart failure associated with SUR2A loss-of-function mutations (Bienengraeber et al. 2004, Olson et al. 2007), and Kir6.1-null and SUR2A-null mice recapitulate the clinical features of human Prinzmetal angina, including baseline hypertension, coronary artery vasospasm, and sudden cardiac death, presumably due to loss of K\textsubscript{ATP} channel activity in either VSM or endothelium (Chutkow et al. 2002, Miki et al. 2002). One gain-of-function mutation in Kir6.1 (S422L) has been found in several individuals with early repolarization syndrome and Brugada syndrome (Barajas-Martinez et al. 2012, Delaney et al. 2012, Haissaguerre et al. 2009, Medeiros-Domingo et al. 2010), although a recent study suggests that S422L may be a benign variant (Veeramah et al. 2013), and mutations in Kir6.1 leading to reduced K\textsubscript{ATP} channel activity have been reported in cases of sudden infant death syndrome (Tester et al. 2011). Recently, multiple different gain-of-function mutations in the gene encoding for SUR2 have been linked to the Cantu syndrome, a complex multiorgan disorder with a myriad of cardiovascular and musculoskeletal features, including hypertrichosis, osteochondrodysplasia, and edema (Harakalova et al. 2012, Nichols et al. 2013, van Bon et al. 2012).

Unlike cardiac K\textsubscript{ATP} channels, pancreatic K\textsubscript{ATP} channels are constitutively active, due to the enhanced stimulatory effects conferred by SUR1 (vs. SUR2A) (Masia et al. 2005). This results in cell membrane depolarization and activation of voltage-gated Ca\textsuperscript{2+} channels, and Ca\textsuperscript{2+}-dependent secretion of insulin granules (Ashcroft 2005). Due to the critical role that these channels play in glucose-stimulated insulin secretion, genetic modifications of Kir6.2 or SUR1 have the potential to cause insulin secretion disorders. Indeed, loss-of-function mutations in pancreatic K\textsubscript{ATP} channel subunits underlie congenital hyperinsulinism (Dunne et al. 2004), and gain-of-function K\textsubscript{ATP} channel mutations lead to transient or permanent neonatal diabetes mellitus (Koster et al. 2005a). The most severe permanent form of the disease extends beyond the pancreas to neuronal or other tissues, such that patients experience motor and intellectual developmental delay, epilepsy, and neonatal diabetes, collectively known as the DEND syndrome (Hattersley and Ashcroft 2005); the extrapancreatic symptoms of DEND have been attributed to K\textsubscript{ATP} overactivity in muscle and/or nerves and brain, which highlights the role of Kir6.2 and SUR1 in tissues outside the pancreas. Mouse models expressing overactive or inducible K\textsubscript{ATP} channels established the K\textsubscript{ATP}-dependent basis of neonatal diabetes (Girard et al. 2009, Koster et al. 2000, Remedi et al. 2009), leading to the realization that sulfonylureas could be used to treat the disease. In early clinical studies, most patients with activating K\textsubscript{ATP} mutations transitioned successfully from insulin to oral sulfonylureas (Koster et al. 2008, Pearson et al. 2006), and these have since become a preferred treatment option for neonatal diabetes (Karges et al. 2012).

18.4.3.4 Mitochondrial K\textsubscript{ATP} channels

A K\textsuperscript{+}-selective, small-conductance channel was first identified in rat liver mitochondrial inner membrane, then reported to be reversibly inhibited by application of ATP and glibenclamide (Inoue et al. 1991), and finally activated by GTP, GDP, and diazoxide (Garlid et al. 1996, Paucek et al. 1996). The pharmacology of heterologously expressed SUR1/Kir6.1 complexes appears to most closely resemble such properties (Hu et al. 1999, Liu et al. 2001b), yet the function of these mitoK\textsubscript{ATP} channels is apparently unaffected in both Kir6.1-null and Kir6.2-null mice (Miki et al. 2002, Suzuki et al. 2002), and whether specific SUR or Kir6 subunits are normally present in mitochondria remains unclear (Cuong et al. 2005, Foster et al. 2008, Hu et al. 1999, Singh et al. 2003, Suzuki et al. 1997, Zhou et al. 2005).

The lack of confirmed presence of canonical SUR or Kir6 subunits in mitochondria has led to alternative hypotheses regarding mitoK\textsubscript{ATP} structure. In addition to opening K\textsubscript{ATP} channels, diazoxide may inhibit succinate dehydrogenase (Hanley et al. 2002) and it has been suggested that this enzyme key to the Krebs cycle and the electron transport chain may be a component of the mitoK\textsubscript{ATP} channel (Ardehali et al. 2004, 2005). Most recently, proteomic analysis and pharmacological studies on bovine mitochondrial inner membranes have implicated a short product of the KCNJ1 (Kir1.1) gene, in the formation of the mitoK\textsubscript{ATP} channel (Foster et al. 2012).

18.4.4 K\textsuperscript{+} TRANSPORT CHANNELS: Kir1.1, Kir4.3, Kir5.1, and Kir7.1

18.4.4.1 Kir1.1

Kirs.1 (encoded by gene KCNJ1), initially described as rat outer medullary K\textsuperscript{+} channel ROMK1, was the first member of the Kir family to be cloned (Ho et al. 1993). Six alternative splicing isoforms of KCNJ1 are known, of which ROMK2, 4, 5, and 6 correspond to the same protein (Kir1.1b), respectively, 19 and 26 amino acids shorter in the NH\textsubscript{2}-terminus than ROMK1 (Kir1.1a) and ROMK3 (Kir1.1c) (Boim et al. 1995, Kondo et al. 1996). Kir1.1 tetrameric assemblies generate very weak inward rectifiers, and this is attributed to the presence of an uncharged amino acid (N171) at the rectification controller site (Leng et al. 2006b). Kir1.1 channels are fundamental for blood and urine salt homeostasis, and mutations that disrupt Kir1.1 function result in an array of renal tubulopathy symptoms collectively known as Bartter’s syndrome (Hebert et al. 2005, Peters et al. 2002).
18.4.4.1 Kir1.1 channel assembly and regulation

Intracellular pH (pHi) modulates the activity of Kir1.1 channels; in particular, acidification closes the channels, with a pKᵢ of ~6.5 (Choe et al. 1997). There is evidence that pHi sensitivity in Kir1.1 channels is controlled by three quartets of interactions, two within the same subunits (a salt bridge between R41 and E318, and a potential hydrogen bond between K80 and A177) and one between adjacent subunits (a salt bridge between E302-R311) (Fakler et al. 1996, Rapedius et al. 2006, Wang et al. 2005). Mutation of any of these six residues alters Kir1.1 pHi sensitivity; for example, R311W and A177T, which underlie and one between adjacent subunits (a salt bridge between E302-R311) (Fakler et al. 1996, Rapedius et al. 2006, Wang et al. 2005). Mutation of any of these six residues alters Kir1.1 pHi sensitivity; for example, R311W and A177T, which underlie Barter’s syndrome, significantly increase the pKᵢ of ROMK channels and reduce open probability in the physiological pH, range (Peters et al. 2003, Schulte et al. 1999). Mutations of K80 that are predicted to disrupt the H⁺ bond lower pH, sensitivity of Kir1.1, and also accelerate the recovery from inhibition induced by acidification. The K80-A177 bond has also been implicated in the control of PIP₂-mediated gating of Kir1.1 channels (Hibino et al. 2010a). Additionally, mutagenesis studies have suggested that the hydrophobic residue L160 may contribute to pHi-dependent gating (Sackin et al. 2005). Finally, G167 and G176 in Kir1.1a and G148 and G157 in Kir1.1b are located close to the cytoplasmic apex of the TM2 helices, and mutation of either residue to Ala results in ROMK channels with increased pKᵢ, again consistent with a role in the mechanisms governing Kir1.1 pHi sensitivity (Sackin et al. 2006).

Kir1.1 channel function is strongly regulated by surface expression. Thus, Kir1.1 has a carboxy-terminal endoplasmic reticulum (ER) retention signal, R368-A369-R370, which undergoes posttranslational modification by intracellular protein kinases and ultimately controls trafficking to the cell membrane (Ma et al. 2001). Kir1.1 channel activity requires PKA-dependent phosphorylation in three sites, namely, S44, S219, and S313, and phosphorylation in S44 by PKA or SGK has been found to increase Kir1.1 surface expression, by suppressing the ER retention signal (Hebert et al. 2005, McNicholas et al. 1994, O’Connell et al. 2005, Yoo et al. 2004). Other serine-threonine kinases, such as WNK1, WNK3, and WNK4, reduce Kir1.1 channel activity by decreasing surface expression (Kahle et al. 2003, Leng et al. 2006a, Wade et al. 2006), and mutations in WNK1 or WNK4 have been associated with Gordon’s syndrome, a type of autosomal-dominant pseudo-hypoaldosteronism with hypertension and hyperkalemia than can be explained by decreased Kir1.1 function (Wilson et al. 2001). Finally, phosphorylation of S219 and S313 by PKA enhances channel activity by increasing sensitivity to PIP₂ (Liou et al. 1999), whereas PKC-dependent phosphorylation of S4 and S201 has been found to inhibit Kir1.1, possibly due to a reduction in membrane PIP₂ concentration (Lin et al. 2002, Wang and Giebish 1991, Zeng et al. 2003).

18.4.4.1.2 Physiology and pathophysiology of Kir1.1 channels

Kir1.1 is expressed in the apical membrane of renal epithelial cells of the thick ascending (TAL) of the loop of Henle, the distal convoluted tubule (DCT), and the cortical collecting duct (CCD), where it plays a crucial role in the regulation not only of K⁺ concentrations in blood and urine, but also of other ions such as Na⁺ and Cl⁻. Kidney TAL cells reabsorb ~25% of all the Na⁺ in the renal filtrate, mainly through the apical membrane Na⁺-K⁺-2Cl⁻ (NKCC) transporter, and Kir1.1 channels provide a route for K⁺ efflux. Kir1.1 channels also facilitate reabsorption of Cl⁻ via NKCC, and Na⁺ and Cl⁻ accumulated in the cytoplasm are returned to the blood by the Na⁺-K⁺-ATPase and the Cl⁻ channels at the basolateral membrane. Thus, functional coupling of Kir1.1 and NKCC results in unidirectional transport of Na⁺ and Cl⁻, sustains the activity of the ATPase, and establishes the transepithelial potential that drives paracellular reabsorption of Na⁺, Ca²⁺, and Mg²⁺ (Hebert et al. 2005). Additionally, the Cl⁻ channel CFTR colocalizes with Kir1.1 in the apical membrane of TAL cells, and may provide Kir1.1 channels with sensitivity to ATP and sulfonylureas through complex protein–protein interactions (Lu et al. 2006, Yoo et al. 2004).

Barter’s syndrome is an autosomal recessive renal tubulopathy characterized by hypokalemic metabolic alkalosis, renal salt wasting, hyperreninemia, and hyperaldosteronism (Barter et al. 1962, Peters et al. 2002). Of the recognized types of the disease, genetic analysis revealed that type II Barter’s syndrome results specifically from mutations in the KCNJ1 gene that affect PKA phosphorylation, pH sensing, channel gating, proteolytic processing, and sorting to the apical membrane. In TAL cells, loss of Kir1.1 function decreases K⁺ supply to the NKCC and loss of the driving force for paracellular cation reabsorption (Hebert et al. 2005). KCNJ1-null null mice recapitulate the symptoms of Barter’s syndrome, and present with hypokalemia and an excess of Na⁺, Cl⁻, and K⁺ in their urine (Lorenz et al. 2002, Lu et al. 2002).

18.4.4.2 Kir4.x and Kir5.1

Kir4.1 (KCNJ10) was independently identified from a brain cDNA library by several groups, and variably referred to as BIR10, Kᵣᵢ₋₂, BIRK-1, and KirL2 (Hibino et al. 2010a). Kir4.1 channels are essential for control of glial function and neuronal excitability, systemic K⁺ homeostasis, and renal salt exchange (Butt and Kalsi 2006, Wagner 2010). Kir4.1 subunits form functional homotetramers, or coassemble with Kir5.1 (KCNJ16, initially termed BIR9) in heterotetramers with distinct physiological properties (Hibino et al. 2004a, Ishii et al. 2003, Tanemoto et al. 2000, Tucker et al. 2000). Kir4.2 (KCNJ15, also named Kir1.3) is found in kidney, liver, embryonic fibrocytes, and microvascular endothelial cells, where it may assemble in functional homotetramers or heterotetramers with Kir5.1 subunits (Pearson et al. 1999, Pessia et al. 2001). Linkage analysis has identified loss-of-function mutations in the KCNJ10 gene that are responsible for an array of neural and somatic symptoms collectively referred to as the SeSAME (seizures, sensorineural deafness, ataxia, mental retardation and electrolyte imbalance) or EAST (epilepsy, ataxia, sensorineural deafness, and tubulopathy) syndrome (Bockenhauer et al. 2009, Sala-Rabanal et al. 2010, Scholl et al. 2009). Recently, antibodies against Kir4.1 have been found in a subset of multiple sclerosis (MS) patients (Srivastava et al. 2012), and thus is possible that some MS cases may also belong to an expanding number of autoimmune disorders caused by ion channel dysfunction (Ehling et al. 2011, Kleopa 2011).
18.4.4.2.1 Channel assembly, structure, and localization

Homomeric Kir4.1 and Kir4.2 channels display intermediate inward rectification, and Kir4.1 channels exhibit an incomplete rectification with a marked punchthrough of polyamines through the channel pore (Kucheryavykh et al. 2007b). Homomeric Kir4.1 channels are inhibited by intracellular acidification with a $pK_a \approx 6$, whereas Kir4.1/Kir5.1 channels are suppressed by only a slight acidification and activated by alkalization, with a $pK_a \approx 7.5$ (Tanemoto et al. 2000, Yang et al. 2000). Homomeric Kir4.2 channels are more sensitive to $pH_i$ than Kir4.1 homomers (Kir4.2, $pK_a \approx 7.1$), but heteromerization with Kir5.1 has only minor effects on $pH_i$ sensitivity (Kir4.2/Kir5.1, $pK_a \approx 7.6$) (Pessia et al. 2001). Several residues in Kir4.1, including E158 in TM2, K67 in the cytoplasmic amino terminus, and H190 in the carboxy terminus, are involved in $pH_i$ sensitivity of Kir4.1 and Kir4.1/Kir5.1 channels, but the mechanism remains elusive (Casassamussa et al. 2003, Xu et al. 2000, Yang et al. 2000). Interestingly, mutation K66M in the NH2 terminus of Kir4.2 renders the channel virtually insensitive to $pH_i$ (Pessia et al. 2001), and this is consistent with the neutralization of the equivalent residue in Kir1.1, K80, which impairs $pH_i$ sensing in these channels (Fakler et al. 1996, McNicholas et al. 1998). Homomeric Kir4.1 channels are found in the microvilli of gastric parietal cells (Fujita et al. 2002), and in the apical membrane of intermediate cells of the cochlear stria vascularis (Hibino and Kurachi 2006). Kir5.1 subunits are found in fibrocytes of the spiral ligament in the inner ear, where they are mostly confined to intracellular compartments (Hibino et al. 2004b). Both Kir4.1 and Kir5.1 subunits are expressed in brain astrocytes and in Müller cells of the retina (Hibino et al. 2010a); heteromeric channels are found in brain and retinal perisynaptic processes, whereas only Kir4.1 homomers are present at the end feet of the Müller cells facing the vitreous humor and the blood vessels (Ishii et al. 2003). Heteromeric Kir4.1/Kir5.1 channels constitute the majority of the basolateral $K^+$ conductance of the distal nephron, and renal epithelial cells do not express homomeric Kir4.1 channels (Lachheb et al. 2008, Lourdel et al. 2002, Wang et al. 2010). The cellular mechanisms governing Kir4.1/Kir5.1 heteromerization, or the preference for Kir4.1/Kir5.1 over homomeric Kir4.1 channels in the kidney, are still unclear. A 44-amino acid stretch [161–205] in the cytoplasmic region just distal to the Kir4.1 TM2 appears to be essential for association with Kir5.1 (Kontras et al. 2003), and PDZ-binding motifs implicated in oligomerization and plasma membrane localization have been identified in the COOH-terminal portion of Kir4.1 (Tanemoto et al. 2004, 2005, 2008).

18.4.4.2.2 Physiology and pathophysiology

In renal tubular epithelia, Kir4.1/Kir5.1 channels are responsible for the negative basolateral membrane potential that helps drive the electrogenic luminal processes of $K^+$ secretion and Na+-reabsorption (Lachheb et al. 2008, Lourdel et al. 2002, Wang et al. 2010). In astrocytic glia, Kir4.1-containing channels account for the spatial buffering of $K^+$ released by neurons during action potential propagation (Butt and Kalsi 2006, Kucheryavykh et al. 2007a, Neusch et al. 2006). Kir4.1 is the predominant $K^+$ channel in retinal Müller glial cells (Bringmann et al. 2006), and in satellite glial cells of sensory ganglia (Tang et al. 2010). Kir4.1 channels in oligodendrocytes are critical for myelination (Kalsi et al. 2004, Neusch et al. 2001), and in the inner ear they play a role in $K^+$ regulation and generation of the endocochlear potential required for normal development of the cochlea and audition (Hibino et al. 2010b, Rozengurt et al. 2003). In the stomach, homomeric Kir4.1 channels have been implicated in acid secretion, as a mechanism of $K^+$ recycling for the H+-K+ pump (Kaufhold et al. 2008). On the other hand, Kir4.2 channels are involved in the migration of mouse embryonic fibrocytes and human microvascular endothelial cells (deHart et al. 2008), which suggests that these channels may play a crucial role in embryogenesis.

Missense and nonsense mutations in KCNJ10 have been identified in SeSAME/EAST syndrome patients (Bockenhauer et al. 2009, Scholl et al. 2009). Individuals affected by this disorder present with an array of both neurological and renal symptoms, including seizures, ataxia, mental retardation, sensorineural deafness, and electrolyte imbalance. All of the SeSAME/EAST mutations have been found to disrupt channel function in Kir4.1 homomers and Kir4.1/Kir5.1 heteromers (Reichold et al. 2010, Sala-Rabanal et al. 2010). In contrast, potential gain-of-function mutations in Kir4.1 have been associated with autism with seizures and intellectual disability (Sicca et al. 2011), and Kir5.1-null mice present with hypokalemia and metabolic acidosis, that is, a renal phenotype essentially opposite to that of SeSAME/EAST, due to a compensatory expression of $pH_i$-insensitive, constitutively active homomeric Kir4.1 in the nephron, which leads to increased salt reabsorption (Paulais et al. 2011). Kir4.1 is highly expressed in glia, where it plays a major role in $K^+$ buffering and neuron repolarization (Haj-Yasein et al. 2011, Kucheryavykh et al. 2007a, Olsen and Sontheimer 2008). Loss of Kir4.1 function may cause reduced astrocyte-mediated $K^+$ clearance, neuronal hyperexcitability, and reduced seizure threshold; alternatively, an increase in Kir4.1 activity during intense neuronal activity may lead to increased and faster $K^+$ influx into glial cells, resulting in a larger and more sustained membrane depolarization that promotes local neuronal synchrony and epileptic activity. In the kidney, loss of Kir4.1/Kir5.1 activity results in a salt-wasting phenotype, presumably by secondarily reducing the activity of the Na+-K+ ATPase (Bandulik et al. 2011).

No pathology has yet been clearly linked to Kir4.2 deficiencies, but the KCNJ15 gene is located close to the locus in chromosome 21 associated with Down syndrome (Gosset et al. 1997), and this suggests a possible linkage between some of the manifestations of the syndrome, which include dysmorphic features, hypotonia, and psychomotor delay, and Kir4.2 channel dysfunction.

18.4.4.3 Kir7.1

Kir7.1 (KCNJ3) is the sole representative of a seventh subfamily, most closely related to Kir4. The cDNA was independently isolated by three groups (Doring et al. 1998, Krapivinsky et al. 1998, Partiseti et al. 1998), and to date, only one isoform of the protein has been described. Kir7.1 is found in epithelial cells of the choroid plexus, thyroid follicular cells, enterocytes, renal epithelial cells of various regions along the nephron, and retinal pigment epithelia (RPE); in all these cells, it colocalizes with
the Na⁺-K⁺-ATPase, and thus may also serve as a mechanism for K⁺ recycling for the establishment and maintenance of the membrane potential (Doring et al. 1998, Nakamura et al. 1999, Ookata et al. 2000). Loss-of-function mutations in KCNJ13 have been found to underlie snowflake vitreoretinal degeneration, an autosomal-dominant, progressive eye disease that causes early-onset cataract, fibrillar degeneration of the vitreous humor, and retinal detachment (Hejtmanek et al. 2008, Pattnaik et al. 2013, Zhang et al. 2013), and this underscores the importance of Kir7.1 channels in normal retinal physiology.

Kir7.1 subunits assemble into functional homotetramers, and no evidence so far has been presented of heteromerization with other Kir family members. Kir7.1 channels are weak inward rectifiers, and this rectification appears to be independent of other Kir family members. Kir7.1 channels are activated by PiP₂, but their binding affinity is lower than in other Kir channels (Rohacs et al. 2003). More notably, Kir7.1 channels are sensitive to pH₄, but their response is bell shaped: thus, maximum activity is observed at pH₄ ~ 7.0 and the current is attenuated at either higher or lower pH₄, and H₂S6 in the cytoplasmic NH₄ terminus appears to control this behavior (Hughes and Swaminathan 2008).

REFERENCES
Inward rectifying potassium channels


Inward rectifying potassium channels


Ion channel families

References


Inward rectifying potassium channels


Inward rectifying potassium channels


Inward rectifying potassium channels


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