Inwardly Rectifying Potassium Channels: Their Molecular Heterogeneity and Function

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Summary: A variety of cells including cardiac myocytes and neuronal cells possess inwardly rectifying K^+ (Kir) channels through which currents flow more readily in the inward direction than outward. These K^+ channels play pivotal roles in maintenance of the resting membrane potential, in regulation of the action potential duration, in receptor-dependent inhibition of cellular excitability, and in the secretion and absorption of K^+ ions across cell membrane. Recent molecular biological dissection has shown that the DNAs encoding Kir channels constitute a new family of K^+ channels whose subunits contain two putative transmembrane domains and a pore-forming region. So far, more than ten cDNAs of Kir channel subunits have been isolated and classified into four subfamilies: 1) IRK subfamily (IRK1–3/Kir1.1–1.3), 2) GIRK subfamily (GIRK1–4/Kir3.1–3.4), 3) ATP-dependent Kir subfamily (ROMK1/Kir1.1, K_{AB} -2/Kir4.1), and 4) ATP-sensitive Kir subfamily (uK_{ATP} -1/Kir6.1, BIR/Kir6.2). *Xenopus* oocytes injected with the

cRNAs of IRKs elicit classical Kir channel currents. GIRKs, as heteromultimers, compose the G protein-gated Kir (K_G) channels, which are regulated by a variety of G_i/G_o -coupled inhibitory neurotransmitter receptors such as m_2 -muscarinic, serotonergic ($5HT_{1A}$), $GABA_B$, somatostatin and opioid (μ , δ , κ) receptors. ROMK1 and K_{AB} -2 are characterized with a Walker type-A ATP-binding motif in their carboxyl termini, and may be involved in K^+ transport in renal epithelial and brain glial cells. uK_{ATP} -1 and BIR form with sulfonylurea receptors, the so-called ATP-sensitive K^+ channels. Thus, it is a feature of the Kir channel family that each subfamily plays a specific physiological functional role. The Na^+ -activated Kir channels identified electrophysiologically in neurons and cardiac myocytes have not yet been cloned. In this review, we overviewed the current understandings of the features of the molecular structures and functions of the four main subfamilies of Kir channels. [Japanese Journal of Physiology, 47, 11–39, 1997]

Key words: inwardly rectifying potassium channel, G protein-gated potassium channel, ATP-sensitive potassium channel, molecular cloning.

The inwardly rectifying K^+ (Kir) channel current was first identified electrophysiologically in high K^+ -depolarized skeletal muscle and was designated an anomalous rectifier K^+ current [1]. Through this K^+ conductance, currents flow more readily in the inward direction than outward (Fig. 1). Because this property is opposite to that of the voltage-dependent delayed-rectifying K^+ channel current in squid giant axon [2], the term “anomalous rectification” was used to describe this peculiar K^+ current in skeletal muscle. The properties of this type of K^+ channel do not follow the

Hodgkin-Huxley kinetics [3]; that is, its kinetics depend not only on the membrane potential but also on the equilibrium potential for K^+ (E_K). The channel conductance increases as the concentration of extracellular K^+ ($[K^+]_o$) increases; that is, it is roughly proportional to the root of $[K^+]_o$. Because of these electrophysiological properties, Kir channels play pivotal roles in maintenance of the resting membrane potential, in regulation of the action potential duration and thus in controlling the excitability of a variety of cells [4–7]. Theoretically, the cells expressing a sufficient

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quantity of Kir channels are expected to have a deep resting potential near to E_K and may not excite automatically. Being excited with stimulation, these cells may exhibit action potentials with a long-lasting plateau when the expression of voltage-gated outward K^+ channels is relatively low. Actually, the Kir channel has been found not only in skeletal muscle but also in a variety of cell types including cardiac myocytes [8–11], neuronal cells [12–17], blood cells [18, 19], osteoclasts [20], and endothelial cells [21]. Thus, this behavior of K^+ channels is an important factor to determine the passive and active electrical properties of various cells.

After the identification of classical Kir channels, a number of K^+ channels have been shown electrophysiologically to possess the inwardly rectifying property (Table 1): the G protein-gated muscarinic K^+ (K_G) [22], ATP-sensitive K^+ (K_{ATP}) [23], and Na^+ -activated K^+ (K_{Na}) channels [24]. K_G channels are activated via pertussis toxin-sensitive G proteins by a variety of inhibitory neurotransmitters [25–27]. K_{ATP} and K_{Na} channels are activated by a decrease of intracellular ATP (ATP_i) and an increase of intracellular Na^+ (Na^+_i), respectively. These Kir channels, thus, can be targets of a variety of cell signaling systems and cell metabolisms. Therefore, Kir channels are also quite important in the receptor- and metabolism-dependent regulation of cellular electrical activity.

In 1993, the molecular structure of Kir channels was disclosed. An ATP-dependent Kir channel, ROMK1/Kir1.1 [28], and a classical Kir channel, IRK1/Kir2.1 [29], were cloned by the expression cloning technique from the outer medulla of rat kidney and a mouse macrophage cell line, respectively. They have a common molecular motif in the primary structure (i.e., two putative membrane-spanning regions (M1 and M2)) and one potential pore-forming region (H5). Thus, the primary structure of these Kir channel subunits resembles that of the S5, H5, and S6 segments of the voltage-gated K^+ (K_v) channels [30–32]. Because the voltage-sensor of the K_v channel subunit exists in its S4 segment, the Kir channel subunits lack a voltage-sensor region. This is consistent with the electrophysiological studies that the kinetics of Kir channels apparently depend on the voltage shift from E_K but not on the membrane potential itself. After the cloning of ROMK1 and IRK1, the cDNAs encoding the main subunits of the K_G and K_{ATP} channels were also cloned (GIRK1/Kir3.1 and uK_{ATP} -1/Kir6.1) [33–35]. All of these channel subunits exhibit the same primary structure. So far, at least 11 cDNAs encoding K^+ channel subunits belonging to the Kir family have been isolated. These cloned Kir channel subunits have been classified into four groups (Table 2): 1) IRK subfamily, classical Kir channels (IRK1, IRK2 [36], and IRK3 [37]); 2) GIRK subfam-

Fig. 1. Steady-state current records of the inwardly rectifying K^+ channel. **A:** Examples of the steady-state recording of patch currents at five membrane potentials indicated in mV at each trace. The concentration of K^+ of the pipette solution is shown at the top of each family in mM. **B:** Plots of the amplitudes of the unitary currents through the channel versus the potential deviation from the resting potential (E_r). Open symbols are values for 75 mM- K^+ in the

pipette ($[K^+]_p$ 75 mM) and filled symbols for $[K^+]_p$ 150 mM. The continuous lines at potentials greater than $E_r + 30$ mV ($[K^+]_p$ 75 mM) or $E_r + 50$ mV ($[K^+]_p$ 150 mM) were drawn by hand. The extrapolation of the straight lines, which were fitted by the least-squares method at potentials less than $E_r + 30$ mV ($[K^+]_p$ 75 mM) or $E_r + 50$ mV ($[K^+]_p$ 150 mM), crossed the zero current level at $E_r + 56$ mV ($[K^+]_p$ 75 mM) or $E_r + 65$ mV ($[K^+]_p$ 150 mM).

ily, G protein-activated K⁺ channels (GIRK1, GIRK2 [38], GIRK3 [38], and GIRK4/CIR [39]); 3) K_{AB} subfamily, ATP-regulated K⁺ channels (ROMK1/K_{AB}-1 and K_{AB}-2/BIR10 [40, 41]); and 4) K_{ATP} subfamily, ATP-sensitive K⁺ channels (uK_{ATP}-1 and BIR [42]).

Recent progress in the molecular biology of Kir channels enables us to understand the structure-function relationship of channel biophysics, physiological regulation, and the pharmacology of these channels at the molecular level. These attempts are now on-going. This review will summarize the current understandings of the molecular properties and functional roles of Kir channels.

I. Molecular Structure of Kir Channels: Comparison with the α -Subunits of Kv Channels

1. Primary structure and multimeric assembly of K⁺ channel subunits

The first K⁺ channel subunit cloned was the *Drosophila* K⁺ channel *Shaker* [43, 44]. The family related to *Shaker* K⁺ channel is thought to encode subunits of voltage-dependent K⁺ (Kv) channels. The Kv channel subunits are grouped into four subfamilies

named after the *Drosophila* genes: *Shaker*, *Shab*, *Shaw*, and *Shal* [45]. Homology screening has led to the identification of a number of counterparts of each subfamily in vertebrates. Currently, these subfamilies are referred to as Kv1.x, Kv2.x, Kv3.x, and Kv4.x, respectively [46]. The α -subunits of the Kv channels consist of six transmembrane segments (S1–S6) and one potential pore-forming region (P or H5) between S5 and S6. Both amino (N-) and carboxyl (C-) termini are supposed to be located intracellularly (Fig. 2B). This structural motif appears four times in the α -subunits of voltage-gated Na⁺ and Ca²⁺ channels (Fig. 2A) [45, 47]. It has been shown by studies on the interaction of a scorpion toxin with coexpressed wild-type and toxin-insensitive mutant *Shaker* K⁺ channels that Kv channels are composed of four subunits [48]. Thus, the structure of Kv channel protein (composed of four subunits) is supposed to be similar to the α -subunits of voltage-gated Na⁺ and Ca²⁺ channels. Because the cDNAs encoding Kv channel subunits are much easier to deal with by molecular biological techniques than those of Na⁺ or Ca²⁺ channel α -subunits, the fundamental properties of voltage-dependent ion channels such as ion selectivity, channel pore and voltage-dependent gate have been examined extensively in Kv channels.

A functional Kv channel can be a tetramer composed of either homo- or heteromeric Kv subunits [48]. The heteromultimeric Kv channel proteins can be formed only by the subunits belonging to the same Kv subfamily, and those from distinct subfamilies fail to assemble with each other. This specific association of Kv subunits is mediated by subfamily-specific interactions between their cytoplasmic N-terminal domains [49, 50]. The ability of different Kv subunits to associate as heteromultimeric complex channel pro-

Table 1. Diversity of native inwardly rectifying K⁺ channels.

Native inwardly rectifying K ⁺ channels	
I _{K1} channel	Classical inwardly rectifying K ⁺ channel
K _G channel	G protein-activated K ⁺ channel
K _{ACh} channel	Receptor-operated muscarinic K ⁺ channel
K _{ATP} channel	ATP-sensitive K ⁺ channel
K _{Na} channel	Na ⁺ -activated K ⁺ channel

Table 2. Nomenclature of inwardly rectifying K⁺ channel subunits.

Subfamilies	Subtypes	Other names	Chandy and Gutman's nomenclature	Variants	References
Classical inwardly rectifying K ⁺ channels	IRK1		Kir2.1		[29, 98, 99]
	IRK2		Kir2.2	Kir2.2v	[36, 104]
	IRK3	BIR11	Kir2.3		[37]
G protein-activated K ⁺ channels	GIRK1	KGA	Kir3.1		[33, 34, 105]
	GIRK2	GIRK2A	Kir3.2	GIRK2B, GIRK2C/K _{ATP} -2	[38, 106–110]
	GIRK3		Kir3.3		[38]
	GIRK4	cK _{ATP} -1, CIR	Kir3.4		[39, 111]
ATP-sensitive K ⁺ channels	uK _{ATP} -1		Kir6.1		[35]
	BIR		Kir6.2		[42, 76]
ATP-dependent K ⁺ channels	ROMK1	K _{AB} -1	Kir1.1a	ROMK2-6/Kir1.1b-f	[28, 112–114, 226]
	K _{AB} -2	BIR10	Kir4.1		[40, 41]
Others	BIR9		Kir5.1		[40]
	Tandem of two pore motifs	TWIK-1	—		[62]

Fig. 2. Hypothetical structures of a main subunit of voltage-dependent Na^+ or Ca^{2+} channels (A), an α -subunit of Kv channels (B), and a main subunit of Kir channels (C). Internal repeats are labeled I, II, III, and IV. Putative transmembrane segments are numbered. P or H5, putative pore forming region; +, voltage-sensing segment.

teins may indicate that a large number of functionally similar but structurally distinct native voltage-dependent K^+ channels exist. The composition of most native K^+ channels *in vivo*, however, remains unknown.

On the other hand, the unit polypeptides which form Kir channels have a common molecular motif of two membrane-spanning domains (M1 and M2), one potential pore-forming region (H5), and cytoplasmic N- and C-terminal domains (Fig. 2C). The membrane topology of Kir subunits resembles that of the S5, H5, and S6 segments of the Kv subunits [30–32]. Yang *et al.* [51] examined the subunit stoichiometry of IRK1 , a member of the classical Kir subfamily, by linking together the coding sequence of multiple-channel subunits, and have shown that the IRK1 channel is com-

posed of four IRK1 subunits. This tetrameric structure has been also suggested for K_G channels composed of GIRK clones [52]. It has been shown that the channel diversity is enhanced by the ability of different Kir subunits to form homomeric or heteromeric channels [39, 53]. In the GIRK subfamily, it was shown that the K_G channel proteins are composed of the heteromeric subunits of GIRK1 and GIRK4 in atrial myocytes and GIRK1 and GIRK2 in some of the brain neurons. In the IRK subfamily, the classical Kir channel proteins are mainly composed of homomeric IRK subunits ([54]; Lily Jan, personal communication). Thus, it seems likely that, as in the case of Kv channels, the heteromeric assembly of Kir subunits also occurs mainly within each subfamily and the physiological

importance of heteromeric assembly may be different among subfamilies.

2. Selective filter and channel pore

In Kv channels, initial studies demonstrated that the H5 region is an important pore-forming structure for Kv channel proteins [55–57]. Recent mutagenesis experiments have proved that the pore is composed of not only the H5 region but also parts of S6 and S4-S5 linker [58, 59]. The H5 region is also present in other K⁺ channels including the Kir, *slo* and *eag* families. An alignment of the H5 regions reveals that these K⁺ channel subunits are highly homologous over a stretch of eight amino acids (TXXTXGYG), corresponding to the sequence TMTTVGYG in the Kv family (Table 3). The H5 region may play an essential role in all K⁺ channels to allow only K⁺ ions to pass through the channel pore (ion-selective filter) [60]. Especially, mammalian K⁺ channel subunits have the signature sequence of GYG with several exceptions, such as GFG in the *eag* family [61] and ATP-sensitive K⁺ channel subfamily [35, 42], and GLG in the second pore domain of TWIK-1, which possesses two pore-forming domains in its primary sequence and exhibits weak inwardly rectifying properties when heterologously expressed [62]. Although the motif of FFG was reported in RACK1 by Suzuki *et al.* [63], its validity has not been established (see III.5.2). Heginbotham *et al.* [60], who examined the effect of point mutations in the eight amino-acid residues of a Kv subunit, *Shaker*, on K⁺ selectivity, demonstrated that Thr at position 8 of the H5 region, Val at position 12,

and Gly at positions 13 and 15 crucially contribute to the ion selectivity filter, whereas other positions are not essential in determining K⁺ selectivity. The H5 region of Kir subunits is also supposed to be the K⁺-selective filter and a part of the pore because of its sequence similarity with the H5 region in Kv subunits.

Although the H5 region appears to be a major determinant of pore function, several other regions may also contribute to forming the aqueous channel pore. In Kv channels, mutations of S4-S5 linker [58, 64] or the S6 region [59, 65] were shown to affect the single-channel conductance and ion selectivity. In Kir channels, Taglialatela *et al.* [66] demonstrated that exchange of the H5 region between IRK1 and ROMK1, which differ markedly from each other in single-channel K⁺ conductance and sensitivity for blocking by intracellular Mg²⁺ (Mg²⁺_i), had no effect on Mg²⁺_i-mediated rectification and little or no effect on the channel conductance. By contrast, they demonstrated that exchange of the C-terminal region but not the N-terminal region transferred the Mg²⁺ blockade and single-channel conductance of IRK1 to ROMK1. Furthermore, it was shown that a single negatively charged residue (Glu²²⁴ of IRK1) in the C-terminal hydrophilic domain as well as that in the middle of the M2 region (Asp¹⁷² of IRK1) are important for the Mg²⁺ and polyamine blocks of outwardly flowing IRK1 currents [67] (see Fig. 6). From these results, the pore of the Kir channel is thought to be composed of not only the H5 region but also its C-terminal and M2 regions.

Table 3. Alignment of the deduced amino-acid sequences of part of the H5 region in various K⁺ channels.

	8	9	10	11	12	13	14	15
Kv channels	T(S)	M	T	T	V(L)	G	Y	G
Mouse <i>slo</i>	T	M	S	T	V	G	Y	G
Mouse <i>eag</i>	S	L	T	S	V	G	F	G
Mouse IRK1 (Kir2.1)	T	Q	T	T	I	G	Y	G
Mouse IRK2 (Kir2.2)	T	Q	T	T	I	G	Y	G
Mouse IRK3 (Kir2.3)	T	Q	T	T	I	G	Y	G
Mouse GIRK1 (Kir3.1)	T	E	A	T	I	G	Y	G
Mouse GIRK2 (Kir3.2)	T	E	T	T	I	G	Y	G
Mouse GIRK3 (Kir3.3)	T	E	T	T	I	G	Y	G
Rat GIRK4 (Kir3.4)	T	E	T	T	I	G	Y	G
Mouse uK _{ATP} -1 (Kir6.1)	V	Q	V	T	I	G	F	G
Mouse BIR (Kir6.2)	V	Q	V	T	I	G	F	G
Rat ROMK1 (Kir1.1)	T	Q	V	T	I	G	Y	G
Rat K _{AB} -2 (Kir4.1)	S	Q	T	T	I	G	Y	G
Rat BIR9 (Kir5.1)	T	Q	T	T	I	G	Y	G
Human TWIK-1(P1)	V	L	S	T	T	G	Y	G
Human TWIK-1(P2)	S	L	S	T	I	G	L	G

3. Voltage sensor and inactivation ball of Kv channels, neither of which Kir channels possess!

The S4 of Kv channels is highly conserved and has net positive charges arising from an Arg/Lys-X-X-Arg/Lys repeat, where the Xs are usually hydrophobic. This segment is also present in a number of other voltage-gated channels including Na⁺ and Ca²⁺ channels (Table 4). Because of its net positive charge and extreme conservation, S4 is thought to be important for sensing the potential difference across cell membrane. Actually, mutations in the S4 of Kv channels have been shown to affect voltage dependence as well as gating current [68, 69]. The fast inactivation of Kv channels is suggested to be achieved by plugging the channel pore with a cytoplasmic inactivation ball composed of a ball-and-chain structure (N-type inactivation; Fig. 3A). This ball-and-chain model was initially proposed for the Na⁺ channel [70, 71]. Rapidly inactivating (A-type) Kv channels, such as Kv1.4 and Kv4.2, have the inactivating ball domain in their N-

Table 4. Alignment of the deduced amino-acid sequences of S4 in voltage-gated channels.

Kv channels																					
<i>Shaker</i>	R	V	I	R	L	V	R	V	F	R	I	F	K	L	S	R	H	S	K	G	L
<i>Shab</i>	Q	V	F	R	I	M	R	I	L	R	V	L	K	L	A	R	H	S	T	G	L
<i>Shaw</i>	E	F	F	S	I	I	R	I	M	R	L	F	K	V	T	R	H	S	S	G	L
<i>Shal</i>	F	V	T	R	V	F	R	V	F	R	I	F	K	F	S	R	H	S	Q	G	L
Na channels	S	A	L	R	T	F	R	V	L	R	A	L	K	T	I	S	V	I	P	G	L
Ca channels	S	V	L	R	C	I	R	L	L	R	L	F	K	I	T	K	Y	W	T	S	L

Positively charged residues (Arg/R and Lys/K) are written in bold-faced letters.

Fig. 3. The inactivation in Kv channels. **A:** The β -subunit binding site is proposed to associate with a cytoplasmic α -subunit domain. The inactivating β -ball domain can occlude the inner mouse (S4-S5 linker) of the open channel. **B:** Alignment of the N-terminal inactivating domain of rat Kv β 1 with those of rat Kv1.4, an A-type Kv channel. Serines and cysteines in serine/cysteine motif are boxed. Both Kv β 1 and Kv1.4 possess arginines and lysines in the cluster of positively charged amino acids, which are shaded. **C:** Alignment of the amino-acid sequences of the S4-S5 linker in rat Kv1.1, a non-inactivating Kv channel, and rat Kv1.4. Asterisks denote a glutamic acid, a negatively charged amino acid.

termini [72, 73]. Non-inactivating Kv channels (delayed rectifiers) can acquire rapid inactivation similar to A-type Kv channels when they are coexpressed with a β -subunit, because the β -subunits of Kv channels are highly homologous to the structure of the inactivation ball in the N-terminus of A-type Kv channel α -subunits (Fig. 3B) [74]. Three subtypes of β -subunits for Kv channels have been cloned. They resemble the "ball" of the N-terminus of inactivating Kv channels, and thus are thought to interact with the negatively charged S4-S5 linker area of the non-inactivating sustained Kv channels [73] (Fig. 3C). Therefore, the inactivating kinetics may not be the fingerprint of some of the inactivating Kv channel *in vivo* as has been assumed. Because Kir channels, on the other hand, do not possess either the voltage-sensor or N-terminal inactivation ball domains, the mechanisms underlying their apparent activation and inactivation properties are different from those of Kv channels: that is, the gating of Kir channels does not consist of "gates" intrinsic to channel proteins but blocks of cur-

rent flow by intracellular substances, such as Mg^{2+} and polyamines, interacting with the receptor sites in the channel as discussed below (section II).

So far, proteins corresponding to the β -subunits of Kv channels have not been identified in Kir channels. But, some of the Kir channels, such as uK_{ATP}-1/Kir6.1 and BIR/Kir6.2, can associate with sulfonylurea receptors, a member of the ATP-binding cassette (ABC) superfamily (Fig. 4), and thus obtain the properties of so-called ATP-sensitive K⁺ channels [42, 75–77]. Not only Kir6.x, but ROMK1 also obtained glibenclamide-sensitivity when co-expressed with SUR1 [77]. Furthermore, it has been shown that the cystic fibrosis transmembrane conductance regulator (CFTR), another of the ABC proteins, can interact with the epithelial Na⁺ channel [78] as well as ROMK2/Kir1.1b [79]. Therefore, interaction of some of the Kir channels and ABC proteins might represent one feature of this channel family. The precise molecular basis responsible for this interaction has not yet been clarified.

Fig. 4. Topology of the K_{ATP} channels.

II. Mechanisms of Inward Rectification of Kir Channels: Intracellular Substance-Mediated Blockade of Outward Currents

The inwardly rectifying property of native Kir channels has been believed to arise from a combination of an intracellular Mg^{2+} -mediated blockade and an intrinsic activation gating process [11, 80–82]. The contribution of each component to inward rectification differs among the distinct types of Kir channels [66, 83–85]. The rectification provoked by intracellular Mg^{2+} (Mg^{2+}_i) is due to induction of the subconducting levels of outwardly flowing K^+ channel currents by this cation. It has also been reported that micromolar concentrations of intracellular Ca^{2+} ions induce subconducting levels of outward Kir channel current and may underlie physiological inward rectification [86]. On the other hand, intrinsic gating reduces the outward currents by decreasing the channel-open probability at potentials more positive than E_K [11, 80–82]. The gating process proceeds more slowly than the Mg^{2+}_i block. In the case of cardiac classical Kir (I_{K1}) channels, for instance, Mg^{2+}_i causes instantaneous inward rectification, which is followed by a time-dependent enhancement of rectification by the intrinsic gating mechanism [82]. Recently, it has been demonstrated that submicromolar polyamines including spermine and spermidine applied to the intracellular surface of excised patch membranes restore the gating of cloned classical Kir channels [87–90] and native cardiac K_G channels [91]. Because polyamines exist at submillimolar concentrations, the putative activation gate is supposed to be attributable to the slow time-dependent blocking and unblocking of the Kir channel current by intracellular polyamines. Upon de-

polarization, the polyamine causes a time-dependent decrease of the outward current of Kir channels, which was previously expressed as “deactivation.” On the other hand, upon hyperpolarization, the inward Kir current increases in a time-dependent manner because of the unblocking of the polyamine effect, which was referred as “activation” of the Kir channel. The kinetic analysis of Mg^{2+} and spermine blocks on the cloned IRK2 current in a mammalian cell line indicates that competition between Mg^{2+} and spermine can be functionally described with an assumption that these substances compete with each other at a single binding site [90] (Fig. 5). Recent studies with site-directed mutagenesis of cloned Kir subunits, however, revealed that Mg^{2+}_i and polyamines can interact with at least two negatively charged amino-acid residues (receptor sites 1 and 2) located in the M2 domain (R1) [67, 85, 88, 89, 91–94] and in the C-terminus (R2) [67] of these channel subunits (Fig. 6). In the case of very strong inward rectifiers (IRKs), these two sites are both negatively charged. Thus, Mg^{2+} and spermine can interact with both R1 and R2. The functional importance of these two binding sites under physiological conditions (e.g., whether both sites are responsible for rectification) have not yet been clarified. For intermediate inward rectifiers such as the GIRK subfamily and K_{AB-2} , only one of the two residues is negatively charged. For weak inward rectifiers such as ROMK1 and the K_{ATP} subfamily, the corresponding sites contain only neutral residues. In fact, mutation from a negatively charged amino-acid residue (Asp¹⁷²) to a neutral one (Asn) at R1 in IRK1 reduces the inward rectification by decreasing the apparent affinity for Mg^{2+}_i and the polyamines. Contrariwise, mutation from Asn to Asp at R1 in ROMK1

Fig. 5. Effects of Mg^{2+} and spermine alone and combined on the IRK2 channel in an excised patch. Command pulses to +40 mV for 200 ms and thereafter to 20 mV for 200 ms were applied from the holding potential of -40 mV. The concentrations of free Mg^{2+} and spermine were fixed at 20 μM and 10 nM, respectively. The internal solution contained 2 mM ATP to prevent channel rundown. The zero current level is indicated by the arrows. Data were obtained at a filter of 3 kHz and a sampling rate of 10 kHz. The leak and capacitive currents were subtracted. **A:** Perfusion with 20 μM free Mg^{2+} alone caused a fast block followed by a minimal slow block of the outward currents through IRK2 channels. Spermine alone blocked outward current in a time-dependent manner. However, adding Mg^{2+} to spermine

slowed the current decay, and subsequently increased the current at the end of the depolarizing pulse, compared to that under application of spermine alone. **B:** Upon hyperpolarizing pulses to -20 mV, an instantaneous inward current was followed by a slowly activating one (time constant, ~1 ms) under application of 20 μM Mg^{2+} alone. The former and the latter represent fast and slow unblocking processes, respectively. In contrast, under 10 nM spermine alone, the hyperpolarizing pulse activated an inward current (time constant, ~0.4 ms) without significant instantaneous components. Under application of their combination, the pulse elicited an instantaneous inward current followed by an activating one which was fitted by the sum of two exponentials (time constants, ~0.4 and ~1 ms, respectively).

Fig. 6. Mg^{2+} and polyamine binding sites. **A:** Mg^{2+} and polyamine-binding amino-acid residues are located in the M2 domain (R1) and in the C-terminus (R2). **B:** Amino-acid residues in the R1 and R2 regions of the Kir channels. N, asparagine; G, glycine; D, aspartic acid; E, glutamic acid; S, serine.

increases inward rectification [89, 92, 93]. These results may indicate that the negatively charged amino acids at these two positions are important for inward rectification.

It may be noteworthy that the "relaxation" of K_G channel currents may not be attributable only to spermine- and Mg^{2+} blocking and unblocking of the current. In the whole cell configuration, an abrupt depolarization of the membrane from potentials negative to E_K to those positive to E_K is followed by the time-dependent decay of an outward K_G channel current. On the other hand, an instantaneous jump of the membrane potential in the opposite direction, from positive to negative potential, results in a very slow time-dependent increase in an inward K_G current [95]. These phenomena occur because the open probability (P_o) of K_G channels relatively slowly relaxes to a new steady-state level following an abrupt change in membrane potential. This relaxation is, however, barely observed in cell-free conditions even in the presence of Mg^{2+} [95]. Spermine, added to the cytoplasmic side, induces a clear time-dependent decrease in ensemble average outward currents, but a time-dependent increase in inward currents is not evident [91]. Actually, the mutation of Phe¹³⁷, a residue in the H5 region of GIRK1, to Ser caused the expression of K^+ currents with nearly time-independent activation, while the mutation of a residue related to Mg^{2+} and polyamine-binding (Asp¹⁷³ to Gln) failed to eliminate the time-dependent activation [96]. These results suggest that the relaxation of the inward currents of K_G channels as well as GIRK1 currents may be due to an intrinsic feature of channel proteins. However, the molecular mechanism has not been elucidated.

III. Phylogenetic Classification of Kir Channels

After ROMK1 and IRK1 were cloned in 1993 [28, 29], a number of members of the Kir subunits (1) IRK

subfamily (Kir2.x) [29, 36, 37, 97–104]; 2) GIRK subfamily (Kir3.x) [33, 34, 38, 39, 105–111]; 3) K_{AB} subfamily: ROMK1–6 (Kir1.1a–f) and K_{AB} -2/BIR10 (Kir4.1) [28, 40, 41, 112–114]; and 4) K_{ATP} subfamily (Kir6.x) [35, 42, 76] have been cloned from various tissues and species. The names in parentheses are those proposed by Chandy and Gutman [115], who have suggested a more uniform terminology, as in Kv channel subunits, to facilitate the comparison of clones identified in different laboratories and/or in different species. The evolutionary tree of the Kir family with two membrane-spanning domains is depicted in Fig. 7. These cloned Kir subunit cDNAs encode proteins between 327 and 501 amino acids in length. The predicted proteins share approximately 30–40% among the Kir subfamilies and >60% within the subfamilies. The highest levels of sequence identity are found in the H5 region and in the proximal C-terminal region (Fig. 8). The level of sequence identity among the subfamilies within the H5 region is 50–60%.

BIR9, which is referred to as Kir5.1, does not produce functional Kir channels when expressed by itself in *Xenopus* oocyte, and may be able to specifically associate with K_{AB} -2 (Kir4.1) to form heteromultimeric Kir channels [53]. However, BIR9 does not have a Walker type-A ATP-binding domain in its C-terminus and has only 36% sequence identity to both ROMK1 and K_{AB} -2, and <42% identity to other Kir channel subunits. Thus, BIR9 may belong to another subfamily of Kir channels. However, neither the mechanism of specific association of Kir5.1 with Kir4.1 nor its physiological functional role have been clarified yet.

1. Classical inwardly rectifying K^+ channel; IRK/Kir2.x subfamily

1) Physiological roles of classical Kir channels.

Kir channels were first discovered in K^+ depolarized muscle by Katz [1], who used the term "anomalous rectification" to contrast their prop-

Fig. 7. The evolutionary tree of the inwardly rectifying K^+ channel family with two membrane-spanning domains.

mGIRK1 (Kir3.1)	MSALR-RKFG DDYQVVTSS SGSGLPQGP GQGPQQQLVP KKHRLRFVVK	49
mIRK1 (Kir2.1)	MSSVTRNRYIS IVSSEEDGMK LATMAVANGF GNGKSKVHTR QQQRSPFVVK	50
mBIR (Kir6.2)	M--L--SRKG IIPSEYVLTIR L-----AEDP AEPR---YRT RERRRPFVSK	38
mKas-2 (Kir4.1)	M-----T SVAKVYYSQT -----TQTE SRPL---VAP GIRRRRLTK	33
mGIRK1 (Kir3.1)	NRCNVQHGN LGSETSRYSLS DLFTTLVDLK MGWNLFTFLL TYTVAMLFMA	99
mIRK1 (Kir2.1)	LGHCNVQFIN VGEKGQRYLA DIFTTCVDIR MRWMLVTFCL AFVLSMLFFG	100
mBIR (Kir6.2)	KNCNVAKHN IREQGRFL-Q DVFTTLVDLK MPTLLLTFTM SFLCSMLLFA	87
mKas-2 (Kir4.1)	LGKSNVMEH IADKRFLLYLK DLWTTFTDMQ WRYKLLIFSA TFAGTWLFLG	83
mGIRK1 (Kir3.1)	SMWVIAATIR GDL-NKAHVQ NYTFCVANVY NFPSAFLFFI ETEATIGMAY	148
mIRK1 (Kir2.1)	CVFWLLIALH GDL-DTSKVS K--ACVSEVN SFTAFLFSI ETQTITIGMCF	147
mBIR (Kir6.2)	MVWVLLIAFAH GDL-APGEGT N-VFCVTSIH SFSSAFLFSI EVOVTIGMFS	135
mKas-2 (Kir4.1)	VWVYLVAAAH GDLLELGPPA NHTFCVQVH TLTCAFLFSL ESQTITIGMCF	133
mGIRK1 (Kir3.1)	RYITDKFEG IILFLFOSIL GSIVDAFLIG CMFIRMSQAK KGAETLAFSE	198
mIRK1 (Kir2.1)	RCVTDECPJA VFMVVFOSIV GCIIDAFIIG AVMAKMAKPK KRNETLVFSA	197
mBIR (Kir6.2)	RMVTDECPJA ILILIVQIV GLMINAIMLG CIFMATAQAH RRAETLIFSK	185
mKas-2 (Kir4.1)	RVISECPJA IVLLIAQVL TITLIEFITG TFLAKIARPK KRAETIRFSQ	183
mGIRK1 (Kir3.1)	HAVISMRIKX LTLNRRVGNL RNSHMVSAQI RCKLLKSRQT HEGEPLHLLQ	248
mIRK1 (Kir2.1)	NAVITAMRIKX LCIMRRVGNL RKSHLVEAHV RAQLLKSRTI SEGENIIFLQ	247
mBIR (Kir6.2)	HAVITLRROR LCFMIRVGD LKSMIISATI HMQVVRKTTT HEGEVVLLIQ	235
mKas-2 (Kir4.1)	HAVVASHNCK FCLMRRVANM RNSLLIGCOV TGKLLQTHQT HEGENIRLQ	233
mGIRK1 (Kir3.1)	LELDVGFTSG A--DQLFLVS PLTICFVMDA KSPFYDLRQR SM-QTEQHEV	295
mIRK1 (Kir2.1)	IDINVGDGSG I--DRIFLVS PTTIVHEIDE DSPLYDLRQR DI-DNADREI	294
mBIR (Kir6.2)	VDIPM--ENG VGGNGIFLVA PLIYHVIDS NSPLYDLAPS DLHHHQDLRI	283
mKas-2 (Kir4.1)	VNVTFQVDTA S--DSHFLIL PLTFYHVIDE TSPKDLPLR S--GEGDFEL	279
mGIRK1 (Kir3.1)	VVILEGIVET TSMTTCARRIS YTEDELWGH RFFPVISL-E EGFFKVMDSQ	344
mIRK1 (Kir2.1)	VVILEGIVEA TAMTTCARRIS YLANEELWGH RYEPVLF-E KHYYKVMDSR	343
mBIR (Kir6.2)	VVILEGIVET TSMTTCARRIS YLANEELWGH RFVETVAE-E DGRYSVDMSK	332
mKas-2 (Kir4.1)	VVILEGIVES TSMTTCARRIS YLPEELWGH EFTPAISLSA SGKYIADPSL	329
mGIRK1 (Kir3.1)	FHATFVPTP PYSVKEQSEM LLMSSPLIAP AITNSKERHN SVECLDGLDD	394
mIRK1 (Kir2.1)	FHKTYEVPTP PLC-SARD-- -LAEEKYI-- -LSNA----N SF-CYE--NE	379
mBIR (Kir6.2)	FNTIRVPTP -LC-TARQ-- -LDEDRSL- -L-----DA	358
mKas-2 (Kir4.1)	FDOVVVYASP S---GLRD-- -STVRYG-- -----DP	352
mGIRK1 (Kir3.1)	ISTKLPSKLQ KITGREDFPK KLLRMSSTTS EKAYSLGDLPL MKLQRISSVP	444
mIRK1 (Kir2.1)	VA----- -LTSKEE-- -----EEDS E-----NGVP	396
mBIR (Kir6.2)	LT----- -LAS----- -L-----	363
mKas-2 (Kir4.1)	EK----- -LK----- -L-----	356
mGIRK1 (Kir3.1)	GNSEELVSK TTKMLSDPMS QSVADLPPLK DKMAGGPTRM EGNLPAKLRK	494
mIRK1 (Kir2.1)	ESTS----- -DSPPGI--LL HNQASVP--L E---PRPLRR	424
mBIR (Kir6.2)	-----S RGP-----L KRKSVAV--A K---APKFSI	383
mKas-2 (Kir4.1)	-----L EES-----L REQAEKE-- ---GSALSV	374
mGIRK1 (Kir3.1)	MNSDRFT	501
mIRK1 (Kir2.1)	-ESE-I	429
mBIR (Kir6.2)	-SPDSL	389
mKas-2 (Kir4.1)	RISNV	379

Fig. 8. Sequence comparison of representative subunits of the different Kir channel sub-families. Positions at which all four amino-acid sequences are identical are boxed. Putative transmembrane segments and pore-forming region are underlined and double underlined, respectively.

erties from those with "normal" delayed rectification. The Kir channels have been best characterized in frog skeletal muscle and in starfish and tunicate eggs in work reviewed by Adrian [116], Hille and Schwarz [117], and Hagiwara and Jaffe [118]. The properties of these channels are K^+ selective, blocked by extracellular Cs^{2+} and Ba^{2+} , and modulated by external K^+ and internal Mg^{2+} . These K^+ channels, which are distinct from other Kir channels such as K_G , K_{ATP} , and K_{Na} channels in their metabolic regulation and degree of rectification, have been termed "classical" Kir channels. The classical Kir channels are not activated by transmitters or voltage but seem to be constitutively active, and their inwardly rectifying property is now believed to arise from a combination of Mg^{2+} -mediated blockade and polyamine (such as spermine

and spermidine)-mediated blockade (see above section II), which contribute to the gating of strong inward rectifiers [88–91].

The Kir conductance in cardiac myocytes, including Purkinje fibers as well as ventricular and atrial tissues [8–11] but not in nodal cells [119], is the dominant component of the resting conductance of these tissues, which was defined as a time-independent background K^+ current, I_{K1} . The single-channel conductance and mean open time at ~ -80 mV of I_{K1} in the ventricular myocytes are 40 pS and 100 ms, respectively [11]. The contributions of I_{K1} to the action potential are illustrated by the current-voltage relationships in Fig. 9. The large inward conductance at membrane potentials below E_K and the relatively large outward conductance at those just above E_K help to

keep the resting membrane potential close to E_K . The lack of outward conductance at plateau-level potentials prevents massive K^+ efflux during the action potential plateau, resulting in the maintenance of depolarization. When repolarization is initiated by activation of the delayed rectifier K_v channel at plateau potential, relatively large outward currents pass through the negative slope region of the I_{K1} channel, resulting in the acceleration of repolarization. Thus, I_{K1} plays an important role in determining the shape of the cardiac action potential: 1) setting the resting potential, 2) maintaining the plateau phase, and 3) causing rapid repolarization.

In the central nervous system, Kir currents are present in neuronal cells including hippocampal neurons [12] and neonatal rat spinal motor neurons [17], and are thought to participate in electrical signaling and information processing.

Fig. 9. Current-voltage relationship and action potential in the ventricular cell. The current-voltage relationship in the voltage clamp experiment for the instantaneous current is indicated by the solid line and that for the steady-state current at 1 s by the broken line. The action potential is drawn sideways to correlate it to the above current-voltage relationship. Shaded area shows I_{K1} currents.

2) Molecular aspects of the classical Kir channel. The features of the IRK subfamily (IRK1–3) closely resemble those of classical Kir channels. In our laboratory, three kinds of IRK subfamily have been cloned from mouse brain [36, 37, 97]. The amino-acid sequence of mouse brain (MB)-IRK1 shares 70 and 61% identity with MB-IRK2 and MB-IRK3, respectively. These three IRKs encode proteins of 428, 427, and 445 amino acids, respectively. The amino-acid sequence is well conserved at M1, M2, and H5. Especially at H5, they show differences from each other at only one amino-acid residue.

The *Xenopus* oocytes injected with cRNAs derived from IRKs expressed strong inwardly rectifying K^+ currents. Hyperpolarizing voltage steps elicit rapidly activated large inward currents which show slowly developed weak voltage-dependent inactivation at hyperpolarizing voltages. The inactivation of IRK2 current is more prominent than that of IRK1 or IRK3. These expressed currents are blocked by external Ba^{2+} and Cs^{2+} in a concentration- and voltage-dependent manner. The single-channel conductances of IRK1, IRK2, and IRK3 are ~ 22 , ~ 34 , and ~ 13 pS with 150 mM $[K^+]_o$, respectively (Fig. 10). The steady-state P_o of the IRK2 channel decreases with hyperpolarization, while those of IRK1 and IRK3 remain constant. The detailed gating kinetic analysis strongly indicates that the increase of long closed gaps (> 200 ms) between clusters of the channel burst openings is mainly responsible for the prominent reduction of steady-state P_o at hyperpolarized potentials in the case of IRK2. The dominant cardiac I_{K1} is reported to have a single-channel conductance of ~ 40 pS with 150 mM $[K^+]_o$ and exhibit voltage-dependent inactivation in the absence of blocking cations [6, 11]. These properties are similar to those of IRK2 [36]. Because IRK subunits seem to assemble as homotetramers, at present, the I_{K1} channel protein is thought to be composed of homotetrameric IRK2 subunits.

3) Localization of IRK channels. We have shown the differential distribution of the mRNAs for three kinds of IRK subfamily in mouse tissues using Northern blot analysis: 1) IRK1 mRNA was detected strongly in the forebrain, heart, and skeletal muscle, less in the cerebellum and not in kidney; 2) IRK2 mRNA was detected strongly in the cerebellum and less in other above described organs; and 3) IRK3 mRNA was detected predominantly in the forebrain.

The distribution of three IRK mRNAs in mouse brain has been further studied using in situ hybridization histochemistry [120] (Fig. 11). All of the IRK mRNAs were detected in neurons but not in glial cells. Their distribution patterns in the brain were,

Fig. 10. Single-channel recordings from cell-attached membrane patches of *Xenopus* oocytes expressing IRK channels. **A:** Membrane current traces recorded at the membrane potential values are indicated to the left of each trace. Arrows to the right of certain traces indicate the patch current level recorded when all channels were closed. Each

of these patches appeared to contain one channel. **B:** Current-voltage relationships of the channel records shown in A.

however, quite divergent. IRK2 mRNA was detected extremely high in granule cells of the cerebellum, relatively high in motor trigeminal nucleus, and moderately in the olfactory bulb, piriform cortex, cerebral cortex, CA1 through CA3 regions of the hippocampus, dentate gyrus, and pontine nucleus. On the other hand, IRK1 mRNA was expressed throughout the whole brain but in particular subsets of neurons, and IRK3 mRNA was expressed in the forebrain. Likewise, in rat brain, these differential distributions of the IRK subfamily have been observed [121]. These results suggest that each IRK channel has a specific function in the regulation of the excitability of brain neurons, although their basic electrical properties are quite similar.

4) Modulation of IRK channels. Collins *et al.* [122] reported that IRK3 is inhibited by the physiological concentration of ATP (K_d of 1.47 mM). This effect is antagonized by ADP, again in the physiological range, implying that this channel is sensitive to the intracellular [ATP]/[ADP] ratio. IRK3 is, however, different from previously known K_{ATP} channels, because K_{ATP} channels have weaker rectification and much higher sensitivity to ATP than IRK3. The inhibition of IRK3 currents by ATP does not require Mg^{2+} and is mimicked by nonhydrolyzable ATP

Fig. 11. Distributions of mRNAs for IRK1, IRK2, IRK3, and G-IRK1 (G-IRK) in mouse brain examined by in situ hybridization. In the color key, red corresponds to relatively high ratios, whereas violet corresponds to relatively low ratios. IRK1 mRNA was detected both in the forebrain and cerebellum, and IRK2 mRNA was detected strongly in the cerebellum and less in the forebrain, while IRK3 mRNA was detected predominantly in the forebrain. G-IRK1 mRNA was detected both in the forebrain and cerebellum.

analogs, indicating that hydrolysis of ATP is not a requisite step. These effects, not observed in IRK1, may be specific to IRK3. On the other hand, Fakler *et al.* [123] showed that IRK1-mediated currents, after run-down upon excision of the patch, can be partly restored by application of 1 mM MgATP with 1 mM free Mg^{2+} but not by the ATP analogs, suggesting that IRK1 is regulated by ATP hydrolysis.

Stanfield *et al.* [124] found that substance P causes slow excitation by suppressing a Kir channel in neuronal cells, although its subunit clone is not identified at the molecular level. This inhibitory effect is supposed to be mediated by protein kinase C [125]. Roy and Southeimer [126] reported that a glial Kir channel, although it may not be a member of the IRK subfamily, is inhibited by intracellular cAMP under β -adrenergic stimulation. These findings suggest that IRK channels may be regulated not only by cations or polyamines but also several intracellular modulators, which might play important functional roles in the control of neuronal excitability.

2. G protein-gated muscarinic K^+ channel; GIRK/Kir3.x subfamily

1) Functional roles of K_G channels. The K_G channels are functionally expressed in the heart [22, 127–130] and various neurons of the central nervous system [26], with the best characterized example being the acetylcholine-activated Kir channel (K_{ACh} channel) expressed in cardiac myocytes. The K_G channels are responsible for the acetylcholine-induced deceleration of heart beat, and may regulate firing rates,

membrane potential, and neurotransmitter responses in the brain. The activation of these Kir channels decreases membrane excitability by hyperpolarizing the membrane potential and slowing membrane depolarization. In the heart, the K_{ACh} channel exhibits strong inward rectification but weaker than the I_{K1} channel. Its single-channel conductance is ~ 50 pS with a high concentration of $[K^+]_o$ [6, 11, 131, 132]. The K_{ACh} channel is directly activated by pertussis toxin-sensitive G proteins.

2) G protein regulation of K_{ACh} channels.

G proteins are membrane-bound proteins which transduce signals from receptors to various effectors such as adenylyl cyclase, phospholipase C and ion channels. They are heterotrimers composed of G_α , G_β , and G_γ [133]. In the absence of an agonist, GDP is bound to G_α . Upon receptor occupation by an agonist, the GDP on G_α is released and GTP binds in exchange. This results in the dissociation of the GTP-bound form of G_α ($G_{\alpha-GTP}$) from $G_{\beta\gamma}$. The $G_{\beta\gamma}$ subunit is a dimer under normal conditions. Theoretically, either $G_{\alpha-GTP}$ or $G_{\beta\gamma}$ may regulate effectors (Fig. 12). G_α has intrinsic GTPase activity and, when GTP on G_α is hydrolyzed to GDP, $G_{\alpha-GDP}$ and $G_{\beta\gamma}$ reassociate in the heterotrimeric form, resulting in the cessation of effector regulation. While G_α is mainly responsible for specific interactions between receptors and G protein, various effectors are regulated by not only G_α but also $G_{\beta\gamma}$ [134, 135]. The activation of the K_{ACh} channel is mediated by a pertussis toxin-sensitive G protein, designated G_K , via a direct membrane-delimited pathway without involving intracellular second messenger sys-

Fig. 12. G protein cycle. R, membrane receptor; α , β , and γ , subunits of the G protein. See text for details.

tems [131, 132, 136, 137]. After the initial controversy over whether G_α subunits [138, 139] or $G_{\beta\gamma}$ [140, 141] heterodimers mediate the membrane-delimited signaling, it now appears clear that $G_{\beta\gamma}$ is responsible for a physiological role in the activation of the cardiac K_{ACh} channel as we have consistently proposed [132, 142, 143]. The K_{ACh} channel can be activated by acetylcholine acting on m2-muscarinic receptors and also by adenosine acting on A_1 -purinergic (adenosine) receptors via the membrane-delimited pathway within the cardiac atrial cell membrane [144]. Actually, adenosine as well as acetylcholine decelerate heart beat and atrioventricular conduction [145] by increasing the Kir current [146]. On the other hand, the K_G channels in the brain can be coupled to not only m2-muscarinic and A_1 -purinergic receptors but also to a variety of other receptors such as serotonergic, opioid, α_2 -adrenergic, D_2 -dopaminergic, somatostatin, and probably $GABA_B$ receptors [25–27] (Table 5). The functional roles of K_G channels in the neuronal network throughout the brain are still not yet fully understood.

3) Molecular aspects of the K_G channels. GIRK1/KGA (Kir3.1), which encodes the main subunit of the K_G channel, was first isolated from rat atrium [33, 34]. From a mouse brain cDNA library, two additional homologs of GIRK1 were further obtained and designated GIRK2 (Kir3.2) and GIRK3 (Kir3.3) [38]. Furthermore, it has been shown that at least three different isoforms of mouse GIRK2 can be generated by alternatively splicing the transcripts of a single gene, and we designated them as GIRK2A, GIRK2B, and GIRK2C in order of identification [106]. GIRK2A and GIRK2C correspond to GIRK2 and GIRK2A designated by Lesage *et al.*, respectively

[147]. These alternatively spliced transcripts share an N-terminal end and a central core, and differ at their C-terminal end. GIRK2B was isolated from a mouse brain cDNA library, and was shown to be ubiquitously distributed among various tissues [106]. Its amino-acid sequence is shorter than that of GIRK2A by 87 amino acids, and the eight amino-acid residues in the C-terminal end of GIRK2B are different from those of GIRK2A. GIRK2C has a C-terminus 11 amino acids longer than that of GIRK2A. GIRK2C was isolated from a hamster insulinoma cDNA library by low-stringency hybridization with a probe coding for GIRK1. At first, it was termed K_{ATP} -2 since it was believed to be K_{ATP} channel subunit [107, 109] because of its sequence similarity to the cK_{ATP} -1 reported by Ashford *et al.* [111]. However, it has been reported that CIR/GIRK4, which is virtually identical to rat cK_{ATP} -1, reconstitutes cardiac $I_{K_{ACh}}$ with GIRK1 and does not contribute to the K_{ATP} channels [39, 148]. Thus, it now appears to be clear that both cK_{ATP} -1 and K_{ATP} -2 are not members of the K_{ATP} subfamily but belong to the GIRK subfamily.

When cRNAs for GIRK1 and the m2-muscarinic receptor are coinjected into *Xenopus* oocytes, Kir currents induced by acetylcholine are observed [33, 34]. The GIRK1 subunit has been used successfully to investigate the molecular mechanism underlying G protein activation of K_G channels. Mutagenesis and immunological studies have shown that $G_{\beta\gamma}$ activates the GIRK1 channel by binding directly to the C-terminal domain of GIRK1, where a region similar to the putative $G_{\beta\gamma}$ -binding domain in β -adrenergic receptor kinase 1 exists [149–152], although the similarity is relatively low [143]. Jan and coworkers [149, 150] further demonstrated that the N-terminal domain of GIRK1 can bind $G_{\beta\gamma}$ and trimeric $G_{\alpha\beta\gamma}$. The trimeric $G_{\alpha\beta\gamma}$ binding to the clone may facilitate the fast rates of activation and deactivation following m2-muscarinic receptor stimulation. It was shown that both the GIRK2A–C and GIRK4 expressed in *Xenopus* oocytes also produce G protein-gated Kir currents [38, 39, 106, 147, 148], suggesting that each GIRK subunit may compose the homomeric K_G channel. However, a recent work [39] proposes that the atrial K_G channel is a heteromultimer of GIRK1 and GIRK4/CIR rather than a homomultimer of the GIRK1 subunits. When GIRK4 is coexpressed with GIRK1 in *Xenopus* oocytes or CHO cells, G protein-gated Kir current is prominently enhanced (Fig. 13). Moreover, the coinjection of GIRK1 plus GIRK2A–C, GIRK1 plus GIRK3, and GIRK2A plus GIRK4 into *Xenopus* oocytes also results in prominent enhancement of current expression [147, 153–155], although

Table 5. Neurotransmitter receptor which activates a K^+ channel via G protein.

Agonist	Receptor type	Tissues
Acetylcholine	m_2	Cardiac nodal and atrial cells Some brain neurons
Adenosine	A_1	Mouse striatum Cardiac nodal and atrial cells
Dopamine	D_2	Rat substantia nigra
GABA	$GABA_B$	Rat hippocampus
5-HT	5-HT $_{1A}$	Rat hippocampus
Noradrenaline	α_{2A}	Rat substantia nigra Rat locus coeruleus
Opioid	μ	Mouse DRG
	δ	Mouse DRG
Somatostatin	?	Pituitary GH $_3$ cells

Fig. 13. Currents recorded from representative *Xenopus* oocytes expressing either mouse (m)-GIRK1 or human (h)-GIRK4 cRNA or both by the two-electrode voltage-clamp method. The m2-muscarinic receptor (m2R) cRNA was always injected in *Xenopus* oocytes. Currents were measured during perfusion with the control bath solution and with a solution containing 10 μ M acetylcholine (ACh). The subtracted ACh-induced current with both m-GIRK1 and h-GIRK4 was significantly greater than that of m-GIRK1 or h-GIRK4 alone.

the coexpression of GIRK3 with GIRK2A suppresses the sole GIRK2A response [106, 110, 147, 153–156]. A differential distribution of the mRNAs for GIRK subunits has been shown. For Northern blot analysis, GIRK1 is mainly expressed in the heart and brain, GIRK2A and GIRK3 in the brain, GIRK2C in the brain and pancreas, and GIRK4 in the heart [33, 34, 39, 107, 111]. *In situ* hybridization (Fig. 11) and reverse transcription-polymerase chain reaction (RT-PCR) analysis have also shown diversity in their distribution patterns, especially within the brain [105, 106, 121, 157–159]. Thus, heteromultimerization among GIRK subunits considerably increases the diversity of native K_G channels in various tissues.

3. ATP-sensitive K^+ channel; K_{ATP} /Kir6.x subfamily

1) Physiological roles of K_{ATP} channels. A defining attribute of K_{ATP} channels is that micromolar concentrations of ATP_i close the channel. The K_{ATP} channel, which was first discovered in heart muscle [23], has also been found in a variety of tissues including pancreatic β -cells, skeletal muscle, smooth muscle, renal tubule cells, and the central nervous system [160–165]. These K_{ATP} channels have been associated with diverse cellular functions such as the shortening of action potential duration and cellular loss of K^+ ions that occur during metabolic inhibition

in the heart, insulin secretion from pancreatic β -cells, smooth muscle relaxation, regulation of skeletal muscle excitability, and neurotransmitter release [166–173].

2) Electrophysiological properties of K_{ATP} channels. The K_{ATP} channels can be distinguished from other K^+ channels based on their biophysical properties. The degree of inward rectification of K_{ATP} channels is much weaker as compared to that of I_{K1} or K_{ACh} channels. The weak inward rectification of K_{ATP} channels is also mediated by Mg^{2+} [174–176]. The K_{ATP} channels are essentially time-independent, and their kinetics usually consist of bursts of channel openings separated by long closed periods [160, 172, 177]. With symmetrical K^+ ions of 140 mM on each side of the membrane, the conductance of the unitary inward current through the K_{ATP} channels in cardiac myocytes is 70–90 pS [23, 83, 160, 174], which is larger than those of I_{K1} and K_{ACh} channels. There has been a considerable variation in the single-channel conductances reported for K_{ATP} channels in various tissues; for example, 40–60 pS in skeletal muscle and 50–75 pS in pancreatic β -cells [165, 172, 174, 175]. Moreover, there appears to be substantial diversity in the single-channel conductance of the K_{ATP} channels among smooth muscle, with the values basically falling into two groups [178]: small to intermediate conductance (15–50 pS in symmetrically high K^+) K_{ATP} channels in the cell membranes of smooth muscle cells from the portal vein [179, 180], cultured coronary artery cells [181], and urinary bladder [182]; and large conductance ($\sim$ 130 pS) K_{ATP} channels from mesenteric arteries [162], rat tail artery [183], and canine aorta [184].

On the other hand, K_{ATP} channels in cortical and hypothalamic neurons and in epithelial cells (nasal polyps, *Amphiuma* distal tubule) did not show inward rectification when 1 mM Mg^{2+} was present in the internal solution [185]. In addition, the K_{ATP} channels of these types were much less sensitive to ATP than the typical K_{ATP} channels, such as cardiac, smooth muscle, or pancreatic K_{ATP} channels, requiring millimolar concentrations to produce significant inhibition. Thus, the K_{ATP} channels in neurons and epithelial cells, although their properties are not yet fully understood, seem to be distinct from the cardiac, smooth muscle, and pancreatic K_{ATP} channels.

3) Regulation of K_{ATP} channels by intracellular nucleotides. K_{ATP} channels are known to be regulated by various intracellular factors such as ATP_i (Fig. 14) and nucleotide diphosphates (NDPs). ATP_i is the main regulator of classical K_{ATP} channels and has two functions: to close the channels, and to maintain

channel activity in the presence of Mg^{2+} [177, 186–188]. The first action of ATP_i is referred to as the “ligand action,” because it is assumed to require the binding of ATP_i to the K_{ATP} channel and persists as long as ATP_i is bound to the channel. Typically, K_{ATP} channels have a very low probability of being open at physiological concentrations of ATP. Half-maximum inhibition of the K_{ATP} channel in cardiac muscle cells is achieved by 20–30 μM of ATP, which is independent of whether ATP is in the free acid form (ATP^{4-}) or is bound to Mg^{2+} ($MgATP$) [160, 174, 189, 190]. In pancreatic β -cells, it has been shown that Mg^{2+} increased half-maximum inhibition of the channel from 4 to 26 μM , suggesting that ATP^{4-} is a more potent inhibitor of the pancreatic K_{ATP} channel than $MgATP$ [191]. Although information about the ATP_i inhibition of K_{ATP} channels in smooth muscle is limited, $MgATP$ is less effective than ATP^{4-} [178, 180]. Half-maximum inhibition of the K_{ATP} channel in the rat portal vein is achieved by 29 μM of ATP^{4-} , whereas $MgATP$ is ineffective.

The second action of ATP_i is referred to as the “hydrolysis-dependent” because it apparently requires the hydrolysis of ATP in the presence of Mg^{2+} , and can last for several tens of minutes after the removal of ATP_i . The effect of ATP_i on K_{ATP} channels depends on the state of the channel protein. When the channels are operative, ATP_i inhibits channel opening. When the channels are not operative, treatment with $MgATP$ restores channel opening.

NDPs are also major regulators of K_{ATP} channel activity. NDPs have two distinct actions: 1) attenuating ATP-induced channel inhibition by competing with

the binding of ATP_i to the K_{ATP} channels, and 2) permitting K_{ATP} channel opening even after rundown [168, 175, 179, 192, 193]. The regulation of channel activity by nucleotides is modulated by factors exogenous to the channel protein such as hormones including galanin and somatostatin, which are known to inhibit insulin secretion, via the activation of G proteins [194, 195]. While the K_{ACh} channels are activated by $G_{\beta\gamma}$, the K_{ATP} channels are activated by a GTP-bound form of $G_{i\alpha}$, at least in the heart [132, 196].

4) Pharmacological regulation of K_{ATP} channels. K_{ATP} channels are the targets of two important classes of drugs: the antidiabetic sulfonylureas which block the channels, and a series of compounds called K^+ channel openers which tend to maintain the channels in an open conformation.

Sulfonylureas including glybenclamide and tolbutamide are hypoglycemic agents that stimulate insulin secretion by blocking the K_{ATP} channel with consequent depolarization and Ca^{2+} influx [168] (Fig. 15). These drugs can also block the K_{ATP} channels in cardiac and smooth muscle cells, though they require higher concentrations than in β -cells [162, 167, 168, 178, 179, 182, 197–199]. K_{ATP} channels are also blocked by less-specific organic K^+ channel blockers such as tetraethylammonium ions and some antiarrhythmic drugs [200, 201].

K^+ channel openers include cromakalim, pinacidil, levcromakalim, nicorandil, diazoxide, and minoxidil sulfate. These agents possess high therapeutic potential in treating various clinical conditions including hypertension, acute and chronic myocardial ischemia, and congestive heart failure, as well as in managing

Fig. 14. Inhibition of K_{ATP} channels by ATP_i . Under the cell-attached configuration of patch-clamp technique (holding potential -75 mV), no activity of the K_{ATP} channel was recorded due to millimolar levels of ATP inside the cardiac cell. After patch excision in an ATP-free solution, several K_{ATP} channels opened immediately. The application of micromolar concentrations of ATP to the cytosolic side of an inside-out patch inhibited K_{ATP} channel opening. Note that the K_{ir} channel current was recorded even in the presence of ATP.

bronchial asthma, urinary incontinence, and certain skeletal muscle myopathies [202–204]. These effects are ascribed to an increase in the open probability of K_{ATP} channels [166, 205, 206].

In the heart, K^+ channel openers enhance K_{ATP} channel activity through different mechanisms and can be classified into three groups on the basis of examinations under various experimental conditions: condition 1, during spontaneous channel activity; condition 2, after rundown of spontaneous channel activity and in the absence of a NDP; condition 3, after rundown of spontaneous channel activity and in the presence of a NDP; and condition 4, during spontaneous channel activity and in the presence of a NDP [174, 192] (Fig. 16). These experimental conditions may reflect the different states of the K_{ATP} channels. The most common profile, type 1 K^+ channel openers (i.e., pinacidil, a cyanoguanidine, levcromakalim and YM-934, two benzopyran derivatives, as well as aprikalim, a carbothioamide) enhance spontaneous channel activity in the absence and presence of ATP (condition 1). After the rundown of spontaneous channel activity and in the absence of a NDP, type 1 drugs cannot activate K_{ATP} channels (condition 2). After rundown of spontaneous activity, when NDP induces the K_{ATP} channel to open, type 1 drugs enhance channel activity in the absence and presence of ATP (condition 3). Thus, type 1 drugs enhance spontaneous or NDP-induced K_{ATP} channel activity by 1) increasing the apparent maximal channel activity and 2) decreasing the apparent sensitivity of the channel toward ATP_i inhibition. Type 2 K^+ channel openers (i.e., HOE-234, a benzopyran, and ER-001533, a phenylalkylamine) enhance spontaneous or nucleotide diphosphate-induced openings only in the presence of ATP-induced K_{ATP}

channel inhibition (conditions 1 and 3). These drugs cannot activate K_{ATP} channels after the rundown of channel activity in the absence of NDPs (condition 2). Thus, type 2 drugs appear to solely antagonize the ATP_i -induced inhibition of K_{ATP} channels. Type 3 K^+ channel openers (nicorandil, a pyridine derivate) en-

Fig. 16. Activation profiles of K^+ channel openers based on the functional model of K_{ATP} channels. **A:** Operative conditions of cardiac K_{ATP} channel. Original current recording from a membrane patch excised from a guinea pig ventricular cell. After formation of the inside-out configuration in an ATP-free solution, K_{ATP} channels appeared and disappeared (rundown). After the rundown of spontaneous channel activity, UDP (a nucleotide diphosphate (NDP)) restored channel openings. Hence, K_{ATP} channels exhibited two operative conditions: 1) spontaneous opening, and 2) NDP-evoked opening after the rundown of spontaneous activity. **B:** Functional model of the K_{ATP} channel. Gate, channel gate; T, transducer unit; A, ATP-binding inhibitory unit; P, presumed phosphorylation site; NDP, NDP-binding site. The K_{ATP} channel is operative when the transducer unit is either phosphorylated or when a NDP is bound to the NDP site. **C:** Functional classification of K^+ channel openers. (+) and (—), respectively, indicate the presence or absence of phosphorylation at the P site or binding of NDP to NDP site. K^+ channel openers were classified into three groups on the basis of examinations under four experimental conditions.

Fig. 15. Effects of tolbutamide on the membrane potential of insulin-secreting cells. The action of tolbutamide on the membrane potential of the mouse insulin-secreting cell line (MIN6) was investigated using the whole-cell current-clamp variation of the patch-clamp technique. The resting membrane potential was determined mainly by the activity of the K_{ATP} channel. The addition of 500 μM tolbutamide to the bath resulted in an increase in membrane potential followed by a burst of action potential, indicating that the agent inhibits the K_{ATP} channel. An expansion of the record is shown in the lower panel.

hance K_{ATP} channel activity in the presence of NDPs, regardless of the presence or absence of ATP (conditions 3 and 4). In the absence of NDPs, a type 3 drug does not significantly activate K_{ATP} channels (conditions 1 and 2). Thus, type 3 drugs apparently require the presence of NDPs to activate K_{ATP} channels or to antagonize ATP_i -induced channel closure. These results suggest that the mechanism of activation of K_{ATP} channel by K^+ channel openers is not uniform and may point to distinct sites of drug-channel activation.

Moreover, K_{ATP} channels in different tissues exhibit considerable variations in response to K^+ channel openers [160, 174, 178, 206, 207]. For example, the pancreatic β -cell K_{ATP} channel is activated by diazoxide and only weakly by pinacidil. The cardiac K_{ATP} channel is activated by pinacidil but not by diazoxide. The smooth muscle K_{ATP} channel is activated effectively by both of these agents. Thus, the properties of K_{ATP} channels vary among tissues, leading to the premise that these channels may be composed of heterogeneous channel proteins.

5) Molecular aspects of the K_{ATP} channel.

Recently, Seino, Bryan, and their colleagues demonstrated that pancreatic β -cell K_{ATP} channels are a complex composed of at least two subunits, a member of the Kir channel family (BIR/Kir6.2) and a sulfonylurea receptor (SUR) [42]. BIR was isolated by screening a human genomic library with uK_{ATP-1} /Kir6.1 as a probe under low-stringency hybridization conditions, and its mouse homolog was also isolated from an insulin-secreting cell line cDNA library [42] (Fig. 4). The predicted amino-acid sequences show that uK_{ATP-1} has roughly 70% identity with BIR and 40–50% identity with other cloned Kir channels [42, 208]. The highly conserved motif in the H5 region (GYG) found in all of the other cloned Kir channels with two transmembrane regions was GFG in both uK_{ATP-1} and BIR. These findings suggest that uK_{ATP-1} is in the same subfamily as BIR. Although Ämmälä *et al.* [77] demonstrated that uK_{ATP-1} also couples to SUR and acquires sulfonylurea sensitivity, other features of the K_{ATP} channels, such as regulation by ATP_i and NDPs and activation by K^+ channel openers, have not been identified on the channel reconstituted from uK_{ATP-1} and SUR. Further studies are needed to clarify whether uK_{ATP-1} can reconstitute K_{ATP} channels.

SUR, a member of the ABC superfamily, was cloned from insulinoma cells by Aguilar-Bryan *et al.* [209]. It has 13 potential transmembrane regions, two nucleotide binding folds with Walker A and B consensus motifs, two N-linked glycosylation sites, and several protein kinase A- and C-dependent phosphorylation sites. The coexpression of hamster (ha)-SUR and

mouse (m)-BIR elicits ATP-sensitive K^+ conductance ($I_{K,ATP}$), which is inhibited by glibenclamide (half-maximal at 1.8 nM) and tolbutamide (half-maximal at 32 μ M) and activated by diazoxide (half-maximal at 60 μ M). The single-channel conductance is 76 pS in symmetrical 140 mM K^+ solution. ATP_i inhibits ha-SUR1/m-BIR channel activity with a half-maximal value of 10 μ M. These properties are characteristic of pancreatic β -cell K_{ATP} channels [168, 207, 210, 211]. Northern blot analysis revealed that mRNA for SUR is expressed in large amounts in pancreatic islets and in small amounts in the brain and heart [42, 209]. Gene mapping data show that both BIR and SUR genes are clustered on human chromosome 11 at position 11p15.1 [42]. Thus, SUR seems to form the pancreatic K_{ATP} channels with BIR. Actually, mutations in SUR protein result in persistent hyperinsulinemic hypoglycemia during infancy, a disease associated with unregulated insulin secretion [212].

Subsequently, two additional homologs of SUR have been isolated. The second member of SUR, designated SUR2, was first cloned from a rat brain by Inagaki *et al.* [75]. We have also cloned a mouse homolog of SUR2 and a novel isoform of SUR from a mouse heart cDNA library [76]. A sequence analysis has indicated that this third member of SUR is essentially the same as mouse SUR2 except for 42 amino-acid residues in the C-terminal ends, suggesting that these two SURs are formed by alternative splicing of a single gene. Based on these results, we proposed to rename pancreatic SUR and SUR2 as SUR1 and SUR2A, respectively, and designated the third member as SUR2B [76]. The amino-acid sequence of rat (r)-SUR1 has 66 and 67% identity with those of m-SUR2A and m-SUR2B, respectively (Fig. 17).

The coexpression of BIR and either SUR2A or SUR2B also elicits $I_{K,ATP}$ with a single-channel conductance of ~ 80 pS in symmetrical high K^+ solution. The r-SUR2A/m-BIR channel activity is inhibited by ATP in a dose-dependent manner with a half-maximal value of 100 μ M and only partially inhibited by glibenclamide at 1 μ M [75]. Moreover, in contrast to the ha-SUR1/m-BIR channel, the r-SUR2A/m-BIR channel is activated by pinacidil and cromakalim but not by diazoxide. These are the features of cardiac and skeletal muscle K_{ATP} channels [166, 167, 174, 197, 198]. On the other hand, the m-SUR2B/r-BIR channel is activated by both pinacidil and diazoxide, suggesting that the features of this channel closely resemble those of the smooth muscle type of K_{ATP} channel [162, 179, 180, 213]. Thus, the pharmacological properties of K_{ATP} channels may be determined by distinct sulfonylurea receptors.

Fig. 17. Structures of sulfonylurea receptors. †, N-terminal glycosylation sites; TM, transmembrane regions; NBF, nucleotide binding folds; Δ, phosphorylation sites of protein kinase A; ▲, phosphorylation sites of protein kinase C. See text for details.

An RT-PCR analysis showed that m-SUR2A mRNA is expressed in the atrium, ventricle, skeletal muscle, cerebellum, eye, and urinary bladder, and that m-SUR2B mRNA is distributed ubiquitously not only in these tissues but also in the forebrain, lung, liver, pancreas, kidney, spleen, stomach, small intestine, colon, uterus, ovary, and fat tissue [76]. These tissue distributions support the idea that these two SURs reconstitute cardiac and smooth muscle K_{ATP} channels, respectively.

4. ATP-dependent Kir (K_{AB} /Kir1.1 and Kir4.1) subfamily which are characterized with a Walker-type ATP-binding motif in their C-termini

1) ROMK (Kir1.1). The Kir channels belonging to this subfamily are characterized with a putative ATP-binding domain in their C-termini. ROMK1 is expressed predominantly in the kidney, where it may play important roles in renal electrolyte transport [28, 114, 214, 215]. An immunohistochemical analysis showed that ROMK1 protein is localized to the apical membrane of the cortical collecting duct in epithelial cells [216, 217]. The ROMK1 expressed in *Xenopus* oocytes forms a weak inwardly rectifying K^+ current with a single-channel conductance of 35–45 pS with 90 mM $[K^+]_o$. The channel requires intracellular MgATP to open, exhibits high open probability at various potentials, and is inhibited by intracellular H^+ . These properties are compatible to those of the low-conductance K^+ channel electrophysiologically identified in the apical membrane of cortical collecting duct cells [28, 218–223]. The expression of ROMK1 in the brain is controversial. Northern blot analysis showed that rat ROMK1 mRNA is expressed in the thalamus, hypothalamus and pituitary, but not elsewhere in the brain [28]. *In situ* hybridization, however, showed that it is expressed in the cortex and hippocampal neurons

[224]. On the other hand, human ROMK1 mRNA has not been detected in the brain [112, 113]. The roles of ROMK1 in the brain are not yet known.

It has been shown that, in rats and humans, the ROMK gene is composed of at least five exons and produces divergent mRNAs as a result of alternative splicing at their 5' regions [112–114]. These five isoforms of ROMK mRNAs have been identified and designated ROMK1-5/Kir1.1a–e [225]. Recently, we have identified a novel ROMK isoform, designated ROMK6/Kir1.1f, from a rat kidney [226]. The divergence of ROMK mRNAs may result in the formation of channel subunit proteins with variable lengths of N-termini. Although these ROMK isoforms produce macroscopic currents indistinguishable from one another when expressed in *Xenopus* oocytes, they are differentially expressed among different segments of renal tubules [227] (Fig. 18A–C), suggesting their distinct functional roles in controlling K^+ secretion in renal tubules.

2) K_{AB} -2 (Kir4.1). The amino-acid sequence of K_{AB} -2/Kir4.1 shares 53% identity to ROMK1 [28, 41]. When expressed in *Xenopus* oocytes, K_{AB} -2 exhibits constitutively active K^+ currents with strong inward rectification [40, 41], while ROMK1 is a weak inward rectifier. This difference might be explained by the charge at two putative binding sites of Mg^{2+} (R1 and R2) and polyamines [67] (Fig. 6); K_{AB} -2 has a negatively charged residue (Glu) at R1, while ROMK1 has only neutral residues at both R1 and R2. In the *Xenopus* oocyte expression system, K_{AB} -2 shows two distinct slopes of conductance, 21 and 36 pS [41]. The distinct conducting states might be produced by heteromeric assembly, because it has been demonstrated that the coinjection of cRNAs encoding K_{AB} -2 and BIR9/Kir5.1, which does not possess an ATP-binding domain, results in much larger K^+ currents and unitary conductance than the injection of

Fig. 18. Distribution of ROMK1 (A), ROMK2 (B), ROMK3 (C), and K_{AB-2} (D) in the kidney. Nephron segments (upper panel) and sites in epithelial cells (lower panel) expressing the Kir channel mRNAs are shaded.

tion of K_{AB-2} cRNA alone [53]. Thus, K_{AB-2} might associate with other Kir channel subunits to form heteromeric channels.

K_{AB-2} mRNA is also expressed in the kidney and brain. However, the distribution of K_{AB-2} within these tissues is significantly different from that of ROMK1. Our immunohistochemical study revealed that K_{AB-2} immunoreactivity is detected specifically in the basolateral membrane of distal tubular epithelia [228] (Fig. 18D). This finding suggests that K_{AB-2} may contribute to supplying K^+ to Na^+-K^+ pumps, which are abundant in the basolateral membrane of distal tubular epithelia, as well as to the maintenance of the deep negative membrane potential of these cells. In the brain, K_{AB-2} mRNA is expressed in the cerebellum, corpus callosum, hippocampus, thalamus, inferior colliculus, and brain stem [41]. Because K_{AB-2} is expressed predominantly in the glial cells of the central nervous system, it might be responsible for a K^+ buffering action.

Müller cells are the glial cells in the retina and are supposed to play an important role in the K^+ siphoning action. Horio *et al.* [229] identified a Kir channel in Müller cells using the cell-attached patch-clamp technique. This channel exhibits strong inward rectification and has a single-channel conductance of 25 pS with 150 mM $[K^+]_o$. The kinetics and conductance of the Kir channel in the Müller cells are identical to those of the K_{AB-2} expressed in the mammalian cell

line HEK 293T. The expression of K_{AB-2} mRNA in Müller cells was confirmed by Northern blot analysis, RT-PCR and *in situ* hybridization. These results suggest that the Kir channel in Müller cells may be composed of K_{AB-2} .

5. Other inwardly rectifying K^+ channels

1) Na^+ -activated K^+ channels. The K_{Na} channel was first described by Kameyama *et al.* in cardiac myocytes [24]. This channel is activated by $Na^+_i > 20$ mM, and shows an inwardly rectifying property and the highest conductance (> 200 pS) among ion channels in the heart. Since the normal physiological Na^+_i concentration is estimated to be around 10 mM or less, the channel is believed to play a role only in pathophysiological conditions, such as ischemia [230, 231], in digitalis intoxication [232], and during perfusion with Ca^{2+} , Mg^{2+} -free medium [233]. Later the channel was shown to be present in neuronal tissue and *Xenopus* oocytes [234, 235]. In neurons, the current has been claimed to play a physiological role as a repolarizing current during the action potential, and is believed to be activated by the intruding Na^+ ions during the upstroke of the action potential [234, 236]. A cDNA clone for the K_{Na} channel has not been isolated yet.

2) RACTK1. Suzuki *et al.* [63] have reported a novel Kir channel, which was isolated from rabbit renal collecting tubule (RACT) cells, and named the

clone RACTK1. RACTK1 was not homologous to other Kir channels (<30%), and was reported to form a pH-sensitive Kir channel in Chinese hamster ovary cells. Northern blot analysis in their study revealed that RACTK1 mRNA was detected only in kidney. Further study on localization of RACTK1 by immunohistochemistry done by Suzuki *et al.* [237] showed that the channel was mainly localized at the apical membrane of the collecting duct epithelial cells, as well as in the vascular smooth muscles of renal, coronary and cerebral arteries, and in pancreatic epithelia and duct cells. In the apical membrane of the collecting duct epithelial cells, two classes of K⁺ channels have been electrophysiologically identified, i.e., APT-inhibitable small conductance (30–40 pS) and Ca²⁺-activated intermediate conductance (40–80 pS) K⁺ channels [218–220]. Suzuki [238, 239] suggested that RACTK1 was the latter type of K⁺ channel, and proposed that RACTK1 exhibited higher homology to a maxi-Ca²⁺-activated K⁺ (K_{Ca}) channel, than to previously cloned Kir channels. Suzuki *et al.* [240] subsequently reported that a cell line stably expressing recombinant RACTK1 exhibited a Ca²⁺-activated and apamin-sensitive K⁺ channel, and also contributed to the database a very highly homologous human RACTK1 cDNA sequence (GenBank U33839).

Recently, Shmukler *et al.* [241] reported that the “RACTK1” polypeptide sequence is deduced from an incorrectly sequenced fragment of *Escherichia coli* genomic DNA. They showed the followings: (1) “RACTK1” open reading frame is a frameshifted translation of the antisense strand of an *E. coli* gene member of a coenzyme A transferase gene family, (2) “RACTK1” mRNA was absent from the tissues free of *E. coli* contamination, and (3) the “RACTK1” gene was undetectable in Southern blots of human and rabbit genomic DNA. Thus, it seems likely that the “RACTK1” channel gene does not exist in mammals.

3) TWIK-1. The tandem of P domains in a weak inward rectifying K⁺ channel (TWIK-1), which encodes 336 amino acids, is the first member of a novel structural and functional group of K⁺ channels isolated from a human kidney cDNA library by Lesage *et al.* [62]. The model deduced from the TWIK-1 hydrophobicity pattern consists of four transmembrane regions. The unique feature of this channel is the presence of two pore motifs in tandem (P1 and P2). The alignment of pore-forming regions of a large group of K⁺ channel sequences indicates that P1 and P2 in TWIK-1 are well conserved. The highly conserved motif, GYG, found in almost all cloned Kir channels is GYG in P1 but GLG in P2. Assuming that four

pore domains are required for a functional K⁺-selectivity filter [48, 51, 52, 242], TWIK-1 is expected to be a dimer. TWIK-1, similar to Kir subunits with two transmembrane regions, does not have a voltage-sensing domain. TWIK-1 channel exhibits weak inward rectification and a single-channel conductance of 34 pS in symmetrical 140 mM K⁺ solution. The inward rectification is eliminated in the absence of Mg²⁺. TWIK-1 is K⁺-selective, and its currents expressed in *Xenopus* oocytes are inhibited by Ba²⁺ and by class I antiarrhythmic agents, quinine and quinidine. Northern blot analysis indicated that TWIK-1 mRNA is widely distributed in human tissues, and is particularly abundant in the brain and heart. Thus, the TWIK-1 channel may be involved in the control of background K⁺ membrane conductance.

Conclusions

The successful cloning of Kir channels has shed considerable light into their structures and functions. Combinations of molecular biological and electrophysiological studies have paved the way to develop general concepts for understanding the properties of these channels, providing evidence that: 1) Kir channel subunits comprise two transmembrane regions and a pore-forming region, but do not possess the voltage sensor or inactivation ball of Kv channels; 2) at least two negatively charged amino-acid residues mediate inward rectification which is caused by Mg²⁺ and polyamines; and 3) Kir channels are composed of multiple genes, distribute differently in various cells and play diverse functional roles. Recent studies have further revealed that K_G channels function as the heteromultimers of two distinct GIRK subunits, and that K_{ATP} channels are a complex composed of Kir channel subunits and ABC proteins such as sulfonylurea receptors. Although the atrial K_G channel appears to be composed of GIRK1 and GIRK4, the assembly of GIRK subunits in other tissues is not clear. The assembly and stoichiometry of K_{ATP} channels is also unknown. In addition, many questions still remain, such as the physiological roles of cloned channels (especially, GIRK3, BIR9, and uK_{ATP}-1) *in vivo*, structural-functional relationships, higher-order structures, clustering mechanisms, and modulation of the channels. To clarify these questions, further studies are needed.

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