G Protein Regulation of Inwardly Rectifying K⁺ Channels

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Inwardly rectifying K⁺ (Kir) channels respond to receptor-stimulated signaling cascades that involve G proteins and other cytosolic messengers. Channel activity is controlled both by direct coupling of G protein subunits and by phosphorylation via protein serine/threonine and tyrosine kinases. The coincidence of both forms of Kir channel signaling may give rise to complex cellular responses.

From a biophysical point of view, inwardly rectifying K⁺ (Kir) channels that preferentially pass current in the inward direction are every bit as complex as voltage-dependent K⁺ (Kv) channels. They are selectively permeable to K⁺, and their gating and conductance are strongly dependent on the K⁺ gradient and voltage across the membrane. When the first Kir channel subunits were cloned in 1993 from kidney, heart, and a macrophage cell line, we were struck by the apparent simplicity of each of the channel-forming subunits. DNA sequence analysis suggested a subunit structure in which a pore-forming region is flanked by two transmembrane α-helical segments. A comparison with Kv subunits revealed that all 15 Kir subunits isolated so far lack four of six transmembrane regions and also other functional domains, e.g., a voltage-sensing element (S4), or a cytosolic “inactivation ball” structure. Mutational analysis, together with the crystal structure of a prokaryotic K⁺ channel (7), suggests that one of the two tilted α-helices of each subunit is connected to the pore loop/selectivity filter facing the pore; the other is pointing to the lipid membrane and gives the tetrameric channel the appearance of an “inverted teepee.” It was found that the most typical channel property, i.e., the apparent gating that gives rise to inward rectification, was not modified by an intrinsic channel structure but by intracellular Mg²⁺ and positively charged polyamines that bind to two major channel residues in a voltage-dependent manner. Various Kir channels are dependent on the cellular metabolism. They respond to changes in ATP hydrolysis, the ATP-to-MgADP ratio, pH, or reactive oxygen species (O₂⁻, H₂O₂, OH⁻) after ischemia, apoptosis, or neurodegeneration, and much is known about the way these molecules and other regulators affect channel activity (Fig. 1). Moreover, a series of central nervous system (CNS) neurotransmitters and their G protein-coupled receptors have been described to converge onto Kir conductances. With data now available from recombinant channels, much effort is focused on the description of the signaling components involved. In the following pages, some of the emerging concepts on this topic are summarized.

Direct activation of Kir3 channels by Gβγ subunits

In many excitable cells, the activation of Kir3 (formerly GIRK) channels by ligand-bound G protein-coupled receptors results in postsynaptic hyperpolarization. It is now well established that stimulated Gβγ subunit dimers, dissociated from their GTP-bound Ga counterparts, exert this action in a membrane-delimited fashion (2, 5). Channels are closed at rest, i.e., in the absence of pertussis toxin (PTX)-sensitive G proteins and their modulators, the basal activity of Kir3 channels is virtually zero. The activity of cardiac KACh channels is enhanced >500-fold by application of purified G protein subunits to the cytoplasmic side in inside-out patches. Thus channel activity is under the obligatory control of free Gβγ subunits without involving any other second messengers (Fig. 2A).

After transmitter action, the rapid activation rate of native and recombinant Kir3 channels is one to two orders of magnitude faster (2–4 s⁻¹) than signaling via cytosolic diffusible mediators. On the basis of the estimated density of receptors, G proteins and channels, and their diffusion coefficients in the cellular environment, quantitative calculations suggest that these rates require the proteins to be within <0.5 µm of each other—far below that predicted by random protein distribution. Receptors, G proteins, and Kir3 channels may therefore exist as discrete, pre-
formed complexes in the membrane. Multiple reports show the direct interaction of Gbg subunits with the Kir3 channel core, as well as the NH2- and COOH-terminal regions, and this action also involves Na+ and phosphatidylinositol 4,5-bisphosphate (PIP2; see Table 1). It is thought that the trimeric G protein binds to the NH2 terminus of each subunit to maintain the closed state of the channel. Receptor stimulation then dissociates Gbg subunits and translocates them to the COOH terminus, which results in removal of the blocking gate or another conformational change, stabilizes the direct interaction with PIP2, and opens the channel.

A kinetic discrepancy in the G protein cycle nonetheless exists for activation of Kir3 channels in native cells. Gbg signaling is terminated by hydrolysis of GaGTP by its intrinsic GTPase activity and reassociation of GaGDP and Gbg subunits (1–2 min\(^{-1}\)). After removal of the receptor ligand, Kir3 currents in atrial myocytes and neurons deactivate ~50-fold faster (1–2 s\(^{-1}\)) than in the recombinant system or than predicted by the GTP hydrolysis rate of Ga subunits. Because Kir3 channels are unlikely to be GTPase-activating proteins (GAPs), a cytoplasmic GAP complexed with the small G protein rasp21 (rasGAP) that apparently uncouples G protein-coupled receptor (GPCR) and G protein has been assumed to accelerate the GTPase rate and Kir channel deactivation. In addition, several isoforms of “regulators of G protein signaling” proteins (RGS1, 3, 4, 8), when heterologously expressed, mimic the temporal properties of onset and termination of Kir3 currents in vivo (6,10). This indicates that RGS proteins that bind to Gbg subunits are crucial determinants in the gating control of Kir3 channels and thus may modulate the temporal properties of “slow” inhibitory postsynaptic potentials.

Signaling via Gbg is exemplified by the muscarinic-gated K\(_{ach}\) channels of the pacemaker cells in sinoatrial and atrioventricular nodes of the heart. The analysis of Kir3.4 knockout mice showed that K\(_{ach}\) currents, as long hypothesized, mediated about one-half of the negative chronotropic effects of vagal stimulation on the heart rate. Native K\(_{ach}\) channels that are also activated by somatostatin, adenosine, epinephrine, platelet-activating factor, neuropeptide Y, calcitonin gene-related peptide, and endothelins are made of Kir3.1 and Kir3.4 subunits (9). From functional analysis of heterologously expressed concatenated subunits, it was deduced that native heart channels have a stoichiometry of (Kir3.1)\(_2\)(Kir3.4)\(_2\) and that optimal function requires positioning of the same subunits opposite each other. It is noteworthy how different subunits assemble and recognize each other. In contrast to Kv channels in which heteromerization is governed by a highly specific 30- to 60-amino acid stretch in the NH2-terminal T1 region, the compatibility between Kir subunits is determined primarily by their core regions. In fact, a single pore residue in Kir3.1 that is responsible for a residual component of slow voltage-
dependent gating controls assembly and function of the heteromeric channels (Fig. 1). Electrophysiological and biochemical evidence now documents that Kir3.1, Kir3.2, and Kir3.3 subunits that are colocalized in many neurons are also capable of assembling in the mammalian brain. From coexpression studies, we have learned that individual Kir3.1 subunits fail to form functional channels, but prominent K⁺ currents are obtained for all combinations with other Kir3 subunits. A still-open question, however, concerns the precise nature and subunit composition of Kir3 channels in identified neurons and their functional contribution to neuronal signal processing. Homomeric channels may also exist in neurons in which only a single Kir3 subunit is present, e.g., Kir3.3 in Purkinje cells.

In *Xenopus* oocytes and mammalian cells, Kir3 channels can be activated by serotonin 1A, m₂ muscarinic, dopamine D₂, D₃, D₄, metabotropic mGluR1a, 2, and 7, GABAₐ, neuropeptide Y1 and Y2, cannabinoid, melatonin, µ-, δ-, and κ-opioid and nociceptin/orphanin receptors, as well as adenosine A₁ receptors. Generally, all receptors that couple to PTX-sensitive Gᵥₒ proteins converge onto Kir3 channels, and it is not fully understood how receptor-G protein-channel specificity is maintained with several of these receptors present in one cell. The assumption that signaling specificity is obtained by the highly selective coupling of receptors, Gβγ subunits, and Kir3 channel isoforms is inadequate to explain how a particular signal is transmitted through the G protein bottleneck. The Gβγ subunit combination itself contributes only limited specificity, because various combinations of Gβ and Gγ isoforms (with a preference to Gβ1/γ2) are capable of activating Kir3 channels. Several other factors, such as synergistic/antagonistic effects of Ga and Gβγ subunits or compartmentalization, may also contribute to signaling specificity. Most importantly, it appears that the Gβγ dimers must dissociate from the relevant heterotrimeric G protein that is complexed with both Gᵥₒ-coupled receptor and Kir3 channel (5).

The significance of Kir3 subunit distribution and channel composition is well shown by the consequences of the weaver mouse mutation. In weaver, a single point mutation in the gene for Kir3.2 subunits (Gly → Ser in the conserved selectivity filter signature; Fig. 1) generates an impaired morphological and functional phenotype with severe cerebellar ataxia. In addition to a decrease in Kir3.2 protein expression and an impaired sensitivity toward Gβγ subunits, mutated Kir channels composed of Kir3.2 subunits also show a decreased selectivity for K⁺ and become permeable to Na⁺. Because Kir3 channels in the absence of G protein signaling are gated by Na⁺ [dissociation constant (Kᵥₒ) = 40 mM], the abnormal Na⁺ influx then provides a regenerative cycle of activation. Consequently,
several neuronal populations, e.g., the dopaminergic neurons in the substantia nigra and the granule cells/Purkinje cells in the cerebellum, degenerate whereas others remain unaffected. It is still unclear why this selective damage results from the mutation of the Kir3.2 subunit that is ubiquitously distributed throughout the brain. Other Kir subunits, e.g., Kir3.3, may compensate for the loss in the unaffected cells. An alternative explanation for the predilection of the substantia nigra is based on the very early expression of Kir3.2 mRNA during development. Kir3.2 mRNA is found during early stages of development in the neuroepithelium and the subventricular zones where the future dopaminergic neurons are localized, possibly indicating that proliferative mitotic precursor cells may be most sensitive to the excessive Na⁺ influx.

Regulation of Kir channels by protein phosphorylation

The response of Kir channels to GTP-primed Gα subunits that act via classic signaling pathways involving phosphorylation by protein ser/threonine kinases is likely to be heterogeneous. Channel activity of the secretory ATP-regulated Kir1.1 (ROMK1) channels in renal epithelia is dependent on phosphorylation of at least two of three potential protein kinase A (PKA) phosphorylation sites (15). On the other hand, the open probability of recombinant Kir2.1 and Kir2.3 is inhibited by both PKA and protein kinase C (PKC) phosphorylation (Fig. 2B). The complete inhibitory pathway, which may be the basis for Kir channel inhibition in many native cells, can be reconstituted in heterologous systems by coexpression of G protein-coupled receptors and Kir2 channels. It was demonstrated that the Kir2.1 protein itself is the substrate for PKA, because mutation of a unique PKA phosphorylation site in the COOH-terminal region suppresses the inhibitory effect of catalytic PKA subunits (14). In analogy to the inactivation gate of A-type Kv channels, it was hypothesized that the COOH terminus of Kir channels represents a gate structure for the closure of the channel pore. In Kir2.1, Kir2.2, and Kir2.3 subunits, the PKA phosphorylation motif is present at the serine at the third-to-last residue near the COOH terminus.

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Table 1. G protein-mediated signaling onto native and recombinant Kir channels

<table>
<thead>
<tr>
<th>Kir Subfamily</th>
<th>Subunits</th>
<th>Mediators/ Modulators</th>
<th>G Protein</th>
<th>Suggested Mechanism of Action</th>
<th>Channel Regulation</th>
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<tr>
<td>Kir1</td>
<td>Kir1.1</td>
<td>PKA</td>
<td>G_s</td>
<td>Phosphorylation</td>
<td>Activation</td>
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<tr>
<td>Kir2</td>
<td>Kir2.1</td>
<td>PKC, PKA</td>
<td>G_s/G₁₁</td>
<td>Phosphorylation</td>
<td>Inhibition</td>
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<tr>
<td></td>
<td>Kir2.3</td>
<td>Gβ1γ2, PKC, Mg²⁺/ATP</td>
<td>G_s/G₁₁</td>
<td>Phosphorylation</td>
<td>Inhibition</td>
</tr>
<tr>
<td>Kir3</td>
<td>Kir3.1/3.4</td>
<td>Gβ1γ2, Gα, PLC, PKA</td>
<td>G_s/G₁₁</td>
<td>Direct coupling</td>
<td>Inhibition</td>
</tr>
<tr>
<td></td>
<td>Kir3.1/3.4</td>
<td>PKA</td>
<td>G_s</td>
<td>Phosphorylation</td>
<td>Activation</td>
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<td></td>
<td>Kir3.1/3.4</td>
<td>PL₁₂₅</td>
<td>G_s</td>
<td>G protein turnover?</td>
<td>Activation</td>
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<td>Kir3.1/3.4</td>
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<td>G_s</td>
<td>Receptor-G protein coupling</td>
<td>Accelerates kinetics</td>
</tr>
<tr>
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<td>Kir3.1/3.2</td>
<td>RGS proteins</td>
<td>G_s</td>
<td>ATP turnover</td>
<td>Acceleration of receptor-induced currents</td>
</tr>
<tr>
<td></td>
<td>Kir3.1/3.4</td>
<td>PIP₂</td>
<td>G_s</td>
<td>Direct coupling and stabilization of Gβγ binding</td>
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<td></td>
<td>Kir3.1/3.4</td>
<td>GRK</td>
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<td>Desensitization</td>
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<tr>
<td>Kir6</td>
<td>Kir6.2/SUR</td>
<td>Gα</td>
<td>G_s</td>
<td>Attenuation of ATP_i-mediated inhibition</td>
<td></td>
</tr>
</tbody>
</table>

GAP, GTPase activating protein; GRK, G protein-coupled receptor kinase; RGS, regulators of G protein signaling; PIP₂, phosphatidylinositol 4,5-bisphosphate; PKI, PK2A, serine-threonine phosphatase 1, 2A; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PL₁₂₅, phospholipase A; ATP_i, intracellular [ATP].

"The complete inhibitory pathway ... can be reconstituted in heterologous systems...."
(Fig. 1), but it is absent in Kir2.4, in which a unique NH$_2$-terminal PKA site could serve similar gate functions. When dephosphorylated, the COOH termini of Kir2.3 and Kir1.2 (Kir4.1) channel interact with PDZ domains of cytoskeletal proteins (e.g., PSD-95; Ref. 4). It might be speculated that this interaction both targets the channel to distinct membrane regions and affects acute channel function via changes in the cellular phosphorylation state.

PKC may inhibit Kir2.1 channels via a similar molecular mechanism, because removal of NH$_2$- and COOH-terminal phosphorylation sites also disrupts inhibition. Suppression of Kir2 channels by PTX-insensitive C$_{b/11}$ receptors such as muscarinic m, receptors (8) and PKC phosphorylation in fact is the likely basis for some slow excitatory potentials in neurons of the nucleus basalis, nucleus accumbens, or sympathetic ganglia, in which transmitter actions have a long latency and last for tens of seconds. Another mechanism for Kir2.3 channel inhibition by m$_1$ receptors that cannot be accounted for by PKC, Ca$^{2+}$, or channel phosphorylation has been described by Chuang et al. (1). Kir2.3 inhibition by acetylcholine persists for a few minutes, and this effect appears to be mediated by free intracellular Mg$^{2+}$. This action of Mg$^{2+}$ as a novel diffusible cytoplasmic messenger, which causes the channel to enter a prolonged inactive state, differs from the known pore-blocking effect of Mg$^{2+}$ by a much slower time course (~1 min), lower affinity, and voltage independence.

Finally, G protein-coupled neurotransmitter receptors, like receptors for surface recognition molecules, growth factors, or cytokines, are also capable of signaling onto Kir channels via tyrosine kinase activity. Recombinant Kir2.1 channels are acutely suppressed by tyrosine kinase activity by a mechanism similar to the one described above, in which a phosphate moiety from ATP is translocated to a residue in the COOH-terminal region that acts as a channel closing gate (13).

Deviations from the dogma and fine-tuning of regulation?

Several deviations from these general paradigms have been described, which likely result from the natural dichotomy of action of G$\alpha$ and G$\beta\gamma$ subunits stimulated by receptor occupancy. When the principle of direct G protein regulation was recognized in the mid- to late 1980s, an intense debate was initially led on the nature of the G protein subunit that activates Kir3 channels. From the robust responses of purified and recombinant subunits, it is now agreed that G$\beta\gamma$ and not G$\alpha$ subunits mediate the activation of Kir3 channels in their native environment. The list of targets for G$\beta\gamma$ subunits has grown constantly since then and now also includes phospholipase A (PLA)$_2$, adenylyl cyclase II–IV, phospholipase C (PLC)$\beta$2, and $\beta$-adrenergic receptor kinase ($\beta$-ARK). Nevertheless, it came as a surprise that G$\beta\gamma$ subunits were also found to interact directly with Kir2.3 channels, resulting in their complete suppression (3). As shown electrophysiologically and by immunoprecipitation, this effect is mediated by G$\beta\gamma$ coupling to the NH$_2$ terminus of Kir2.3 (Fig. 2B) and may explain experimental data in which receptor stimulation results in fast inhibitory responses of native Kir channels. Residual evolutionary conservation of the gating mechanisms between Kir3 and Kir2 subunits may be a possible interpretation for this action.

What function can then be assigned to free G$\alpha$ subunits in Kir3 channel regulation? Schreibmayer et al. (11) have shown that G$\beta\gamma$2-activated Kir3 currents ($I_{Kir3}$) are potently inhibited by GTP-bound G$\alpha_q$ and partially by G$\alpha_q$ and G$\alpha_i$, suggesting that this antagonistic interaction may contribute to G protein-channel coupling (Fig. 2A). On the other hand, receptor coupling to Kir3 channels was found to be under the control of G$\alpha$-mediated PKA and PKC pathways, although this is still controversial. In Xenopus oocytes, recombinant Gq/PLC-coupled metabotropic mGluR1a,5 receptors were found to inhibit Kir3 channel activity via G$\alpha_q$ subunits and PKC-µ (12), and this may also be the case in hippocampal and locus ceruleus neurons. The amino acid sequences of all Kir3 subunits show multiple putative PKC phosphorylation sites. However, final evidence for a direct interaction of PKC with Kir channels is still lacking. In contrast, Kir3 channels are positively modulated by PKA both in vivo and in the recombinant system, despite the lack of PKA phosphorylation sites. It was shown that $\mu$-opioid receptor desensitization of Kir3 channels in Xenopus oocytes can be reversed by promoting the PKA pathway and that in mammalian cells the activity of different Kir3 subunit combinations is enhanced through PKA phosphorylation. Upon application of receptor ligands, a transient or biphasic response may occur as a result of 1) the fast activation by G$\beta\gamma$ subunits and 2) the pronounced reduction of $I_{Kir3}$ amplitudes upon activation of G$\beta\gamma$-coupled receptors that inhibit adenylyl cyclase and PKA activity. If PKA is targeted by scaffold or anchoring proteins (AKAPs) in the vicinity of receptor-G protein-Kir3 channel modules, a tight temporal and spatial control of Kir3 channel activity by PKA phosphorylation/dephosphorylation is to be
expected that may even predominate receptor action in the case of unsuccessful completion of direct Gβγ channel gating (caused by locally low protein density).

Owing to their coupling to different signaling pathways, we see that the functional consequences of the activity of Kir2 and Kir3 channels differ greatly. Membrane-bound Gβγ subunits are limited in their lateral diffusion rate. Activation of neuronal Kir3 channels results in brief, localized inhibitory postsynaptic potentials (IPSPs) and thus synaptic transmission at an intermediate rate (1–2 s\(^{-1}\)). On the other hand, signal transduction via freely diffusible cytoplasmic mediators that cause a change in Kir2 conductances results in a more generalized spread of the signal. Because of the phosphorylation process, these excitatory potentials may last tens of seconds, minutes, or hours. In addition, a great degree of fine-tuning may be mediated by coincident signaling inputs utilizing both of these major forms of signaling. Because Kir2 and Kir3 channels may be expressed in the same cellular microcompartment, interact with the same receptors, and respond to signal transduction via Gα and Gβγ subunits, this could generate complex cellular output functions in response to a neurotransmitter. Signal transduction onto Kir channels thus perfectly illustrates the complexity of the mechanisms that have been developed during evolution to serve the coordination of cellular systems in the brain.

Because of space limitations, only a brief selection of original citations in this field is provided, and I apologize to all colleagues whose work was not cited appropriately. I thank all other group members for their intellectual input and their contributions to the studies described in this review article.

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References


Science becomes dangerous only when it imagines that it has reached its goal.

George Bernard Shaw, The Doctor’s Dilemma, 1911