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New insights into the therapeutic potential of Girk channels

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Abstract

G protein-dependent signaling pathways control the activity of excitable cells of the nervous system and heart, and are the targets of neurotransmitters, clinically-relevant drugs, and drugs of abuse. G protein-gated inwardly-rectifying potassium (K^+) (Girk/Kir3) channels are a key effector in inhibitory signaling pathways. Girk-dependent signaling contributes to nociception and analgesia, reward-related behavior, mood, cognition, and the heart rate regulation, and has been linked to epilepsy, Down syndrome, addiction, and arrhythmias. Here, we discuss recent advances in our understanding of Girk channel structure, organization in signaling complexes, and plasticity, as well as progress on the development of subunit-selective Girk modulators. These findings offer new hope for the selective manipulation of Girk channels to treat a variety of debilitating afflictions.

Introduction to Girk signaling

Signal transduction involving inhibitory ($G_{i/o}$) G proteins titrates the excitability of neurons, cardiac myocytes, and endocrine cells, actions crucial for regulating mood and cognition, nociception and antinociception, reward, energy homeostasis, motor activity and coordination, hormone secretion, and cardiac output. Not surprisingly, dysregulation of $G_{i/o}$-dependent signaling has been linked to a number of neurological and cardiac disorders. Given this, and since the efficacy of many clinically-relevant and abused drugs relates to their actions on $G_{i/o}$-dependent signaling, it is important that we understand with cellular, subcellular, and molecular detail how such signaling is organized, how it is regulated, and how and when it goes awry.

G protein-gated inwardly-rectifying potassium (K^+) (Girk/Kir3) channels are a common effector for $G_{i/o}$-dependent signaling pathways in the heart and nervous system [1, 2]. Studies of mutant mice and a more limited set of linkage analyses have suggested that dysregulation of Girk signaling may contribute to certain human diseases and disorders

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(Table 1). While this work suggests that therapeutic approaches targeting Girk channels may prove beneficial in some settings, there is legitimate concern that manipulation of Girk signaling would trigger profound and widespread off-target effects. The goal of this review is to highlight recent developments related to our understanding of Girk channel diversity, compartmentalization, and plasticity. These studies suggest new opportunities for selective manipulation of Girk signaling, efforts that could eventually lead to novel treatments for debilitating human afflictions.

Girk channel structure

Girk channels are tetramers formed by differential multimerization among the products of four genes: Girk1/Kir3.1/Kcnj3, Girk2/Kir3.2/Kcnj6, Girk3/Kir3.3/Kcnj9, and Girk4/Kir3.4/Kcnj5 [1, 2] (Figure 1A). Each Girk subunit possesses intracellular N- and C-terminal domains, and two transmembrane segments that flank a hydrophobic pore domain. Random assembly theoretically allows for the formation of many distinct Girk channel subtypes, and alternative splicing of the *Girk1* and *Girk2* genes potentially adds an additional spice of diversity (*e.g.*, [3, 4]). There are, however, two observations that suggest that a more limited number of Girk channel subtypes exist *in vivo*: 1) Girk1 does not form functional homomultimeric channels [5, 6], an observation attributable at least in part to the absence of an endoplasmic reticulum (ER) export signal that is found in Girk2 and Girk4 [7]. Surprisingly, however, introduction of a single point mutation into the pore domain of Girk1 (F137S) is sufficient to yield functional homomeric channels [8]. Collectively, these findings suggest that while ER export signals are important, other factors such as protein folding and stability may also influence the trafficking of Girk channels. 2) The four Girk subunits exhibit overlapping but distinct expression patterns, which is particularly evident in the nervous system [9, 10]. Indeed, work in the last two decades has clarified the cell type-specific expression patterns of Girk subunits (Box 1). Moreover, recent crystallography studies have begun providing insight into the three-dimensional structure of Girk channels, with resolution of the membrane-spanning and large portions of the intracellular domains of homomeric channels [11–16] (Figure 1B).

Box 1

Cellular and sub-cellular diversity of Girk channels

While Girk channel expression has been reported in neuroendocrine cells and more recently in some cancer cells (*e.g.*, [114–117]), their expression and relevance is far better understood in the heart and nervous system. Girk1/4 heteromers comprise the muscarinic-gated atrial K^+ channel I_{KACh}, a critical mediator of the parasympathetic regulation of heart rate [5, 86]. While the prototypical neuronal Girk channel is thought to be the Girk1/2 heteromer, multiple Girk channel subtypes exist within the rodent nervous system. Indeed, *Girk3* is expressed throughout the central nervous system [9], and while *Girk4* expression is not prominent in the brain, it is found in a few regions including the hypothalamus and cerebellum [105, 118]. *Girk1*, *Girk2*, and *Girk3* are co-expressed in many neuron populations, including hippocampal pyramidal neurons [9, 10]. In contrast, dopamine neurons of the VTA and substantia nigra *pars compacta* (SNc) display Girk2/3 and Girk2a/c heteromers, respectively [27, 119]. The cerebellum exemplifies the molecular diversity that can be achieved via differential subunit expression; seven distinct Girk expression patterns were discerned within the various neuronal subtypes in this brain region [118].

Girk channels are distributed mainly in the somato-dendritic compartment of neurons (*e.g.*, [10, 96, 118, 120]). This distribution is consistent with most studies showing that Girk channels selectively mediate the postsynaptic inhibitory effects of neurotransmitters

and related drugs, while making little or no contribution to their presynaptic inhibitory effects [121]. Some ultrastructural and functional data, however, support the contention that Girk channels contribute to presynaptic inhibition in some neurons [122–124]. Girk channels of distinct subunit composition can also exist within specific subcellular compartments of the same neuron. For example, Girk1 and Girk2 show extensive co-localization across the different dendritic layers of the hippocampus [120]. Their expression levels vary among dendritic regions innervated by distinct synaptic inputs in CA1 pyramidal cells, showing a significant increase from proximal to distal dendrites. In contrast, Girk3 is more uniformly distributed along the cell surface of pyramidal cells, including the presynaptic terminal [10]. In addition, Girk2 and Girk3 are evenly distributed along the postsynaptic density (PSD) of pyramidal cells and Purkinje cells, whereas Girk1 is absent from excitatory synapses and only observed at perisynaptic sites [10, 96, 120, 123]. Finally, in the sub-population of parvalbumin-expressing interneurons consisting mainly of basket and chandelier cells, Girk1, Girk2 and Girk3 share the same localization at very similar densities [125]. Collectively, these data argue that distinct Girk channel subtypes exist in a tissue/cell-type and subcellular compartment-dependent manner, a scenario that enhances the prospects for selective and efficacious therapeutic manipulation of Girk signaling.

Functional implications of Girk channel diversity

In expression systems, Girk channels of various composition – including heteromers (Girk1/2, Girk1/3, Girk1/4, Girk2/3, Girk2/4) and homomers (Girk2 and Girk4) – display K^+ selectivity, inward rectification, and G protein-dependent gating [1, 2]. While it cannot form a functional homomer, Girk1 is an integral subunit of most neuronal Girk channels and the cardiac Girk channel I_{KACh} [5, 17]. Girk1 confers robust receptor-dependent activity to Girk heteromers, attributable in part to unique residues found in the pore and second transmembrane helix that enhance single-channel conductance and open probability [8, 18–20]. The intracellular C-terminal domain also contributes to the potentiating influence of Girk1 on GPCR-dependent heteromeric channel activity, likely due to the presence of unique Gβγ, Gα, and PIP_2 interaction domains and phosphorylation sites [20–26].

The functional relevance of Girk channel subunit composition is nicely illustrated in the ventral tegmental area (VTA). VTA dopamine neurons express Girk2 and Girk3, while VTA GABA neurons express Girk1, Girk2, and Girk3 [27, 28]. VTA dopamine neurons are significantly less sensitive than VTA GABA neurons to direct $GABA_B$ receptor-dependent inhibition [27, 28], consistent with the relatively low sensitivity of recombinant Girk2/3 heteromers to Gβγ-dependent activation [29]. Interestingly, ectopic expression of Girk1 or genetic ablation of *Girk3* enhanced the sensitivity of the Girk channel in VTA dopamine neurons to $GABA_B$ receptor-dependent inhibition [28]. The negative influence of Girk3 on the sensitivity of Girk2/3 heteromers to Gβγ- and $GABA_B$ receptor-dependent activation may be linked to intrinsic structural elements that weaken its interaction with Gβγ or the coupling between Gβγ binding and channel gating, an explanation supported by the behavior of recombinant Girk2/3 heteromers [29]. Alternatively, Girk3-specific interactions with negative regulatory proteins expressed in VTA dopamine neurons (Rgs2 and/or sorting nexin 27 [28, 30], discussed below), interactions that are presumably precluded or mitigated by the presence of Girk1, may explain the differential sensitivity of Girk channels in VTA dopamine and GABA neurons to $GABA_B$ receptor activation. Regardless of the mechanism, the molecular and cellular diversity of Girk channels shapes the sensitivity of VTA dopamine output to $GABA_B$ receptor activation, and helps explain the intriguing pharmacodynamics differences between the $GABA_B$ receptor agonists baclofen (an anti-craving compound) and gamma hydroxybutyric acid (GHB, a drug of abuse) [28].

Macromolecular organization of Girk signaling

Girk channels are thought to exist in multi-protein complexes that include G protein-coupled receptors (GPCRs), G proteins, and regulatory proteins [1, 2, 31], a consensus that has emerged despite the fact that relatively few protein-protein interactions involving Girk channels have been demonstrated using classical biochemical approaches or native systems. Indeed, most reported interactions have involved over-expression of Girk subunits and putative binding partners in cell types that do not normally express Girk channels. Nevertheless, data obtained using these approaches has been valuable in supporting a conceptual model that aligns with key functional properties of GPCR-Girk signaling (*e.g.*, $G_{i/o}$ coupling specificity, signaling kinetics).

A core signaling complex: GPCR-Gαβγ-Girk

The interaction with Gβγ represents, from a structural and functional perspective, the best understood of the protein-protein interactions involving Girk channels. Gβγ binds to Girk channels, strengthening the interaction between the channel and phosphatidylinositol-4,5-bisphosphate (PIP_2), a required co-factor for channel gating [32, 33]. While biochemical and structure-function approaches have suggested multiple interaction domains for Gβγ in all four Girk subunits [1, 34], clear resolution of this critical interaction was obtained recently with the crystallization of a complex formed by Gβγ and the Girk2 homomer [16] (Figure 1B). Girk2 homomers possess four binding sites for Gβγ found at the well-conserved cytoplasmic interfaces between adjacent subunits that contribute to formation of the extended cytoplasmic pore; Gβγ promotes a “pre-open” state of the channel that is intermediate between the closed state of the channel and the open conformation of a constitutively-active Girk2 homomer [15, 16].

Girk channels also interact with $G\alpha_{i/o}$. The inactive heterotrimeric G protein ($G\alpha_{i/o}$-GDP/Gβγ), as well as $G\alpha_{i/o}$-GDP and $G\alpha_{i/o}$-GTP, can bind to intracellular domains of Girk channels [25, 26, 35–38]. Interactions between Girk channels and $G\alpha_{i/o}$-GDP (either alone or in the context of the inactive heterotrimeric G protein) suppress basal activity of Girk channels, while enhancing their G protein-dependent activation [25, 39, 40]. Moreover, the selective association between Girk channels and $G\alpha_{i/o}$ likely explains in part the strict coupling specificity between Girk channels and $G_{i/o}$-dependent signaling pathways *in vivo* [36, 41]. This specificity may be reinforced further by selective pre-coupling between certain GPCRs and $G\alpha_{i/o}$-GDP/Gβγ [42], and/or by direct GPCR-Girk interactions [31]. Indeed, several GPCRs that couple to Gi/o G proteins, including D_2 and D_4 dopamine receptors and $GABA_B$ receptors, have been shown to interact directly with Girk channels [31, 43, 44].

Collectively, these studies support the vision of a compact core Girk signaling complex, wherein minor conformational changes triggered by agonist binding to GPCR unveils key protein-protein interaction interfaces [45]. Organization of signaling elements within a multi-protein complex affords several advantages over random, collision-based designs. The close spatial proximity of the relevant molecules allows for fast and efficient signaling, and the noise or “cross-talk” that might otherwise occur with non-specific collision events is minimized, creating a tailored intracellular response to an external stimulus. Moreover, the strength or sensitivity of the signaling pathway can theoretically be titrated, by altering macrocomplex composition, to suit the needs of the cell under specific circumstances. With respect to this latter point, two Girk-interacting proteins warrant further discussion.

Sorting nexin 27 (SNX27)

Girk2c and Girk3 possess identical C-terminal Class 1 interaction motifs for PDZ domain-containing proteins (-ESKV) (Figure 1A). Using the distal C-terminal domain of Girk3 as bait in an unbiased proteomic screen, sorting nexin 27 (SNX27) was identified as a Girk-interacting protein [30]. SNX27 regulates the trafficking between cell surface and endosome of an array of neuronal signaling proteins [46], and has been implicated in Down syndrome and addiction [47, 48]. SNX27 is the only member of the sorting nexin family that has a PDZ domain, and it recognizes class I PDZ-binding motifs [49].

Amino acids immediately upstream from the ESKV motif in Girk3 are crucial for promoting the Girk3-SNX27 interaction, and preclude its interaction with PSD95 [30, 50]. SNX27 also contains a Ras association (RA) and a lipid-binding phox-homology (PX) domain. All three functional domains are critical for the proper function of SNX27, which in the context of Girk signaling involves targeting Girk3-containing channels to early endosomes, effectively reducing the surface expression of Girk channels and enhancing the excitability of host neurons [30, 50, 51] (Figure 2). Interestingly, while SNX27 can bind to Girk2c, the surface distribution of homomeric Girk2c channels is unaffected by SNX27 expression, suggesting that another factor(s) may influence the trafficking fates of Girk channels [52].

The up-regulation of b isoform of SNX27 (SNX27b) in response to *in vivo* exposure to the psychostimulants cocaine and amphetamine is particularly intriguing in light of recent observations that $GABA_B$-Girk signaling is weakened by acute psychostimulant exposure in VTA dopamine and GABA neurons, and following chronic cocaine treatment in Layer 5/6 pyramidal neurons of the medial prefrontal cortex (mPFC) [47, 53–55] (discussed below). Similarly, the association between loss of SNX27 and Down syndrome [48] is interesting given that Girk signaling is enhanced in the hippocampus and cortex of a mouse model of Down syndrome [56, 57], and since Girk2 triploid mice recapitulated many of the neurological phenotypes associated with this syndrome [58]. While altered SNX27 expression and/or function will certainly impact a wide array of cell signaling pathways, these studies argue that SNX27-dependent alterations in Girk signaling may contribute to some of the cellular and behavioral deficits linked to psychostimulant addiction and Down syndrome.

R7 RGS proteins

GPCR-Girk signaling is negatively-modulated by Regulator of G protein Signaling (RGS) proteins [1, 2]. RGS proteins enhance the GTPase activity of Gα subunits, accelerating the deactivation of G protein signaling following agonist removal [59]. Accordingly, RGS proteins accelerate receptor-induced Girk current deactivation kinetics, among other influences [60]. Discrete protein modules confer unique functionality to specific RGS proteins [59], and these – together with their unique cell/tissue expression patterns – appear to promote interactions with Girk channels. For example, the selective expression of *Rgs2* in VTA dopamine neurons, and its preferential association with Girk3, weakens the coupling between $GABA_B$ receptors and the Girk2/3 heteromer [28]. Interestingly, changes in the expression of *Rgs2* in response to chronic GHB (and morphine) treatment correlated with altered $GABA_B$-Girk coupling in VTA dopamine neurons, and this neuroadaptation may contribute to the development of tolerance to GHB and other drugs of abuse.

Recent data show that Girk channels are modulated by RGS proteins in the R7 subfamily, which includes Rgs6, Rgs7, Rgs9, and Rgs11. The R7 RGS proteins possess a domain resembling the G protein Gγ subunit (G gamma-like domain, or GGL) that promotes association with the atypical 5^{th} member of the G protein Gβ family, Gβ5 [61]. The crystal structure of the Rgs9-Gβ5 complex reveals that the interaction between GGL and Gβ5

resembles that observed in conventional Gβγ dimers [62]. Not surprisingly, therefore, complexes formed between R7 RGS proteins and Gβ5 (Rgs/Gβ5) bind to Girk channels and modulate the kinetics of m_2 muscarinic receptor/Girk signaling in atrial cardiomyocytes (Rgs6/Gβ5) [63, 64] and $GABA_B$ receptor/Girk signaling in hippocampal CA1 pyramidal neurons (Rgs7/Gβ5) [44, 65]. Moreover, Gβ5 ablation enhanced the sensitivity of Girk channels to $GABA_B$ receptor stimulation in hippocampal neurons, indicating that Rgs/Gβ5 complexes can also negatively influence GPCR-Girk coupling efficiency [65]. Interestingly, Rgs6/Gβ5 complexes appear to modulate $GABA_B$-Girk signaling in cerebellar granule cells [66], showing that complexes containing either Rgs6 or Rgs7 are relevant to neuronal Girk signaling. In addition, the Rgs/Gβ5-Girk channel interaction may be controlled by the R7 RGS-binding protein R7BP [67], providing another layer of fine regulation of GPCR-Girk signaling and another potential therapeutic target.

Loss of the Rgs6/Gβ5-dependent modulation of Girk signaling in the heart correlates with bradycardia and enhanced parasympathetic influence [63, 64]. As dysregulation of the parasympathetic control of cardiac output has been linked to sick sinus syndrome, heart failure, and arrhythmia [68], selective targeting of the Rgs6/Gβ5-Girk axis may prove beneficial in clinical settings involving cardiac disorders. While the relevance of the Rgs7/Gβ5-dependent modulation of $GABA_B$-Girk signaling in the hippocampus is not understood, mice lacking Gβ5 were more sensitive to the sedative effect of the $GABA_B$ agonist baclofen [65]. Moreover, mice lacking R7BP exhibited enhanced thermal nociceptive thresholds and augmented analgesic effects of opioids and baclofen, consistent with its proposed role as a facilitator of the Rgs/Gβ5-dependent regulation of GPCR-Girk signaling [67]. As enhanced Girk signaling has been linked to depotentiation [69], a particular form of excitatory synaptic plasticity, and cognitive deficits associated with Down syndrome [58], it will be particularly important to explore the relationship between the Rgs7/Gβ5-regulation of Girk signaling and hippocampal-dependent learning and memory. Moreover, it will be interesting to probe the involvement of Rgs/Gβ5 complexes in other neuronal GPCR-Girk signaling pathways.

Plasticity of Girk signaling

Recent work has shown that the strength and sensitivity of neuronal Girk signaling is titratable and subject to regulation by multiple stimuli. The first clear example of Girk signaling plasticity came with the demonstration that stimulation protocols that evoked NMDA receptor-dependent long-term potentiation (LTP) of glutamatergic neurotransmission in hippocampal CA1 neurons also strengthened synaptic $GABA_B$-Girk signaling [70]. Subsequent work in cultured hippocampal pyramidal neurons revealed that neuronal activity triggered by NMDA receptor activation lead to a rapid increase in the levels of Girk channels (Girk1/2) on the somatodendritic membrane, and enhanced Girk signaling via the A_1 adenosine receptor [71]. Pharmacologic or genetic ablation of Girk signaling suggested that this neuroadaptation is critical for the depotentiation of excitatory LTP [69].

Drugs of abuse

As documented in Table 1, Girk signaling shapes many of the behavioral effects of drugs of abuse, including opioids, psychostimulants (cocaine), and ethanol. Exposure to drugs of abuse can alter neuronal Girk signaling in durable fashion in the reward circuitry, the core of which consists of interconnected neurons in the VTA, mPFC, and nucleus accumbens (NAc) [72]. For example, a single non-contingent exposure to cocaine suppressed $GABA_B$-Girk signaling by 50% in VTA dopamine (but not SNc dopamine) neurons for several days [53], paralleling the better-understood enhancement of glutamatergic neurotransmission occurring in the same neurons [72]. Acute psychostimulant exposure also persistently suppressed

$GABA_B$-Girk signaling in VTA GABA neurons [54]. Finally, repeated cocaine exposure suppressed $GABA_B$-Girk signaling in Layer 5/6 glutamatergic pyramidal neurons of the mPFC [55], the main source of glutamatergic input to the VTA and NAc. This neuroadaptation was specific for pyramidal neurons in the prelimbic cortex (as compared to pyramidal neurons in the infralimbic and motor cortices), and persisted for more than a month after the final cocaine injection. Moreover, persistent suppression of Girk signaling in Layer 5/6 of the mPFC pre-sensitized mice to the motor-stimulatory effects of acute cocaine. While more work is required to understand the behavioral relevance of drug-induced adaptations in Girk signaling in the reward circuitry, these early insights, along with the anatomic and cellular specificity of the neuroadaptations, and their durability, suggest that they drive and/or contribute to the persistent expression of addictive behaviors including drug-seeking, craving, and relapse.

Mechanism(s) of plasticity

Plasticity of Girk signaling triggered by neuronal activity and drugs of abuse involves the redistribution of Girk2-containing channels to and from the surface membrane (Figure 3). Enhanced Girk signaling in hippocampal pyramidal neurons triggered by NMDA receptor activation was linked to enhanced trafficking of Girk2-containing channels from recycling endosomes to the cell surface [71]. In contrast, increased intracellular labeling at the expense of surface labeling was seen for Girk2 in VTA dopamine and GABA neurons following acute cocaine and methamphetamine treatment [53, 54], respectively, and in Layer 5/6 pyramidal neurons following repeated cocaine [55]. Interestingly, a similar redistribution of the $GABA_B$ receptor from the surface to inside the cell was observed in VTA GABA and Layer 5/6 mPFC pyramidal neurons, but not in VTA dopamine neurons, suggesting that different mechanisms mediate Girk signaling plasticity in different cell types. Consistent with this premise, the methamphetamine-induced adaptation in VTA GABA neurons and cocaine-induced adaptation in Layer 5/6 mPFC pyramidal neurons were both blocked by pretreatment with a D_1 dopamine receptor antagonist, while the cocaine-induced adaptation in VTA dopamine neurons was D_2 dopamine receptor-dependent. Moreover, $GABA_B$-Girk signaling (but not somatodendritic inhibitory signaling via D_2 dopamine or α_2 adrenergic receptors, or somatodendritic $GABA_B$-dependent signaling that did not involve Girk channels) was suppressed by repeated cocaine in Layer 5/6 mPFC pyramidal neurons, while Girk signaling via both the $GABA_B$ and D_2 dopamine receptor was suppressed by acute cocaine in VTA dopamine neurons. These observations are reminiscent of the selective enhancement by neuronal activity of Girk signaling via the A_1 adenosine (but not $GABA_B$) receptor [69], and suggest that some forms of Girk signaling plasticity are compartmentalized within neurons, and presumably driven by the GPCR or other proteins within the signaling macrocomplex.

The trafficking of Girk channels and GPCR-Girk complexes to and from the cell surface that underlies the forms of plasticity described above appears to be regulated by phosphorylation. For example, the potentiation of synaptic $GABA_B$-Girk signaling triggered in parallel with LTP of glutamatergic neurotransmission was dependent on CaMKII activity [70]. And in cultured hippocampal neurons, CaMKII activation (via prolonged morphine treatment, activation of metabotropic glutamate receptors, or a constitutively-active CaMKII mutant) shifted the distribution of Girk2 from dendritic shafts to spines, leading to enhanced Girk signaling via 5-HT receptors and decreased $GABA_B$-Girk signaling [73]. While the direct molecular target of CaMKII was not determined in these studies, Girk2(Ser9) is a reasonable candidate. Phosphorylation of Girk2(Ser9), which sits upstream of a unique internalization motif (VL) [7], suppresses surface trafficking of Girk2-containing channels [71]. Conversely, dephosphorylation of Girk2(Ser9) via protein phosphatase 1 (PP1) promotes surface trafficking of Girk2-containing channels from recycling endosomes, explaining the

activity-dependent potentiation of Girk signaling linked to the depotentiation of LTP [69, 71]. While dephosphorylation of Girk2(Ser9) promotes enhanced surface distribution of Girk channels, psychostimulant-induced suppression of $GABA_B$-Girk signaling in VTA GABA and Layer 5/6 mPFC neurons is more likely related to the phosphorylation status of the $GABA_B$ receptor [54, 55]. Surprisingly, despite the durable nature of the drug-induced suppression of $GABA_B$-Girk signaling in these pyramidal neurons, acute intracellular treatment with the PP1/PP2a inhibitor okadaic acid restored normal Girk signaling.

Pharmacologic manipulation of Girk channels

Given the broad distributions and roles of Girk channels in the nervous system, and their contributions to cardiac and endocrine physiology, global and direct pharmacologic manipulation of Girk signaling should evoke an array of consequences, many undesirable (Table 1). Indeed, global constitutive ablation of *Girk2* triggers an array of phenotypes, most notably a shortened lifespan due to spontaneous lethal seizures [74]. While constitutive ablation of other Girk subunits is correlated with less severe phenotypes, the full therapeutic potential associated with inhibiting or enhancing Girk signaling will likely not be achieved without regional and/or Girk subunit-selective manipulation. For example, an agonist with selectivity for Girk2/3 heteromers, the Girk channel subtype that appears to be uniquely expressed in VTA dopamine neurons [28], could be useful as an anti-craving compound. In addition, drugs that selectively activate or inhibit Girk1/4 heteromers, and which cannot pass the blood-brain barrier, could represent efficacious therapies for certain types of arrhythmias. Indeed, two drugs that can inhibit Girk1/4 heteromers (NTC-801 and NIP-151) showed promise in preclinical studies in the treatment of atrial fibrillation [75, 76].

While Girk channels are activated in a G protein-independent manner by ethanol [77, 78], volatile anesthetics [79, 80], and the flavonoid naringin [81], and are blocked by an array of psychoactive compounds (many of which are clinically-relevant) (*e.g.*, [82]), most of the compounds have other primary molecular targets and/or there is little evidence for Girk subtype specificity or pharmacokinetic advantages. Recently, however, a new class of subunit-selective, efficacious, potent, and direct-acting Girk channel agonists and antagonists was identified [83–85]. The prototype (ML297) is strikingly selective for Girk1-containing heteromers, and was efficacious in mice in delaying seizure onset in a maximal electroshock model of epilepsy, and preventing convulsions and lethality in a chemically-induced epilepsy model [84]. Accordingly, this family of compounds and derivatives should afford an excellent opportunity to investigate the potential therapeutic benefits associated with direct activation or inhibition of Girk1-containing channels, and may serve as a platform for the identification of compounds that can discriminate across a wide range of channel sub-types.

Conclusions

Efforts by many research groups over the last two decades have revealed key functional, structural, and regulatory features of Girk channels. This body of evidence, combined with our evolving understanding of Girk channel contributions to physiology and disease, and a continually-improving capacity for pharmacologic manipulation, sets the stage for an exciting future of investigations into the therapeutic potential of this interesting and important channel class. Such efforts hold the promise of yielding novel therapeutic approaches to the treatment of many forms of neurological, cardiovascular, and neuroendocrine disorders.

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OUTSTANDING QUESTIONS

Pharmacology

The recent identification of the subunit-selective, efficacious, and potent direct modulators of Girk signaling represents an important step forward in this field, and should provide a foundation on which efforts in synthetic chemistry, molecular modeling/simulations, and crystallography synergize to yield new compounds with optimized pharmacodynamic and pharmacokinetic properties. When available, these compounds will greatly facilitate efforts directed at understanding the physiological relevance of Girk channels, permitting us to move beyond studies in mutant mice.

Macrocomplex formation

While a large body of evidence supports the existence of discrete signaling complexes containing Girk channels *in vivo*, gaps in our understanding of Girk channel trafficking and regulation argue that there are additional proteins that influence Girk function - either via direct or indirect physical interaction - remaining to be discovered. Moreover, understanding the molecular determinants of macrocomplex formation will be helpful as it can potentially provide the means to selectively manipulate Girk signaling in a subtle manner.

Physiological relevance

Further investigation into the physiological relevance of Girk channels is required to better understand opportunities for beneficial therapeutic manipulation. The development of novel pharmacological tools that can discriminate among the various Girk channel subtypes existing *in vivo* will facilitate this process, and will complement next-generation genetic approaches that can give region and cell-type dependent insights into the function of Girk channels formed by distinct combinations of subunits (including specific splice isoforms). In addition, efforts that build on the growing evidence linking mutations or polymorphisms in GIRK genes to human disease will be invaluable.

Plasticity

We are just beginning to understand the triggers and mechanisms underlying the plasticity in Girk signaling. Going forward, it will be important to understand more about the mechanisms involved in the dynamic modulation of Girk channel trafficking, including differences that may exist across distinct drugs of abuse, neuron populations, Girk channel subtypes, and GPCR-Girk combinations. It will be particularly interesting to explore the potential relationship between SNX27, the phosphoregulation of Girk trafficking, and the drug-induced adaptations in Girk signaling seen in the reward circuitry. Of course, understanding the physiological (and perhaps pathophysiological) relevance of the adaptations is the ultimate goal.

Highlights

- Girk channels are novel targets for therapeutic interventions in a broad array of human nervous system disorders.
- Girk channels exist in multi-protein complexes whose molecular composition can differ to suit the cell needs under specific circumstances.
- Girk signaling shapes many of the behavioral effects of drugs of abuse, including opioids, cocaine, methaamphetamine, and ethanol.
- Plasticity of Girk signaling triggered by neuronal activity and drugs of abuse involves the subcellular redistribution of the channel.

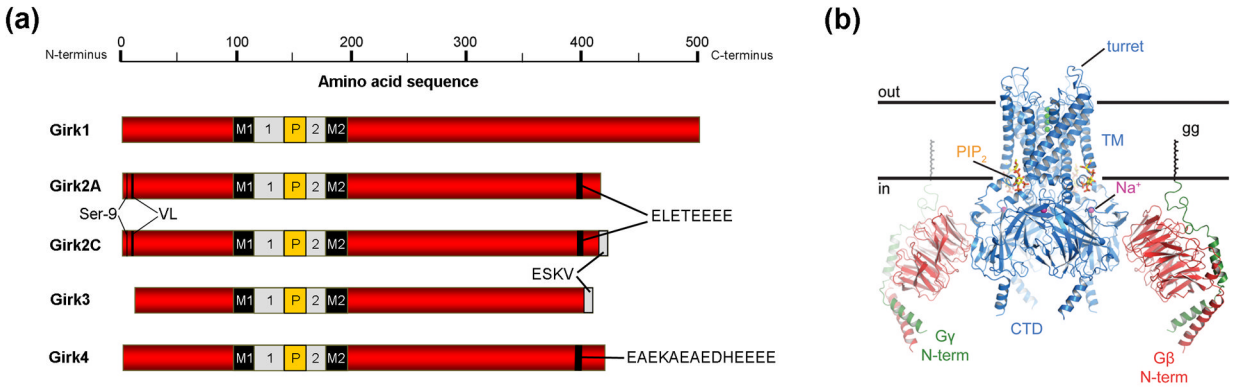

Figure 1. Girk channel structure

(a) Linear depiction of Girk channel subunits, including two prevalent Girk2 splice variants expressed in the mouse nervous system. The four Girk subunits exhibit a high degree of homology in the membrane-spanning (M1, M2), extracellular (1, 2), and pore (P) domains, with most inter-subunit differences observed in the distal N- and C-terminal domains. The Girk2 splice variants and Girk4 contain ER export signals (ELETEEEE and EAEKAEAEDHEEEE, respectively) not found in the other subunits [7]. Girk2 also exhibits an N-terminal internalization motif (VL), whose influence on channel trafficking is precluded by phosphorylation of Girk2(Ser9) [71]. Girk2C and Girk3 possess identical C-terminal PDZ interaction motifs (-ESKV). **(b)** Structure of the Girk-Gβγ complex from a side-view, with colors highlighting Girk2 (blue), Gβ (red), Gγ (green, with associated geranyl-geranyl (gg) lipid modification), PIP_2 (yellow/orange sticks), Na^+ ions (purple spheres), K^+ ions (green spheres within the pore). Note that there are 4 Gβγ binding sites per channel, and the Gβγ facing the viewer has been removed for clarity [16].

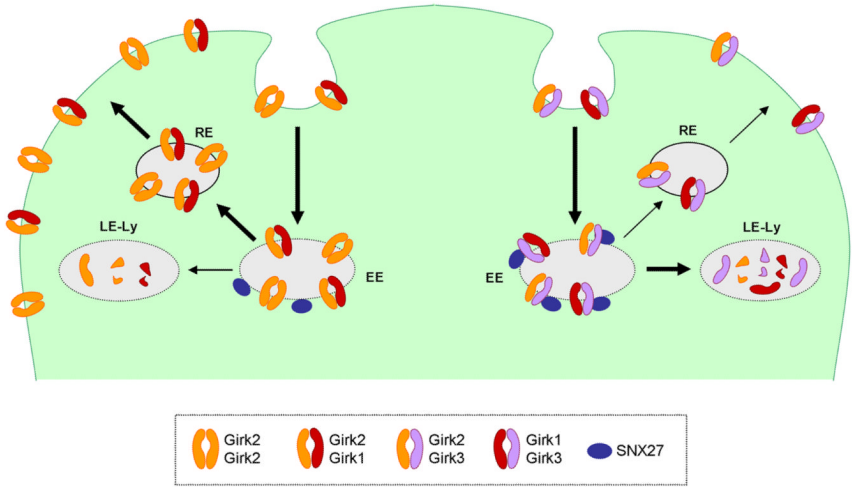

Figure 2. Subunit-dependent regulation of Girk channel trafficking by SNX27

Neuronal Girk channels are thought to internalize into early endosomes (EE), at which point they can be recycled back to the cell surface via recycling endosomes (RE) or diverted to late endosomes/lysosomes (LE-Ly) for degradation. Via a selective physical and functional association with Girk3, SNX27 enhances the trafficking of Girk3-containing channels to early endosomes, leading ultimately to increased lysosomal degradation and consequently, reduced numbers of Girk channels on the cell surface (right side of schematic). In contrast, SNX27 does not influence the trafficking of Girk2 homomers or Girk1/2 heteromers (left side of schematic).

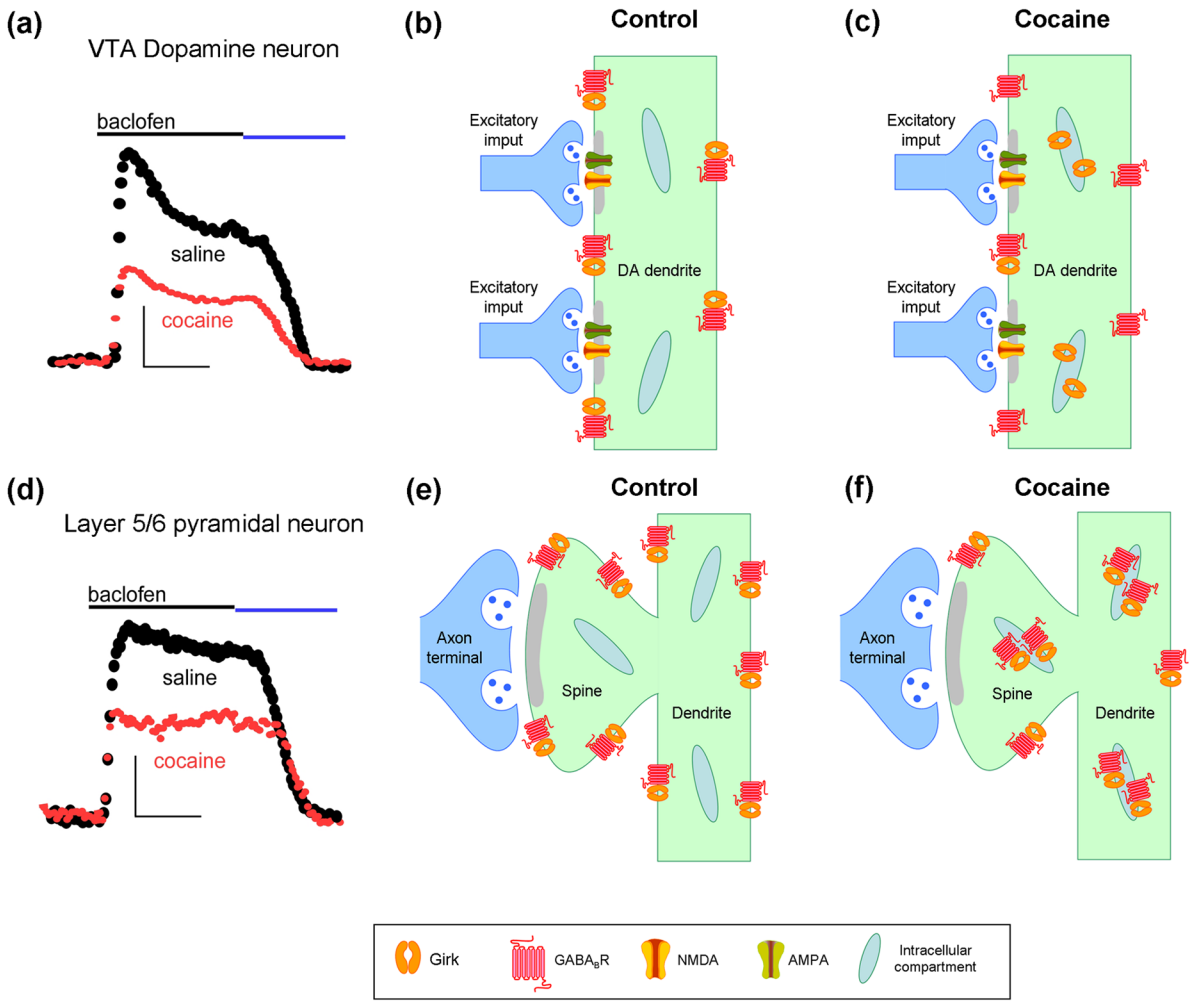

Figure 3. Plasticity of neuronal Girk signaling

Cocaine-induced suppression of Girk signaling in VTA (**a–c**) and mPFC (**d–f**). **a**) A single cocaine injection reduced baclofen-induced ($GABA_B$ receptor-dependent) Girk currents in VTA dopamine neurons, an adaptation that persisted for up to 5 days, required activation of D_2 dopamine receptors, and correlated with a redistribution of Girk2-containing channels (but not $GABA_BR$) from the cell surface to intracellular sites (**b,c**) [53]. **(d)** Repeated cocaine administration suppressed baclofen-induced Girk currents in Layer 5/6 pyramidal neurons, an adaptation that persisted for more than a month, required activation of D_1 dopamine receptors, and correlated with a phosphorylation-dependent redistribution of Girk2-containing channels and $GABA_B$ receptors from the cell surface to intracellular sites (**e,f**) [55]. Blue line in the current traces shows that the baclofen-induced current was reversed by a $GABA_B$ receptor antagonist.

Table 1

Physiological and pathophysiological relevance of Girk signaling

	Girk gene(s)	Mutation	Phenotype	References
Cardiovascular physiology				
heart rate	*Girk1, Girk4*	null	resting tachycardia	[86, 87]
parasympathetic regulation	*Girk1, Girk4*	null	decreased chronotropic response	[86, 87]
heart rate variability	*Girk4*	null	decreased variability	[86]
arrhythmia	*GIRK1, GIRK4*	multiple	Long QT, atrial fibrillation	[88–90]
hypertension	*GIRK4*	multiple	associated with aldosteronism	[91, 92]
Nociception				
thermal	*Girk1, Girk2*	null	hyperalgesia	[93, 94]
mechanical	*Girk2*	null	hyperalgesia	[93]
chemical	*Girk2*	null	hyperalgesia	[93]
Antinociception				
opioid	*Girk1, Girk2*	null	decreased analgesia	[93–97]
	Girk3	null	decreased sensitivity	[98]
	GIRK2	polymorphism	increased dosing requirement	[99, 100]
α_2 adrenergic	*Girk2,Girk3*	null	decreased analgesia	[93, 95, 98]
$GABA_B$	*Girk2*	null	decreased analgesia	[95]
cholinergic	*Girk2*	null	decreased analgesia	[95]
cannabinoid	*Girk2, Girk3*	null	decreased sensitivity	[95, 98]
Reward				
motor activity	*Girk1, Girk2*	null	enhanced basal and cocaine-induced	[101–103]
natural rewards	*Girk2, Girk4*	null	elevated responding for food	[104, 105]
	Girk2	triploid	enhanced sucrose intake	[58]
self-administration	*Girk2, Girk3*	null	decreased (cocaine)	[102]
	Girk2	null	enhanced consumption (ethanol)	[106]
dependence/withdrawal	*Girk2 & Girk3*	null	decreased opioid withdrawal	[97]
	Girk3	null	decreased sedative-hypnotic withdrawal	[107]
	GIRK2	polymorphism	association with ethanol intake and stress	[108]
Learning/memory				
spatial learning/memory	*Girk4*	null	decreased recall	[109]
fear conditioning	*Girk2*	triploid	decreased contextual recall	[58]
Anxiety	*Girk2*	null	anxiolysis	[104, 110]
Schizophrenia	*GIRK1*	polymorphism	genetic association	[111]
Seizure/epilepsy	*Girk2*	null	increased spontaneous and PTZ-induced	[74]
Energy homeostasis	*Girk4*	null	late-onset obesity	[105]
Thermoregulation	*Girk2*	null	decreased drug-induced hypothermia	[112]
Neurodevelopment	*Girk2*	*weaver*	loss of granule and dopamine neurons	[113]

The table lists outcomes from behavioral studies involving mice harboring mutant Girk subunits (null/knockout, triploid, or *weaver*) or from human linkage studies (*GIRK* gene in all capital letters) that identified polymorphisms or mutations in *GIRK* genes.