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Diverse Regulation of the Neuronal G-Protein Gated K^+ Channel (GIRK), GIRK1 and GIRK2 by Gα and Gβγ

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G-protein activated K^+ channels (GIRK, Kir3) mediate postsynaptic inhibitory effects of neurotransmitters via activation of G-protein coupled receptors, followed by activation of $G_{i/o}$ proteins and direct binding of Gβγ. The main neuronal GIRK channel is composed of GIRK1 and GIRK2 heteromers. These channels express in various regions of the brain. Unlike GIRK1, GIRK2 can also form function homomeric channels predominantly expressed in the substantia nigra. *In vitro* protein interaction studies showed that the binding of the whole cytosolic domain of GIRK1 to $G\alpha_{i3}^{GDP}$ or $G\alpha_{i3}^{GTP}$ was enhanced by the presence of Gβγ. This increment was not observed with GIRK2, implying subunit specific modulated by G-proteins.

Functional implication of these diversions were explored using homomeric GIRK1* (a pore mutant that forms functional homomers) and GIRK2 channels in *Xenopus* oocytes. In addition, two $G\alpha_{i3}$ mutants were utilized to simulating the GTP/GDP bound states: ''constitutively active'' $G\alpha_{i3}$Q204L (''QL''; poor GTPase) and ''constitutively inactive'' $G\alpha_{i3}$G203A (''GA'') which forms a stable complex with Gβγ. GIRK2 behaved like a ''classical'' Gβγ effector, demonstrating very low basal activity and strong Gβγ-dependent activation, while Gα expression was without effect. GIRK1* exhibited large basal currents and no response to coexpressed Gβγ, whilst retaining activation by agonist. Furthermore, in excised patches GIRK1* homomers displayed a reverse correlation between the basal activity and the Gβγ evoked currents. $G\alpha_{i3}$GA restored the ability of Gβγ to activate GIRK1*, whereas $G\alpha_{i3}$QL elicited no effect.

These results suggest a specific role for GIRK1 as the scaffold for $G\alpha_i\beta\gamma$ within GIRK-G-protein signaling complex, while GIRK2 is the Gβγ sensitive, responsive subunit. Moreover, we suggest that GIRK1/2 may contribute to regulation of resting potential and excitability in neurons, whereas GIRK2 homomers serves as a high-gain neurotransmitter-induced inhibitory relay.

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Both "Constitutively-active" and "Inactive" $G\alpha_{i3}$ Mutants Interact with GIRK1/2 Heterotetramer

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Biophysical and imaging studies of the G-protein-inward-rectifying-K^+-channel (GIRK), point towards the existence of multiprotein complexes of channel, G-proteins and occasionally G-Protein-coupled-receptors (GPCR). It is established that GIRK directly interacts with the Gβγ subunits, before and after receptor activation, whereas GIRK's interaction with the $G\alpha_i$ subunit remains unresolved. We previously reported that GIRK is closely regulated by the $G\alpha_i$ subunit. Biochemically, we find that the full cytoplasmic domain of the channel (G1NC) binds $G\alpha_i$. This interaction is enhanced in the presence of Gβγ, with either GDP or GTPγS. Our findings demonstrate that both N- and C- termini of the channel, when associated with Gβγ, form a favorable 3D binding domain for the active and the inactive $G\alpha_{i3}$ subunit.

We assessed the interaction between GIRK and G proteins with fluorescence resonance energy transfer (FRET), using a doubly-labeled channel (DL-GIRK1), showing that the DL-GIRK channel acts as a sensitive reporter for the presence of G-proteins. DL-GIRK1 coexpressed with Gβγ displayed an increase in FRET, implying the nearing of N- and C-termini. Both phosducin and $G\alpha_{i3}$GA (''constitutively-inactive'' mutant, $G\alpha_{i3}$G203A) caused a dramatic decrease in both currents and FRET, probably by deviating Gβγ from its activation site. Nevertheless, coexpression of $G\alpha_{i3}$GA and Gβγ restored the channel's open conformation as reported by FRET and currents, whereas phosducin did not. An observed increase in FRET, with coexpressed $G\alpha_{i3}$Q204L (''constitutively-active'' $G\alpha_{i3}$ mutant) and Gβγ, strongly supports our biochemical findings of constitutive interaction. We tested the effect of Gαβγ on a doubly-labeled G-protein-insensitive-inward-rectifier-K^+-channel, Kir2.1 (DL-IRK1). DL-IRK1 failed to demonstrate any changes in FRET with coexpressed G-proteins.

Our findings imply that GIRK acts as the nucleator of the GIRK-Gα-Gβγ complex. Both active and inactive $G\alpha_{i3}$ remain bound to the channel, ensuring fast and specific activation and termination of the signal.

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Structural alterations in the cytoplasmic region of G protein-gated inward rectifier potassium channel, Kir3.2

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G protein-gated inward rectifier potassium (K_G) channel underlies the deceleration of the heartbeat upon vagal nerve stimulation and the formation of slow inhibitory postsynaptic membrane potential in neurons. The K_G channels are tetramers and either heteromeric or homomeric assembly of Kir3.1-Kir3.4 subunits and their splicing variants. Like the other inward rectifiers, the K_G channel possesses two distinct domains, a transmembrane domain and a cytoplasmic domain. The cytoplasmic domain of K_G channel containing either Kir3.2 or Kir3.4 is thought to interact with channel activators such as G protein βγ subunit, intracellular Na^+ and PIP_2, and control the channel gating at the transmembrane domain. However, it is not clear how such activators interact and cause the structural alteration in the cytoplasmic region of K_G channel. In this study, we prepared protein crystals of the cytoplasmic region of K_G channel subunit Kir3.2 in the presence or absence of its channel activator Na^+, and then compared both crystal structures. Essential conformational changes between two structures were observed around aspartate 228 on CD loop which was in the vicinity of the plasma membrane. The change in the structure affected on the interaction between N- and C-termini and yielded the different positions of β strand in N-terminus. The aspartate 228 is thought to be responsible for the Na^+-dependent activation, and the interaction between N- and C-termini is also known to be crucial for the regulation in the channel gating of inward rectifiers. These observations suggested that the K_G channel activator Na^+ caused structural alterations restricted at the membrane-facing area in the cytoplasmic region, leading to the regulation of the channel gating at the transmembrane domain.

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Analysis of GIRK Subunit Intracellular Domain Association and Channel Function

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The G protein coupled inwardly rectifying potassium (GIRK) channels are key mediators of cell excitability. In previous studies, we have identified important GIRK1 subunit residues that are critical to channel function and N- and C-terminal domain association. To further explore the specificity of these interactions and the functional implications, we have focused on determining if the nature of these associations is dependent upon a hydrophobic region in the N- and C-terminal domains of the GIRK1 and GIRK4 subunits. Mutations of the GIRK1 N- or C-terminal residue to the complementary residue did not significantly alter the carbachol-induced channel activation whereas mutation to a charged arginine greatly altered the association of domains and channel function. Similarly, mutation of the homologous GIRK4 subunit N- and C-terminal residues alters channel function and affects association between domains. Further analysis confirms that the C-terminal mutation to the complementary N-terminal residue preserved the termini association while mutation to a charged residue disrupts the association suggesting that this region is critical for interaction.

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Unique Role For The -5′ Position In The Carboxyl Terminus Of GIRK3 Channel In Determining Binding Specificity To The PDZ Domain Of Sorting Nexin 27

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We recently established that sorting nexin 27 (SNX27) regulates trafficking of neuronal GIRK2/3 but not the closely related IRK1 channels. This regulation is achieved by the interaction of the C-terminal PDZ binding motif (-SKV) of GIRK3 with the PDZ domain of SNX27. We also found that IRK1(-SEI) binds to PSD95-PDZ2 but not to SNX27-PDZ. Due to the similarity in the PDZ binding motif between IRK1 and GIRK3, we hypothesized that amino acids in the -4′ and -5′ position are important for determining PDZ binding specificity. Using in vitro binding assays, we discovered that exchanging the these amino acids, IRK1(-ESESKV) and GIRK3(-RRESKV), reversed the binding specificity, suggesting a critical role for -5′ glutamate for binding SNX27 and -5′ arginine for binding PSD95. Further mutagenesis in IRK1 revealed that aspartate or glutamine substitution at -5′ position, IRK1(-DRESKV) and IRK1(-QRESKV), promotes binding to SNX27-PDZ. Surprisingly, substitution with the large, hydrophobic tryptophan enhanced SNX27 binding more than the wild-type glutamate; IRK1(-WRESKV) was 5 ± 1.3 fold greater than IRK1(-ESESKV). We next investigated the consequence of altering the PDZ binding specificity using a clustering assay. CFP-tagged IRK1 or GIRK3 were co-expressed with YFP-tagged PSD95 or SNX27 in COS7 cells. Using TIRF microscopy to visualize