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Crystal Structure of the Mammalian GIRK2 K^+ Channel and Gating Regulation by G Proteins, PIP_2, and Sodium

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SUMMARY

G protein-gated K^+ channels (Kir3.1–Kir3.4) control electrical excitability in many different cells. Among their functions relevant to human physiology and disease, they regulate the heart rate and govern a wide range of neuronal activities. Here, we present the first crystal structures of a G protein-gated K^+ channel. By comparing the wild-type structure to that of a constitutively active mutant, we identify a global conformational change through which G proteins could open a G loop gate in the cytoplasmic domain. The structures of both channels in the absence and presence of PIP_2 suggest that G proteins open only the G loop gate in the absence of PIP_2, but in the presence of PIP_2 the G loop gate and a second inner helix gate become coupled, so that both gates open. We also identify a strategically located Na^+ ion-binding site, which would allow intracellular Na^+ to modulate GIRK channel activity. These data provide a structural basis for understanding multiligand regulation of GIRK channel gating.

INTRODUCTION

G protein-gated K^+ (GIRK) channels are members of the inward rectifier (Kir) channel family, so named because the outward flow of K^+ ions is inhibited by intracellular polyamines and Mg^{2+}, which block the pore in a voltage-dependent manner. Kir channels play an essential role in many physiological processes, including neuronal signaling, kidney function, insulin secretion, and heart rate control. Mutations of Kir channels underlie numerous diseases including primary aldosteronism, Andersen syndrome, Bartter syndrome, and congenital hyperinsulinism (Choi et al., 2011; Hibino et al., 2010).

All Kir channels share the same basic topology: four subunits combine to form a canonical K^+ pore-forming transmembrane domain (TMD) and a large cytoplasmic domain (CTD) (Figure 1A). It is thought that ion conduction may be regulated by two gates in series: one formed by the inner helices of the TMD (Doyle et al., 1998; Jiang et al., 2002), and the other by the G loop at the apex of the CTD (Nishida et al., 2007; Pegan et al., 2005). Various regulatory molecules are thought to control these gates, but the control mechanisms are still unknown.

The anionic lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) is essential for the activation of all Kir channels (Hibino et al., 2010). GIRK channels (Kir3.x) are unique in that they also require G proteins, in combination with PIP_2, for activation (Huang et al., 1998; Logothetis et al., 1987; Reuveny et al., 1994; Sui et al., 1998; Wickman et al., 1994). Certain GIRK channel subtypes are also modulated by intracellular Na^+ ions (Ho and Murrell-Lagnado, 1999a, 1999b; Lesage et al., 1995; Sui et al., 1996; Sui et al., 1998). GIRK channel activation elicits the flow of K^+ ions across the cell membrane and thus drives the membrane voltage toward the Nernst potential for K^+. Near the K^+ Nernst potential, voltage-dependent Na^+ and Ca^{2+} channels tend to be silenced and therefore electrical excitation is diminished. This is an important signaling mechanism by which hormone and neurotransmitter stimulation of G protein-coupled receptors (GPCRs) regulates many essential physiological processes (Lüscher and Slesinger, 2010). For example, acetylcholine secreted by the vagus nerve controls heart rate through stimulation of muscarinic GPCRs in cardiac pacemaker cells (Logothetis et al., 1987; Pfaffinger et al., 1985).

Electrophysiological studies have sought to understand how the various ligands—G proteins, PIP_2, and Na^+—interact simultaneously with GIRK channels to regulate their gating. These studies suggest that G proteins and Na^+ function in a codependent manner with PIP_2 to open GIRK channels (Huang et al., 1998; Sui et al., 1998; Zhang et al., 1999). Here, we present crystal structures of a quiescent, closed GIRK2 channel and of a point mutant that is constitutively active, independent of G protein stimulation. Further, both structures are determined in the absence and presence of PIP_2. These structures render a molecular mechanistic description of multi-ligand regulation of GIRK channels.

RESULTS

Closed Structure of GIRK2

Four GIRK channel isoforms (Kir3.1–Kir3.4) associate into various homo-/heterotetrameric complexes. GIRK2 (Kir3.2) forms functional homotetramers and is thus a good candidate

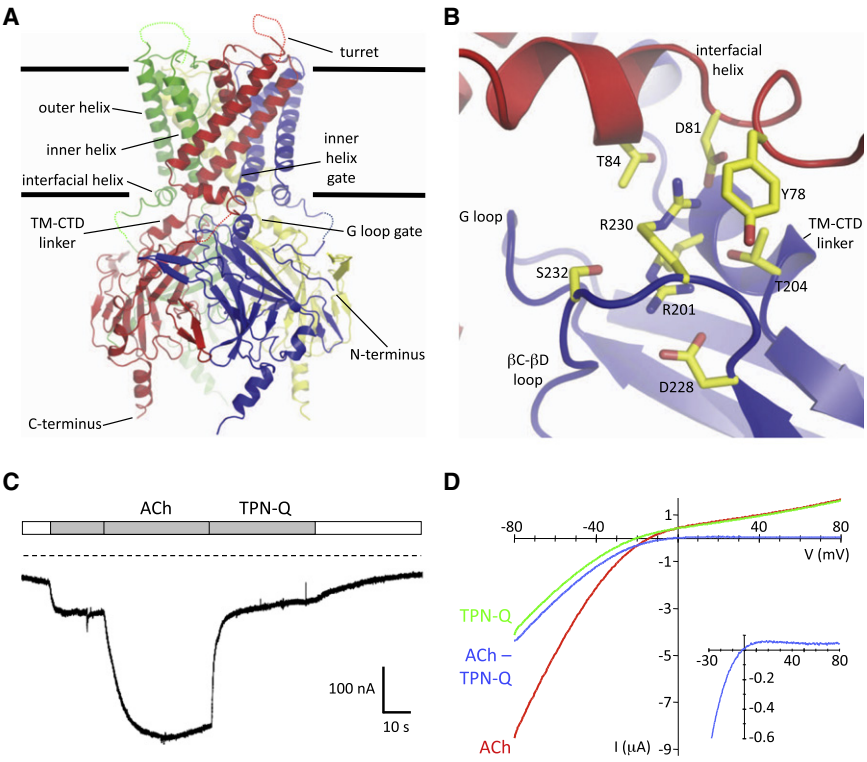

Figure 1. Structure and Function of the GIRK2 Channel

(A) Cartoon diagram of the GIRK2 structure. Each subunit of the tetramer is a different color. Unmodeled segments of the turret and N-terminal linker are drawn with dashed lines. The approximate boundary of the phospholipid bilayer is indicated by the thick black lines. The extracellular surface is on top.

(B) A cartoon diagram of key residues that mediate the contacts at the interface between the cytoplasmic and transmembrane domains. The same coloring scheme as in (A) is used.

(C) Representative example of a two-electrode voltage-clamp recording from *Xenopus laevis* oocytes expressing the truncated GIRK2 construct used for crystallography, held at −80 mV. The white bar indicates a physiological extracellular solution containing 96 mM NaCl and 2 mM KCl, whereas the gray bars represent a solution of 98 mM KCl only. The application of 10 μM acetylcholine (ACh) or 1 μM tertiapin-Q (TPN-Q) is also indicated. The dashed line represents zero current—all traces under this line represent negative, inward currents.

(D) Voltage ramps of oocytes expressing the same truncated GIRK2 construct. Currents were measured using two-electrode voltage clamp with an extracellular solution of 98 mM KCl, in the presence of 1 μM ACh (total current, red) or 1 μM ACh + 100 nM TPN-Q (background current, green). GIRK2-specific current (total minus background) is shown as the blue trace. The inset graph is the same data, but with a different scale for the axes to illustrate the degree of rectification.

See also Figure S1 and Table S1.

for crystallographic structure determination (Kofuji et al., 1995). To obtain suitably diffracting crystals, we modified the mouse GIRK2 complementary DNA (cDNA) to remove unstructured regions of the N and C termini (Nishida et al., 2007; Nishida and MacKinnon, 2002; Pegan et al., 2005; Tao et al., 2009). The resulting channel differs from the corresponding human ortholog by only one amino acid near the structured end of the C terminus (Asn377 is Ser in human GIRK2) (Figure S2 available online). This channel with unstructured regions of the N and C termini removed, which we refer to as wild-type in this study, exhibits the fundamental characteristics of the full-length GIRK2: G protein activation, inhibition by tertiapin-Q, and a strongly rectifying current-voltage curve (Figures 1C and 1D).

Wild-type GIRK2 crystals diffracted X-rays to 3.6 Å resolution. Initial phases were determined by molecular replacement with a GIRK2 CTD structure, and a model was built and refined to working and free residuals (R_w/R_f) of 26.0%/27.3% (Figures 1A and 1B and Table S1) (Inanobe et al., 2007). The overall architecture of GIRK2 is similar to the G protein-independent "classical inward rectifier" channel Kir2.2 (Tao et al., 2009), but has two significant differences. First, the turrets surrounding the extracellular entryway to the pore form a wider, more open vestibule in GIRK2 (Figures S1B and S1C). This structural difference may provide a simple explanation for pharmacological differences between classical inward rectifiers and GIRK channels. Many GIRK channels, including GIRK2, are inhibited by certain pore-blocking toxins such as tertiapin, as shown in Figure 1C, whereas classical inward rectifier channels are not (Jin and Lu, 1999). The more open turrets in GIRK2 would allow tertiapin to fit into the vestibule, whereas the more restrictive turrets in classical inward rectifiers appear to prevent toxin binding. The second structural difference occurs at the interface between the TMD and CTD. In Kir2.2 the CTD is extended away from the TMD in the absence of PIP_2, whereas in GIRK2 the two components are tightly juxtaposed (Figures 1A and 1B and Figure S1A) (Tao et al., 2009).

The TMD-CTD interface in GIRK2 is mediated by both hydrophilic and hydrophobic interactions between the interfacial helices of the TMD, the TM-CTD linker, and the βC-βD loop of the CTD (Figure 1B). These interactions were absent in the more extended Kir2.2 structure (Tao et al., 2009). It seems likely that they play an important role in the control of GIRK2 channel activity because they are in close proximity to the two constrictions along the ion pathway that have been hypothesized to function as gates. One gate—the inner helix gate—is formed by the inner helices of the TMD, just inside the membrane, above the level of the interfacial helix (Figure 1A and Figure S1A). Another gate—the G loop gate—is formed by the G loop at the apex of the CTD, just outside the membrane, below the level of the interfacial helix (Figure 1A and Figure S1A). In this structure of GIRK2, both gates are tightly closed.