Specificity and Diversity in \(G_{i/o}\)-Mediated Signaling: How the Heart Operates the RGS Brake Pedal

Thomas Wieland, Stefan Herzig

\(G\)-protein–coupled receptors (GPCRs) are involved in the regulation of virtually every physiological process. These receptors operate by catalyzing the GDP/GTP exchange at a coupled heterotrimeric G protein (Gaβγ), thereby promoting the dissociation of the heterotrimer into a free GTP-liganded Ga-subunit and a Gβγ dimer. Both Ga and Gβγ-dimer then regulate the activity of effectors, eg, second-messenger producing enzymes and ion channels. The duration of G protein activation is primarily controlled by the intrinsic GTPase activity of Ga. On GTP hydrolysis, Ga returns to the GDP-bound conformation and reassembles with the Gβγ dimer. More than 100 different GPCRs have been detected in cardiovascular cells, some of which are coupled to members of the pertussis-toxin (PTX)-sensitive \(G_{i/o}\) subfamily of heterotrimeric G proteins. An intense focus of investigation has been the mechanism(s) by which such a wide array of specific signals can be channeled through a very limited number of multifunctional G protein subunits, and yet retain specificity when reaching their ultimate molecular targets, such as enzymes or ion channels. In recent years, such “preferential coupling” has been attributed to spatially restricted signaling complexes, formed in lipid rafts and caveolae, and held together by anchoring or scaffolding proteins.

Within this context, regulators of G protein signaling (RGS) proteins are of particular interest. RGS proteins were first identified as GTPase Activating Proteins (GAPs) which speed proteins to and from their catalytic state. However, GAPs also play a role in Gi/o-mediated signaling. They contribute to the complexity in Gi/o-mediated signaling due to several factors: (1) Certain RGS2, which contain an acidic CaM binding domain, and RGS5, with a conserved homology domain, which contains the GTPase accelerating \(G_{i/o}\)-GAP activity, but during depolarisation \(Ca^{2+}\) enters the cell, and level of sympathetic (\(\beta_2\)-adrenoceptor) tone. The ACh-activated inward rectifier potassium current \(I_{\text{K,ACH}}\) is carried by a potassium–regulated potassium channel (GIRK, a tetramer consisting of Kir3.1 and Kir3.4 subunits). For opening, it requires Gβγ dimers released from activated Gi/Go proteins.7 Inhibition of cardiac \(I_{\text{K}}\) (HCN1,2 and 4 tetramers, see reference 10), and of \(I_{\text{Ca,L}}\) (a heterotrimer of a Cav1.2 pore and accessory \(\beta_1\)- and \(\delta\)-subunits) depend on inhibition of \(CAMP\) production (direct or PKA-mediated effects, respectively) through \(G_{\alpha}\) and \(G_{\alpha}\), as revealed by their PTX-sensitivity and, more specifically, in knockout mice.11-13

Given this functional diversity of bradycardic signals and mechanisms, what is the role of G protein regulating RGS proteins? Instead of laboriously creating knockout mice of the many, and likely functionally redundant, RGS proteins (note that despite 10 years of RGS protein research only 2 knockout mice14,15 have been published), Fu et al6 took advantage of a very simple molecular switch that specifically prevents any RGS GAP effect on specific mutants of \(G_{i/o}\). Using a knock-in of such an RGS-insensitive \(G_{i/o}\) mutant, they demonstrate a major sensitization of rate regulation by muscarinic agonist on elimination of RGS protein control of \(G_{i/o}\), both in stem cell–derived cardiocytes and living mice. Of note, this sensitization is entirely abolished by a blocker of GIRK, arguing for an RGS-sensitive preferential coupling chain of \(M_{2}\)–\(G_{i/o}\)–Gβγ–GIRK. Similarly, the authors show involve-

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ment of RGS proteins in preferential A₂Gαₐ coupling, and β₂Gαq (Gαq) coupling. Because pharmacological blockers remain imperfect tools, the ion channel species encountered have not been directly identified in this study. However, the strength of the current approach lies in the ingenious method, which preserves the natural arrangement and stoichiometry of signaling proteins, and can be scrutinized from molecular to in vivo levels. The concentration dependence of agonist effects confirms that under physiological conditions, there is no exclusive coupling through specific Gαo proteins. However, in the classical case of muscarinic control of inward rectifier potassium channels, the role of RGS proteins deserves as much attention as the discoveries of nondissociation of activated Gaβγ, or coregulation of GIRK by Gaq.

In a next step, thorough understanding of RGS protein function may be achieved by double-mutant approaches, specifically rescuing one pair of RGS protein and Gα subunit species. Indeed, such an approach has been reported for the interactions of RGS16 and RGS4 with Gαo and Gαq, respectively. A highly conserved Glu residue was replaced by Lys on the RGS protein. The complementary Lys residue on the Gα subunit which forms an interacting salt bridge at the RGS–Gαo interaction surface was changed to a Glu, producing RGS and Gα mutants which exhibit significantly reduced interactions with their “natural” counterparts. Both mutants, however, form a fully functional RGS protein–Gα subunit pair as proven by in vitro GAP activity and functional inhibition at the cellular level. By applying such mutant pairs to the system described by Fu et al., divergent functions of apparently redundant Ga isoforms and RGS proteins could be addressed at a new level of scientific rigor and physiological significance. Embryonic stem cell–derived cardiocytes hold promise as an excellent system for use of the molecular-genetic toolbox in modern cell biology and electrophysiology, provided that their developmental biology, eg, of signal transduction, is taken into account. Ultimately, the creation of transgenic animals, as done by Fu et al., and the possibility of crossing of such transgenic animals will provide fertile ground for novel and unsuspected insights into signaling specificity, with possible therapeutic relevance beyond cardiovascular diseases.

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