Multi-Tasking RGS Proteins in the Heart
The Next Therapeutic Target?

Evan L. Riddle, Raúl A. Schwartzman, Meredith Bond, Paul A. Insel

Abstract—Regulator of G-protein–signaling (RGS) proteins play a key role in the regulation of G-protein–coupled receptor (GPCR) signaling. The characteristic hallmark of RGS proteins is a conserved ~120-aa RGS region that confers on these proteins the ability to serve as GTPase-activating proteins (GAPs) for $G_s$ proteins. Most RGS proteins can serve as GAPs for multiple isoforms of $G_s$ and therefore have the potential to influence many cellular signaling pathways. However, RGS proteins can be highly regulated and can demonstrate extreme specificity for a particular signaling pathway. RGS proteins can be regulated by altering their GAP activity or subcellular localization; such regulation is achieved by phosphorylation, palmitoylation, and interaction with protein and lipid-binding partners. Many RGS proteins have GAP-independent functions that influence GPCR and non-GPCR–mediated signaling, such as effector regulation or action as an effector. Hence, RGS proteins should be considered multifunctional signaling regulators. GPCR-mediated signaling is critical for normal function in the cardiovascular system and is currently the primary target for the pharmacological treatment of disease. Alterations in RGS protein levels, in particular RGS2 and RGS4, produce cardiovascular phenotypes. Thus, because of the importance of GPCR-signaling pathways and the profound influence of RGS proteins on these pathways, RGS proteins are regulators of cardiovascular physiology and potentially novel drug targets as well. (Circ Res. 2005;96:401-411.)

Key Words: RGS protein ■ regulator of G-protein signaling ■ GPCR ■ heart

G-protein–coupled receptors (GPCRs) are found ubiquitously through the body and are involved in virtually every physiological process. Cardiovascular cells possess >100 different GPCRs.1 GPCRs bind a heterotrimeric G-protein complex of $\alpha$, $\beta$, and $\gamma$-subunits. G-Proteins are divided into four families (eg, $G_s$, $G_i$, $G_q$, and $G_12$) based on similarity of $\alpha$-subunits among individual family members;2 at least 20 $\alpha$-subunits are found in mammalian cells.3 Agonist binding of GPCRs promotes G-protein activation. This activation is achieved by catalyzing GDP–GTP exchange on the $\alpha$-subunit. A conformational change in the GTP-bound $\alpha$-subunit leads to a dissociation of $G_s$ from the $\beta\gamma$-subunits. GTP-bound $G_s$-subunits and dissociated $\beta\gamma$-dimers regulate downstream effectors. These signaling events are terminated as a consequence of intrinsic GTPase activity of the $G_s$-subunit, which hydrolyzes bound GTP to GDP, resulting in a reassociation of the G-protein heterotrimer. The intrinsic GTPase activity of $\alpha$-subunits is generally insufficient to
correlate with physiological rates of G-protein inactivation, but this activity can be accelerated by the presence of GTPase-activating proteins (GAPs), such as regulator of G-protein–signaling (RGS) proteins.

The discovery of RGS proteins suggested the possibility of a specific RGS protein for each Gα subtype. However, this idea was discarded when it was discovered that the number of RGS proteins (now >30) was greater than that of Gα subunits, along with the recognition that individual RGS proteins could act as GAPs for multiple families of Gα.

However, as understanding of RGS function increases, evidence has emerged for Gα specificity of certain RGS proteins, especially in the in vivo setting, as well as their modulation of distinct GPCR-mediated signaling pathways. Although a substantial amount is known regarding identity and expression of RGS proteins, much remains to be elucidated regarding their function, localization, and regulation. Their GAP activity is a primary characteristic, but RGS proteins use a variety of mechanisms to regulate signaling. Several excellent reviews have provided insight on the structure and function of RGS proteins.4–7 However, numerous recent developments related to RGS proteins have important implications, especially for cardiovascular physiology and pathophysiology. In this review, we first provide an overview of RGS structure, emphasizing non-RGS domains. Second, we discuss mechanisms for the regulation of RGS proteins. Finally, we bring the reader up to date on current knowledge of the importance of these “multitasking” RGS proteins in the cardiovascular system.

RGS Protein Structure

Figure 1 summarizes key features of RGS genes and proteins. Many RGS genes produce different isoforms (eg, rgs6 encodes 36 distinct transcripts). A description of each isoform is beyond the scope of this review. RGS proteins are diverse, ranging in size from 152 (RGS21) to 1376 (RGS12) amino acids. Many, indeed most, RGS proteins identified to date exhibit GAP activity toward the Gi and Gq families of G-proteins. However, it is important to note that even though an RGS protein may be able to serve as a GAP for a particular...
Gᵢ₃-subunit, particular GPCR signaling pathways involving the identical Gᵢ₃-subunit can show receptor-selective regulation by RGS proteins.⁹

On the basis of sequence similarity of the RGS domain, RGS proteins can be classified into one of five subfamilies: R4, R7, R12, RZ, and RL (Figure 1). Although some RGS proteins consist almost exclusively of the RGS domain, a conserved ≈120-aa region, others contain additional domains. The RGS domain is necessary and sufficient to confer GAP activity;¹⁰ however, other domains, to be discussed subsequently, can increase specificity, determine cellular localization, and provide additional activities.

**PSD-95 Disk-Large ZO-1 Domain**

PSD-95 disk-large ZO-1 (PDZ) domains, ≈90-aa regions with a highly conserved four-residue GLGF sequence, are involved in the clustering of multiprotein signaling complexes.¹¹ RGS12 and a variant of RGS3, PDZ-RGS3, contain PDZ domains. The PDZ domain of PDZ-RGS3 binds the non-GPCR E-phriment receptor, thereby providing a possible link between GPCR signaling and E-phriment signaling.¹² In addition, PDZ-RGS3 could be important for E-phriment-regulated cardiovascular development.¹³

The PDZ domain of RGS12 binds selectively to the interleukin-8 receptor B (CXCR2), a Gᵢ₃-coupled GPCR.¹⁴ Although RGS12 has GAP activity toward Gᵢ₃, it remains to be determined whether RGS12 alters CXCR2-mediated G-protein signaling. The interaction of RGS12 with CXCR2 has the potential to influence myocardial viability during ischemia reperfusion.¹⁵

**G-Protein γ Subunit–Like Domain**

RGS6, RGS7, RGS9, and RGS11 contain G-protein γ subunit–like (GGL) domains, a 64-aa region with a high level of similarity to the Gγ-subunit, can form dimers with particular G-protein subunits (ie, Gβ⁵) but not others (eg, Gβ1 to Gβ3).¹⁶ This RGS/Gβ⁵ interaction appears to influence RGS and Gβ⁵ protein stability.¹⁷,¹⁸ GAP activity of RGS proteins,¹⁶,¹⁹ and subcellular localization of RGS and Gβ⁵.⁸,²⁰

**GoLoco Domain**

RGS12 and RGS14 contain GoLoco domains, a 19-aa Gα, binding motif that acts as a guanine nucleotide dissociation inhibitor (GDI) by binding and stabilizing GDP-bound Gα, inhibiting the rate of exchange of GDP for GTP in a GAP-independent manner.²¹ The RGS and GoLoco domains of RGS14 can independently inhibit Gα₉ signaling, but both domains are necessary for maximal inhibition.²² The GoLoco domain of RGS14 exhibits GDI activity toward Gα₉ and Gα₁₃, but not Gα₂ or Gα₁, even though the GAP activity is not G-protein subtype selective.²³

**PX Domain**

RGS-PX1, the only RGS protein thus far identified with GAP activity for Gαᵢ₃, contains a PX domain, an ≈120-aa phosphoinositide-binding domain involved in membrane targeting.²⁴,²⁵ RGS-PX1 delays lysosomal degradation of the EGF receptor, most likely as a result of the sorting nexin function of the PX domain.²⁴ The relationship between RGS-PX1 and the EGF receptor suggests a possible role for RGS-PX1 in angiotensin II (Ang II)–induced cardiac hypertrophy.²⁶

**Disheveled EGL-10 Pleckstrin Domain**

The 70-aa disheveled EGL-10 pleckstrin (DEP) domains are present in various signaling proteins, including RGS6, RGS7, RGS9, and RGS11, but little is known about their function. The DEP domain of RGS9 appears to be critical for interaction with R9AP (RGS9 anchoring protein) and subcellular targeting of RGS9 to the rod outer segment.²⁷ The DEP domain of RGS7 can bind snapin, a protein that interacts with synaptosomal-associated protein of 25 kDa, a component of the soluble N-ethylmaleimide–sensitive factor attachment protein receptor complex, suggesting a role for RGS7 in synaptic vesicle exocytosis.²⁸ Interestingly, snapin was discovered recently to bind adenylyl cyclase type 6 (AC6),²⁹ a highly expressed isoform of adenylyl cyclase in the heart,³⁰ providing a possible link for RGS7 in the regulation of cAMP levels.

**Regulation of RGS Proteins**

RGS proteins have the potential to decrease or stop GPCR-mediated signaling. This important action implies that activity and expression of RGS proteins must be regulated. Indeed, RGS proteins are highly regulated through various mechanisms such as alterations in expression levels, subcellular localization, post-translational modifications, and binding partners. In addition to direct regulation of RGS proteins, the effects of RGS proteins can be regulated by post-translational modification of the G-protein α-subunit.

**Regulation of RGS Expression**

Increases or decreases in cellular levels of RGS proteins have the potential to be critical for RGS-induced regulation of G-protein signaling. Increasing evidence demonstrates that RGS protein and mRNA levels are dynamically altered by various drugs, second messengers, and disease states. Although numerous RGS proteins show dynamic expression, RGS2 provides an excellent example of a highly regulated RGS protein. Dopamine D₁ receptor agonists increase RGS2 mRNA, whereas a decrease in RGS2 mRNA occurs with agonism of the dopamine D₂ receptor.³¹ Increases in cAMP levels by forskolin appear to increase RGS2 protein levels.³² Alterations in RGS2 levels show pathophysiological relevance because an overabundance of RGS2 protein is seen in individuals with Bartter’s/Gitelman’s Syndrome.³³ and RGS2 knockout mice exhibit a severe cardiovascular phenotype.³⁴ as is discussed subsequently. Chronic pharmacological therapy, as commonly used in the treatment of cardiovascular disease, likely alters RGS expression. The impact of such alterations on signal transduction has not been well studied but could lead to a myriad of effects, including sensitization or desensitization of signaling pathways, side effects, tolerance, dependence, etc.

**Subcellular Localization**

In order for an RGS protein to actively serve as a GAP, it must be localized in a region in the cell where it can bind its target Gᵢ₃-subunit. Localization of RGS proteins in a partic-
ular subcellular compartment could increase the specificity of an RGS protein for particular G-proteins and GPCRs or pathway even though the GAP activity of an RGS protein may have multiple potential Gα targets.

On G-protein activation, RGS3 is translocated from the cytosol to the plasma membrane.35 In addition, the phosphorylation of RGS10 promotes its translocation from the plasma membrane and cytosol to the nucleus.36 The subcellular localization of RGS2 and RGS4 are dependent on specific G-proteins and GPCRs.37 In human embryonic kidney 293 cells, transfected RGS2 and RGS4 localize to the nucleus and cytosol, respectively. Whereas RGS2 translocates to the plasma membrane when cotransfected with Gq or Gi2 but not Gi1 RGS4 translocates to the plasma membrane when cotransfected with Gi2 but not Gq or Gs. A similar translocation profile is observed when GPCRs have been expressed that preferentially interact with specific G-protein family members. For example, the Ang II receptor (Gq-coupled) and β2-adrenergic receptor (Gs-coupled) promoted plasmalemmal translocation of RGS2, whereas RGS4 was only translocated by expression of the M2 muscarinic cholinergic receptor (mAChR; Gi-coupled). Unlike RGS3, the translocation of RGS2 and RGS4 does not appear to depend on G-protein activation.

Colocalization of proteins in a signaling pathway may be critical for signaling. Membrane microdomains, such as lipid rafts and caveolae, allow for the clustering and compartmentation of signaling molecules and are likely important for integrating GPCR-mediated signaling pathways.38 Many GPCRs move into or out of lipid rafts on agonist stimulation,39,40 an effect that could move the GPCR closer to or away from an RGS protein. Little work has been performed to determine whether RGS proteins are found in membrane microdomains. However, RGS16 localizes to lipid rafts on palmitoylation,41,42 which may be important for it to exert GAP activity for a particular signaling pathway.

### Phosphorylation and Palmitoylation

Phosphorylation and palmitoylation, which reportedly can occur for multiple RGS proteins, produce a variety of effects, including alterations in subcellular localization, protein stability, and alterations in GAP activity. However, the physio-

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PKC indicates protein Kinase C; PKA, protein Kinase A; SRC, Src family kinases; ERK2, extracellular signal-regulated Kinase 2.

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ND indicates not determined; *dependent on assay systems used.
logical importance of these modifications remains to be determined. Tables 1 and 2 provide a summary of RGS proteins known to be influenced by phosphorylation and palmitoylation, respectively. It is likely that most, if not all, RGS proteins are regulated by phosphorylation or palmitoylation; however, much work needs to be done in this area.

Phosphorylation and palmitoylation of \( G_{\text{protein}} \)-subunits can affect RGS GAP activity, in particular, decreasing such activity.

**Protein and Lipid-Binding Partners**

Several RGS protein-binding partners have been identified. Of particular interest are 14-3-3 proteins, which bind target proteins at phosphorylated residues. This binding is inhibited by tumor necrosis factor-\( \alpha \), which can decrease serine 434 phosphorylation. In contrast, 14-3-3 binds RGS3 at phosphoserine 264 in a region outside the RGS domain but still reduces the potency of RGS3 in the inhibition of G-protein signaling.

The GAP activity of many RGS proteins is inhibited by phosphatidylinositol-3,4,5-trisphosphate (PIP3), phosphatidic acid (PA), and lysophosphatidic acid, effects reversed by \( \text{Ca}^{2+}/\text{calmodulin} \). PIP3 and PA can bind RGS4 and additively inhibit GAP activity, which suggests multiple binding sites. The binding of \( \text{Ca}^{2+}/\text{calmodulin} \) can occur on two sites of RGS4 to reverse PA- and PIP3-mediated GAP inhibition without affecting GAP activity alone. The physiological significance of PIP3-mediated inhibition of RGS proteins includes regulation of \( \text{G-protein} \)-gated \( K^+ \) channels in cardiac myocytes, as is discussed subsequently.

**Receptor Selectivity**

As noted above, some RGS proteins can be very “promiscuous” in that they have GAP activity in vitro for many subtypes of \( G_{\text{protein}} \)-subunits. However, it has become apparent that RGS proteins are highly regulated and can be extremely specific in modulating distinct signal transduction pathways. In addition to the various methods of RGS regulation discussed above, RGS proteins may selectively alter GPCR signaling in a receptor-specific manner, as summarized in Table 3.

Many of the methods used to determine receptor selectivity involve overexpression of GPCRs or RGS proteins. Overexpression can lead to artificial results by producing “unnatural” protein levels. Alternatively, specific knockdown of proteins with ribozymes, antisense oligonucleotides, RNA interference, etc, avoids this problem, albeit introducing reagents with other potential sets of actions. Even so, one imagines that “knockdown strategies” can facilitate study of RGS/GPCR interactions in a setting with less potential for nonspecific perturbation than overexpression.

It is clear that the ability of a RGS protein to show GAP activity for a specific \( G_{\text{protein}} \)-subunit in vitro does not allow for the prediction of the precise signaling pathways that the RGS protein regulates in vivo. Because there are many factors that influence RGS activity or subcellular localization, it is not unreasonable to imagine cell-specific effects where a particular GPCR expressed in different cell types is differentially regulated by RGS proteins.

In addition to RGS selectivity for receptor signaling, Bernstein et al. have recently demonstrated differential physical binding of RGS proteins to mACHRs. RGS1, RGS2, RGS4, and RGS16 were tested for interaction with the third intracellular loops of each of the five mACHR subtypes. RGS2, but not RGS1 or RGS16, bound the \( M_3 \) mACHR, whereas none bound the \( M_1 \) mACHR. Moreover, RGS2, but not RGS16, colocalized with the \( M_3 \) mACHR at the plasma membrane and inhibited \( M_3 \) mACHR-induced phosphoinositide hydrolysis.

**RGS and G-Protein Selectivity**

As shown in Figure 1, most RGS proteins are GAPs for the \( G_i \) and \( G_o \) families of G-proteins. However, many RGS proteins show \( G_i \) subtype-selective activity, as demonstrated by the following. (1) RGS19 interacts with \( G_{i1} \), \( G_{i3} \), and \( G_{o2} \) but not \( G_{o3} \); (2) RGS4 shows selectivity for \( G_{o2} \) and \( G_{o1} \);
over Go_{\alpha_1} and Go_{\alpha_{12}}.^{60} (3) GPCR kinase 2 (GRK2) binds Go_{\alpha_1}, Go_{\alpha_{12}}, and Go_{\alpha_{13}} but not Go_{\alpha_{16}}.^{61} (4) RGS14 inhibits guanine nucleotide exchange on Go_{\alpha_1} and Go_{\alpha_3} but not Go_{\alpha_2}.^{25} and (5) A splice variant of RGS20, RGSZ1, is 100-fold selective for Go_{\alpha_2} over Go_{\alpha_1}.^{45}

Only one RGS protein, RGS-PX1, has thus far been found to exhibit GAP activity for the G_{i} family.^{24} Even though other RGS proteins do not regulate G_{i}-mediated signaling pathways via GAP activity, GAP-independent regulation occurs. Initial evidence demonstrated that RGS2 and a truncated form of RGS3, RGS3T, inhibited cAMP production.^{62,63} Later, Sinnarajah et al.^{64} showed that RGS1, RGS2, and RGS3, but not RGS4 or RGS5, decreased odorant-induced cAMP production in olfactory membranes. Those workers also demonstrated that RGS2 inhibits cAMP production from AC3, AC5, AC6, but not AC1 or AC2. This inhibition is achieved by the N terminal of RGS2 directly binding the C(1) domain of AC5.^{64,65}

p115Rho–guanine nucleotide exchange factor (GEF) is an RGS protein that shows selective GAP activity for members of the G_{i} family of G-proteins, Go_{\alpha_12} and Go_{\alpha_{13}}, but also has an additional functional role.^{66} Activated Go_{\alpha_1} stimulates p115Rho-GEF and enhances its GEF activity for the monomeric G-protein Rho.^{67} Rho is involved in a variety of cellular responses, such as actin stress fiber formation, gene transcription and transformation. Of particular importance to the cardiovascular system, Rho induces hypertrophic responses in isolated cardiac myocytes,^{68} and cardiac overexpression of Rho in mice results in the development of ventricular failure.^{69} Because p115Rho-GEF activates (via GEF activity on Rho) and prevents activation (via GAP activity on Go_{\alpha_{12}}) of Rho, it has a unique dual role in Rho signaling that may be critical for cardiovascular function. Although no other RGS proteins have been reported to be GAPs for Go_{\alpha_{12}}, RGS16 is involved in the inhibition of Go_{\alpha_{12}} signaling via a GAP-independent mechanism. RGS16 binds Go_{\alpha_{12}} and translocates it to detergent-resistant membranes, presumably lipid rafts, preventing effector interaction and therefore inhibiting Go_{\alpha_{12}} signaling.^{70}

RGS Proteins and the Cardiovascular System

GPCRs, and in turn, RGS proteins, are important regulators of cardiovascular signaling. Figure 1 demonstrates that the gene expression of virtually all known RGS proteins has been detected in the mammalian heart. Emerging evidence demonstrates that RGS proteins are needed for normal cardiovascular function and are altered in various cardiovascular disease states.

RGS2

Many GPCRs in the cardiovascular system responsible for vasoconstriction are coupled to G_{i}. More than half of the currently known RGS proteins exhibit GAP activity toward G_{i} and therefore have the potential to influence vascular tone. RGS2 shows some selectivity toward G_{i} and may be its most potent GAP.^{54,71} RGS2 has emerged as a potentially critical regulator of cardiovascular function because its GAP activity for G_{i} antagonizes G_{i}-mediated vasoconstriction. Although RGS2 can regulate G_{i} and G_{s} signaling (through GAP and non-GAP mechanisms), its potent regulation of G_{s} signaling appears to produce the most significant physiological effects.

Ang II is a vasoconstrictor, the effects of which are predominantly mediated through AT_{1}, a GPCR coupled to G_{s}. In cultured vascular smooth muscle cells, Ang II stimulates the gene expression of RGS2,^{72} a response that may serve as negative feedback regulation, because RGS2 could inactivate this pathway via its GAP activity on G_{s}. In mice deficient for the RGS2 gene, a strong hypertensive phenotype is observed.^{34} In anesthetized mice, this phenotype appears to be attributable exclusively to the Ang II signaling pathway because it can be reversed with an AT_{1} antagonist or blockade of Ang II production with an angiotensin-converting enzyme inhibitor. Interestingly, rgs2+/− and rgs2−/− mice exhibit a similar hypertensive phenotype, demonstrating that both copies of the gene are essential for normal cardiovascular function.

RGS2 is also regulated through a GPCR-independent pathway. NO is a potent vasodilator that induces the activation of cyclic GMP–dependent protein kinases (PKG). PKGs promote vascular relaxation through a variety of mechanisms including activation of RGS2.^{74} In particular, cGMP-dependent protein kinase I-α binds directly to and phosphorylates RGS2, which increases its GAP activity on G_{s} and results in vasodilation. In RGS2 knockout mice, this pathway is disrupted. Aortas from RGS2-deficient mice show increased vasoconstriction in vitro in response to G_{s}-coupled agonists and decreased relaxation in response to cyclic GMP.^{54}

RGS4

RGS4 is a key regulator of the G-protein–gated K+ (K_{c}) channels. Although it was discovered >80 years ago that acetylcholine (ACh) released from stimulation of the vagus nerve causes bradycardia,^{75} it was not until the recent discovery and characterization of RGS proteins that the kinetic mechanisms behind ACh-induced heart deceleration could be explained more fully (Figure 2). K_{c} channels found in sino-atrial node cells decrease heart rate when activated by G_{\beta}\gamma. At a resting diastolic state (with low intracellular Ca^{2+}) in the cardiac myocyte, PIP_{2} binds RGS4 within its RGS domain, preventing GAP activity.^{51–54} Because GAP activity is prevented, an ACh-bound M_{2} mACHR causes the heterotrimERIC G-protein complex to dissociate, allowing the G_{\beta}\gamma-subunit to bind and activate K_{c} channels, which leads to K^{+} efflux and cellular hyperpolarization. On depolarization and subsequent Ca^{2+} influx, the Ca^{2+}/calmodulin complex binds RGS4, relieving the PIP_{2} inhibition. This allows RGS4 to accelerate the GTPase activity of the α-subunit, promoting reassociation of the heterotrimeric complex and inactivation of the K_{c} channel. Because intracellular Ca^{2+} decreases, Ca^{2+}/calmodulin dissociates from RGS4, allowing PIP_{2} to again bind and inhibit RGS4. RGS proteins thus appear to speed the activation and deactivation of K_{c} channels.^{77}

In order for the kinetics of K_{c} channel activation and deactivation to approximate native conditions, RGS proteins must be present; however, it is still a subject of controversy whether physiological appropriate rates can be obtained.^{78}
perhaps as a consequence of assembly of different tetrameric KG channels. Increased deactivation can be explained by RGS GAP activity promoting reassociation of the heterotrimeric G-protein complex. RGS-induced increases in channel activation are more difficult to explain with no conclusive data available thus far.

Muslin et al.79–81 have characterized an additional role for RGS4 in influencing cardiac hypertrophy. Reversible exercise-induced cardiac hypertrophy is not detrimental, whereas chronic hypertrophy is associated with cardiac arrhythmias, congestive heart failure, and death.82–84 Initially, the overexpression of RGS4 in neonatal cardiac myocytes was observed to inhibit phenylephrine- and endothelin-1–induced hypertrophy.80 A second study used transgenic mice overexpressing RGS4 in ventricular tissue to study cardiac physiology.79 Overexpression of RGS4 did not affect basal cardiac function but significantly reduced the ability of the heart to adapt to an increase in cardiac afterload induced by transverse aortic constriction. Compared with littermate controls, the transgenic mice exhibited reduced ventricular hypertrophy, left ventricular dilation, depressed systolic function, and increased mortality in response to transverse aortic constriction. Thus, ventricular RGS4 overexpression appears to block beneficial compensatory hypertrophic mechanisms of the heart in response to an acute increase in cardiac afterload. Such results possibly suggest that increased cardiac RGS4 expression/activity would be unfavorable. However, a third study demonstrated favorable effects of RGS4 overexpression in mice that co-overexpress Gq.79 Transgenic mice overexpressing Gq in the heart exhibit a phenotype similar to human cardiac hypertrophy,85,86 but co-overexpression of RGS4 and Gq delays the Gq-mediated onset of cardiac hypertrophy.79

GRK2
GRKs decrease β-adrenergic receptor signaling via phosphorylation of the activated receptor and by antagonizing G-protein signaling. In addition to its kinase activity, GRK2 (also known as βARK1) is an RGS protein that shows weak GAP activity for Gq.87 However, GRK2-mediated inhibition of Gq signaling is likely attributable to its binding and sequestration of activated Gq instead of the weak GAP activity.87 GRK2 activity and expression are increased in human hypertension and heart disease, including heart failure.88–91 GRK2 inhibition can help prevent and blunt heart failure in animal models.92,93 In addition, the RGS domain of a kinase-inactivated mutant of GRK2 decreases endothelin-1 and Ang II signaling.94 Such data suggest that GRK2 has a dual role, perhaps in serving as a negative RGS (via its GAP activity and its sequestration of Gq) and in addition, as a receptor-desensitizing kinase for

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**Figure 2.** Schematic of the role of RGS4 in K+ channel activity. A, The muscarinic M2 GPCR (M2) is activated by the binding of Ach, which activates the associated G-proteins by catalyzing the exchange of GDP for GTP on the α-subunit. This causes the dissociation of the βγ-dimer, which binds and activates the K+ channel. RGS4 is inhibited by PIP3.3,4,5 B, As calcium (Ca2+) levels rise in the cell after depolarization, the Ca2+/calmodulin complex binds RGS4 to relieve the PIP3-mediated inhibition. This allows RGS4 to exert its GAP activity on the α-subunit, resulting in the hydrolysis of GTP. C, The GDP-bound α-subunit promotes the reassociation of the heterotrimeric G-protein complex, leading to an inactivation of the K+ channel. As intracellular Ca2+ levels decrease, Ca2+/calmodulin dissociates from RGS4, allowing PIP3 to bind and inhibit the GAP activity of RGS4.
GPCRs. The ability of GRK2 to interact with caveolin may also contribute to its localization and actions, including its RGS activity.95

**Human Heart Failure**

Although variable results have been obtained, the expression profile of RGS2, RGS3, and RGS4 are altered in failing human hearts. Mittmann et al96 observed an increase in RGS4 mRNA but no change in RGS2 or RGS3 in such hearts, whereas Owen et al97 found an apparent upregulation of RGS3 and RGS4 protein and mRNA in human heart failure, and Takeishi et al96 identified an apparent decrease in RGS2 protein. These alterations in RGS expression have the potential to significantly alter cardiac physiology, as demonstrated in animal studies that show cardiovascular abnormalities in mice overexpressing RGS479 or deficient for RGS2.34

**RGS Proteins as Drug Targets**

Approximately 50% of all drugs target GPCRs,99,100 a fact that underscores the importance of GPCR signaling in the treatment of disease. However, there are many cases in which the current drugs targeted to GPCRs are inadequate. A good example is targeting of β-adrenergic receptors. β2-Adrenergic receptor agonists are widely used for the treatment of bronchospasm, but these drugs can stimulate cardiac β-adrenergic receptors as well. β-Adrenergic receptor antagonists are commonly used for the treatment of various cardiovascular disorders. Difficulties can arise when an individual requires a β-agonist for bronchospasm but has associated cardiovascular disease.101 Conversely, nonselective β-blockers can be contraindicated in asthma and chronic obstructive pulmonary disease because of induced bronchoconstriction, and even β2-selective antagonists can cause problems in some patients.102 At higher doses, the selectivity of the “cardioselective” β-blockers is lost and an increase in pulmonary side effects are seen. In the treatment of asthma, the use of β1-antagonists leads to an increase in heart rate and is associated with an increased risk of adverse cardiovascular events.101

Thus, although β-adrenergic receptors are important targets for the treatment of bronchospasm and cardiovascular disorders, treatment of one disease can precipitate dangerous side effects in the other organ system, thereby complicating therapy directed to the GPCR. RGS proteins, as potent regulators of GPCR signaling, potentially provide a new target for the regulation of GPCR signaling in such settings. A possible target could be RGS1, which shows strong mRNA expression in the lung but not the heart.103 In addition, RGS4 is found at moderate levels in the heart but low levels in the lung.103,104

In some reconstituted and in vitro systems, RGS proteins can exhibit GAP activity toward a variety of Gα, β-subunits. However, data in more physiological systems suggest that RGS proteins act with much more specificity. As the unique specificity profile of each RGS protein is determined, in particular in the in vivo setting, pharmacological manipulation of RGS proteins seem likely to become more enticing. Pharmacological regulation of RGS proteins could: (1) potentiate or attenuate the actions of an endogenous agonist; (2) complement a GPCR pharmacological agonist or antagonist, thereby reducing the dose needed; or 3) combat the drug-induced side effects produced by GPCR agonism or antagonism.

The mechanistic effects of pharmacological manipulation of RGS proteins can be grouped into two categories: (1) alteration of RGS activity; and 2) addition or removal of an RGS protein from a particular pathway, perhaps by altering subcellular localization. Drugs developed to alter RGS activity may be preferred to drugs that alter localization because drugs with the latter action have the potential to promote RGS protein interaction with other signaling pathways.

RGS proteins of the R4 subfamily are the most studied because of their “simple” structure. However, these “simple” proteins exert profound physiological effects and, perhaps as a consequence, are highly regulated via a variety of mechanisms. For example, RGS4 is regulated by phosphorylation, palmitoylation, PIP3, Ca2+/calmodulin, G-proteins, and GPCRs. Other RGS proteins with a more complex structure would presumably be subject to an even larger number of regulatory factors. The complexity of RGS protein structure or regulation may present problems in developing drugs that selectively alter a specific pathway without influencing other pathways. However, from another perspective, the numerous methods of regulation could provide additional drug targets for the manipulation of RGS activity.

One could argue that every physiological process uses a unique subset of signaling events. The challenge in developing drugs to treat disease is in finding unique targets within a specific signaling pathway that would yield efficacy without toxicity. Drugs targeted to GPCRs have proven to be extremely important for the treatment of many diseases, in part as a consequence of unique patterns of tissue expression and accessibility on the cell surface. RGS proteins appear to offer an alternative target to alter GPCR and G-protein–mediated signaling. With a tissue distribution different from that of GPCRs, RGS proteins may prove to be useful targets. Work is already under way to develop agents that alter RGS function. Mosberg et al105 have begun developing RGS4 inhibitors that could be useful in treatment of cardiovascular disorders.79 However, RGS proteins may not prove immune to specificity problems, and accessibility of drugs to key sites on the proteins will need to be achieved. In the case of RGS4, it would be important that RGS4 activity in the brain is not altered because it may lead to schizophrenic symptoms.106

The “gaps” in current understanding of the unique mechanisms by which each RGS protein alters signaling make it challenging to predict the impact that drugs targeted to RGS proteins would have on human cardiovascular physiology. However, in our view, the significance of RGS proteins in the regulation of cell signaling warrants drug discovery efforts.

**Acknowledgments**

This work was supported by National Institutes of Health (NIH) grants HL007261 (to E.L.R.), and NIH AG16613 and NIH HL62562 (to M.B.), American Heart Association postdoctoral fellowship (to R.S.), and NIH grants NIH HL69758, HL66941, HL58120, and HL53773 (to P.A.I.).

**References**


Multi-Tasking RGS Proteins in the Heart: The Next Therapeutic Target?
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_Circ Res._ 2005;96:401-411
doi: 10.1161/01.RES.0000158287.49872.4e
_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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