Regulators of G protein signalling: a spotlight on emerging functions in the cardiovascular system

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Regulator of G protein signalling (RGS) proteins are GTPase-activating proteins for heterotrimeric G protein α subunits, and are therefore physiologically and pathophysiologically important negative regulators of G-protein-coupled receptor signalling in the cardiovascular system. Owing to the functional redundancy of many of the 20 RGS, and more than 20 RGS-like, proteins even within a single cell, animal models shedding light on the functions of individual RGS proteins are often missing. Nevertheless, RGS2 is a member of this protein family, for which specific functions in the vasculature and the heart are now emerging. Recent data show that the 519-amino acid RGS3, the only RGS protein with an additional G protein βγ dimer binding domain, largely alters the signalling of G_i proteins to the monomeric GTPases Rac1 and RhoA in cardiomyocytes. In addition, an alternative approach using transgenic animals expressing RGS-resistant G protein α subunits now highlights the contributions of RGS proteins to distinct signalling pathways in the heart.

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Introduction

G-protein-coupled receptors (GPCRs) are involved in the regulation of virtually every physiological process. They catalyse GDP/GTP exchange at a coupled heterotrimeric G protein (Gαβγ), thereby promoting dissociation of the heterotrimer into a free, GTP-liganded Gα-subunit and a Gβγ dimer. Both Gα and the Gβγ dimer then regulate the activity of various effectors. Regulator of G protein signalling (RGS) proteins were first identified as GTPase-activating proteins (GAPs), which accelerate the intrinsic GTPase activity of Gα proteins. As the duration of the G protein activation cycle is primarily controlled by GTP hydrolysis, RGS proteins are important regulators for GPCR-induced signalling. All RGS proteins share a 120-amino-acid RGS homology domain, which mediates the GTPase-accelerating activity at Gα subunits. In mammals, 20 distinct genes for 'classical' RGS proteins have been identified and divided into four subfamilies (*RZ/A*: RGS17, 19 and 20; *R4/B*: RGS1–5, 8, 13, 16 and 21; *R7/C*: RGS6, 7, 9 and 11; *R12/D*: RGS10, 12 and 14). All RGS proteins are GAPs for $G\alpha_{i/o}$ family members and many also act on $G\alpha_{q/11}$ proteins, but none affect the GTPase rates of either $G\alpha_{12/13}$ or $G\alpha_s$ family members [1–3]. Most R7/C and R12/D proteins have additional functional domains, whereas the RZ/A and R4/B proteins do not. R7/C family members contain a Gα-like domain and form requisite dimers with the atypical Gβ subunit $G\beta_5$. RGS12 and RGS14 contain a GoLoco motif [4], which binds to isolated $G\alpha_i$ and impedes GDP dissociation in solution, although it could also confer other activities [5]. One splice variant of the atypical R4/B family member RGS3 is able to bind free Gβγ dimers [6]. In addition, there are also approximately 20 related 'RGS-like' proteins, of which some have GAP activity [1–3]. Notably, the three members of the p115RhoGEF Rho guanine nucleotide exchange factor subfamily are the only RGS-like proteins with GAP activity for $G_{12/13}$ proteins.

Is there functional redundancy of RGS proteins in the cardiovascular system?

All mammalian cells (e.g. cardiomyocytes [7]) express at least a few different RGS proteins with GAP activity for $G_{i/o}$ and $G_{q/11}$. Given that RGS proteins tend to produce their effects over comparable concentration ranges (generally mid-to-high nanomolar *in vitro* [1–3]), the abundance of an RGS protein should correlate with its contribution to the total GAP activity within a cell. Therefore, questions regarding promiscuous and redundant functions of RGS proteins have been raised in the past. Nowadays, there is increasing evidence that specific interactions occur between certain signalling systems and RGS proteins. RGS proteins are able to bind to GPCRs, effector molecules, scaffold proteins and regulators that govern their availability and or activity [8•,9•]. Taking into account that the expression of several RGS proteins (e.g. RGS2, RGS4 and RGS16) can be rapidly induced by different stimuli [10–12], further specific actions of RGS proteins can be anticipated. Nevertheless, only two viable knockout mice for RGS proteins with specific phenotypes have been published. The first protein, RGS9-1 [13], has a specific function in regulating photoreceptor signal transduction;

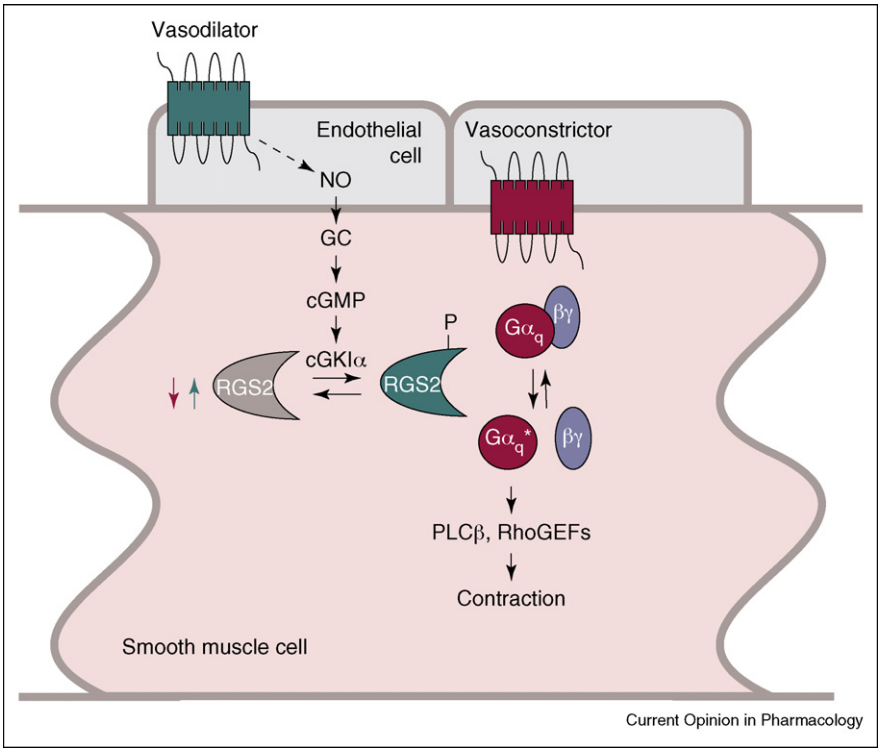
the second, RGS2 [14], is of interest in the cardiovascular system and will be discussed below.

The lack of further reported phenotypes of knockout mice argues for considerable redundancy among RGS protein family members. Furthermore, in contrast to the rapid induction of RGS proteins mentioned above, antidromic regulation might occur if the respective stimuli persists for longer periods of time (e.g. downregulation of RGS2 in cardiac hypertrophy [15•], and upregulation of RGS4 in cardiac hypertrophy [16] and human heart failure [17,18]). Therefore, the physiological and pathophysiological role of a specific RGS protein in cardiovascular tissues is often obscure. This short review highlights, firstly, the role of RGS2 and RGS3 (see also Update) in the cardiovascular system and, secondly, an interesting model that has recently been developed as a result of transgenic mice carrying $G\alpha_{i2}$ subunits resistant to the GAP function of RGS proteins [19••,20••]. This method is ingenious at circumventing methodological problems caused by the tandem arrangement of many RGS genes on one chromosome [21], which makes it difficult to create mice carrying more then one depletion of closely related RGS genes.

Role of RGS2 in cardiovascular function

RGS2 is unique owing to its preferential interaction with $G\alpha_{q/11}$ (and $G\alpha_s$) and its low affinity for $G\alpha_i$ [22,23]. RGS2 binds either directly (M_1 muscarinic receptor [24], or α_{1A}- [25,26] and β_2-adrenoceptors [27]) or indirectly via interaction with a scaffold protein (e.g. at the α_{1B}-adrenoceptor [28•]) to GPCRs. In addition, it has been shown that RGS2 directly binds to $G\alpha_s$ (without displaying GAP activity for this Gα subunit [29]) and to different adenylyl cyclase (AC) isoforms [30,31]. Both might contribute to the reported inhibition of AC activity by RGS2 [27,29–31]. Through its unique G protein selectivity for $G\alpha_{q/11}$, RGS2 appears to play a key role in cardiovascular pathophysiology, in which deleterious processes are often initiated via $G_{q/11}$-coupled GPCRs; for example, in blood vessels, many contractile responses are mediated via $G\alpha_q$ (Figure 1). Furthermore, the expression of RGS2 is upregulated in response to angiotensin II stimulation in vascular smooth muscle cells [11,32]. Furthermore, the GAP activity of RGS2 is increased in the vasculature by the nitric oxide–cGMP pathway via phosphorylation by cGMP-dependent kinase Iα [18,33•,34•]. A vascular role for RGS2 is also implied by

Figure 1

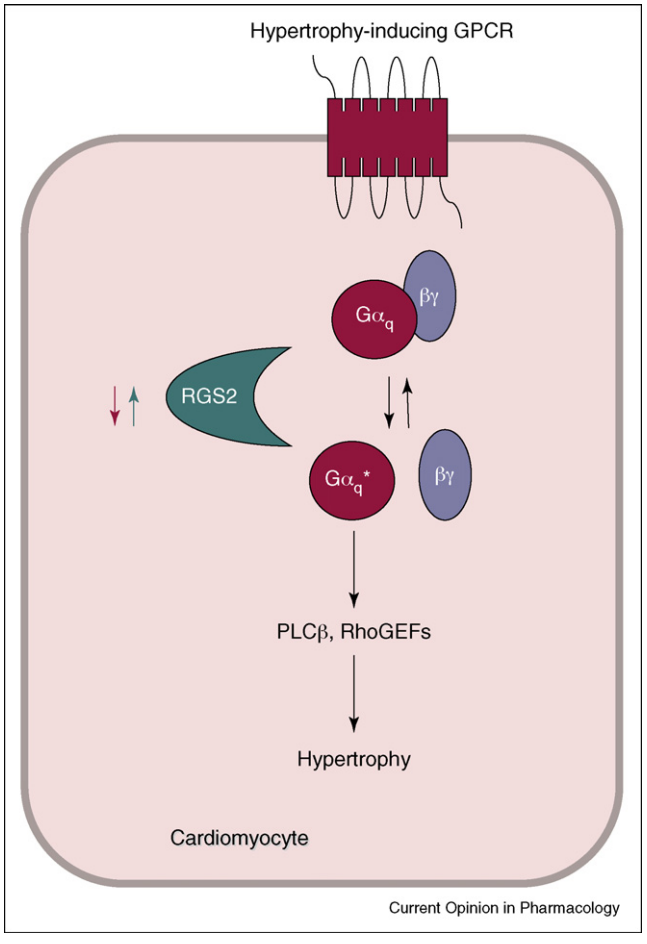


Role of RGS2 in regulation of vascular tone. RGS2 negatively regulates G_q-coupled receptors for vasoconstrictors (e.g. angiotensin II, vasopressin, noradrenaline) by accelerating the GTP hydrolysis of activated $G\alpha_q$ ($G\alpha_q^*$) in vascular smooth muscle cells. Thereby, the activity of the known $G\alpha_q$ effectors phospholipase C β (PLCβ) and $G\alpha_q$-regulated Rho guanine nucleotide exchange factors (RhoGEFs) is lowered. The activity of RGS2 appears to be controlled via phosphorylation by cGMP-dependent kinase Iα (cGKIα). Thereby, RGS2 activity is under control of vasodilators (e.g. bradykinin, acetylcholine, endothelin-2) that induce nitric oxide (NO) production in endothelial cells and thus stimulation of soluble guanylyl cyclase (GC) in the smooth muscle cell. Enhanced RGS2 expression (↑) that increases negative regulation occurs in response to prolonged angiotensin II exposure and is observed in hypotensive patients. By contrast, decreased RGS2 expression occurs in hypertension (↓).

the observed correlation between the elevated and reduced expression of RGS2 and hypo- and hyper-tension, respectively, in humans [35,36•]. Correspondingly, RGS2 knockout mice exhibit an elevation in mean arterial blood pressure, which could reflect enhanced and/or prolonged vascular contractile responses to α_1-adrenoceptor and angiotensin II receptor stimulation [18,37]. RGS2 knockout animals also display moderate hypertrophy of the aorta and renal vessels in these studies [18,37].

Several studies have indicated that RGS2 might be important in cardiac hypertrophy initiated by $G_{q/11}$-coupled receptors (Figure 2). RGS2 mRNA is selectively upregulated in neonatal rat cardiomyocytes by the α_1-adrenoceptor agonist phenylephrine [11]. A recent report additionally demonstrates enhanced expression of RGS2 in response to sustained activation of the AC–cAMP system in osteoblasts [38•], a response that might similarly occur in cardiomyocytes. The overexpression of RGS2 in cardiomyocytes essentially eliminated any increases in cell size and genetic markers of hypertrophy in response to α_1-adrenoceptor stimulation [11,39]. However, the exact mechanisms through which RGS2 produces its observed protective effects in cardiomyocytes remain to be determined, as both GTPase acceleration and scavenging ('effector antagonism') of activated $G\alpha_q$ has been observed with RGS2 [24,25]. Other GPCR signals associated with hypertrophy, including those mediated via endothelin-1, angiotensin II and protease-activated receptors, might also be blocked by RGS2, as these signals have all been shown to be inhibited by RGS2 in cardiomyocytes [39,40]. In contrast to the acute protective effects of RGS2 observed in isolated neonatal cardiomyocytes [11], a recent study found RGS2 to be selectively downregulated during the early onset of cardiac hypertrophy *in vivo* in two different models with enhanced $G_{q/11}$ signalling [15•]. Corresponding experiments examining hypertrophy and $G\alpha_q$ signalling in isolated neonatal rat cardiomyocytes showed that the small interfering RNA-mediated suppression of RGS2 expression increased both the hypertrophic effect and phospholipase Cβ stimulation induced by α_1-adrenoceptors and endothelin-1 [15•].

Figure 2



Role of RGS2 in regulation of cardiac hypertrophy. In cardiomyocytes, RGS2 negatively regulates G_q-coupled receptors that induce cardiac hypertrophy (e.g. α_1-adrenoceptors, endothelin receptors and protease-activated receptors) either by accelerating GTP hydrolysis or by scavenging activated $G\alpha_q$ ($G\alpha_q^*$). Again, the activity of the known $G\alpha_q$ effectors phospholipase C β (PLCβ) and $G\alpha_q$ regulated Rho guanine nucleotide exchange factors (RhoGEFs) is lowered. Enhanced RGS2 expression (↑), which increases negative regulation, occurs in response to cardiomyocyte stimulation with hypertrophy-inducing GPCR agonists (e.g. phenylephrine). Animal models with persistent G_q activation (i.e. cardiac overexpression of $G\alpha_q^*$) displayed reduced cardiac RGS2 expression (↓).

Somewhat surprisingly, no overt changes were seen in the cardiac function of RGS2−/− mice up to 6 months of age [37]. Therefore, it seems likely that RGS2 knockout animals develop compensatory mechanisms in pathways that are normally repressed by this RGS protein in the myocardium. Consistent with this notion, a study on pancreatic acinar cells from RGS2−/− mice showed $G\alpha_q$-mediated intracellular calcium transients to be largely normalized owing to decreased calcium influx into the cytosol from the ER and extracellular space, as well as increased calcium efflux through pumps in the ER membrane and plasma membrane [41]. Taken together, the available evidence implies a protective role for RGS2 against hypertrophy-associated GPCR signals in the heart, although clearly more studies are needed to elucidate the factors that control RGS2 expression during this disease process.

RGS-resistant Gα subunits to dissect cardiovascular signalling pathways

As mentioned above, several RGS proteins have overlapping specificities, and their role in distinct pathways is often difficult to dissect owing to their functional redundancy. Similarly, even without consideration of the influence of RGS proteins, the contribution of an individual

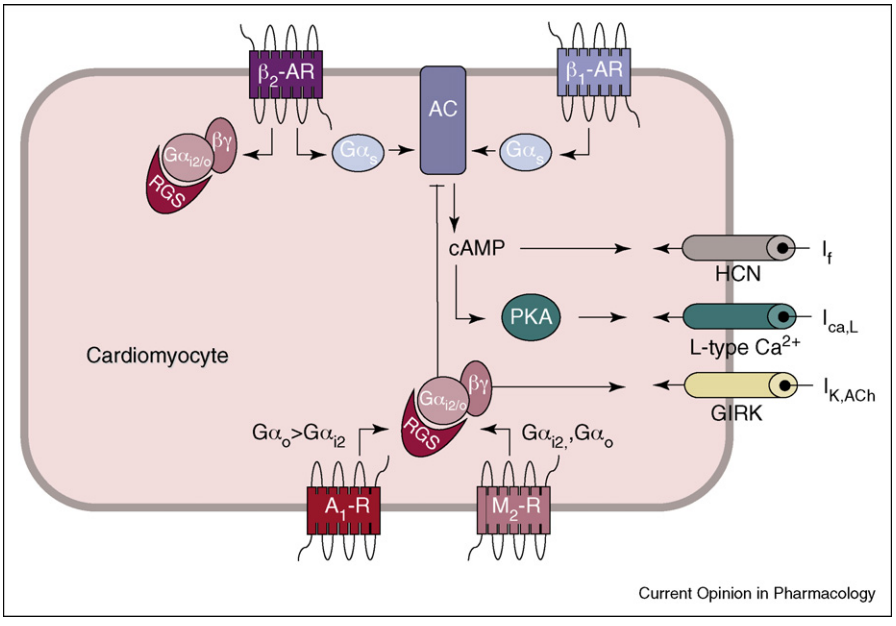
G protein α-subunit isoform to a specific physiological response is often difficult to analyse.

For example, it has been shown that the negative chronotropic effect of G_i-coupled receptors (e.g. M_2 muscarinic and A_1 adenosine receptors) involves several members of the $G_{i/o}$ subfamily (G_{i2}, G_{i3} and different splice variants of G_o [42–44]). In addition, at least three different ion currents ($I_{K,ACh}$, I_f and $I_{Ca,L}$) are regulated via this pathway. The $I_{K,Ach}$ current is caused by $G_{i/o}\beta\gamma$-mediated activation of G-protein-regulated potassium channels (GIRKs) [45]. The inhibition of cardiac hyperpolarisation-activated cyclic nucleotide-regulated cation channels carrying the I_f current [46] depends upon the decrease of cAMP production, which also contributes to the inhibition of $I_{Ca,L}$ by lowering the protein kinase A (PKA)-dependent phosphorylation of L-type calcium channels.

A recent study [19••] used the knock-in of RGS-insensitive mutants to analyse the individual contributions of $G\alpha_{i2}$ and $G\alpha_o$ to the negative chronotropic effects of M_2 muscarinic and A_1 adenosine receptors. The strength of this approach lies within the method, which mimics the natural arrangement and stoichiometry of signalling proteins. The concentration dependence of the agonist effects confirmed that, under physiological conditions, there is an impartial overlap with respect to the coupling of the studied GPCRs to specific $G_{i/o}$ proteins. The authors demonstrated major sensitisation of the carbachol-induced negative chronotropy by elimination of RGS protein control on $G\alpha_{i2}$. As this sensitisation was entirely abolished by a blocker of GIRK, a preferential coupling of M_2 muscarinic receptors to GIRK via G_{i2}-derived $G\beta\gamma$ dimers can be assumed (Figure 3). Unexpectedly, A_1 adenosine receptors diminished heart rate largely through $G\alpha_o$-dependent inhibition of AC and subsequent inhibition of I_f and $I_{Ca;L}$.

Furthermore, the authors provided new insights into the controversially discussed coupling of $G_{i/o}$ family members to β_2-adrenoceptors. It is well-known that, in regular heart function, β_2-adrenoceptor stimulation in concert with the more abundant β_1 adrenoceptors exerts positive chronotropic and inotropic effects through activation of the $G\alpha_s$–AC–PKA pathway. In heart failure, however, the potential coupling of β_2-adrenoceptors to G_i proteins is assumed to be cardioprotective [47,48]. Surprisingly, the use of RGS-resistant mutants revealed profound coupling of the β_2-adrenoceptors to $G\alpha_{i2}$ and $G\alpha_o$, even under physiological conditions. In the presence of the $G\alpha_{i2}$

Figure 3



RGS proteins control $G_{i/o}$-mediated negative chronotropic responses in cardiomyocytes. In cardiomyocytes, three different currents, I_f, $I_{Ca,L}$ and $I_{K,Ach}$, which are carried by hyperpolarisation-activated cyclic nucleotide-regulated cation (HCN), L-type Ca^{2+} and G-protein-regulated inward rectifying potassium (GIRK) channels, respectively, contribute to negative chronotropic effects of $G_{i/o}$-coupled receptors. The I_f is directly, whereas the $I_{Ca,L}$ is indirectly (via phosphorylation by PKA), regulated by cellular cAMP levels. AC activity is stimulated by G_s-coupled β_1-adrenoceptors (β_1-AR) and β_2-adrenoceptors (β_2-AR). The $G_{i/o}$-coupled M_2 muscarinic acetylcholine (M_2-R) and adenosine A_1 (A_1-R) receptors exert negative chronotropic effects by inhibiting both AC and the activation of GIRK by $G_{i/o}$-derived $G\beta\gamma$ dimers. A_1-R-mediated negative chronotropic actions are largely dependent upon $G\alpha_o$ and minimally involve regulation of GIRK currents. By contrast, the M_2-R utilizes $G\beta\gamma$ dimers released from both $G\alpha_{i2}$ and $G\alpha_o$ to activate GIRK, with this being the main mechanism mediating its negative chronotropic response. Both pathways are controlled by RGS proteins. Interestingly, the potential effects occurring via the coupling of β_2-ARs to $G_{i/o}$ proteins appear to be completely supressed by RGS proteins under physiological conditions.

mutant, β_2-adrenoceptor stimulation primarily induced a negative chronotropic response, which was replaced by an increase in the heart rate at higher agonist concentrations. These data indicate that the naturally occurring coupling of β_2-adrenoceptors to $G_{i/o}$ proteins under physiological conditions is blunted by RGS proteins, and thus $G\alpha_s$-mediated activation of AC prevails. These data indicate a completely unanticipated function of cardiac RGS proteins that awaits further analysis.

The authors meanwhile analysed the transgenic $G\alpha_{i2}$ (G184S) mutant mice on a broader basis [20••]. They demonstrated a complex phenotype affecting multiple organ systems (i.e. heart, myeloid, skeletal muscle and central nervous system). Even heterozygotes (with one intact $G\alpha_{i2}$ allele) exhibited reduced viability and decreased body weight. The transgenic animals showed stunted growth of long bones, a markedly enlarged spleen, elevated neutrophil counts, an enlarged heart, and behavioural hyperactivity. The authors therefore concluded that the loss of RGS action on a single Gα subunit (i.e. $G\alpha_{i2}$) produces dramatic and pleiotropic alterations. This phenotype is much more severe than those typically seen for individual RGS protein knockouts.

In future studies, a thorough understanding of RGS protein function in the cardiovascular system and other organs might be achieved by a double-mutant approach, rescuing specifically one pair of RGS and Gα species. Indeed, such a strategy has been reported for the interactions of RGS16 and RGS4 with $G\alpha_{i1}$ and $G\alpha_q$, respectively [49]. In this approach, a highly conserved Glu residue on the RGS protein, and a highly conserved Lys residue on Gα, are substituted by a Lys and a Glu, respectively. Whereas one of these substitutions yielded significantly reduced RGS–Gα interactions with wild-type partners, the two complementary point mutations together form an interacting salt bridge at the RGS–Gα interaction surface. Thereby, RGS and Gα mutants were developed that exhibit significantly reduced interactions with their 'natural' counterparts, but which, together, form a fully functional RGS–Gα pair as proven by *in vitro* GAP activity and functional inhibition at the cellular level. By applying such mutant pairs to transgenic animals [20••], divergent functions of apparently redundant Gα isoforms [48,50] and RGS proteins [51,52] can be addressed at a new level of scientific rigour and (patho)physiological significance.

Conclusions

A significant amount of work on RGS proteins has been performed in recent years that has clearly provided evidence for important functions of these molecules in signalling cascades in almost every cell-type. Certainly, RGS and RGS-like proteins are more than GAPs for Gα subunits; they interact with GPCRs, effector molecules, scaffold proteins and additional regulators. Some of these proteins (e.g. the p115RhoGEF family members) unify effector and GAP activities within same protein [1–3]. Despite all of the data on RGS proteins which have been accumulated so far, our knowledge of the specific function(s) of a distinct family member in the physiology and pathophysiology of the cardiovascular system is still limited. Owing to functional redundancies of several highly related RGS proteins and also experimental obstacles, new approaches have to be applied to future research on RGS proteins to indeed make them the 'the next therapeutic target', as anticipated in a recent review [3].

Update

RGS3 is an abundant RGS protein in the heart that exists in several splice variants. At least two of these variants exhibit an extended amino-terminal domain in addition to the RGS domain [53]. Out of these, the 519-amino acid isoform of RGS3 (RGS3L) binds Gβγ-dimers with a unique interaction domain located between amino acids 319 and 458 [6]. It thereby acts as a Gβγ scavenger and inhibits the Gβγ-induced effector activation independently of its GAP activity. A recent manuscript now reports that, by binding to Gβγ, RGS3L acts as molecular switch regulating the activation of Rho GTPases by prototypical G_i-coupled receptors in cardiomyocytes [54••]. At a low expression level of RGS3L, these receptors induce the activation of Rac1 using the canonical $G_i\beta\gamma$- and phosphoinositide 3′-kinase-dependent pathway. In contrast, at increased expression levels of RGS3L, these receptors activate RhoA but not Rac1, apparently through the same signalling pathway. The expression of RGS3L in cardiomyocytes is under control of fibroblast growth factor 2, a known cardioprotective stimulus. Therefore, the differential activation of Rac1 and RhoA, depending upon RGS3L expression and the distinct downstream signalling pattern, might contribute to these cardioprotective effects.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hollinger S, Hepler JR: **Cellular regulation of RGS proteins: modulators and integrators of G protein signaling**. *Pharmacol Rev* 2002, **54**:527-559.

2. Wieland T, Mittmann C: **Regulators of G-protein signalling: multifunctional proteins with impact on signalling in the cardiovascular system**. *Pharmacol Ther* 2003, **97**:95-115.

3. Riddle EL, Schwartzman RA, Bond M, Insel PA: **Multi-tasking RGS proteins in the heart: the next therapeutic target?** *Circ Res* 2005, **96**:401-411.

4. Willard FS, Kimple RJ, Siderovski DP: **Return of the GDI: the GoLoco motif in cell division**. *Annu Rev Biochem* 2004, **73**:925-951.

5. Hepler JR, Cladman W, Ramineni S, Hollinger S, Chidiac P: **Novel activity of RGS14 on $G_o\alpha$ and $G_i\alpha$ nucleotide binding and hydrolysis distinct from its RGS domain and GDI activity**. *Biochemistry* 2005, **44**:5495-5502.

6. Shi CS, Lee SB, Sinnarajah S, Dessauer CW, Rhee SG, Kehrl JH: **Regulator of G-protein signaling 3 (RGS3) inhibits $G\beta_1\gamma_2$-induced inositol phosphate production, mitogen-activated protein kinase activation, and Akt activation**. *J Biol Chem* 2001, **276**:24293-24300.

7. Doupnik CA, Xu T, Shinaman JM: **Profile of RGS expression in single rat atrial myocytes**. *Biochim Biophys Acta* 2001, **1522**:97-107.

8. • Abramow-Newerly M, Roy AA, Nunn C, Chidiac P: **RGS proteins have a signalling complex: interactions between RGS proteins and GPCRs, effectors, and auxiliary proteins**. *Cell Signal* 2006, **18**:579-591.

This review summarizes nicely the increasing evidence for specific interactions of RGS proteins with GPCRs, effectors, scaffolds and other proteins.

9. • Benians A, Nobles M, Hosny S, Tinker A: **Regulators of G-protein signaling form a quaternary complex with the agonist, receptor, and G-protein: a novel explanation for the acceleration of signaling activation kinetics**. *J Biol Chem* 2005, **280**:13383-13394.

Herein, the authors provide evidence for an interaction between RGS proteins and $G\alpha$ subunits independent of the activation state of $G\alpha$. They hypothesize that the formation of a quaternary complex between the heterotrimeric G protein and a RGS protein is relevant for the acceleration of the activating kinetics of GIRK seen with RGS proteins and distinct GPCRs.

10. Patten M, Bünemann J, Thoma B, Krämer E, Thoenes M, Mittmann C, Wieland T: **Endotoxin induces desensitization of cardiac endothelin-1 receptor signaling by increased expression of RGS4 and RGS16**. *Cardiovasc Res* 2002, **53**:159-164.

11. Grant SL, Lassegue B, Griendling KK, Ushio-Fukai M, Lyons PR, Alexander RW: **Specific regulation of RGS2 messenger RNA by angiotensin II in cultured vascular smooth muscle cells**. *Mol Pharmacol* 2000, **57**:460-467.

12. Zou MX, Roy AA, Zhao Q, Kirshenbaum LA, Karmazyn M, Chidiac P: **RGS2 is upregulated by and attenuates the hypertrophic effect of α_1-adrenergic activation in cultured ventricular myocytes**. *Cell Signal* 2006, **18**:1655-1663.

13. Chen CK, Burns ME, He W, Wensel TG, Baylor DA, Simon MI: **Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1**. *Nature* 2000, **403**:557-560.

14. Tang KM, Wang GR, Lu P, Karas RH, Aronovitz M, Heximer SP, Kaltenbronn KM, Blumer KJ, Siderovski DP, Zhu Y, Mendelsohn ME: **Regulator of G-protein signaling-2 mediates vascular smooth muscle relaxation and blood pressure**. *Nat Med* 2003, **9**:1506-1512.

15. • Zhang W, Anger T, Su J, Hao J, Xu X, Zhu M, Gach A, Cui L, Liao R, Mende U: **Selective loss of fine tuning of $G_{q/11}$ signaling by RGS2 protein exacerbates cardiomyocyte hypertrophy**. *J Biol Chem* 2006, **281**:5811-5820.

The authors demonstrate in this report that RGS2 is downregulated during the onset of hypertrophy in mice with cardiac overexpression of constitutively active $G\alpha_q$. In isolated cardiac myocytes, RGS2 depletion increased $G_{q/11}$ signalling in response to phenylephrine and endothelin-1.

16. Zhang S, Watson N, Zahner J, Rottman JN, Blumer KJ, Muslin AJ: **RGS3 and RGS4 are GTPase activating proteins in the heart**. *J Mol Cell Cardiol* 1998, **30**:269-276.

17. Mittmann C, Chung CH, Höppner G, Michalek C, Nose M, Schuh A, Eschenhagen T, Weil J, Pieske B, Hirt S, Wieland T: **Expression of 10 RGS proteins in human myocardium: Functional characterization of an upregulation of RGS4 in heart failure**. *Cardiovasc Res* 2002, **55**:778-786.

18. Owen VJ, Burton PB, Mullen AJ, Birks EJ, Barton P, Yacoub MH: **Expression of RGS3, RGS4 and $G_i\alpha_2$ in acutely failing donor hearts and end-stage heart failure**. *Eur Heart J* 2001, **22**:1015-1020.

19. •• Fu Y, Huang X, Zhong H, Mortensen RM, D'Alecy LG, Neubig RR: **Endogenous RGS proteins and $G\alpha$ subtypes differentially control muscarinic and adenosine-mediated chronotropic effects**. *Circ Res* 2006, **98**:659-666.

This paper uses the expression of RGS-resistant mutants in mouse embryonic stem cells and transgenic animals to explore the contribution of $G_{\alpha i2}$ and $G_{\alpha o}$ to the negative chronotropic effects of cardiac G_i-coupled receptors. Expected, as well as unknown, complexities in G-protein-mediated signalling are reported.

20. •• Huang X, Fu Y, Charbeneau RA, Saunders TL, Taylor DK, HankensonKD, Russell MW, D'Alecy LG, Neubig RR: **Pleiotropic phenotype of a genomic knock-in of an RGS-insensitive G184S Gnai2 allele**. *Mol Cell Biol* 2006, **26**:6870-6879.

This paper is an extension of the work reported in [19••]. It analyses the complex phenotypes created by the knock-in of RGS-resistant $G_{i\alpha2}$ subunits in mice. Again, unexpected results are presented and discussed.

21. Sierra DA, Gilbert DJ, Householder D, Grishin NV, Yu K, Ukidwe P, Barker SA, He W, Wensel TG, Otero G *et al.*: **Evolution of the regulators of G-protein signaling multigene family in mouse and human**. *Genomics* 2002, **79**:177-185.

22. Heximer SP, Srinivasa SP, Bernstein LS, Bernard JL, Linder ME, Hepler JR, Blumer KJ: **G protein selectivity is a determinant of RGS2 function**. *J Biol Chem* 1999, **274**:34253-34259.

23. Cladman W, Chidiac P: **Characterization and comparison of RGS2 and RGS4 as GTPase-activating proteins for M_2 muscarinic receptor-timulated G_i**. *Mol Pharmacol* 2002, **62**:654-659.

24. Bernstein LS, Ramineni S, Hague C, Cladman W, Chidiac P, Levey AI, Hepler JR: **RGS2 binds directly and selectively to the M_1 muscarinic acetylcholine receptor third intracellular loop to modulate $G_{q/11}\alpha$ signaling**. *J Biol Chem* 2004, **279**:21248-21256.

25. Anger T, Zhang W, Mende U: **Differential contribution of GTPase activation and effector antagonism to the inhibitory effect of RGS proteins on G_q-mediated signaling *in vivo***. *J Biol Chem* 2004, **279**:3906-3915.

26. Hague C, Bernstein LS, Ramineni S, Chen Z, Minneman KP, Hepler JR: **Selective inhibition of α_{1A}-adrenergic receptor signaling by RGS2 association with the receptor third intracellular loop**. *J Biol Chem* 2005, **280**:27289-27295.

27. Roy AA, Baragli A, Bernstein LS, Hepler JR, Hebert TE, Chidiac P: **RGS2 interacts with G_s and adenylyl cyclase in living cells**. *Cell Signal* 2006, **18**:336-348.

28. • Wang X, Zeng W, Soyombo AA, Tang W, Ross EM, Barnes AP, Milgram SL, Penninger JM, Allen PB, Greengard P, Muallem S: **Spinophilin regulates Ca^{2+} signalling by binding the N-terminal domain of RGS2 and the third intracellular loop of G-protein-coupled receptors**. *Nat Cell Biol* 2005, **7**:405-411.

This paper describes the novel function of spinophilin as a scaffold protein that mediates the specific function of RGS2 (and other RGS proteins) on distinct GPCRs.

29. Roy AA, Lemberg KE, Chidiac P: **Recruitment of RGS2 and RGS4 to the plasma membrane by G proteins and receptors reflects functional interactions**. *Mol Pharmacol* 2003, **64**:587-593.

30. Sinnarajah S, Dessauer CW, Srikumar D, Chen J, Yuen J, Yilma S, Dennis JC, Morrison EE, Vodyanoy V, Kehrl JH: **RGS2 regulates signal transduction in olfactory neurons by attenuating activation of adenylyl cyclase III**. *Nature* 2001, **409**:1051-1055.

31. Salim S, Sinnarajah S, Kehrl JH, Dessauer CW: **Identification of RGS2 and type V adenylyl cyclase interaction sites**. *J Biol Chem* 2003, **278**:15842-15849.

32. Li Y, Hashim S, Anand-Srivastava MB: **Angiotensin II-evoked enhanced expression of RGS2 attenuates G_i-mediated adenylyl cyclase signaling in A10 cells**. *Cardiovasc Res* 2005, **66**:503-511.

33. • Sun X, Kaltenbronn KM, Steinberg TH, Blumer KJ: **RGS2 is a mediator of nitric oxide action on blood pressure and vasoconstrictor signaling**. *Mol Pharmacol* 2005, **67**:631-639.

This report demonstrates that the activity of RGS2 in the vasculature is under the control of the nitric oxide–cGMP pathway via phosphorylation by cGMP-dependent kinase Iα.

34. Obst M, Tank J, Plehm R, Blumer KJ, Diedrich A, Jordan J,
• Luft FC, Gross V: **NO-dependent blood pressure regulation in RGS2-deficient mice**. *Am J Physiol* 2006, **290**:R1012-R1019.
In this report, evidence for the dependence of vasoconstrictor actions on nitric oxide-mediated RGS2 activation is presented by telemetric analyses of wild-type and RGS2-deficient mice. In addition to increased peripheral vasoconstrictor actions, an increased central activation of the sympathetic system is proposed in RGS2−/− mice.

35. Calo LA, Pagnin E, Davis PA, Sartori M, Ceolotto G, Pessina AC, Semplicini A: **Increased expression of regulator of G protein signaling-2 (RGS-2) in Bartter's/Gitelman's syndrome. A role in the control of vascular tone and implication for hypertension**. *J Clin Endocrinol Metab* 2004, **89**:4153-4157.

36. Semplicini A, Lenzini L, Sartori M, Papparella I, Calo LA, Pagnin E,
• Strapazzon G, Benna C, Costa R, Avogaro A *et al.*: **Reduced expression of regulator of G-protein signaling 2 (RGS2) in hypertensive patients increases calcium mobilization and ERK1/2 phosphorylation induced by angiotensin II**. *J Hypertens* 2006, **24**:1115-1124.
This is the first report directly correlating the loss of RGS2 expression with enhanced signalling via angiotensin II receptors in human hypertension.

37. Heximer SP, Knutsen RH, Sun X, Kaltenbronn KM, Rhee MH, Peng N, Oliveira-dos-Santos A, Penninger JM, Muslin AJ, Steinberg TH *et al.*: **Hypertension and prolonged vasoconstrictor signaling in RGS2-deficient mice**. *J Clin Invest* 2003, **111**:445-452.

38. Roy AA, Nunn C, Ming H, Zou MX, Penninger J, Kirshenbaum LA,
• Dixon SJ, Chidiac P: **Up-regulation of endogenous RGS2 mediates cross-desensitization between G_s and G_q signaling in osteoblasts**. *J Biol Chem* 2006, **281**:32684-32693.
This paper is the first to demonstrate cross desensitisation of $G_{q/11}$ coupled receptors by enhanced expression of RGS2 via increased cAMP signalling. Although performed in osteoblasts, it raises the possibility of this being a general mechanism that also occurs in the cardiovascular system.

39. Hao J, Michalek C, Zhang W, Zhu M, Xu X, Mende U: **Regulation of cardiomyocyte signaling by RGS proteins: differential selectivity towards G proteins and susceptibility to regulation**. *J Mol Cell Cardiol* 2006, **41**:51-61.

40. Snabaitis AK, Muntendorf A, Wieland T, Avkiran M: **Regulation of the extracellular signal-regulated kinase pathway in adult myocardium: differential roles of $G_{q/11}$, G_i and $G_{12/13}$ proteins in signalling by α_1-adrenergic, endothelin-1 and thrombin-sensitive protease-activated receptors**. *Cell Signal* 2005, **17**:655-664.

41. Wang X, Huang G, Luo X, Penninger JM, Muallem S: **Role of regulator of G protein signaling 2 (RGS2) in Ca^{2+} oscillations and adaptation of Ca^{2+} signaling to reduce excitability of RGS2−/− cells**. *J Biol Chem* 2004, **279**:41642-41649.

42. Chen F, Spicher K, Jiang M, Birnbaumer L, Wetzel GT: **Lack of muscarinic regulation of Ca^{2+} channels in $G_{i2}\alpha$ gene knockout mouse hearts**. *Am J Physiol* 2001, **280**:H1989-H1995.

43. Nagata K, Ye C, Jain M, Milstone DS, Liao R, Mortensen RM: **$G\alpha_{i2}$ but not $G\alpha_{i3}$ is required for muscarinic inhibition of contractility and calcium currents in adult cardiomyocytes**. *Circ Res* 2000, **87**:903-909.

44. Valenzuela D, Han X, Mende U, Fankhauser C, Mashimo H, Huang P, Pfeffer J, Neer EJ, Fishman MC: **$G\alpha_o$ is necessary for muscarinic regulation of Ca^{2+} channels in mouse heart**. *Proc Natl Acad Sci USA* 1997, **94**:1727-1732.

45. Kurachi Y, Ishii M: **Cell signal control of the G protein-gated potassium channel and its subcellular localization**. *J Physiol* 2004, **554**:285-294.

46. Michels G, Er F, Khan I, Sudkamp M, Herzig S, Hoppe UC: **Single-channel properties support a potential contribution of hyperpolarization-activated cyclic nucleotide-gated channels and I_f to cardiac arrhythmias**. *Circulation* 2005, **111**:399-404.

47. Lohse MJ, Engelhardt S, Eschenhagen T: **What is the role of beta-adrenergic signaling in heart failure?** *Circ Res* 2003, **93**:896-906.

48. Foerster K, Groner F, Matthes J, Koch WJ, Birnbaumer L, Herzig S: **Cardioprotection specific for the G protein G_{i2} in chronic adrenergic signaling through β_2-adrenoceptors**. *Proc Natl Acad Sci USA* 2003, **100**:14475-14480.

49. Wieland T, Bahtijari N, Zhou XB, Kleuss C, Simon MI: **Polarity exchange at the interface of regulators of G protein signaling with G protein α-subunits**. *J Biol Chem* 2000, **275**:28500-28506.

50. Ivanina T, Varon D, Peleg S, Rishal I, Porozov Y, Dessauer CW, Keren-Raifman T, Dascal N: **$G\alpha_{i1}$ and $G\alpha_{i3}$ differentially interact with, and regulate, the G protein-activated K^+ channel**. *J Biol Chem* 2004, **279**:17260-17268.

51. Jaen C, Doupnik CA: **Neuronal Kir3.1/Kir3.2a channels coupled to serotonin 1A and muscarinic M_2 receptors are differentially modulated by the 'short' RGS3 isoform**. *Neuropharmacology* 2005, **49**:465-476.

52. Mutneja M, Berton F, Suen KF, Luscher C, Slesinger PA: **Endogenous RGS proteins enhance acute desensitization of $GABA_B$ receptor-activated GIRK currents in HEK-293T cells**. *Pflugers Arch* 2005, **450**:61-73.

53. Kehrl JH, Srikumar D, Harrison K, Wilson G, Shi CS: **Additional 5′ exons in the RGS3 locus generate multiple mRNA transcripts, one of which accounts for the origin of human PDZ-RGS3**. *Genomics* 2002, **79**:860-868.

54. Vogt A, Lutz S, Rümenapp U, Han L, Jakobs KH, Schmidt M,
•• Wieland T: **Regulator of G protein signalling 3 redirects prototypical G_i-coupled receptors from Rac1 to RhoA activation.** *Cell Signalling* 2007, in press.
This manuscript demonstrates that increased expression of the 519-amino acid isoform of RGS3 (RGS3L) switches the signalling of M_2 muscaranic acetylcholine and adenosine A_1 receptors in cardiomyocytes from Rac1 to RhoA activation by binding of RGS3L to $G_i\beta\gamma$ dimers. Apparently, this is a novel, unique feature of RGS3L with (patho)physiological implications.