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Review

The therapeutic potential of targeting cardiac RGS4

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***Abstract*:** G protein-coupled receptors (GPCRs) play pivotal roles in regulation of cardiac function and homeostasis. To function properly, every cell needs these receptors to be stimulated only when a specific extracellular stimulus is present, and to be silenced the moment that stimulus is removed. The regulator of G protein signaling (RGS) proteins are crucial for the latter to occur at the cell membrane, where the GPCR normally resides. Perturbations in both activation and termination of G protein signaling underlie numerous heart pathologies. Although more than 30 mammalian RGS proteins have been identified, each RGS protein seems to interact only with a specific set of G protein subunits and GPCR types/subtypes in any given tissue or cell type, and this applies to the myocardium as well. A large number of studies have provided substantial evidence for the roles various RGS proteins expressed in cardiomyocytes play in cardiac physiology and heart disease pathophysiology. This review summarizes the current understanding of the functional roles of cardiac RGS proteins and their implications for the treatment of specific heart diseases, such as heart failure and atrial fibrillation. We focus on cardiac RGS4 in particular, since this isoform appears to be selectively (among the RGS protein family) upregulated in human heart failure and is also the target of ongoing drug discovery efforts for the treatment of a variety of diseases.

Keywords: arrhythmias, atrial fibrillation, cardiac myocyte, cyclic AMP, G protein-coupled receptor, G proteins, heart failure, regulator of G protein signaling, signal transduction

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Introduction

G protein-coupled receptors (GPCRs) are the single largest class of pharmaceutical targets, with over 35% of the currently FDA-approved drugs directly acting on these receptors.[1] GPCRs are crucial regulators of almost every cellular physiological process, from vision to cardiovascular function and blood pressure.[2] This is largely because they always reside at the plasma membrane, thereby mediating the signal from the vast majority of extracellular stimuli that cannot pass across the cell membrane (e.g. ionized or not lipophilic enough molecules). Therefore, GPCR abnormalities result oftentimes in various pathologies, depending on the physiology of the dysfunctional receptor. Dysfunction of cardiovascular GPCRs lead to cardiovascular diseases, such as heart failure (HF), cardiomyopathies, cardiac hypertrophy, hypertension, angina, and so on.[3,4] All GPCRs share a common core motif of seven largely hydrophobic α helices, each spanning the entire plasma membrane [seven transmembrane (TM)-spanning or heptahelical receptors].[5] The heptahelical motif is essential for interaction with G proteins only upon agonist binding on the extracellular side of the receptor.[6–10] The receptor-Gα subunit interaction activates, in turn, the heterotrimeric G protein, causing guanine nucleotide exchange and the separation of Gα from the Gβγ subunits.[11–14] However, regulation of the duration of a GPCR signal is of paramount importance for cellular homeostasis and the cell utilizes various ways to terminate the GPCR signal, starting with two major processes at the level of the

cell membrane. One of them operates on the receptor itself and involves GPCR phosphorylation by GPCR-kinases (GRKs), followed by arrestin binding (homologous or agonist-dependent receptor desensitization).[15,16] Phosphorylation by second messenger-dependent kinases, such as protein kinase A (PKA), is also supposed to terminate G protein signaling (the so-called 'heterologous' or agonist-independent receptor desensitization), but whether arrestin binding follows second messenger kinase-mediated phosphorylation is still a matter of intense debate. It is more likely that second messenger-dependent kinases switch the coupling of a particular receptor to a different G protein, such as the case of PKA-induced Gs to Gi coupling switch of the β_2-adrenoceptor (reviewed in Ref. 16). The other process, perhaps even more important, operates on the active G protein. The main mechanism for G protein signaling termination is guanosine triphosphate (GTP) hydrolysis to guanosine diphosphate (GDP) by the intrinsic GTPase activity of the Gα subunit.[12] As soon as GTP is converted to GDP, GDP-bound Gα subunit regains its affinity for the Gβγ subunits (switch II region loses its contacts with the guanine nucleotide and binds Gβ again) and the G protein heterotrimer reassociates, no longer being able to transduce signals (i.e. neither Gα nor Gβγ can interact with effectors now).[12]

Unlike the monomeric Ras-like G proteins, all 16 human Gα subunits of heterotrimeric G proteins, that is, the two members of the G_s, the eight members of the $G_{i/o}$, the four members of the $G_{q/11}$, and the two members of the G_{12} family, possess intrinsic GTPase activity.[12,17] Nonetheless, rates of GTP hydrolysis vary considerably for the various Gα subunits, with certain isoforms (Gαq, Gαz) being extremely slow at converting GTP to GDP.[12,18,19] Importantly, the GTP hydrolysis rates for all heterotrimeric G protein Gα subunits measured in vitro appear slow and probably incompatible with in vivo functions.[20–22] This is why the cell utilizes 'regulator of G protein signaling (RGS)' domain-containing proteins, a ~120-amino acid-long domain that can bind the Gα subunit and dramatically accelerate GTP hydrolysis.[20–30] The proteins that contain this RGS domain, first discovered in yeast and in the nematode worm *Caenorhabditis elegans*, are called RGS proteins.[20–30] GTP hydrolysis is enormously (up to 2000 times higher) accelerated by RGS proteins, and both the amplitude and duration of Gα and free Gβγ subunit signaling are markedly reduced.[12,31] Every protein with a functional RGS domain is categorized into a subfamily, designated by a letter (A–F) and the name of a representative member of that particular subfamily (next to the letter 'R').[31–35] For instance, the A/RZ subfamily is named after the representative RGSZ protein member.[36] Some RGS proteins, for example, RGS4 or RGS2, also interfere with the interaction of active (GTP-bound) Gα subunits with downstream effectors.[26] By acting as GTPase-activating proteins (GAPs) on Gα subunits, RGS proteins also accelerate free Gβγ signaling termination, since the heterotrimer reassembles.[26,27] It was initially thought that there might be a specific RGS protein for each of the 16 different Gα subunits but we now know that this could not have been further from the truth.[27] Not only do the RGS proteins outnumber the Gα subunits, but also several of them can act upon more than one Gα type/family (e.g. RGS4 inactivates both $G\alpha_{i/o}$ and $G\alpha_{q/11}$ subunits). Furthermore, Gαs is not a substrate for any RGS protein, and it is still an open question whether $G\alpha_{12/13}$ are. However, it seems that most (if not all) RGS proteins inactivate G proteins in a cell type- and GPCR-specific manner, that is, they do not inactivate their Gα subunit substrates at all times or under any circumstances.[27] The identity of the receptor that has stimulated the G protein seems to play a crucial role in whether that G protein serves as a substrate for the RGS protein. For example, RGS4 inactivates angiotensin II type 1 receptor (AT_1R)-stimulated Gαq but not gonadotropin-releasing hormone receptor-stimulated Gαq subunits.[37,38] This is extremely important to consider because it bestows RGS protein functions with exceptional receptor-G protein signaling pathway specificity that can be exploited for therapeutic purposes.

In the present review, we first discuss the current literature on the regulation of cardiac GPCRs by RGS proteins in the context of heart physiology but also of heart disease, followed by a closer look at cardiac RGS4, which has been documented to be implicated in HF and atrial fibrillation (AFib). Our review focuses exclusively on the cardiac effects of the B/R4 family of RGS proteins (RGS1–5, RGS8, RGS13, RGS16, RGS18, and RGS21), the smallest mammalian RGS protein family members that function primarily (if not exclusively) as G protein GAPs, that is, are bona

fide RGS proteins. Other proteins that contain RGS homology domains but serve other primary functions (e.g. GRKs, which are serine/threonine kinases), as well as a thorough discussion of the biology and physiology of RGS proteins in tissues outside the heart, are beyond the scope of the present review.

Cardiac RGS proteins and regulation of GPCR signaling pathways

RGS1, RGS2, and RGS3 are expressed in both cardiac myocytes and fibroblasts. RGS2 is also robustly expressed in both vascular smooth muscle and endothelial cells.[32,39] RGS4 is highly expressed in the brain, heart, and adrenal glands.[24,30,31] RGS5 displays mainly vascular expression.[34,40,41] RGS8, RGS13, and RGS18 are mainly expressed in immune cells although RGS18 is also present in platelets. RGS16 and RGS21 are expressed in the heart.[34,42–44] RGS3 exists in multiple isoforms,[34] of which the PDZ-containing one is expressed in cardiac atria and both its long and short isoforms are abundant in the ventricles.[37] In human aortic smooth muscle cells, RGS3 regulates sphingosine 1-phosphate receptor, endothelin-1 (ET-1) receptor, and AT_1R signaling.[37] Cardiac-specific overexpression of RGS3 blocks maladaptive hypertrophy and fibrosis and improves cardiac function.[45] RGS3 is also upregulated in spontaneously hypertensive heart failure (SHHF) rat hearts.[46] However, in a SHHF rat model that developed congestive HF over time, RGS3 was found downregulated in the myocardium.[46] Consistent with these findings, RGS3 appears elevated in myocardial samples from human end-stage HF patients, suggesting a role in human chronic and advanced HF.[47] Nevertheless, the specific GPCRs or signaling mechanisms affected by the RGS3 expression changes in human HF are unknown, so it is unclear at present whether these RGS3 changes are causative or not.

Cardiac RGS4 is most abundant in the sinoatrial (SA) and atrioventricular (AV) nodal regions, as well as throughout the atria.[48,49] It is also expressed in aorta and in ventricles.[37,46,47] Its functions in the heart are discussed in detail in the following sections below. RGS2 plays a critical role in vascular tone regulation but has been shown to affect cardiac compensation to pressure overload.[50] It also appears to be involved in the counter-regulatory effects of atrial natriuretic factor against AT_1R-induced hypertrophy.[51] Notably, RGS2 is the only RGS protein reported to date to directly oppose Gs protein signaling, albeit not by acting as a GAP for Gαs but rather by interacting with adenylyl cyclase (the effector of Gαs) and inhibiting it.[52–54] No RGS protein acting as Gαs-GAP has been reported to date.[36] RGS5 has also been reported to participate in cardioprotection against pressure overload, although no specific receptors were examined in that study.[55] RGS5 or RGS2 knockouts lead to worsened pressure overload-induced cardiac fibrosis in mice.[50,55] $G_{q/11}$-coupled receptors AT_1R endothelin type A receptor (ET_AR) are major profibrotic mediators in human cardiac fibroblasts.[56–58] RGS2 opposes AT_1R signaling-dependent cell proliferation and collagen synthesis in ventricular fibroblasts.[59] However, cardiomyocyte-residing RGS2, acting in a paracrine fashion, may have contributed to these anti-fibrotic effects of cardiac RGS2.

RGS13 is one of the two RGS proteins (the other one being RGS2) that typically localizes in the cell nucleus.[60] Indeed, upon cyclic 3′,5′-adenosine monophosphate (cAMP) synthesis and cAMP-dependent protein kinase (PKA) activation, RGS13 translocates to the nucleus and interacts with the PKA-phosphorylated transcription factor cAMP response element-binding (CREB) protein, inhibiting gene transcription downstream of CREB.[61] However, this may not occur in the heart, given the very low RGS13 expression in the myocardium.[31] RGS16 is present in both cardiac myocytes and fibroblasts[31,62,63] and is one of the very few RGS proteins identified to date that act as $G\alpha_{12/13}$-GAPs.[64] Bacterial lipopolysaccharide (LPS) endotoxin impairs cardiac contractility and precipitates acute septic HF.[65] Treatment of cardiomyocytes with LPS or ET-1 upregulates RGS16 transcriptionally, lowering phospholipase C (PLC)-β activation by ET-1-activated ET_ARs in cardiac myocytes.[32,66]

Therapeutic potential of cardiac RGS4

Cardiac RGS4 and HF

Exogenous overexpression of RGS4 in cardiomyocytes attenuates ET_AR signaling through PLCβ activation, thereby reducing contractility but also hypertrophy.[67–69] Indeed, RGS4 overexpression in murine cardiac myocytes inhibits compensation for aortic banding-induced afterload increase.[68] Cardiac RGS4-overexpressing

mice also suffered from increased postoperative mortality following aortic banding.[68] This could have occurred because of reduced Gq signaling-dependent adaptive hypertrophic/inotropic responses.[67] Surprisingly however, positive inotropy in response to dobutamine was preserved in the RGS4-overexpressing mice,[68] so β-adrenergic-dependent contractility was intact. Perhaps the excess mortality happened because of RGS4-mediated blockade of $G_{i/o}$ protein signaling, which is essential for anti-apoptosis in the myocardium.[70,71] Importantly, RGS4 overexpression ameliorated cardiac hypertrophy in the survivors by inhibiting the 'fetal' gene program activation induced by the Gq protein/calcium signaling pathway.[68] The salutary effects of RGS4 in Gq-dependent hypertrophy induction were also observed in transgenic mice overexpressing both RGS4 and Gαq in the same hearts.[69] Thus, RGS4 was established more than 20 years ago as cardioprotective against hypertrophic signals and increased afterload courtesy of its Gq signaling inhibition. Corroborating this role for RGS4 is the fact that it is found upregulated in an experimental rat model of cardiac hypertrophy as well.[46]

Importantly, cardiac RGS4 was found upregulated in advanced human HF in two different populations.[47,72] In a German study, RGS4 was found selectively upregulated, that is, the only 1 out of 10 RGS proteins examined, at both the mRNA and protein levels, in human dilated or ischemic cardiomyopathy-related end-stage HF.[72] In the English study, RGS4 mRNA and protein levels were increased in both end-stage and acute human HF.[47] Additionally, RGS4 dampened PLC activity in human left ventricular membranes, along with terminating ET_AR-dependent Gq/PLC/Ca^{2+} signaling.[72] In conclusion, cardiac RGS4 appears to be cardioprotective and its upregulation in the failing human heart may very well serve as a compensatory mechanism in the face of excessive hypertrophic and maladaptive (metabolically demanding) Gq/PLC/Ca^{2+} signaling by certain cardiac GPCRs.

Consistent with this, we recently uncovered that RGS4 also opposes the $G_{i/o}$ protein signaling of the short-chain free fatty acid receptor (FFAR)-3 in cultured cardiomyocytes.[73] FFAR3 is activated mainly by gut microbial metabolites propionate and butyrate, but also by other free fatty acids with a shorter than six carbon atoms-long chain.[74,75] Like the other three human FFARs (FFAR1, FFAR2, FFAR4), FFAR3 is a $G_{i/o}$-coupled GPCR that promotes inflammation through interleukin (IL)-6 and IL-1β induction, transforming growth factor (TGF)-β-dependent fibrosis, and increased norepinephrine release (via $G_{i/o}$-derived free Gβγ-activated PLCβ activation and subsequent Ca^{2+} signaling).[76–79] RGS4 was found to be essential for the blockade of cardiac FFAR3-mediated inflammation and fibrosis, as well as for neuronal FFAR3-dependent sympatholysis that preserved cardiac βAR reserve (cardiomyocyte β-adrenergic receptor (AR) membrane density).[73] These findings suggest a protective role for cardiac RGS4 in reverse remodeling and in mitigation of sympathetic nervous system hyperactivity induced by gut microbiota-derived nutrient metabolites, such as propionic and butyric acids.[73,77] Of note, ketone bodies like β-hydroxybutyrate have been reported to antagonize FFAR3,[76,80] so it appears that RGS4 can mimic (at least some of) the beneficial actions of ketone bodies in the heart.

Another signaling mechanism that could potentially endow RGS4 with therapeutic benefit potential in human HF is the positive regulation of cardiac cAMP levels it may exert courtesy of its GAP activity at Gαi subunits (Figure 1). As has been suggested for RGS4 in pancreatic beta cells and other tissues,[81,82] termination of Gαi subunit signaling by RGS4 would relieve adenylyl cyclase from Gαi inhibition, thereby indirectly promoting cAMP synthesis (and PKA activation) by Gs-coupled GPCRs, such as the cardiac βARs (Figure 1). The fact that the response of the RGS4-overexpressing mice to dobutamine post-aortic banding was normal also argues in favor of this scenario.[68] This mechanism might be particularly important in the setting of human HF, given that Gαi (but not Gαs or Gαq) is known to be selectively upregulated in the failing human heart, regardless of the type of failure (acute or chronic end-stage) or etiology (ischemic or dilated cardiomyopathy)[83–88] (Figure 1). This Gαi upregulation is driven by norepinephrine overstimulation of cardiac β_1ARs, which transcriptionally upregulate Gαi *via* the Gs protein/cAMP/PKA signaling axis, and thus probably serves as a feedback, counter-regulatory mechanism against catecholaminergic overdrive of the failing heart.[84,85] However, increased Gαi activity means that basal and hormone-activated adenylyl cyclase activities

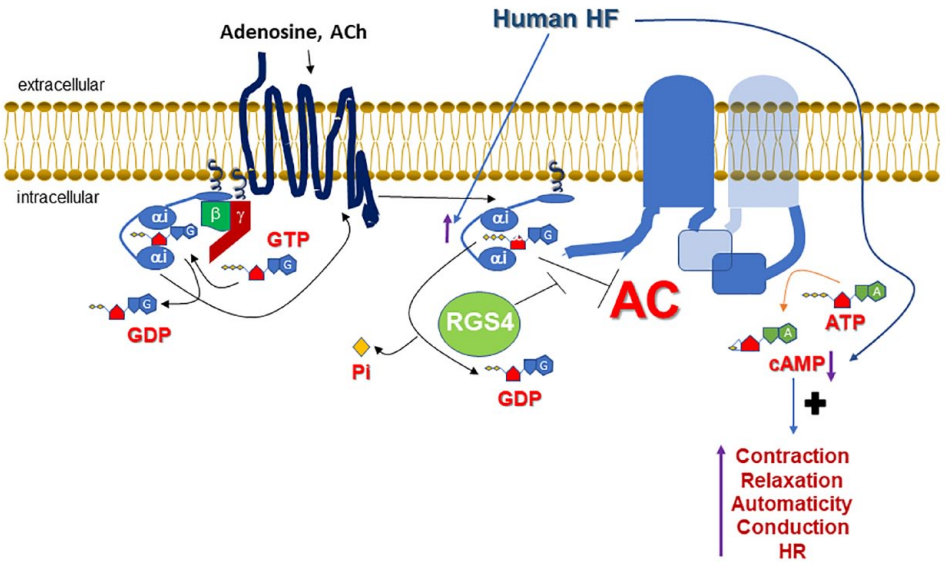

Figure 1. Role of cardiomyocyte RGS4 in the context of human HF. Basal and hormone induced (e.g. by adenosine A_1 and A_3 or M_2 muscarinic cholinergic receptors) Gαi activity is elevated, so cAMP levels are low in human HF. RGS4, by accelerating GTP hydrolysis on Gαi, functionally opposes/terminates Gαi actions, thereby (indirectly) promoting AC activation and cAMP synthesis. cAMP exerts multiple effects in the heart crucial for cardiomyocyte function, such as contraction followed by relaxation, automaticity, and positive chronotropy and dromotropy (conduction). Thus, RGS4 can potentially reverse part of the molecular abnormalities present in the failing human myocardium.
A, adenine; AC, adenylyl cyclase; ATP, adenosine triphosphate; cAMP, 3′,5′-adenosine monophosphate; G, guanine; HF, heart failure; P, phosphorylation; Pi, inorganic phosphate; RGS, regulator of G protein signaling. See text for more details and all other molecular acronym descriptions.

are suppressed, leading to chronically low cAMP levels in the failing human heart (Figure 1). Indeed, several lines of evidence point to the fact that cAMP levels are low and cAMP synthesis is deficient in the failing human heart.[89–92] Although this might initially serve as an adaptive response of the failing myocardium to protect itself from excessive norepinephrine stimulation (the developing sympathetic nervous system overdrive), low cAMP levels can become maladaptive over time, because cAMP is essential not only for the contractile (systolic) function of the heart but also for its relaxation (diastolic) function.[86,93] In addition to inotropy, automaticity, and dromotropy, cAMP increases lusitropy of the myocardium, as well. This is mainly achieved by a combination of PKA-dependent phosphorylations that primarily activate sarco(endo)plasmic reticulum calcium adenosine triphosphatase (SERCA) in the sarcoplasmic reticulum (SR) (*via* phospholamban phosphorylation) to remove Ca^{2+} from the cytoplasm back into the SR,[94] the sodium pump in the plasma membrane (*via* phospholemman phosphorylation) to drive sodium/calcium exchanger-mediated Ca^{2+} extrusion out of the cardiomyocyte,[95] and even accelerate actomyosin filament relaxation (via myosin-binding protein-C3 phosphorylation).[96,97] All these actions combined reverse the intracellular free [Ca^{2+}] elevation induced by cAMP during contraction and allow for the myocardium to relax and fill with blood during diastole.[98,99] It is thus quite plausible that RGS4 is selectively (among all RGS proteins expressed in the human heart) upregulated in the failing human heart as a compensatory mechanism for the myocardium in an effort to counterbalance the Gαi upregulation and maintain some basic level of adenylyl cyclase activity and cAMP synthesis necessary for proper cardiomyocyte homeostasis (Figure 1). In fact, one of the first articles reporting the Gαi upregulation in human HF, by Böhm and colleagues in 1990, concluded with the quote: 'Inactivation of Giα could be a potential target for the medical treatment of chronic heart failure'.[83] The RGS proteins discovered a few years later, specifically

RGS4, could fill this role perfectly. Nevertheless, this RGS4 upregulation is evidently insufficient to increase cAMP levels in the failing human heart to a substantial extent, given that the cAMP levels measured in advanced human HF are still low.[89,90] Thus, RGS4 upregulation alone does not suffice to halt (let alone reverse) the progressive deterioration of cardiac function in humans with chronic HF. Finally, it is worth noting that the fact that Gαi is elevated in the failing human heart means that interventions such as GRK2 inhibition, aimed at increasing βAR-elicited Gs protein signaling that is depressed in human HF due to elevated GRK2-dependent desensitization,[100,101] would probably be ineffective at sufficiently improving cAMP levels and, consequently, cardiac function.

Cardiac RGS4 and AFib

Apart from its putative roles in regulation of cardiac inotropy and lusitropy, RGS4 has been documented to play a crucial role in cardiac chronotropy regulation.[48] Cholinergic regulation of heart rate (HR) is mainly mediated by the $G_{i/o}$-coupled M_2 muscarinic cholinergic receptor (mAChR).[89,102] The underlying mechanism for acetylcholine (ACh)-induced bradycardia is activation of $G_{i/o}$-derived free Gβγ subunits, which help open atrial G protein-coupled inwardly rectifying K^+ (GIRK) channels, resulting in ACh-induced potassium hyperpolarizing currents (IKACh).[27,48,102] M_2 mAChR-stimulated, as well as adenosine receptor-stimulated, Gαi-dependent inhibition of adenylyl cyclase also contributes to cholinergic (and adenosinergic) slowing of HR since cAMP is essential for the operation of hyperpolarization-activated cyclic Nucleotide-gated (HCN)-4 channels, responsible for the generation of the pacemaker 'funny' current (If) in SA nodal pacemaker cells.[103,104] cAMP also enhances depolarizing Ca^{2+} influx currents in AV nodal cells (*via* PKA-mediated phosphorylation and opening of L-type calcium channels and of ryanodine receptor 2 channels), which is responsible for propagation of electrical conduction throughout the atria, AV node, and over to the ventricles (Purkinje fibers and Hiss bundle).[26,27,94,105] In other words, cAMP lowering reduces automaticity and induces negative dromotropy in the heart. RGS4 and RGS6 have long been known to function as key regulators of cholinergic control of HR.[106–108] RGS4 or RGS6 genetic deletion results in severe bradycardia from vagal stimulation in vivo.[106–108] However, RGS6 may use a different mechanism for slowing HR, since, unlike RGS4, RGS6 can directly interact with Gβ5 via its Gγ-like domain and form a complex that suppresses IKACh.[106] In fact, the role of RGS4 in negative regulation of normal, basal IKACh currents in the SA node has been challenged by several studies.[109,110] Indeed, it appears that, under normal basal vagal tone conditions, RGS6 and RGS10 are mainly responsible for IKACh desensitization.[109,111] In conditions that enhance vagal tone, however, RGS4 takes over and suppresses the excess IKACh currents that promote AFib development secondary to physical exercise or other AFib-precipitating stimuli.[108,110] Further supporting a cardioprotective role for RGS4 against AFib pathogenesis is the fact that RGS4 is essential for suppression of pro-arrhythmogenic Ca^{2+} signaling by $G_{q/11}$ protein-coupled receptors, primarily the endothelin ET_A and angiotensin II AT_1 receptors, in the heart[112] (Figure 2). Indeed, RGS4 knockout atrial myocytes developed AFib more frequently and exhibited higher endothelin-dependent Ca^{2+} spark frequencies than controls.[112] Thus, RGS4 protects against AFib induced by uncontrolled $G_{q/11}$-PLCβ/inositol trisphosphate (IP_3)/Ca^{2+} signaling, causing abnormal beats/electrical events.[112] Finally, RGS4 has been shown to suppress PLC activity (and subsequent Ca^{2+} signaling), both basally and upon ET-1 stimulation, in human cardiomyocyte membranes.[72] In conclusion, RGS4 appears essential for suppression of excessive Ca^{2+} and excessive cholinergic IKACh signaling in human atria, both of which are arrhythmogenic and can lead to AFib development (Figure 2). This strongly suggests that pharmacological interventions to enhance cardiac RGS4 expression and/or activity might have significant therapeutic value in AFib treatment and prevention, especially since RGS4 does not seem to negatively affect normal vagal HR regulation, which would be arrhythmogenic on its own and also appears to be protective against pathological cardiac hypertrophy.[113]

Conclusions and future perspectives

A lot of progress has been made over the past 20 years in elucidating the signaling actions and biological effects of RGS proteins in the heart, as in other organs and organ systems. RGS proteins could be attractive therapeutic targets in diseases of the heart, the kidneys, the central nervous

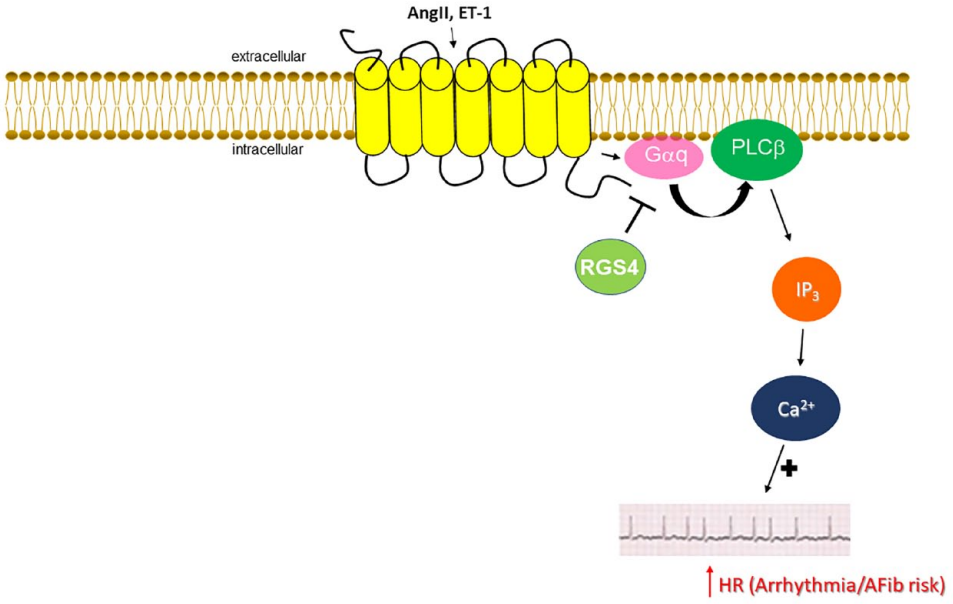

Figure 2. Role of (atrial) cardiomyocyte RGS4 in the context of human AFib. RGS4 terminates Gq protein signaling induced by AngII and ET-1 receptors, thereby attenuating pro-arrhythmic calcium signaling and reducing risk of AFib development.
ACh, acetylcholine; AFib, atrial fibrillation; AngII, angiotensin II; ET-1, endothelin-1; HR, heart rate; IP_3, inositol 1′,4′,5′-trisphosphate; RGS, regulator of G protein signaling. See text for more details and all other molecular acronym descriptions.

system, but also in oncology and other disease areas. The major question that needs to be answered for each disease and each RGS protein is whether its inhibition or potentiation is therapeutically desirable, which, of course, depends on each individual tissue type and disease setting in question. RGS protein inhibition generally enhances GPCR signaling, which can theoretically be beneficial for reducing dosage (and side effects) of other drugs that act as GPCR agonists (e.g. β_2-adrenergic agonists in asthma). Moreover, by blocking activation of certain effectors by certain G proteins (e.g. RGS2-mediated blockade of adenylyl cyclase activation, RGS4-mediated blockade of PLCβ) RGS protein inhibitors fine-tune GPCR signaling in response to GPCR agonist drugs. On the flip side, RGS protein stimulation can be desirable in many pathological conditions characterized by aberrant G protein signaling and low RGS protein activity or expression.

Although a considerable amount of work still needs to be done to fully elucidate its function in the heart and in other organs, RGS4 already emerges as a potential therapeutic target in both human AFib and HF. Coupled with its potential in treatment of kidney injury/disease,[114] cancer,[115,116] asthma,[117] and diabetes,[82] not to mention its already substantiated potential as a genetic risk factor for psychiatric disorders,[118] development of a pharmacological 'magic bullet' based on RGS4 activity augmentation in the future will not be surprising.

Interestingly, a number of small molecule inhibitors for RGS4 have been developed over the past 10–15 years,[40] largely for the purpose of delineating the effects of this protein in vivo, that is, as an alternative to RGS4 knockouts (see O'Brien *et al.*[40] for an excellent recent review on the chemistry and pharmacology of RGS protein-targeting compounds). Indeed, in vivo experiments with the RGS4 small molecule inhibitor CCG-50014 confirmed the crucial role RGS4 plays in modulating analgesia, including opioid receptor-mediated pain relief, which was significantly enhanced by coadministration of this RGS4 inhibitor.[40] However, while RGS4 inhibition may be therapeutically advantageous in analgesia or in brain disorders, the findings from cardiovascular studies discussed above strongly argue for RGS4 potentiation being advantageous in HF and AFib. Unfortunately, design and development of RGS protein

enhancers is inherently more difficult than that of RGS protein inhibitors. Besides, a Gαi or a Gαq inhibitor can (in theory at least) do the same job as an RGS4 enhancer. An interesting, alternative approach toward augmentation of RGS4 expression/activity could be protein stabilization, that is, inhibition of RGS4 proteasomal degradation via ubiquitination.[119] Indeed, pharmacological inhibition of the N-end rule pathway that degrades several R4 RGS proteins including RGS4[40,119] with the neurostimulant agent para-chloroamphetamine has been shown to increase RGS4 protein stability/levels.[120] Pharmacological augmentation of RGS4 levels/activity is thus feasible.

Admittedly, our present review has several limitations, such as relying on in vitro and animal model studies; focusing exclusively on the myocardium and on cardiomyocytes without taking into account the complex interplay between cardiac myocytes, fibroblasts, and endothelial cells, as well as, of course, between the heart, blood vessels, and neurons that innervate the myocardium; and, finally, focusing specifically on RGS4, while it is almost certain that RGS4 works in concert with other RGS proteins and G protein-interacting partners to produce its biological effects in the heart and in other tissues/organs. Nevertheless, we attempted herein to document a case for the beneficial effects of RGS4, and hence, for its pharmacological potentiation being potentially therapeutic, specifically in human HF and AFib. The arrival of better isoform-specific small organic molecules and of other molecular tools that modulate activity or expression or subcellular localization of RGS proteins in the near future will be instrumental in defining the appropriate place of each individual RGS protein, RGS4 included, on the map of targets for the current and future therapeutic arsenals for cardiac hypertrophy, HF, AFib, arrhythmias, hypertension, and other cardiovascular diseases.

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Giselle Del Calvo: Investigation; Writing – original draft.

Teresa Baggio Lopez: Investigation; Writing – original draft.

Anastasios Lymperopoulos: Conceptualization; Investigation; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing.

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