Regulator of G-Protein Signaling Subtype 4 Mediates Antihypertrophic Effect of Locally Secreted Natriuretic Peptides in the Heart

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Background—Mice lacking guanylyl cyclase-A (GC-A), a natriuretic peptide receptor, have pressure-independent cardiac hypertrophy. However, the mechanism underlying GC-A–mediated inhibition of cardiac hypertrophy remains to be elucidated. In the present report, we examined the role of regulator of G-protein signaling subtype 4 (RGS4), a GTPase activating protein for Gq and Gi, in the antihypertrophic effects of GC-A.

Methods and Results—In cultured cardiac myocytes, treatment of atrial natriuretic peptide stimulated the binding of guanosine 3′,5′-cyclic monophosphate-dependent protein kinase (PKG) I-α to RGS4, PKG-dependent phosphorylation of RGS4, and association of RGS4 and Gq. In contrast, blockade of GC-A by an antagonist, HS-142-1, attenuated the phosphorylation of RGS4 and association of RGS4 and Gq. Moreover, overexpressing a dominant negative form of RGS4 diminished the inhibitory effects of atrial natriuretic peptide on endothelin-1–stimulated inositol 1,4,5-triphosphate production, [3H]leucine incorporation, and atrial natriuretic peptide gene expression. Furthermore, expression and phosphorylation of RGS4 were significantly reduced in the hearts of GC-A knockout (GC-A-KO) mice compared with wild-type mice. For further investigation, we constructed cardiomyocyte-specific RGS4 transgenic mice and crossbred them with GC-A-KO mice. The cardiac RGS4 overexpression in GC-A-KO mice significantly reduced the ratio of heart to body weight (P<0.001), cardiomyocyte size (P<0.01), and ventricular calcineurin activity (P<0.05) to 80%, 76%, and 67% of nontransgenic GC-A-KO mice, respectively. It also significantly suppressed the augmented cardiac expression of hypertrophy-related genes in GC-A-KO mice.

Conclusions—These results provide evidence that GC-A activates cardiac RGS4, which attenuates Gq and its downstream hypertrophic signaling, and that RGS4 plays important roles in GC-A–mediated inhibition of cardiac hypertrophy. (Circulation. 2008;117:2329-2339.)

Key Words: calcineurin ■ hypotrophy ■ natriuretic peptides ■ regulators of G-protein signaling proteins ■ remodeling

Cardiac myocytes respond to mechanical stress and neurohumoral factors by undergoing a hypertrophic response, which is characterized by increases in cell size and protein synthesis, and by activating programs for a specific set of genes, such as atrial natriuretic peptide (ANP), β-myosin heavy chain (MHC), and α-skeletal actin (reviewed in References 1 to 3). Although some of this hypertrophy is adaptive, much of it is maladaptive and can ultimately result in cardiac failure.4 Calcineurin, a calcium/calmodulin-activated serine-threonine phosphatase that is activated by G-protein–coupled receptor (GPCR) agonists, such as angiotensin (Ang) II and endothelin (ET)-I,5–7 has emerged as a key mediator of cardiac hypertrophy.

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ANP and brain natriuretic peptide (BNP) are cardiac hormones that act through guanylyl cyclase-A (GC-A) to lower blood pressure (BP), induce diuresis/natriuresis, and dilate blood vessels.8,9 Cardiac synthesis of ANP and BNP is increased during cardiac hypertrophy associated with various
cardiovascular diseases. Recently, we reported that in situ activation of cardiac GC-A by locally secreted natriuretic peptides protects the heart from cardiac hypertrophy by guanosine 3′,5′-cyclic monophosphate-dependent protein kinase (PKG)–mediated inhibition of calcineurin and its downstream mediator, nuclear factor of activated T cells (NFAT). However, the molecular mechanism underlying GC-A–mediated inhibition of the calcineurin-NFAT pathway remains to be elucidated.

GTG-activating proteins for Go have recently been identified and named regulator of G-protein signaling (RGS) proteins. RGS proteins terminate GPCR signaling by accelerating the rate of GTP hydrolysis by Go (reviewed in References 11 to 13). Among >30 RGS proteins, RGS2, RGS3, and RGS4 have been implicated in cardiovascular pathophysiology. Interestingly, it has been reported that PKG binds to, phosphorylates, and activates RGS2, attenuating GPCR-mediated vascular contraction. In addition, inhibitory effects of RGS proteins on Go mediates cardiac hypertrophy have been reported. Therefore, it is tempting to speculate that RGS proteins might mediate the effects of PKG in tissues other than blood vessels, especially the heart. In the present study, we investigated the role of RGS in GC-A–mediated inhibition of cardiac hypertrophy.

Methods
Experimental procedures are described in the online-only Data Supplement.

Statistical Analysis
All values are shown as mean±SEM. Statistical significance between the 2 groups was determined with the use of the unpaired t test or Mann–Whitney U test. For multiple comparisons, the data were subjected to 1-way or 2-way ANOVA followed by Fisher multiple post hoc tests. Probability values of <0.05 were considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Posttranslational and Transcriptional Regulation of RGS4 by ANP
Because inhibitory effects of RGS2 and RGS4 on Go-mediated cardiac hypertrophy have been reported, we first analyzed the localization of RGS2 and RGS4 in the hearts of wild-type (WT) C57BL/6 mice. As shown in Figure 1A, left panel, RGS2 was expressed primarily in coronary artery smooth muscle cells but was rarely observed in cardiac myocytes. In contrast, RGS4 was not expressed in vessels but was observed in cardiac myocytes (Figure 1A, right panel). Therefore, we focused on RGS4 in subsequent experiments.

Using cultured cardiac myocytes, we first examined whether GC-A activation affects the state of RGS4. As shown in Figure 1B, ANP induced binding of PKG/Go to RGS4. Because serine phosphorylation of RGS4 is critical for its association with Go and for its GTPase activity, we next evaluated RGS4 phosphorylation. ANP stimulated phosphorylation of RGS4 (142% versus control; Figure 1C). In addition, ANP stimulated RGS4 association with Go (149% versus control; Figure 1D). Pretreatment of cardiac myocytes with the PKG inhibitor KT5823 significantly blocked ANP-stimulated RGS4 phosphorylation (Figure 1C) and RGS4 association with Go (Figure 1D). ANP significantly elevated RGS4 gene (139% versus control; Figure 1E) and protein (169% versus control; Figure 1F) expression. Pretreatment of cardiac myocytes with the KT5823 significantly blocked ANP-stimulated RGS4 gene (Figure 1E) and protein expression (Figure 1F). In Figure 1E, RNA values were obtained from Northern blot analysis, and these data were corroborated by real-time polymerase chain reaction (data not shown). These results suggest that, in cardiac myocytes, exogenous ANP induces RGS4 phosphorylation, accelerates RGS4 association with Go, and elevates gene and protein expression of RGS4 through a primarily PKG-dependent mechanism.

Because ANP and BNP are synthesized from the heart, we next investigated the contribution of locally secreted natriuretic peptides on the state of RGS4 using a natriuretic receptor antagonist, HS-142-1. As shown in Figure 1G and 1H, treatment of cardiac myocytes with HS-142-1 significantly reduced RGS4 phosphorylation (47% versus control) and significantly inhibited the association of RGS4 with Go (73% versus control). Moreover, as shown in Figure 1I, HS-142-1 treatment significantly decreased the RGS4 protein expression (81% versus control). These results suggest that not only exogenous but also endogenous locally secreted natriuretic peptides are intimately involved in the activation and expression of RGS4.

RGS4 Is Required for the Antihypertrophic Effects of ANP
We next examined whether RGS4 was required for the inhibitory effects of ANP on cardiac hypertrophy induced by ET-1, a potent GPCR agonist. ET-1–mediated activation of Go stimulates phospholipase C-β (PLC-β), which leads to production of inositol 1,4,5-triphosphate (IP3) and mobilization of intracellular calcium. Therefore, we evaluated the contribution of RGS4 to ANP-mediated inhibition of IP3 production in cultured cardiac myocytes using a dominant negative form of RGS4 (RGS4DN). In advance, we confirmed that ANP inhibits ET-1–stimulated IP3 production via a PKG-dependent mechanism (Figure I in the online-only Data Supplement). We also confirmed that exogenous expression of a truncated mutant of RGS4 [RGS4(1-58)] works in a dominant negative fashion to RGS4 specifically (Figure I in the online-only Data Supplement). RGS4(1-58) completely blocked WT RGS4-induced suppressive effect of the IP3 production, whereas it did not block the effect of RGS2. As shown in Figure 2A to 2C, the inhibitory effect of ANP on ET-1–stimulated IP3 production, [3H]leucine incorporation, and elevation of ANP gene expression was abolished in cells overexpressing RGS4DN. These results suggest that endogenous RGS4 is required for ANP to exert its potent antihypertrophic action.

We recently demonstrated that the calcineurin-NFAT pathway contributes importantly to the establishment of cardiac hypertrophy in GC-A knockout (GC-A-KO)
mice. As shown in Figure 2D, whereas ANP significantly decreased ET-1–mediated elevation of calcineurin activity, the inhibitory effect of ANP was abolished in cells overexpressing RGS4DN. Next, we evaluated the role of RGS4 in the suppressing effect of ANP on dephosphorylation of NFATc3, a direct downstream effector of calcineurin in the heart. As shown in Figure 2E, ANP significantly reversed ET-1–mediated dephosphorylation of NFATc3, and the effect of ANP was abolished in cells overexpressing RGS4DN. The gene expression of modulatory calcineurin-interacting protein (MCIP) 1 (same as RCAN1), which is augmented by NFAT activation, was also increased in cells treated with ET-1, and ANP significantly decreased ET-1–induced elevation of MCIP1 gene expression (Figure 2F). Again, overexpression of RGS4DN abolished the inhibitory effect of ANP (Figure 2F). Thus, these results suggest that RGS4 is necessary for ANP-mediated inhibition of the calcineurin-NFAT pathway in cardiac myocytes. To better visualize the effects of RGS4DN on ANP-mediated actions, the data shown in Figure 2 were expressed as percent inhibition in Figure III in the online-only Data Supplement.
Expression and Phosphorylation of RGS4 Were Diminished in Hearts of GC-A-KO Mice

As shown in Figure 3A, at 16 weeks of age, RGS4 expression was significantly diminished in the hearts of GC-A-KO mice compared with WT mice (48% versus WT). As shown in Figure 3B, there was a significant decrease in RGS4 phosphorylation (when assessed as the ratio of phospho-RGS4 to total RGS4) in GC-A-KO ventricles. Total RGS4 protein expression was also significantly diminished in GC-A-KO ventricles compared with WT (Figure 3B, upper middle panel).

Because we previously found that the augmented Ang II system plays an important role in the progress of cardiac remodeling in GC-A-KO mice,27 we next assessed the regulation of RGS4 expression and phosphorylation in an Ang II–induced cardiac hypertrophy model. Ten days of subcutaneous administration of Ang II (2 mg/kg per day) in 16-week-old WT mice caused significant increase in systolic BP (control, 103±4 versus Ang II, 143±2 mm Hg [P<0.01]) and ratio of heart to body weight (control, 3.8±0.3 versus Ang II, 5.2±0.2 [P<0.01]). As shown in Figure 3C, top panel, RGS4 expression was significantly diminished in the hearts of Ang II–administrated mice (49% versus control).

In contrast, ANP gene expression was significantly augmented (7.7-fold; P<0.001; Figure 3C, upper middle panel). As shown in Figure 3D, top panel, there was a significant increase in RGS4 phosphorylation in the hearts of Ang II–administrated mice. However, total RGS4 protein expression was significantly diminished (Figure 3D, upper middle panel). These results suggest that the major cause of downregulation of RGS4 gene expression in the hearts of GC-A-KO mice could likely be a chronic activation of the Ang II system rather than a deficiency of direct positive regulation of endogenous ANP on RGS4 expression and also indicate that the central role of ANP in the regulation of RGS4 status is a posttranslational phosphorylation rather than transcriptional regulation in in vivo pathological situations.

Overexpression of RGS4 Attenuates Cardiac Hypertrophy in GC-A-KO Mice

RGS4 is required for the inhibitory effects of ANP on cardiac hypertrophy and the calcineurin-NFAT pathway (Figure 2). On the other hand, the expression and phosphorylation of RGS4 are significantly downregulated in GC-A-KO mouse hearts (Figure 3). Therefore, we hypothesized that cardiac hypertrophy in GC-A-KO mice is caused at least in part by a reduction of RGS4
function, which may lead to the excessive activation of $G_{\alpha}$
signaling, including the calcineurin-NFAT pathway. Thus, we
sought to determine whether exogenous expression of the RGS4
gene in GC-A-KO mouse heart could inhibit cardiac hypertro-
phy and the calcineurin-NFAT pathway.

Using the $\alpha$-MHC promoter, we generated transgenic (Tg)
mice expressing mouse RGS4 in a heart-specific manner
(Figure 4A).28 As shown in Figure 4B, 6 lines of Tg mice
were successfully established. We used high-expression lines
(lines 2, 3, and 4) for the following experiments.

To investigate whether overexpression of RGS4 in the
heart could rescue cardiac hypertrophy in GC-A-KO mice,
we crossbred RGS4-Tg mice with GC-A-KO mice and also
with WT mice. At 16 weeks of age, consistent with a previous
report,19 no significant differences were observed in body
weight, systolic BP, or heart rate between RGS4-Tg and
control WT mice (Table 1). Likewise, RGS4-Tg/GC-A-KO
mice showed no difference in these physiological parameters
(Table 1). In the WT background, cardiac overproduction of
RGS4 did not affect the ratio of heart to body weight in either
male (Figure 4C) or female (Figure 4D) mice. In contrast,
cardiac overproduction of RGS4 significantly attenuated the
increase in ratio of heart to body weight on the GC-A-KO
background in both male (non-Tg-GC-A-KO, 6.13±0.28
versus Tg-GC-A-KO, 4.90±0.33 [Figure 4C]) and female
mice (non-Tg-GC-A-KO, 4.96±0.26 versus Tg-GC-A-KO,
4.33±0.31 [Figure 4D]). Because similar tendencies in the
attenuation of ratio of heart to body weight by cardiac-
specific overproduction of RGS4 were observed in male and
female mice on the GC-A-KO background, we performed the
following experiments using male mice. As we reported
previously,10 echocardiographic analysis demonstrated an
increase in the thickness of the interventricular septum and
the left ventricular posterior wall and an increase in the left
ventricular diastolic dimension in the GC-A-KO mice com-
pared with WT. Fractional shortening did not differ signifi-
cantly between these genotypes. Interestingly, on the GC-
A-KO background, cardiac overproduction of RGS4
significantly attenuated the increases in thickness of the
interventricular septum, thickness of the left ventricular
posterior wall, and left ventricular diastolic dimension,
whereas it did not affect fractional shortening (Table 2). In
contrast, on the WT background, cardiac overproduction of
RGS4 did not affect these parameters (Table 2). Representa-
tive images of the M-mode echocardiogram are shown in
Figure 4E. In hematoxylin and eosin–stained sections, we
observed an increase in cross-sectional myocyte area and
length in GC-A-KO mice compared with WT mice. Interest-
ingly, on the GC-A-KO background, the increase was significantly abrogated by cardiac overproduction of RGS4 (Figure 4F and 4G). In contrast, overproduction of RGS4 did not affect myocyte area and length on the WT background. Representative photomicrographs of hematoxylin and eosin–stained sections are shown in Figure 4H.

**Figure 4.** Cardiac myocyte–specific RGS4 transgenic overexpression inhibited hypertrophic remodeling in GC-A-KO mouse hearts. A, Schematic diagram of the transgenic construct used to generate RGS4-Tg mice. The construct contains the α-MHC gene promoter, full-length mouse RGS4 cDNA, and the human growth hormone (hGH) polyadenylation sequence. B, Representative image of Western blot analysis of RGS4 protein from ventricles of WT mice and RGS4-Tg mice (lines 1 to 6). C and D, Transgenic RGS4 overproduction in cardiac myocytes (RGS4-Tg) significantly decreased ratio of heart to body weight (HW/BW) in both male (C) and female (D) GC-A-KO mice, n=8 animals per group. E, Representative images of M-mode echocardiograms. F and G, Transgenic RGS4 overproduction in cardiac myocytes (RGS4-Tg) significantly decreased both cardiomyocyte area (F) and length (G) in GC-A-KO mice. n=4 animals per group. H, Representative photomicrograph of cardiomyocytes in sections stained with hematoxylin and eosin (magnification ×400). Values are expressed as mean±SEM. *P<0.05 vs non-Tg WT male; **P<0.05 vs non-Tg WT female; †P<0.05 vs non-Tg KO male; ††P<0.05 vs non-Tg KO female.

Effect of Overexpression of RGS4 on Cardiac Hypertrophy–Related Gene Expression in GC-A-KO Mice

As shown in Figure 5, ventricular expression of the MCIPI, ANP, and BNP genes was significantly elevated in GC-A-KO mice compared with WT mice, as we reported.

### Table 1. Body Weight, Systolic BP, and Heart Rate in Each Experimental Group

<table>
<thead>
<tr>
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<th>WT Male</th>
<th>WT Female</th>
<th>KD Male</th>
<th>KD Female</th>
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<tr>
<td></td>
<td>Non-Tg</td>
<td>Tg</td>
<td>Non-Tg</td>
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<tr>
<td>Body weight, g</td>
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<td>Systolic BP, mm Hg</td>
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<td>103±2</td>
<td>103±2</td>
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<td>Heart rate, bpm</td>
<td>619±8</td>
<td>621±2</td>
<td>624±7</td>
<td>629±7</td>
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</table>

Data are mean±SEM. Tg indicates α-MHC-RGS4 transgenic; KO, GC-A-knockout. n=8 animals per group. 
*P<0.05 vs non-Tg WT male. 
†P<0.05 vs Tg WT male. 
‡P<0.05 vs non-Tg WT female. 
§P<0.05 vs Tg WT female.
previously. Cardiac-specific overproduction of RGS4 significantly suppressed the expression of MCIP1, ANP, and BNP in GC-A-KO mice, whereas it had no effect in WT mice. In addition, elevated ventricular expression of the \(\beta\)-H9251-skeletal actin and \(\beta\)-H9252-MHC genes was observed in GC-A-KO mice; this was markedly suppressed by overproduction of RGS4. There were no significant differences in the expression levels of \(\beta\)-MHC and sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) 2 mRNA levels in each group.

**Overexpression of RGS4 Rescues Excessive Activation of the Calcineurin-NFAT Pathway in the Absence of GC-A**

We next evaluated the effects of RGS4 overexpression on the calcineurin-NFAT pathway in the hearts of GC-A-KO mice. As we reported previously, calcineurin activity, phosphorylation of NFATc3, and GATA4 DNA-binding activity was significantly increased, attenuated, and enhanced in GC-A-KO (non-Tg) mouse hearts compared with WT (non-Tg) mice (Figure 6A through 6C). RGS4 overexpression significantly inhibited calcineurin activity, increased phosphorylated NFATc3, and reduced GATA4 DNA-binding activity on the GC-A-KO background but not on the WT background (Figure 6A through 6C).

Recently, transient receptor potential channel subfamily C (TRPC) members have been reported to promote cardiomyocyte hypertrophy through activation of calcineurin signaling.\textsuperscript{29–32} It has also been reported that hypertrophic GPCR agonists stimulate the expression of TRPC3 and TRPC6 through activation of the calcineurin-NFAT pathway.\textsuperscript{30,32} Interestingly, as shown in Figure 6D, TRPC3 gene expression was dramatically elevated in GC-A-KO mouse hearts. Cardiac-specific overexpression of RGS4 significantly suppressed the expression of TRPC3 in GC-A-KO mice, whereas it had no effect in WT mice. There were no significant differences in TRPC6 gene expression in each group (data not shown). As shown in Figure 6E, treatment of cardiac myocytes with HS-142-1 significantly elevated TRPC3 gene expression. The concomitant addition of the calcineurin

**Table 2. Echocardiographic Characteristics in Each Experimental Group**

<table>
<thead>
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<th>WT Non-Tg</th>
<th>Tg</th>
<th>KO Non-Tg</th>
<th>Tg</th>
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<tr>
<td>IVSth, mm</td>
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<td>0.53±0.01</td>
<td>0.94±0.04*</td>
<td>0.68±0.03†</td>
</tr>
<tr>
<td>LVPWth, mm</td>
<td>0.58±0.01</td>
<td>0.53±0.01</td>
<td>0.86±0.06*</td>
<td>0.66±0.03†</td>
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<tr>
<td>LVEDD, mm</td>
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<td>3.81±0.08</td>
<td>4.11±0.11*</td>
<td>4.11±0.11†</td>
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<tr>
<td>LVESD, mm</td>
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<td>2.45±0.09</td>
<td>2.85±0.12*</td>
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<td>Fractional shortening, %</td>
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<td>35.8±1.7</td>
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<td>38.7±1.8</td>
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<tr>
<td>Heart rate, bpm</td>
<td>287±12</td>
<td>278±8</td>
<td>288±6</td>
<td>297±7</td>
</tr>
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</table>

Data are mean±SEM. Tg indicates \(\alpha\)-MHC-RGS4 transgenic; KO, GC-A-knockout; IVSth, thickness of the interventricular septum; LVPWth, thickness of the left ventricular posterior wall; LVEDD, left ventricular end-diastolic diameter; and LVESD, left ventricular end-systolic diameter. n=8 animals per group. *\(P<0.05\) vs non-Tg WT. †\(P<0.05\) vs non-Tg KO.

**Figure 5.** Cardiac myocyte-specific RGS4 transgenic overexpression attenuated overexpression of hypertrophy-related genes in GC-A-KO mice. A, Representative images of Northern blots. B, Quantitative analysis of the Northern blots. Shown are mRNA levels relative to non-Tg WT normalized by GAPDH mRNA levels. n=4 animals per group. Values are expressed as mean±SEM. *\(P<0.05\) vs non-Tg WT; †\(P<0.05\) vs non-Tg KO.
inhibitor FK506 or cyclosporine A almost completely blocked HS-142-1–dependent induction of the TRPC3 gene, indicating that locally secreted natriuretic peptides inhibit TRPC3 gene expression through inhibition of the calcineurin-NFAT pathway in an autocrine manner. As shown in Figure 6F, consistent with a previous report,30 ET-1 significantly elevated TRPC3 gene expression. ANP significantly reduced ET-1–induced elevation of TRPC3 gene expression. The concomitant addition of KT5823 or overexpression of RGS4DN completely abolished the inhibitory effect of ANP,
suggesting that the effect of ANP is dependent on PKG-mediated activation of RGS4.

Discussion
As has been reported previously, GC-A plays a primary role in moderating cardiac hypertrophy in vivo independent of its effects on BP regulation.33–35 We have shown previously that GC-A exerts its antihypertrophic action by antagonizing the calcineurin-NFAT pathway through a PKG-dependent mechanism.10 Because GPCR agonists such as Ang II provoke calcineurin activation by stimulating G\(\alpha_q\),5,36 it was hypothesized that GC-A signaling negatively interacts with G\(\alpha_q\) signaling via the activation of PKG. However, the precise mechanism linking the natriuretic peptide/GC-A/PKG pathway and the G\(\alpha_q\) signaling remained to be elucidated.

RGS proteins play key roles in the inhibitory regulation of GPCR signaling by accelerating GTPase activity.11–13 Menden and coworkers16 demonstrated an inhibitory effect of RGS2 on ET-1- and phenylephrine-induced cardiac myocyte hypertrophy. However, although they exhibit hypertension, cardiac hypertrophy was not shown in mice deficient for RGS2,14,15 suggesting only a minor role for RGS2 in the physiological regulation of cardiomyocyte size. On the other hand, Muslin and coworkers18,37 reported RGS4 gene and protein expression in adult rat heart and found an inhibitory effect of RGS4 on ET-1- and phenylephrine-induced cardiac myocyte hypertrophy.

In this study, we focused on RGS4 and examined its role in GC-A-mediated antihypertrophic action. These results suggest that RGS4 is required for GC-A-mediated antihypertrophic action in the heart and also suggest that the reduced activation of RGS4 causes excessive activation of the calcineurin-NFAT pathway, which results in cardiac hypertrophy in mice deficient for GC-A. A schematic diagram depicting these signaling mechanisms is shown in Figure 7.

In regard to upstream factor(s) that activate the calcineurin-NFAT pathway in GC-A-KO mouse hearts, we suggest that the Ang II system may contribute to calcineurin activation. Like many other calcium-mobilizing GPCRs, Ang II type 1A receptor is coupled to G\(\alpha_q\), which activates PLC-\(\beta\) and stimulates IP$_3$/calcium signaling.24 It has been reported that the selective Ang II type 1A receptor blocker attenuated cardiac calcineurin activity and the development of cardiac hypertrophy in hypertensive rats, even at a dose that did not lower BP.38 We previously reported that, in the absence of GC-A, the intracellular signaling downstream of Ang II type 1A receptor becomes hypersensitive to ligand activation.37 Taking the results herein into account, we suggest that locally secreted natriuretic peptide–induced activation of RGS4 inhibits G\(\alpha_q\) signaling coupled to Ang II type 1A receptor and, thereby, downstream hypertrophic transduction.

In the present study, we have demonstrated the importance of RGS4 as a target of cardiac natriuretic peptides to exert antihypertrophic effects. However, our findings do not exclude contributions of other RGS proteins in inhibition of GC-A–mediated inhibition of cardiac hypertrophy. In fact, gene and protein expressions of RGS2, RGS3, and RGS5 have been detected in the heart.39

Other molecules downstream of PKG also merit consideration for a role in the antihypertrophic action of natriuretic peptide/GC-A signal transduction. Recently, Kilic et al40 reported that GC-A moderates cardiac growth response to pressure overload by preventing excessive activation of the Na$^+$/H$^+$ exchanger NHE-1 and subsequent increases in Ca$^{2+}$/calmodulin-dependent kinase II as well as Akt. This demon-
strated that not only calcineurin-NFAT but also other signal-
ing pathways contribute to cardiac hypertrophy in GC-A-KO mice. Nevertheless, the results herein, in conjunction with our previous data, demonstrate a dominant role of reduction in RGS4 function and subsequent excessive activation of the calcineurin-NFAT pathway in cardiac remodeling in the mouse.

Nakayama et al previously described a functional mutation in the 5′-flanking region of the human GC-A gene that is associated with essential hypertension and cardiac hypertrophy. GC-A gene expression is most likely diminished in these patients because of the mutation, predisposing them to cardiac hypertrophy similar to that seen in GC-A-KO mice. In such patients, it is possible that inhibition of the calcineurin-NFAT pathway by RGS4 would be a useful treatment for the prevention of cardiac remodeling.

In conclusion, our findings indicate that GC-A activates RGS4 via PKG in cardiac myocytes; this attenuates Goq and downstream hypertrophic signaling. These findings provide new insights into endogenous mechanisms for protection of the heart by natriuretic peptide/GC-A signaling and predict that RGS4 is a potential therapeutic target to restrain cardiac remodeling.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

Cardiac myocytes respond to mechanical stress and neurohumoral factors by undergoing a hypertrophic response. Although some of this hypertrophy is adaptive, much of it is maladaptive and can ultimately result in cardiac failure. On the other hand, apart from acting as circulating hormones, atrial natriuretic peptide and brain natriuretic peptide have some functionality as autocrine and/or paracrine factors. Recently, we reported that in situ activation of cardiac guanylyl cyclase-A (GC-A), a natriuretic peptide receptor, by locally secreted natriuretic peptides protects the heart from cardiac hypertrophy by guanosine 3',5'-cyclic monophosphate-dependent protein kinase (PKG)–mediated inhibition of calcineurin and its downstream mediator, nuclear factor of activated T cells. However, the molecular mechanism underlying GC-A–mediated inhibition of the calcineurin–nuclear factor of activated T cells pathway remains to be elucidated. GTPase-activating proteins for Gα have recently been identified and named regulator of G-protein signaling (RGS) proteins. In the present study, we investigated the role of RGS in GC-A–mediated inhibition of cardiac hypertrophy. Our findings indicate that GC-A activates RGS4 via PKG in cardiac myocytes; this attenuates Gα and downstream hypertrophic signaling. Nakayama et al previously described a functional mutation in the 5'-flanking region of the human GC-A gene that is associated with essential hypertension and cardiac hypertrophy. GC-A gene expression is most likely diminished in these patients because of the mutation, predisposing them to cardiac hypertrophy similar to that seen in GC-A knockout mice. In such patients, it is possible that inhibition of the calcineurin–nuclear factor of activated T cells pathway by RGS4 would be a useful treatment for the prevention of cardiac remodeling.
Regulator of G-Protein Signaling Subtype 4 Mediates Antihypertrophic Effect of Locally Secreted Natriuretic Peptides in the Heart
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