Gq-Coupled Receptor Agonists Mediate Cardiac Hypertrophy Via the Vasculature

Janelle R. Keys, Emily A. Greene, Walter J. Koch, Andrea D. Eckhart

Abstract—The Gq-coupled receptor-signaling pathway has been implicated in the cardiac hypertrophic response to stress, but little is actually known about the contributions of Gq signaling in either the heart or the vasculature. Therefore, we developed a line of transgenic mice that express a peptide inhibitor of Gq (GqI) in vascular smooth muscle to determine if vascular Gq signaling was important in the cardiac hypertrophic response. After chronic administration of the Gq agonists phenylephrine, serotonin, and angiotensin II, we observed an attenuation of mean arterial blood pressure and an inhibition of cardiac hypertrophy in the transgenic mice with vascular-specific GqI expression. In contrast, cardiac GqI peptide expression did not attenuate the hypertension or the cardiac hypertrophy. Importantly, all mice were capable of cardiac hypertrophy, because direct β-adrenergic receptor stimulation induced a similar level of hypertrophy in both lines of transgenic mice. This clearly suggests that after chronic Gq-coupled receptor agonist administration, it is the hypertensive state induced by vascular Gq activation that mediates remodeling of the heart, rather than direct stimulation of cardiac Gq-coupled receptors. Thus, the contribution of vascular Gq-coupled signaling to the development of cardiac hypertrophy is significant and suggests that expression of the GqI peptide is a novel therapeutic strategy to lower Gq-mediated hypertension and cardiac hypertrophy. (Hypertension. 2002;40:660-666.)

Key Words: hypertrophy ■ hypertension, experimental ■ mice, transgenic ■ vasculature ■ receptors, adrenergic

G protein–coupled receptors (GPCRs) are important components in the regulation of the cardiovascular system. Hormones such as norepinephrine (NE), epinephrine (EPI), angiotensin (Ang) II, and serotonin bind to GPCRs on the surface of both cardiomyocytes and vascular smooth muscle (VSM) to stimulate G protein–signaling cascades. Ang II, serotonin, and α-adrenergic receptors (ARs) primarily couple to the heterotrimeric G protein Gq, which elicits the accumulation of diacylglycerol and inositol 1,4,5-triphosphate and activates protein kinase C (PKC) and mitogen-activated protein kinases (MAPKs).1 Ligand binding to Gs-coupled receptors, such as EPI and NE to βARs, stimulates adenylyl cyclase and induces accumulation of cAMP and activation of protein kinase A.1 Although in the vasculature, these pathways have opposing effects to one another, the Gq cascade results in the constriction of blood vessels, whereas the Gs signal mediates vasodilation. Both pathways have been shown to mediate hypertrophy in cardiac cells.2

Several recent studies have implicated Gq as a critical component in the hypertrophic response of the stressed heart. First, stimulation of the Gq pathway via chronic administration of agonists that activate α1ARs—such as NE,3 phenylephrine (PE),4 and Ang II5—can lead to increases in myocardial mass. Moreover, recent molecular studies in transgenic mice have demonstrated that cardiac overexpression of wild-type Gq itself,6 or a constitutively active mutant form of Gaq,7 leads to myocardial hypertrophy that may progress to ventricular dilatation and early death owing to heart failure. Interestingly, transgenic mice that overexpress the Gq-coupled α1B AR in the heart do not present with cardiac hypertrophy.8 However, recent studies have suggested that these mice are “primed” for a hypertrophic response because chronic α1AR stimulation by PE resulted in increased mortality associated with exaggerated cardiac hypertrophy, fibrosis, and biochemical characteristics of heart failure.9 Interestingly, this phenotype after PE administration was similar to the phenotype displayed by mice with cardiac overexpression of a constitutively active mutant form of the α1B AR.8 Importantly, to demonstrate that Gq is a final common trigger for the initiation of pressure overload hypertrophy, transgenic mice with myocardial-targeted expression of a peptide that specifically inhibits Gq-coupled signaling (GqI), developed significantly less left ventricular hypertrophy than did control mice after transverse aortic constriction (TAC).11

Chronic administration of the Gs-coupled receptor agonist isoproterenol (ISO) induces increased cardiac hypertrophy along with characteristic downregulation of the signaling pathway, including lower βAR density and a decrease in adenylyl cyclase activity.4,12 Thus, it appears that both Gq and Gs signals have the potential to mediate alterations in cardiac structure and mass. However, in the physiological milieu of
the intact animal, it is likely that events leading to hypertrophy are more complex than direct stimulation of receptors by a single ligand and a single G protein. Indeed, to complicate the distinction between Gq and Gs stimulation, it has been shown that one may lead to the other. Administration of α-adrenergic agonists and Ang II, acting via Gq, induce increases in blood pressure. Elevated blood pressure has been associated with a release of the sympathetic catecholamines NE and EPI, which then stimulate both Gq- and Gs-coupled receptors in an attempt by the body to maintain vascular tone and normalize blood pressure. Additionally, ISO administration, which acts via the Gs-coupled βAR, has been shown to activate the Gq-coupled circulatory renin-angiotensin system.

To delineate the contribution of Gq signaling in the vasculature and heart to the development of cardiac hypertrophy, after exposure to Gq-coupled receptor agonists, we have developed a line of transgenic mice that express the GqI exclusively in VSM cells under the control of the SM22α promoter. Herein, we describe the physiological implications of inhibiting Gq-signal transduction specifically in either the vasculature or myocardium, and determine its impact on blood pressure and cardiac hypertrophy.

Methods

Transgene Construction and Development of Transgenic Mice

A 300-bp fragment, encoding the amino acids 305 to 359, of murine GqI (Gq),11 was ligated into a previously described plasmid containing a portion of the SM22α promoter and the SV40 intron poly(A+) signal.15 Also used in this study were mice previously described (C57BL/6 background mice bred in-house at DUMC), which had the GqI targeted to the heart via the α-myosin heavy-chain (αMHC) gene promoter (αMHC-GqI). Adult animals (2 to 12 months old) were used for all studies, along with their nontransgenic littermate controls (NLC mice). Institutional review board approval for all experiments was obtained from Duke University. Positive transgene expression was confirmed by reverse transcription–polymerase chain reaction,13 on isolated ventricular myocytes and cultured VSM cells.

Analysis of SM22-GqI Mice

MAPK (extracellular-regulated kinases 1/2 [ERK1/2] and c-jun kinase [JNK]) activity was assessed using Western blotting for activated proteins. Histology and morphology, in vivo physiology, and heart weight/body weight ratio were all assessed as previously described.

Chronic Agonist Treatment

Mini-osmotic pumps (Alzet Model 2002) were implanted in mice anesthetized with ketamine (100 mg/kg body weight) and xylazine (5 mg/kg body weight).16 Pumps were filled with PE, PE and metoprolol, serotonin, Ang II, ISO, NE, or vehicle (0.002% ascorbic acid in PBS) and were set to deliver PE at 100 mg/kg per day, metoprolol at 10 mg/kg per day, serotonin at 30 mg/kg per day, Ang II at 10 mg/kg per day, ISO at 30 mg/kg per day, and NE at 30 mg/kg per day for 7 days each. Mice that received ISO were administered with an intraperitoneal injection of PE (8 μg/kg) immediately after pump implantation to offset acute mortality. After 7 days of treatment, mice were anesthetized, and mean arterial pressure (MAP) was determined via carotid catheterization. Mice were then killed, and heart/body weight ratio was determined.

Catecholamine Analysis

Blood samples were taken immediately after death via the inferior vena cava. Ethylenediaminetetraacetic acid–plasma was isolated from the samples and frozen. Samples were sent to AniLytics Inc for analysis of EPI and NE levels present in the plasma.

Statistical Analysis

Data are expressed as mean±SEM. Data were analyzed using 2-way ANOVA or unpaired Student’s t test as indicated. P<0.05 was considered significant.

An expanded Methods section can be found in an online supplement available at http://www.hypertensionaha.org.

Results

Transgenic Mice Expressing GqI in the Vasculature

To generate mice with targeted VSM expression of the GqI transgene, we used a portion of the SM22α promoter as previously described, along with a 300-bp section of the Gq gene that has been characterized as the GqI.11 The GqI peptide has been shown to specifically attenuate signaling through Gq-coupled receptors but does not alter other G protein signals, such as Gs or Gi, when expressed in the heart (αMHC-GqI mice).11 Positive GqI mRNA expression was seen only in the aortae of transgenic mice and not in samples from NLC mice or in the heart (Figure 1A).

To study signaling consequences of vascular GqI expression, we generated primary VSM cells cultured from aorta of SM22-GqI mice along with cells from NLC mice. Figure 1B is a representative immunoblot of the ability of PE to induce MAPK activation. As shown, there is a dose-dependent increase in the amount of phosphorylated ERK1/2 (ie, activated) present in VSM cells from NLC mice in response to PE. In contrast, there was an attenuated ERK1/2 activation in the cells isolated from SM22-GqI mice, although the total amounts of unphosphorylated enzyme were similar to those found in NLC mice (Figure 1B). In addition, we found that in NLC mice, treatment with PE (10−6, 5 minutes) increased JNK activity 2.6±0.1-fold over baseline (normalized to total JNK levels). However, in VSM cells from SM22-GqI mice, this increase in activity was significantly inhibited (0.9±0.1; P<0.05; data not shown). Thus, GqI transgene expression is sufficient in these SM22-GqI VSM cells to functionally affect Gq-coupled signaling.

Initial insight into the phenotype of SM22-GqI mice was found by assessing the vascular wall thickness of isolated aortae, as chronic VSM expression of the GqI results in a significant reduction of aortic thickness (Figure 1C). Importantly, this vascular irregularity is apparent only in the VSM layer, and there do not seem to be any differences in collagen and elastin deposition between the lines. Thus, it appears that VSM growth may be retarded in these mice, perhaps by chronic attenuation of Gq-mediated MAPK signals (Figure 1B).

To examine the effect of GqI on in vivo vascular function, we examined the blood pressure of anesthetized mice by use of an indwelling fluid-filled carotid artery catheter. To determine whether the GqI may affect pressor responses, we measured in vivo blood pressure responses in anesthetized mice to agonists for 2 different Gq-coupled receptors, α1-ARs...
and Ang II receptors. The response to the α1AR agonist PE was attenuated 75% at the maximum dose of PE (Figure 1D). The SM22-GqI transgenic mice also exhibited a dose-dependent attenuation of Ang II signaling, as vasoconstriction and subsequent pressor response were inhibited 90% at the maximum dose (Figure 1D). Importantly, there was no change in the βAR responsiveness to ISO, as SM22-GqI mice relaxed equally as well as the NLC mice did (Figure 1D). Therefore, the GqI peptide is acting specifically and selectively in vivo against Gq-coupled receptors in the vasculature of these mice.

Cardiac and Hemodynamic Responses to Chronic Gq-Coupled Receptor Stimulation

Anesthetized MAP was not altered in SM22-GqI mice compared with NLC mice (Figure 2A). In addition, neither systolic nor diastolic pressure was affected by GqI expression (Table), and heart rate was unchanged between the groups (data not shown).

To determine the role of cardiovascular Gq in response to chronic exposure to Gq-coupled receptor agonists, we subcutaneously implanted mini-osmotic pumps that released PE over 7 days. As expected, NLC mice demonstrated a significant increase in their heart weight/body weight ratio after treatment (Figure 2B). Expression of the GqI peptide in VSM inhibited PE-induced hypertrophy, however, GqI in the heart did not, as αMHC-GqI hearts were as hypertrophic as those of NLC mice (Figure 2B). We also measured blood pressure in these mice, and NLC mice demonstrated significant hypertension (46±4% over baseline) after PE treatment, and the αMHC-GqI mice also had a 46±10% rise in blood pressure, whereas the SM22-GqI mice exhibited only a...
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25 ± 5% increase in MAP (Figure 2A). These data are all
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in MAP that are concordant with changes in both SBP and DBP.

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and calculated
MAP (MAP = DBP + 1/3(SBP − DBP)) for all treatments. The data reflect changes
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(Table). The results demonstrate that PE-induced hypertrophy is principally mediated by Gq-coupled α1Rs in the vasculature and subsequent rise in MAP.

To confirm that these results were generalized to Gq-mediated signaling and not specifically to α1Rs, we chronically stimulated mice with another Gq-coupled receptor agonist, serotonin. This treatment produced the same pattern as did PE, with cardiac hypertrophy elicited by serotonin in both NLC mice and αMHC-GqI mice, whereas no hypertrophy was evident in the mice with VSM GqI expression (Figure 2B). Blood pressure responses mimicked that of the α1AR agonist (Figure 2A and Table). Interestingly, MAP increases and hypertrophy were less for 5-HT compared with PE. To confirm this pattern of hypertrophic development, mice were also chronically treated with the Gq-coupled receptor agonist Ang II. Similar to PE and serotonin, we observed a pattern of heart weight/body weight ratio that was elevated in NLC (4.7 ± 0.2 mg/g) and αMHC-GqI (5.0 ± 0.1 mg/g) mice after agonist exposure and was not altered in the SM22-GqI mice (3.7 ± 0.2 mg/g). These data confirm the Gq specificity of serotonin and Ang II and, like the PE data, suggest that it is the VSM Gq signal that mediates cardiac hypertrophy in response to these agents and not direct stimulation of cardiac Gq-coupled receptors.

To investigate the maintained hypertrophic response when Gq-coupled receptors are blocked in the heart, we turned to βAR signaling because there could be feedback release of NE and EPI induced by the rise in blood pressure after chronic PE, serotonin, and Ang II exposure. To do this, we simultaneously treated NLC and αMHC-GqI mice with PE and the β1AR-antagonist metoprolol for 7 days. Although blockade of any potential βAR signal by metoprolol did not affect blood pressure responses to PE (Figure 2C), it did significantly attenuate the hypertrophic response in αMHC-GqI mice by 40 ± 2% (Figure 2D). This suggests that βAR stimulation can play a role in cardiac hypertrophy induced by PE treatment. Interestingly, this effect, presumably via catecholamines, is only evident when cardiac Gq-coupled receptors are blocked.

Release of Circulating Catecholamines by Chronic Gq-Coupled Receptor Stimulation
To further determine if endogenous catecholamines do indeed play a role in the Gq-coupled receptor agonist–mediated hypertrophy in the αMHC-GqI mice versus SM22-GqI mice, we obtained blood samples after chronic treatment with serotonin and analyzed them for levels of circulating EPI and NE (Figure 3). Interestingly, there was a significant difference in circulating EPI levels between the treated mice, with a 1.7-fold elevation observed in the αMHC-GqI compared with the SM22-GqI mice, which had values similar to PBS-treated mice (81.7 ± 8.1 ng/mL). There was no difference in the NE values attained. These data confirm that endogenous catecholamines do play a role in the hypertrophy seen in the αMHC-GqI mice.

Cardiac and Hemodynamic Responses to Chronic Gs-Coupled Receptor Stimulation
To confirm the Gq specificity of the GqI peptide, we chronically treated transgenic mice with the Gs-coupled βAR agonist ISO. NLC mice demonstrated a significant increase in heart weight/body weight ratio after treatment (Figure 4A). As expected, this was not inhibited by the GqI expressed in either VSM (SM22-GqI) or myocardium (αMHC-GqI) (Figure 4A). Surprisingly, ISO treatment induced an increase in blood pressure (Figure 4B), although this has been shown previously with catecholamine administration.3,4 The substantial increase in heart rate elicited by chronic treatment with this Gs-coupled receptor agonist (untreated mice, 242 ± 22 bpm; ISO-treated mice, 577 ± 29 bpm) likely mediates a mechanical mechanism to increase blood pressure and override the vasodilatory signals of the ligand. The hyperten-
admission. However, the critical finding of this study is that inhibition of Gq-mediated signaling prevented cardiac hypertrophy only when the peptide was expressed in VSM and not in the heart. This result clearly suggests that it is the hypertensive state, and its physiological and biochemical ramifications induced by vascular Gq activation, that mediates remodeling of the heart rather than direct stimulation of cardiac Gq-coupled GPCRs, which is a provocative and novel result.

The findings that PE-, serotonin-, and Ang II–induced hypertrophy proceeds via VSM Gq-coupled receptors are in contrast to the induction of cardiac hypertrophy by experimental pressure overload induced by TAC, in which myocardial Gq appears to be the common trigger for this response.11 Cardiac GqI expression not only attenuates pressure overload hypertrophy but also prevents chronic cardiac remodeling and heart failure long-term after TAC.17 However, in the present study, chronic administration of Gq-coupled receptor agonists, which can raise MAP significantly, do not induce cardiac hypertrophy via direct stimulation of the cardiomyocyte Gq pathway because αMHC-GqI expression had no effect on PE-, serotonin-, or Ang II–induced hypertrophy. However, these agonists did not elicit any hypertrophy whatsoever when VSM Gq-signaling was inhibited as in SM22-GqI mice. TAC may be more representative of cardiac hypertrophy that is induced by direct cardiac mechanical strain, whereas in the present study, increased total peripheral resistance induced by hormones/growth factors (eg, NE) resulted in cardiac hypertrophy. In fact, even for TAC, although the majority of the cardiac effects associated with it occur through Gq, there was non-Gq-mediated activation of p38 MAPK.18 Thus, other signaling pathways are probably also induced by TAC and by hypertension.

Initial analysis of the data suggested that the primary mechanism for induction of cardiac hypertrophy in the present study might be an increased secretion of catecholamines in response to the high blood pressure via feedback mechanisms in an attempt to reestablish basal resting vascular tone. In essential hypertension, elevated arterial pressure has been associated with increased central sympathetic output.19 In addition, the use of an animal model lacking the biosynthetic ability to convert dopamine to NE and EPI led to the conclusion that endogenous levels of these hormones are required, to a large extent, for the development of cardiac hypertrophy after TAC.20 These data may correlate with the inhibition of TAC-induced hypertrophy by the GqI, as NE acting via cardiac α1ARs may be the principal mechanism.

Our data also suggest that endogenous catecholamine release may be one of the mechanisms that lead to cardiac hypertrophy in our present model, because the addition of the βAR-blocker metoprolol partially inhibited the hypertrophy induced by PE in the αMHC-GqI mice. Analysis of circulating levels of EPI and NE confirmed the involvement of the catecholamine pathway in the present study. The elevated levels of EPI in the αMHC-GqI mice suggest that metoprolol was effective in reducing hypertrophy in these mice by blocking the βAR stimulation by catecholamines. It is possible that because our blood pressure increases are peripherally induced, EPI release from the adrenal medulla may be an
important mediator, in contrast to local NE release in TAC, thus having a greater impact on the cardiac βAR signaling system. Metoprolol may have only produced a partial attenuation of cardiac hypertrophy because it is more selective for β1AR versus β2AR. Moreover, blood pressure increases were maintained by PE with the addition of metoprolol; therefore, we did not reduce the primary hypertrophic stimulus. Interestingly, we did not see any decrease in hypertrophy in the NLC mice treated with PE and metoprolol. This may be because of cross-talk between certain Gq-coupled receptors and βARs, which has been suggested previously by Dorn and colleagues. Accordingly, this cross-talk would be blocked by αMHC-GqI expression and thus so would the decrease in hypertrophy. Further studies are warranted to investigate this potential exciting and novel mechanism of GPCR signaling the heart.

Further evidence that the catecholamines act directly via the βARs on the heart is that neither VSM nor cardiac Gq inhibition prevented hypertrophy induced by NE or ISO at the concentrations used in our experiments. Previous studies have also shown that the development of cardiac hypertrophy after chronic NE administration seems to occur directly via myocardiary ARs rather than via hemodynamic changes. Indeed, ISO has repeatedly been shown to induce cardiac hypertrophy directly via the βAR pathway, as evidenced by concomitant alterations in its signaling components.

It has also been demonstrated that chronic infusion of ISO is associated with elevation of the circulatory renin-angiotensin system, likely via renal βAR stimulation, although it has been shown that renin-angiotensin system did not play a major role in the cardiac trophic responses to ISO. This would concur with our results, as the GqI expressed in the cardiomyocytes would have inhibited Ang II signaling. αMHC-GqI mice not only had sustained hypertrophy in the face of increased MAP caused by chronic PE and serotonin administration but also had exposure to Ang II itself, emphasizing the lack of direct Gq-coupled receptor stimulation being involved. Importantly, Ang II elevation may go on to influence the production and release of hormones, from other cell-types including fibroblasts, in which Gq signaling is not blocked in our model, and this may then impact cardiomyocytes via autocrine and paracrine action. These hormones include aldosterone, as the final component of the renin-angiotensin system, endothelin-I, and transforming growth factor-β1 (TGF-β1), which act via myocyte-mediated non-Gq mechanisms or on cardiac fibroblasts to indirectly mediate cardiac hypertrophy.

Perspectives

The results of this study represent an important step in deciphering the in vivo physiological paradigms that are associated with hypertension and cardiac hypertrophy, particularly that which is mediated by the Gq-mediated signal transduction cascade. The finding that the cardiac Gq pathway does not mediate hypertrophy during chronic agonist-mediated Gq stimulation, implies a complex physiological response to increased blood pressure. Sympathetic activity is implicated in the cardiac remodeling observed; however, other factors might also be involved. Importantly, if we inhibit Gq-induced elevations in blood pressure, using VSM GqI expression, we can prevent the development of cardiac hypertrophy. These findings support other studies that have shown that agents that lower blood pressure, no matter what the mechanism, all appear to eventually be able to reverse hypertrophy. These findings, coupled with our experiments, suggest that peripheral resistance, and its physiological and biochemical adaptations, is the principal determinant of cardiac hypertrophy in states of hypertension. In addition, our studies have shown that expression of the GqI peptide in VSM is a novel therapeutic strategy to lower Gq-mediated hypertension and cardiac hypertrophy.

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References


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