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Original article

Inhibition of angiotensin II Gq signaling augments β-adrenergic receptor mediated effects in a renal artery stenosis model of high blood pressure ☆

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ABSTRACT

Chronic ventricular pressure overload states, such as hypertension, and elevated levels of neurohormones (norepinephrine, angiotensin II, endothelin-1) initiate cardiac hypertrophy and dysfunction and share the property of being able to bind to Gq-coupled 7-transmembrane receptors. The goal of the current study was to determine the role of endogenous cardiac myocyte Gq signaling and its role in cardiac hypertrophy and dysfunction during high blood pressure (BP). We induced renal artery stenosis for 8 weeks in control mice and mice expressing a peptide inhibitor of Gq signaling (GqI) using a 2 kidney, 1 clip renal artery stenosis model. 8 weeks following chronic high BP, control mice had cardiac hypertrophy and depressed function. Inhibition of cardiomyocyte Gq signaling did not reverse cardiac hypertrophy but attenuated increases in a profile of cardiac profibrotic genes and genes associated with remodeling. Inhibition of Gq signaling also attenuated the loss of cardiac function. We determined that Gq signaling downstream of angiotensin II receptor stimulation negatively impacted β-adrenergic receptor (AR) responses and inhibition of Gq signaling was sufficient to restore βAR-mediated responses. Therefore, in this study we found that Gq signaling negatively impacts cardiac function during high BP. Specifically, we found that inhibition of AT1-Gq signaling augmented βAR mediated effects in a renal artery stenosis model of hypertension. These observations may underlie additional, beneficial effects of angiotensinogen converting enzyme (ACE) inhibitors and angiotensin receptor antagonists observed during times of hemodynamic stress.

1. Introduction

Hypertension induces a chronic pressure overload that can cause the heart and its myocytes to enlarge or hypertrophy to maintain cardiac output against a persistent afterload. Increased plasma and local levels of hormones such as catecholamines and angiotensin II (AngII) are elevated. Importantly, these ligands bind to G protein-coupled receptors, some of which couple to the Gq heterotrimeric protein. Initially, these alterations are thought to be compensatory. However, chronic exposure and persistent activation of these hormone receptors usually makes the heart transition from compensated hypertrophy to a progressively dysfunctional state. The mechanisms underlying this transition remain unclear.

Enhanced Gq signaling in the heart has been linked to both hypertrophy and cardiomyopathy [1–3]. In vitro, it is clear that Gq coupled ligands, such as phenylephrine [4] and AngII [5] result in hypertrophy of neonatal rat cardiomyocytes. In vivo, the role of Gq signaling in adult cardiac myocytes is less well understood. Cardiac myocyte expression of either wild-type or a constitutively active mutant of Gαq from birth results in hypertrophy and cell death [6,7]. In contrast, other studies find when Gq expression is increased in adulthood, it results in dilated cardiomyopathy accompanied by reversible morphological changes [2] that rapidly progress to heart failure [3]. Importantly, when Gq signaling is increased in adulthood, there is an increase in heart size without concomitant individual cardiac myocyte hypertrophy [3]. Therefore, especially in adults under stress conditions that increase Gq signaling, the role of cardiac myocyte Gq signaling with respect to hypertrophy and function needs to be better elucidated.

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Previously, we generated high blood pressure (BP) in mice using chronic administration of individual ligands binding to Gq-coupled receptors such as phenylephrine and AngII [8]. If high BP was attenuated, the concomitant cardiac hypertrophy was prevented. Others have also documented in a simple BP model of AngII infusion that cardiac hypertrophy also follows increased BP [9]. In contrast, when we generated high BP using a renal artery stenosis model (2K1C) that is thought to more closely approximate human hypertension, cardiac hypertrophy persisted even with vascular smooth muscle specific reversal of the high BP [10]. In pressure overload generated by transverse aorta constriction, inhibition of cardiac myocyte Gq signaling attenuated cardiac hypertrophy, decreased re-expression of ventricular atrial natriuretic peptide and improved cardiac function [11] despite increases in wall stress [12]. The goal of the current study was to elucidate the role of endogenous cardiac myocyte Gq signaling in hypertrophy and dysfunction in the setting of induced hypertension.

2. Methods

2.1. Characterization of mice

Transgenic mice (C57Bl/6J) expressing GqI in cardiac myocytes and vascular smooth muscle have been previously characterized [8,11]. In the current study we used male and female mice, 8–20 weeks of age. Littermate mice not expressing the GqI transgene were used as controls. We verified persistent expression of cardiomyocyte GqI by performing transverse aorta constriction (TAC) as previously described [11–13] and harvesting the RNA from the left ventricle 1 week following surgery. We confirmed using Real-time PCR that ANP levels were increased 2.6-fold in control mice (control sham: 1.0±0.2, $n=5$ (where ANP expression in one control left ventricle was arbitrarily set to 1.0 and the other 4 hearts were compared to expression in the first and normalized to expression of 28S mRNA) versus control TAC: 2.6±0.1 as compared to control sham ANP expression, $n=6$, $P<0.05$ one-way ANOVA, Bonferroni multi-comparison post-test). ANP expression was unchanged with TAC in the cardiac myocyte GqI expressing mice (GqI sham: 0.7±0.6 as compared to control sham versus GqI TAC: 0.6±0.3 as compared to control sham, $n=3$). In addition, ANP mRNA expression in GqI TAC mice was significantly less than control TAC mice ($P<0.05$, one-way ANOVA, Bonferroni multi-comparison post-test). These results are similar to what was documented previously [11] and gave us confidence the mice express cardiomyocytes GqI to a similar extent.

2.2. 2K1C surgery

2K1C surgery was performed as previously described [10]. Briefly, mice were anesthetized with ketamine (50 mg/kg) and xylazine (2.5 mg/kg) and a 0.12 mm stainless steel U-shaped clip was placed around the left renal artery. The incision was closed with 5-0 vicryl suture. Right kidney to left kidney weight ratios were recorded to ensure successful surgery. If the right kidney to left kidney weight ratio was ≤1.2, the animal was excluded from the study. Sham operations were identical except that the clip was removed before closure of the incision. Mice were treated with 20 mg/kg ibuprofen via the drinking water for 24 h after surgery. All protocols were approved and performed in accordance with Thomas Jefferson University IACUC regulations.

2.3. Measurement of conscious blood pressure

Mean arterial BP (MBP) was obtained as previously described in detail [10]. Briefly, MBP was measured in mice that were conscious and unrestrained via radiotelemetry implanted into the left common carotid artery (Data Sciences International) 8 weeks after surgery. Measurements were recorded 4 days post implantation of the device and averaged over a 24-h time period. Mice were treated with 20 mg/kg ibuprofen via the drinking water for 24 h after surgery. BP was also considered during light and dark periods during a 24 h cycle.

2.4. Histology

Histological methods have been described previously in detail [10,14]. Masson's trichrome stain was used. Cross-sectional area (CSA) was calculated by using an Olympus confocal microscope in the brightfield setting. Individual myocytes were outlined and FluoView software was used to calculate CSA. At least 100 myocytes were counted from each heart section by a blinded observer.

2.5. Echocardiography

Echocardiography methods have been described previously [10]. Briefly, mice were anesthetized with isoflurane (1–2%) at a level where heart rate was consistent for control purposes. Images were acquired in M-mode echocardiography and measurements were determined by calculations within the Vevo Software (Visual Sonics).

2.6. Vascular reactivity

Abdominal aorta were dissected and 2.5-mm segments hung on a force pressure transducer as described previously [8,14,15]. Briefly, segments were denuded of endothelial cells and loss of endothelial cells was verified by lack of response to 10^{-5} mol/L acetylcholine. Rings were also stimulated with 60 mmol/L KCl and were discarded if there was no response. Increasing doses of isoproterenol were administered in 3 min time intervals. Importantly, doses were normalized to an EC_{50} dose of phenylephrine. We also stimulated with 3×10^{-7} mol/L AngII, which provides a maximal contraction in control and GqI aortic rings [10].

2.7. Quantitative RT-PCR

mRNA levels were measured as previously described [15]. Total RNA was isolated from whole hearts (Ultraspec) and 1 μg was converted into cDNA (BioRad Iscript) and used as a template for polymerase chain reaction using the fluorophore SYBR green (BioRad). Genes of interest were compared to levels of a housekeeping gene, 28S (Fwd: TTG AAAATCCGGGGGAGAG, Rev: CATTGTTCCAA CATGCCAG); Adamts2 (Fwd: GTCTCGCTACTGCTCCATCC, Rev: GGGTGATCTT CAGTGGCAT); Tenascin C (Fwd: GCTACTGCCAGGCATCTTTC, Rev: GAAGCTCCCACTGGAC TCTG); thrombospondin-1 (Fwd: GGGGCAG-GAAGAC TATGACA, Rev: CTCCCCGTTTTGTCTGTGT); periostin (Fwd: GAGACAGTG GGCTTCCG, Rev: GCCAGCAAAGTGTATTCTC); MMP3 (Fwd: GATCGATGTGC CATTTCTA, Rev::ACACTGGAAAGTCCTCAGC).

3. Results

3.1. Renal artery stenosis causes similar increases in BP and cardiac hypertrophy in control and mice with inhibition of Gq signaling in cardiac myocytes

Eight weeks following 2K1C surgery, MBP as determined in conscious mice increased more than 20% in both controls (122.6±5.3 mmHg, $n=6$) and mice with cardiac myocyte expression of an inhibitor of Gq signaling, GqI (126.8±8.5 mmHg, $n=6$) compared to sham-operated mice (control: 97.5±5.8, $n=6$ and GqI: 100.5±4.2 mmHg, $n=4$) (Fig. 1A). When considered with respect to light: dark cycles, there were no differences in systolic or diastolic BP between sham control and GqI mice or 2K1C control and GqI mice (Fig. 1B). Heart rates were also measured during the BP reading and were not different between shams and 2K1C mice (Control sham:

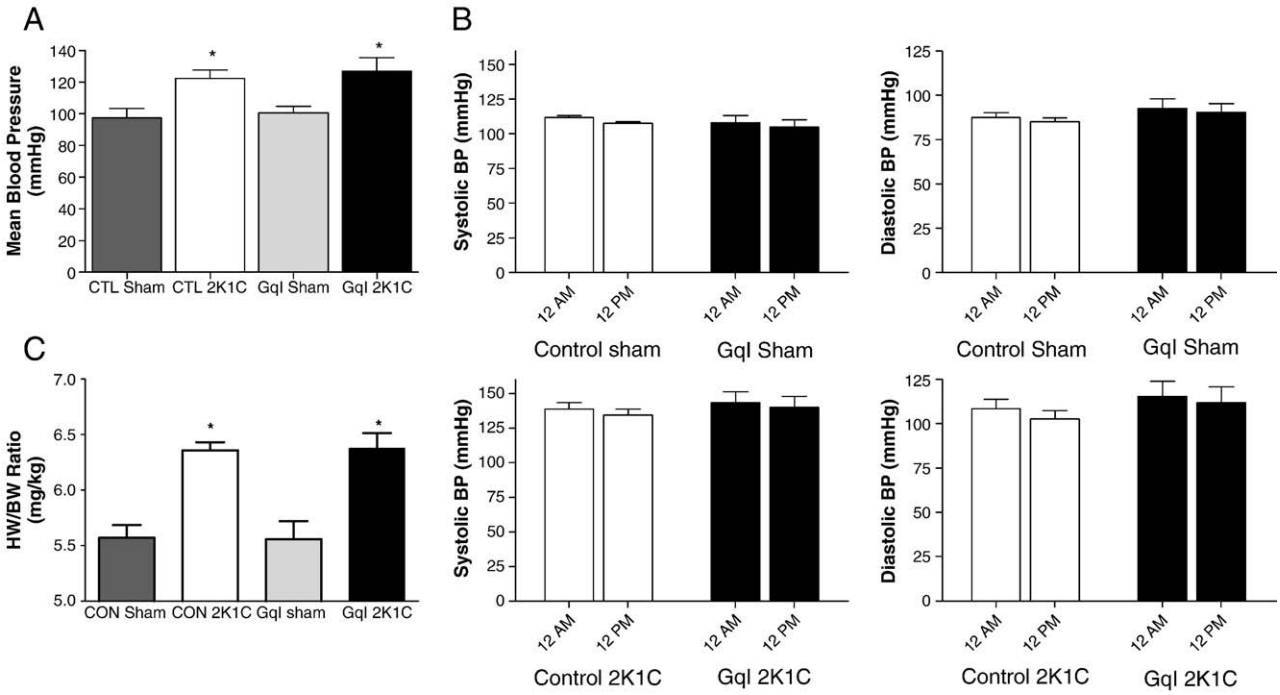

Fig. 1. Renal artery stenosis increases BP and cardiac hypertrophy equally in control (CTL) mice and mice with cardiac myocyte expression of a peptide inhibitor of Gq (GqI) signaling. (A) Eight weeks following 2K1C surgery, there was an approximate 25% increase in mean BP in control and GqI mice. *$P<0.05$, One-way ANOVA, Bonferroni multiple comparison post-test sham versus 2K1C ($n=6, 6, 4, 6$). (B) Systolic and diastolic BP was similar between sham and 2K1C control ($n=6, 6$) and GqI ($n=4, 6$) mice regardless of light (12p) or dark (12a). (C) Heart-weight-to-body weight ratio in control mice and mice with cardiac myocyte GqI expression 8 weeks following sham or 2K1C surgery. *$P<0.05$, One-way ANOVA, Bonferroni multiple comparison post-test sham versus 2K1C ($n=19, 22, 10, 22$).

598±29, $n=6$, Control 2K1C: 638±43, $n=6$, GqI sham: 597±33, $n=4$, GqI 2K1C: 632±32, $n=6$).

Eight weeks following 2K1C, cardiac hypertrophy, as determined using heart weight to body weight (HW/BW) ratios, was increased similarly in control (sham: 5.57±0.11, $n=19$, 2K1C: 6.36±0.07, $n=22$) and GqI mice (sham: 5.56±0.16, $n=10$, 2K1C: 6.37±0.14, $n=22$) (Fig. 1C).

We also used M-mode echocardiography to assess in vivo cardiac function and morphology in sham and 2K1C mice. 2K1C hypertension increased cardiac hypertrophy as indicated by the increase in posterior wall thickness (PWT) (Table 1).

Therefore, our data suggest that inhibition of Gq signaling in cardiac myocytes does not prevent gross cardiac hypertrophy in a renal artery stenosis model of hypertension. However, the heart is comprised of numerous cell types in addition to myocytes including fibroblasts and vascular smooth muscle. Unlike previous studies, we were interested in the effect of increased afterload directly on the cardiac myocyte therefore we isolated cardiac myocytes and investigated hypertrophic status of the myocyte alone.

Table 1
Increased cardiac function, but no alterations in cardiac hypertrophy, when Gq signaling is inhibited in cardiac myocytes during chronic hypertension

		CTL Sham (12)	GqI Sham (6)	CTL 2K1C (18)	GqI 2K1C (15)
PWT (mm)	Basal	0.75±.04	0.74±.06	0.75±.02	0.72±.03
	8 weeks	0.80±.02	0.75±.05	1.02±.04*	0.92±.04*
EDD (mm)	Basal	3.07±.07	3.23±.15	3.27±.07	3.14±.07
	8 weeks	3.35±.14	3.49±.15	3.69±.14	3.79±.12
HR (bpm)	Basal	378±8	383±13	384±6	392±8
	8 weeks	402±8	394±14	423±9	390±8

Echocardiography was performed on anesthetized animals at given time points. PWT=posterior wall thickness, EDD=end diastolic diameter, HR=heart rate. (number of animals in parantheses) *$P<0.05$ vs. respective sham.

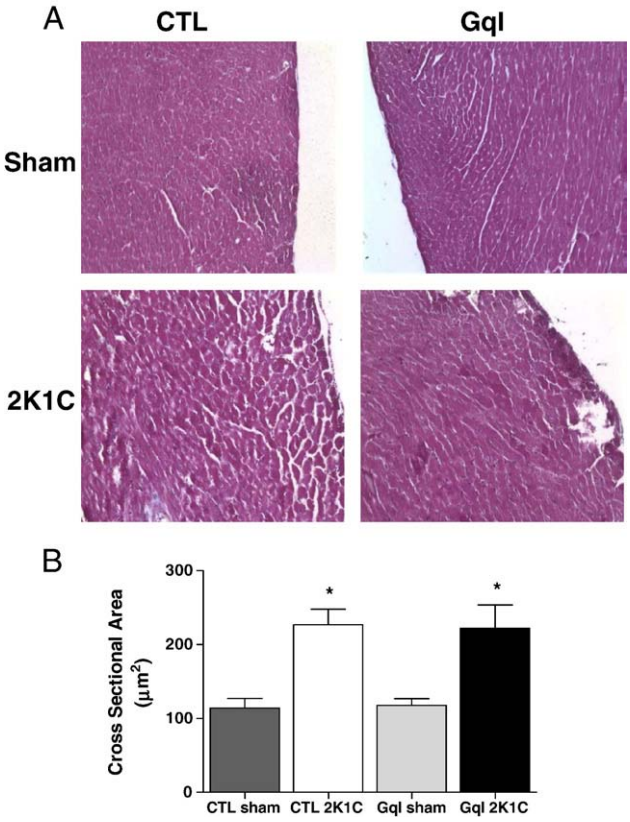

Fig. 2. Renal artery stenosis induces cardiac and myocyte hypertrophy to a similar extent in both control mice and mice with cardiac myocyte expression of GqI. (A) Representative histology images from control and GqI hearts 8 weeks following sham or 2K1C surgery using Masson's trichrome stain. (B) Average data showing that following 2K1C there is a similar increase in cross-sectional area (CSA) in control and GqI hearts. *$P<0.05$, One-way ANOVA, Bonferroni multiple comparison post-test sham versus 2K1C ($n=3–6$).

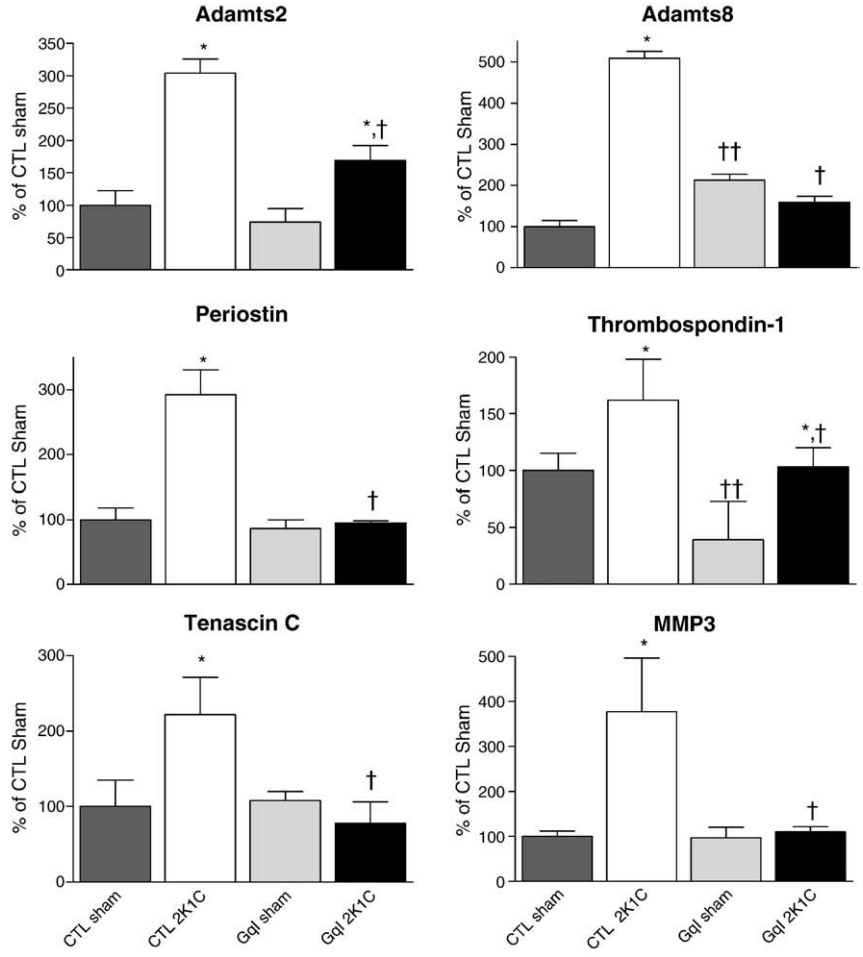

Fig. 3. Inhibition of cardiac myocyte Gq signaling decreases extracellular matrix and profibrotic gene expression following renal artery stenosis induced hypertension. These data represent six prominent genes upregulated in control hearts following 2K1C. Inhibiting Gq signaling in cardiac myocytes decreases the expression of these genes following 2K1C. *P<0.05 vs. sham, †P<0.05 vs. NLC 2K1C, ††P<0.05 vs. NLC sham n=6–8).

3.2. Cardiac myocyte Gq signaling does not confer myocyte hypertrophy in the 2K1C model of increased afterload

Previous data suggest that Gq signaling may [6,7] or may not [2] be involved in adult cardiac myocyte hypertrophy. To measure cardiac myocyte size, we performed histology on hearts fixed 8 weeks following sham or 2K1C surgery. Representative images are shown in Fig. 2A. Myocyte cross-sectional area (CSA) was measured using a confocal microsope in bright field. Eight weeks following 2K1C surgery there was a similar increase in myocyte CSA in both control (sham: 114.2±12.9 μm^2, n=3, 2K1C: 227.0±21.2 μm^2, n=6) and GqI (sham: 117.5±9.2 μm^2, n=6, 2K1C: 222.2±31.4 μm^2, n=3) hearts (Fig. 2B). These data suggest that myocyte hypertrophy is similar between control and GqI hearts and that it occurs independent of

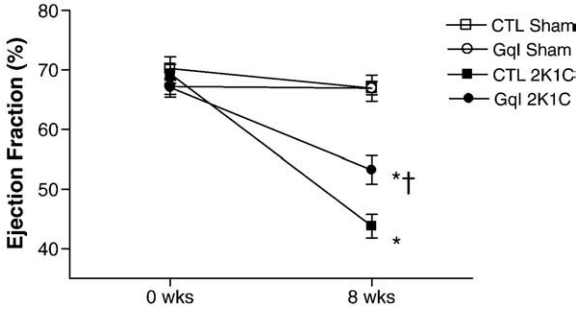

Fig. 4. Hypertension decreases cardiac function after 8 weeks and inhibition of Gq signaling attenuates the loss of function. Echocardiography was performed on mice basally and 8 weeks following sham or 2K1C surgery. There was no difference in ejection fraction (EF) basally (0 weeks) between sham control mice and mice expressing GqI or 8 weeks later. After 8 weeks, GqI mice had improved cardiac function compared to control mice. (n=12 control sham, n=6 GqI sham, n=18 CTL 2K1C, n=15 GqI 2K1C). *P<0.05 versus respective sham, two-tailed unpaired Student's t-test, †P<.05 vs. CTL 2K1C.

Table 2
β-adrenergic receptor blockade decreases cardiac function in mice expressing GqI following chronic hypertension

		CTL 2K1C+metoprolol (6)	GqI 2K1C+metoprolol (6)
PWT (mm)	Basal	0.74±.02	0.80±.03
	8 weeks	0.91±.05*	0.93±.06*
HR (bpm)	Basal	445±16	429±16
	8 weeks	422±20	424±13
MAP (mm Hg)		133±6	130±10
HW/BW ratio		6.53±0.2	6.5±0.5
Conscious HR (bpm)		536±64	576±32

Metoprolol (10 mg/kg/day) was given in the drinking water of mice for 8 weeks. Echocardiography was performed at given time points. Mean arterial pressure (MAP) was acquired in conscious mice by radiotelemetry. PWT=posterior wall thickness, HR=heart rate, HW/BW ratio= heart weight to body weight ratio. *P;0.05 versus basal.

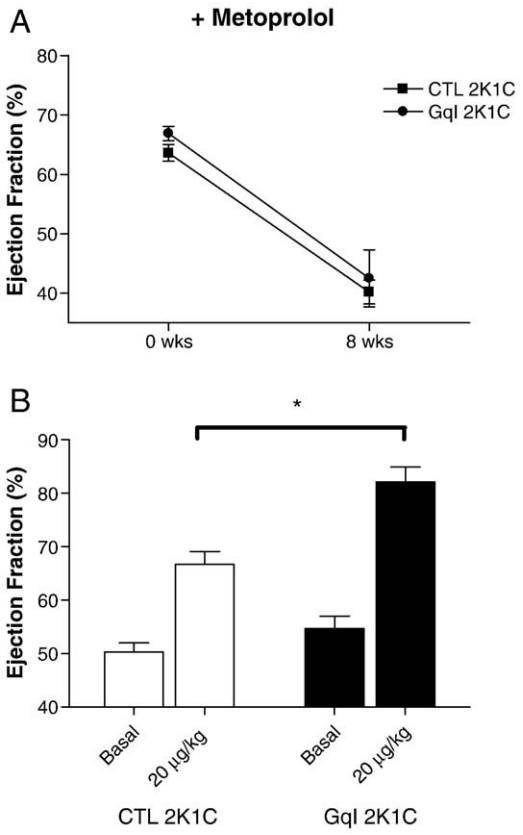

Fig. 5. Chronic inhibition of βAR stimulation abrogates the improvement in cardiac function imparted by Gq inhibition and alternatively, Gq inhibition augments acute in vivo βAR stimulation. (A) 10 mg/kg/d Metoprolol was given in the drinking water of mice for 8 weeks. Echocardiography was performed basally and 8 weeks following sham or 2K1C surgery. $n=6$ for both control and GqI mice. (B) EF was measured using M-mode echocardiography in mice following 2 weeks of renal artery stenosis. Basal EF was similar between control mice and mice expressing GqI. Mice were injected intraperitoneally with 20 μg/kg isoproterenol. EF was recorded after 2 min. There was no difference in basal cardiac function or after a maximal dose of isoproterenol (30 mg/kg) (Control 2K1C EF=90.1±1.7%, $n=7$, GqI 2K1C EF=90.7±0.8%, $n=7$). *$P<0.05$, two-way ANOVA, Bonferroni multiple comparison post-test.

cardiac Gq signaling in this model. Although we were unable to quantify it, we did note that there appeared to be an increase in extracellular space surrounding the myocytes following 2K1C in control but not GqI hearts (Fig. 2A) similar to what has been described previously [2]. Therefore, we were interested in determining whether cardiac myocyte Gq inhibition affected cardiac expression of profibrotic genes and genes associated with remodeling such that there was a change in the composition of cardiac extracellular matrix.

3.3. Inhibition of cardiac myocyte Gq signaling decreased expression of a profile of profibrotic genes

Initially, we performed oligo microarray analysis for extracellular matrix and adhesion molecules (data not shown). We identified genes of interest and further pursued them using quantitative RT-PCR (Fig. 3). High BP induced by renal artery stenosis increased expression of numerous profibrotic genes and genes associated with remodeling including Adamts2, Adamts8, periostin, thrombospondin-1, tenascin C and matrix metalloproteinase 3 (MMP3). Although inhibition of Gq signaling did not significantly prevent individual cardiac myocyte hypertrophy, it did attenuate the increase in mRNA expression of profibrotic and remodeling genes (Fig. 3) although at time point we investigated we were unable to histologically detect any differences (Fig. 2). These data suggest that cardiac myocyte Gq signaling may play a role in the maintenance of the extracellular matrix by influencing other cell types of the heart which potentially include fibroblasts, endothelial cells and other circulating cells although this remains to be further elucidated.

3.4. Inhibition of cardiac myocyte Gq signaling in hypertension improves in vivo cardiac function

Using M-mode echocardiography on mice before surgery and 8 weeks following sham or 2K1C surgery, we found no difference in any basal parameters between control and GqI mice (Table 1). There were no significant changes in either control or GqI sham mice over the 8 week period. Following 8 weeks of hypertension, cardiac function, as measured using ejection fraction, decreased approximately 40% in control mice. Inhibition of Gq signaling significantly attenuated this loss of function (Fig. 4).

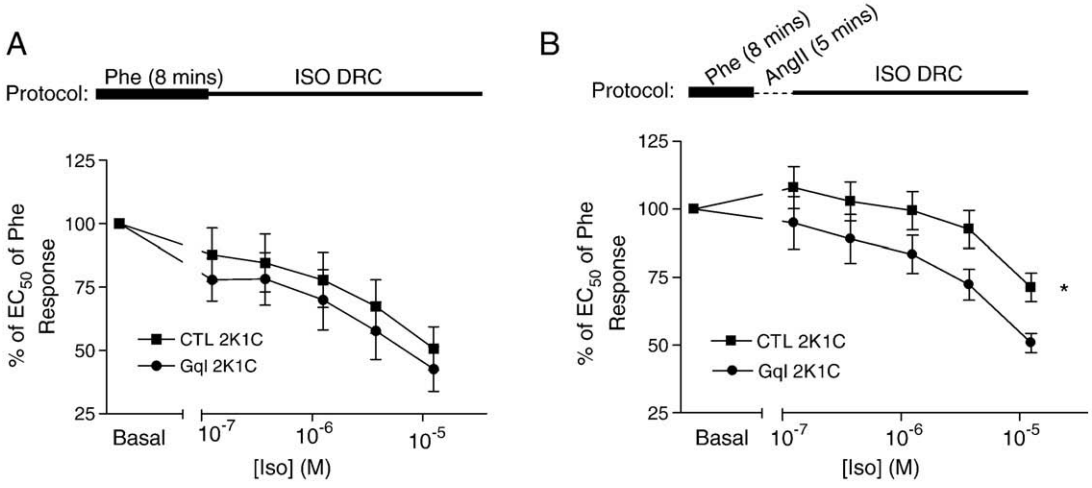

Fig. 6. Inhibition of Gq signaling in the setting of hypertension alleviates AngII attenuation of βAR-mediated response. (A) Abdominal aortic rings from control and GqI 2K1C mice were preconstricted with phenylephrine (Phe) (using an EC_{50} dose of 3×10^{-7} mol/L) for 8 min . Subsequently, a dose response curve to isoproterenol (Iso) was performed. There was no difference between Control (CTL) (■) and GqI (●) βAR-mediated decrease in tension. (B) Rings were preconstricted with Phe (3×10^{-7} mol/L) for 8 min , then stimulated with AngII (3×10^{-7} mol/L) for 5 min . There was a peak additional vasoconstriction to AngII then tension returned to baseline established with Phe preconstriction within 5 min (data not shown). Subsequently an Isoproterenol dose response was performed. Vessels with inhibition of Gq signaling had a greater decrease in tension in response to Iso than Control rings. *$P<0.05$, two-way ANOVA, Bonferroni multiple comparison post-test Control versus GqI, ($n=5$, 6).

3.5. βAR signaling is involved in the improvement in cardiac function following inhibition of Gq signaling during hypertension

Next, we wanted to determine whether a common clinically prescribed therapeutic for cardiac dysfunction, βAR antagonists, could further improve cardiac function in GqI mice following 2K1C. Metoprolol (10 mg/kg/day) was given to a subset of control and GqI mice following 2K1C surgery for 8 weeks and echocardiography was performed before surgery and following 8 weeks (Table 2). Chronic metoprolol treatment reversed the improvement in cardiac function in the GqI mice (Fig. 5A). These data suggest that alterations in βAR signaling may underlie improved cardiac function in GqI mice following 2K1C.

Chronic βAR inhibition attenuated the cardiac myocyte GqI-mediated improvement in function. Next, we wanted to test whether direct βAR stimulation in these hearts was improved. We performed in vivo stress echocardiography on both control and GqI mice by administering isoproterenol acutely (intraperitoneal) while determining cardiac function. We examined this response 2 weeks following renal artery stenosis to understand initial changes. We previously verified that BP was increased at this time point [10]. Two weeks following 2K1C, control (50.3±1.7%, $n=7$) and GqI (54.7±2.3%, $n=7$) mice had similar cardiac function (Fig. 5B). A low, submaximal dose of isoproterenol (100 μL, 20 μg/kg) increased cardiac function in GqI mice 50% (82.1±2.8%, $n=7$) whereas cardiac function was increased less than 30% in control mice (66.7±2.4%, $n=7$) with isoproterenol. PBS vehicle control had no response in either control or GqI mice. When control and GqI mice were injected with a large, maximal dose of isoproterenol (30 mg/kg), both had similar increases in cardiac function suggesting that a similar response is possible and that it is unlikely that changes in βAR expression is conferring the difference in response.

3.6. βAR-mediated responses are diminished in the presence of AngII and Gq inhibition restores this response

An important aspect of the 2K1C model of hypertension is that it relies on the renin-angiotensin system (RAS) to initiate increases in BP. Previous studies suggest that there is a direct interaction between AngII receptors and βARs [16]. We were unable to achieve a detectable, consistent influence of AngII signaling on isolated mouse cardiac myocytes (data not shown). Therefore, we took advantage of another mouse model at our disposal that expresses GqI in vascular smooth muscle [8,10] to determine interactions between AngII receptors and βAR receptors in the setting of high BP induced by renal artery stenosis. We subjected control mice and mice with vascular smooth muscle expression of GqI [8,10] to 2K1C surgery. 2 weeks later, we isolated abdominal thoracic aorta segments, scraped off endothelial cells and performed vascular reactivity studies.

Segments of abdominal aorta were preconstricted with an EC_{50} concentration of phenylephrine for 8 min and then an isoproterenol dose response curve was performed (Fig. 6A). In the abdominal aorta, a blood vessel which we had not previously examined, there was no significant difference in the constriction in response to the EC_{50} concentration of phenylephrine between control and GqI mice (data not shown). These findings were somewhat surprising but may have to do with the αAR subtype expressed and the possibility that the αAR subtype couples to another G protein such as G12/13 as it is pertussis toxin insensitive (data not shown) although this remains to be determined.

There were no differences in βAR mediated relaxation between control and GqI vessels (Fig. 6A). In our model of high BP, in vivo AngII levels are elevated therefore, we recapitulated this in vivo aspect in situ. Vascular segments were preconstricted with an EC_{50} concentration of phenylephrine then stimulated with AngII (3×10^{-7} mol/L) to determine whether concurrent stimulation of the Gq coupled AngII AT1 receptors could influence βAR-mediated vasodilation. Subsequently, vasodilation in response to isoproterenol was measured (Fig. 6B). Following 2 weeks of high BP mediated by renal artery stenosis, in the presence of AngII, blood vessels expressing GqI had enhanced βAR-mediated relaxation compared to controls. These data suggest that AngII Gq signaling can negatively regulate βAR signaling during high BP. These data also suggest that inhibition of Gq signaling improves βAR-mediated responses and therefore cardiac function in the setting of hypertension through the alleviation of the negative effects of AngII signaling on βARs.

4. Discussion

Increased plasma and local levels of catecholamines and AngII play a critical role in modulating cardiac function following hemodynamic stress such as hypertension. The objective of the current study was to determine if inhibiting cardiac Gq signaling during hypertension could prevent cardiac hypertrophy and dysfunction. Our data show that although cardiac hypertrophy was similar in hearts from control and GqI mice, cardiac myocyte expression of GqI improved expression of a panel of profibrotic and remodeling genes. Inhibition of cardiac myocyte Gq signaling also improved cardiac function following 2K1C induced hypertension. An important finding in our study was that βAR blockade prevented the improved cardiac function in GqI mice following 2K1C, suggesting an interaction between Gq and βAR mediated contractility. We provide in vivo data showing that GqI mice have a greater increase in cardiac function following acute isoproterenol injection. Furthermore, we show that following simultaneous AngII and isoproterenol stimulation, inhibition of Gq signaling enhances βAR mediated responses which should lead to an increase in cardiac function. These data suggest in hypertension, Gq mediated signaling, and in particular AngII-mediated signaling, negatively impacts βAR responses such that cardiac function is compromised.

Our present data are consistent with previous studies showing that GqI improved cardiac function following TAC [12]. It also corroborates with another study that showed mice with global gene ablation of G11 and cardiac myocyte specific gene deletion of Gq had improved cardiac function following TAC [17]. Similar to our study, the previous GqI study found no alterations in the thickness of the left ventricular wall following TAC [12]. In contrast to the current study, they note a decrease in left ventricular EDD in GqI mice, which is probably responsible for the decrease in left ventricle to body weight (LV/BW) ratio [12]. The decreased LV/BW ratio, at least at 7 days post TAC, was associated with decreased activation of ERK and JNK [13]. The differences between the previous studies and the current study are most likely due to the differences in pressure overload models since we did verify persistent and consistent GqI expression. TAC induces a large, acute pressure overload, whereas renal artery stenosis hypertension is a more slowly developing subtle, chronic increase in afterload. Other investigators have also documented discrepancies in findings between TAC and hypertension. Klein et al performed TAC on PKCε knockout mice [18]. Although overexpression of PKCε caused cardiac hypertrophy, TAC in mice with gene ablation of PKCε had a similar amount of cardiac hypertrophy as wildtype mice. In contrast, Inagaki et al used a Dahl salt-sensitive hypertensive rat model and pharmacologically inhibited PKCε. In contrast to the study using TAC, they found that PKCε attenuated cardiac fibrosis and dysfunction in the hypertension induced heart failure [19]. The latter study attributed the differences due to etiology of pressure overload.

It is well established that there are increased plasma levels of renin in the 2K1C model as well as increased expression and activity of angiotensin converting enzyme (ACE) within the heart [20,21]. The role of AngII in TAC induced pressure overload is not well understood although studies have shown that Losartan, an AngII Type 1 receptor (AT1) antagonist, prevents cardiac hypertrophy following TAC in mice [22]. Conversely, when cats were subjected to pulmonary artery banding, which induces pressure overload more gradually than TAC in

the mouse, it was found that increased hemodynamic load itself is capable of stimulating cardiac growth, independent of the renin-angiotensin II system [23]. A previously published study from our lab further supports the hypothesis that BP elevation is responsible for cardiac hypertrophy following infusion of Gq coupled agonists [8]. We have also shown that decreasing BP in the 2K1C model did not decrease cardiac hypertrophy following 2K1C [10]. Also of note, mice lacking G11 had the most significant decrease in cardiac hypertrophy with TAC and the additional removal of cardiac Gq concurrent with this contributed to much less to further decreases in TAC induced hypertrophy suggesting G11 may be more critical to cardiac hypertrophy that Gq although this remains to be determined [17]. Our data suggest that cardiac myocyte hypertrophy following 2K1C induced hypertension occurs independent of cardiac myocyte Gq signaling. We cannot rule out the role of G11 and this is a focus of future studies. In addition, non-Gq coupled hormones are also increased in 2K1C that may be involved in the cardiac hypertrophy including aldosterone [24] and (pro)renin.

In this study, we identified that inhibition of Gq signaling was sufficient to reverse a panel of cardiac profibrotic and remodeling genes. AngII is linked to fibrosis [25] and AngII can induce TGFβ through TSP-1 and MMPs and this induction is a significant factor in the development of fibrosis [26]. AngII, by itself, can also increase profibrotic genes. Our data would suggest these genes are upregulated in hypertension and they are activated by a Gq coupled pathway. Whether AngII is the only endogenous hypertension associated ligand remains to be determined. In addition, whether this gene profile represents early stage remodeling in the progression of heart failure also remains to be determined. We were unable to document any differences in collagen deposition following 8 weeks of high BP. Whether longer time points are required to document fibrotic changes in the heart induced by the 2K1C model and reversed by cardiac GqI expression remains to be determined.

Our data suggest in the setting of high BP, inhibiting Gq signaling improves βAR response. This is in contrast to other studies that found there was no difference between control and GqI mice either basally or following agonist-stimulation with dobutamine [12]. When hemodynamic analysis was performed 7 days following TAC, GqI mice had almost an 2-fold increase in cardiac function when stimulated with dobutamine (78% vs. 41%).[12] Additionally, conscious echocardiography was studied and undoubtedly, endogenous catecholamines are most likely elevated within the animal and may be contributing to improved function [12]. It was hypothesized that the improved βAR signaling was due to a lack of PI3K activation and that PI3K may play an important role in the transition from hypertrophy to failure [12]. Overall, this indicates that PI3K regulation downstream of Gq signaling could be responsible for the observations in the current study although it remains to be determined. The precise molecular mechanism in which Gq signaling attenuates βAR mediated effects in the setting of hypertension is a focus of future studies. It is known that the GqI peptide competitively inhibits Gq signaling and decreases activation of IP_3 and DAG, subsequently leading to less PKC activation [11]. PKC has been shown to phosphorylate and desensitize βARs, particularly the β_1AR [27]. Therefore, a potential mechanism is that GqI prevents PKC activation during chronic AngII stimulation occurring during 2K1C, which leads to βAR uncoupling and less downstream signaling. It needs to be determined whether the mechanism of action is indirect through signaling molecules or direct through other mechanisms such as heterodimerization [28].

To our knowledge, our current study is the first to look at an interaction of AngII Gq-coupled and βAR signaling in a high BP model. Decreases in sympathetic vasoregulation in skeletal muscle following 2K1C hypertension have been observed indicating a potential interaction between elevated AngII and βAR regulation [29]. Other studies have shown that AngII enhances βAR mediated relaxation due to other cAMP forming pathways [30,31]. However, in contrast to our studies in hypertension, these studies were performed under normal physiological conditions. It was also shown in control normal hearts AT1Rs can directly interact with both β_1AR and β_2ARs and that this interaction elicited a phenomenon by which selective βAR blockade inhibited signaling of AT1Rs, whereas selective AT_1R blockade inhibited downstream signaling of βARs [16]. These studies were also performed on control "normal" hearts whereas our studies were performed during disease conditions.

Numerous alterations occur during pressure overload that could potentially affect both AngII and βAR signaling in the heart. The AT1 receptor binds to both Gαq and GαI [32]. Although, the GqI peptide inhibits Gq signaling [8,11], the impact on the ability of the receptor to couple to Gαi is not known. GqI is specific to Gq inhibition [11] but it is unknown whether Gq inhibition could facilitate increased signaling through other G proteins. Studies have reported increases in Gαi protein expression and subsequent decreases in adenylyl cyclase activity following the volume dependent, 1 kidney, 1 clip model of hypertension [33,34]. Increases in Gαi would decrease the formation of cAMP due to βAR stimulation and would attenuate βAR responses which is in opposition to our results. In addition, Gαs adenylyl cyclase activation is impaired in the vasculature during hypertension but impaired Gαs-adenylyl cyclase activity is found less often in cardiac myocytes [35,36]. This is likely not the primary defect in the current study since we obtain similar βAR mediated effects when AngII is not present. Another common feature associated with the development of heart failure is downregulation or desensitization of βARs [37]. Heart failure is associated with increased levels of G protein receptor kinase 2 (GRK2), which uncouples βARs and AT1 receptors [38,39]. Although we cannot rule out alterations in GRKs, it is likely not the cause since AngII initiated a similar amount of vasoconstriction at the dose we studied and βAR responses were similar in the absence of AngII.

The present study suggests that cardiac myocyte AT1-Gq signaling decreases βAR-mediated responses and contributes to depressed cardiac function and remodeling during renal artery stenosis induced hypertension. The current study shows that hypertension induced by 2K1C can alter in vivo cardiac responses to βAR stimulation and blockade. Additionally, this study shows that inhibiting AT1-Gq signaling can augment βAR mediated effects in the 2K1C model of hypertension. These observations may underlie additional, beneficial effects of ACE inhibitors and angiotensin receptors antagonists observed during times of hemodynamic stress.

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