iment 2 demonstrates that in noun-phrase production the syntactic gender of a noun is retrieved before its abstract phonological properties.

By comparing the go LRP with the no-go LRP obtained in experiment 1, we can estimate the length of the time interval in which syntactic but no phonological information of the noun was available. Two time points are of interest from this comparison (Fig. 3). First, the go and no-go LRPs started to develop at about 370 ms after picture onset, so at that moment, syntactic gender was available to select the correct response hand. Second, at about 410 ms after picture onset the go and no-go LRPs diverged sharply. While the go LRP continued to develop, the no-go LRP gradually returned to the base line. This indicates that there was already enough phonological information available at 40 ms after LRP onset to make the go or no-go distinction. Thus, in noun-phrase production it takes only about 40 ms to retrieve a noun's initial phoneme once its syntactic gender has been retrieved.

These data provide fine-grained temporal information about the moments at which distinct word representations are retrieved from the mental lexicon during the real-time process of speaking. The empirical approach that we have presented opens the way for further, temporally fine-grained neurophysiological analyses of the uniquely human skill of speaking.

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4. Thirty-two right-handed students between 21 and 29 years of age took part in the experiments. All were native speakers of Dutch and had normal or corrected-to-normal vision, and none had any neurological impairment or had experienced any neurological trauma according to their answers to a questionnaire.
5. The materials consisted of 48 colored line drawings depicting objects and animals with morphologically simple names. Half of the picture names had common gender, and the other half had neuter gender. The words were matched for word frequency, number of syllables, and word length. There were no clear semantic differences between the sets of common and neuter gender words. Two sets of pictures were constructed. In one set the names of the pictures started with the phonemes /b/ and /s/, in the other set the picture names started with the phonemes /k/ and /v/. Each of the phonemes was represented equally often in the picture sets. To control for material-specific effects, we rotated the assignment of the four response types to the different picture categories across participants in such a way that each picture contributed equally to each of the response types. Each target picture was presented to each participant four times in naming-only trials and six times in trials that required the additional classification task. A trial started with the presentation of a fixation cross. After 1500 ms a picture was presented for 2500 ms in either the color yellow or red. Participants were instructed to name the colored picture as quickly as possible using a noun phrase without a determiner. On half of the trials a frame appeared around the picture at 150 ms after picture onset, signaling that the classification task had to be carried out and naming had to be delayed. The syntactic classification involved a decision about the noun's definite article (either "de" or "het"), and the phonological classification involved a decision about the word's initial phoneme (for example, /b/ or /s/). For go trials, participants made a hand response by pressing with their index finger either the button on the left side or the button on the right side of the chair in which they were seated. For no-go trials participants did not press any of the buttons. At 1650 ms after picture onset the frame disappeared, and participants had to produce the appropriate no-determiner noun phrase.
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11. Trials on which participants produced utterances other than the appropriate ones or gave an incorrect hand response were eliminated from the data. Incorrect hand responses were defined as go trials in which electromyogram (EMG) activity was detected on the incorrect response side, and no-go trials in which EMG activity occurred. In experiment 1 error rates were 4.8 and 1.5% for go and no-go trials, respectively. In experiment 2 error rates were 4.9 and 3.1% for the go and no-go trials, respectively. All single-trial waveforms containing movement artifacts in the time window of 200 ms before picture onset to 1500 ms after picture onset were removed from the data. Per participant, the minimum number of trials left for averaging was 35 per condition. For each single-trial waveform the average voltage in the 200-ms period preceding picture onset was subtracted from the voltage at all following time points. LRPs were derived separately for the go and no-go conditions. To test for the presence of an LRP and to estimate its onset, we performed analyses on 50-ms intervals, starting from frame onset in sequential steps of 10 ms (for example, 150 to 200 ms, 160 to 210 ms, and so on). For each window a one-tailed t test with a 95% confidence interval was performed to test whether the mean voltage within the window exceeded the mean voltage within the base-line interval. An LRP was defined to be present if five or more consecutive windows resulted in a significant t value. The onset of the first of these windows was taken to be the LRP onset latency. To determine the point of divergence between the go and no-go LRPs, we subtracted the average voltage at each individual time point of the no-go waveform from the average voltage at the corresponding time points of the go waveform. We performed one-tailed t tests to test whether the mean go and no-go difference scores differed significantly from zero, using the same procedure as described for the individual LRP waveforms.

12. We thank M. Coles, M. Kutas, W. Levelt, A. Roelofs, and H. Schriefers for valuable suggestions; H. Clark, J. Marshall, and A. Martin for their comments on the manuscript; and I. Doehring for preparing the figures. Supported by a stipend from the Max Planck Society to M.v.T. and by grant 400-56-384 from the Netherlands Organization for Scientific Research to P.H. and C.M.B.

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Targeting the Receptor-G_q Interface to Inhibit in Vivo Pressure Overload Myocardial Hypertrophy

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Hormones and neurotransmitters may mediate common responses through receptors that couple to the same class of heterotrimeric guanine nucleotide–binding (G) protein. For example, several receptors that couple to G_q class proteins can induce cardiomyocyte hypertrophy. Class-specific inhibition of G_q-mediated signaling was produced in the hearts of transgenic mice by targeted expression of a carboxyl-terminal peptide of the α subunit $G\alpha_q$. When pressure overload was surgically induced, the transgenic mice developed significantly less ventricular hypertrophy than control animals. The data demonstrate the role of myocardial G_q in the initiation of myocardial hypertrophy and indicate a possible strategy for preventing pathophysiological signaling by simultaneously blocking multiple receptors coupled to G_q.

Myocardial hypertrophy is an adaptive response to various mechanical and hormonal stimuli and represents an initial step in the pathogenesis of many cardiac diseases that ultimately progress to ventricular failure. The mechanisms by which cardiac hypertrophy is initiated and how this condition eventually progresses to heart failure are poorly understood. Several independent signaling pathways have been implicated in the activation of the hypertrophic response in vitro (*1*). The G protein G_q is thought to be important in this process because various ligands, such as phenylephrine, angiotensin II (AngII), and endothelin I, that activate G_q-coupled receptors can trigger hypertrophic responses in cultured myocytes (*2*). In vivo studies with G_q-coupled receptor antagonists have also implicated G_q-mediated signaling in pressure-overload ventricular hypertrophy (*3*), and transgenic mice with cardiac overexpression of either $G\alpha_q$, α_1-

adrenergic receptors (ARs), or AngII receptors present with myocardial hypertrophy (*4*–*6*). Although these data indicate that chronic stimulation of G_q-coupled receptors is sufficient to induce myocardial hypertrophy, they do not define the contribution of G_q to the physiological hypertrophic response to ventricular pressure overload.

We examined the possibility of class-specific G protein inhibition through targeting the receptor-G_q interface, thereby simultaneously eliminating responses from multiple receptors that couple to G_q. This therapeutic strategy would potentially eliminate the need for multiple receptor antagonists in a variety of diseases including pressure overload hypertrophy. We designed a COOH-terminal peptide of $G\alpha_q$ that contains the region of the $G\alpha$ subunit that interacts with the intracellular domains of agonist-occupied receptors (*7*) and created transgenic mice with myocardial-targeted overexpression of this peptide.

Cellular expression of the third intracellular domain (3i) of the α_{1B}-AR antagonizes in vitro α_{1B}-AR–mediated signal transduction, apparently through competition between the 3i peptide and the activated receptor for binding sites on $G\alpha_q$ (*8*). We sought to determine whether the expression of peptides derived from the COOH-terminus of $G\alpha_q$ would similarly antagonize receptor-mediated signaling. Two $G\alpha_q$ "minigene" constructs were created that correspond to the COOH-terminal peptide sequence of $G\alpha_q$, residues 305 to 359, and the NH_2-terminal peptide sequence of $G\alpha_q$, residues 1 to 54. COS-7 cells were transiently transfected with plasmid DNA encoding the $G\alpha_q$ minigenes, and expression of these peptides was demonstrated by protein immunoblotting (Fig. 1A). Coexpression of α_{1B}-ARs with the intact $G\alpha_q$ subunit led to enhancement of epinephrine-stimulated inositol phosphate (IP) production compared with that in cells expressing equal numbers of receptors alone. In contrast, coexpression of $G\alpha_q$(305-359) resulted in a marked inhibition (47.8 ± 4.4%) of maximal α_{1B}-AR–mediated IP production (Fig. 1B). Coexpression of the $G\alpha_q$ NH_2-terminus [$G\alpha_q$(1-54)] had no effect. Inhibition by $G\alpha_q$(305-359) was apparently specific for G_q-coupled receptors because neither α_{2A}-AR–mediated IP production (G_i-coupled) nor dopamine D_{1A} receptor–mediated cAMP production (G_s-coupled) were inhibited, whereas signaling through both the G_q-coupled α_{1B}-AR and M1 muscarinic acetylcholine receptor (AChR) were attenuated (Fig. 1C). Thus, the expression of $G\alpha_q$(305-359) specifically uncouples G_q-coupled receptors.

To study the effects of this peptide on G_q-mediated signaling pathways in vivo, we created transgenic mice with cardiac-specific expression of $G\alpha_q$(305-359). This G_q inhibitor transgene (G_qI) was targeted to the myocardium by linking it with the murine α-myosin heavy chain (αMyHC) promoter (*9*, *10*). Five founder lines that transmitted the transgene were established (TG G_qI-8, -10, -11, -26, and -38). The TG G_qI-10 line had the greatest transgene expression as shown by Northern (RNA) analysis (*11*), so we used heterozygous (+/−) animals of this line in all further studies. At 10 weeks of age, G_qI peptide expression was documented by protein immunoblot analysis of myocardial extracts from the TG G_qI-10 line (Fig. 2A). These transgenic mice were normal in size, appearance, and behavior compared with

Table 1. Myocardial sarcolemmal membrane adenylyl cyclase activity. ISO, isoproterenol. Activity is presented as picomoles of cAMP per minute per milligram of protein and is the mean ± SEM of $n = 6$ for each group.

Hearts	Basal	ISO (10^{-6} M)	ISO (10^{-4} M)	NaF (10^{-2} M)
Control (NLC)	43.3 ± 6.5	61.3 ± 5.5*	63.4 ± 5.3*	343 ± 16
TG G_qI	40.9 ± 3.3	61.8 ± 5.6*	61.1 ± 4.6*	333 ± 21

*$P < 0.005$ ISO-stimulated values versus basal (ANOVA with post-hoc Scheffe test). *P*, not significant for TG G_qI values versus NLC (two-way repeated measures ANOVA).

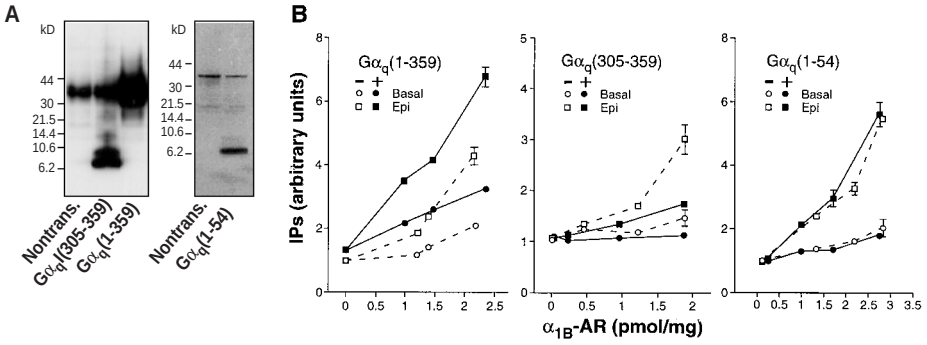

Fig. 1. Selective in vitro inhibition of G_q-receptor coupling by expression of the $G\alpha_q$(305-359) peptide. (**A**) COS-7 cells were transiently transfected with plasmid DNA encoding either empty vector (nontransfected), intact $G\alpha_q$(1-359), $G\alpha_q$I(305-359) (left panel), or $G\alpha_q$(1-54) (right panel) (*22*, *23*). Expression of the $G\alpha_q$ minigene products was determined by protein immunoblot analysis (*24*). (**B**) COS-7 cells were transiently transfected with plasmid DNA encoding the α_{1B}-AR (0.01 to 1.0 μg of DNA per well) and either intact $G\alpha_q$, $G\alpha_q$(305-359), or $G\alpha_q$(1-54) (2.0 μg of DNA per well) (*23*). Basal (circles) and epinephrine-stimulated (Epi, squares) IP production was determined as described (*8*) (solid lines). Responses from control cells transfected with the receptor plasmid plus empty vector are shown in each panel (dashed lines). Data are presented in arbitrary units such that one unit equals the basal amount of IPs measured in cells transfected with empty vector alone. Data shown represent mean ± SEM values for triplicate determinations in one of four separate experiments. (**C**) COS-7 cells were transiently transfected with plasmid DNA encoding the G_q/11-coupled α_{1B}-AR, the M_1 AChR, the G_i-coupled α_{2A}-AR, or the G_s-coupled D_{1A} dopamine receptor (0.1 μg of DNA per well), and either the $G\alpha_q$(305-359) minigene (black bars) or empty vector (2.0 μg per well) (open bars). Basal and agonist-stimulated IP or adenosine 3′,5′-monophosphate (cAMP) production was determined (*8*). Data are presented in arbitrary units such that one unit equals the basal amount of IP or cAMP measured in unstimulated cells transfected with empty vector alone. Each panel represents mean ± SEM values for three separate experiments performed in triplicate. *$P < 0.05$ versus control stimulation [analysis of variance (ANOVA)].

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nontransgenic littermate control (NLC) animals.

Stimulation of G_q-coupled receptors leads to the activation of phospholipase C and the generation of the second messengers inositol trisphosphate and diacylglycerol. As a direct measurement of the state of endogenous G_q signaling in these mice, we measured basal left ventricular diacylglycerol content (*12*). The diacylglycerol content in the TG G_qI mice was significantly depressed compared with that in NLC mice (Fig. 2B). This finding indicates that basal G_q signaling is decreased in the transgenic hearts, verifying the in vivo G_q-inhibitory properties of the transgene.

We also studied p42/44 mitogen-activated protein (MAP) kinase activity in response to endogenous myocardial G_q-coupled receptor stimulation. In anesthetized transgenic and NLC mice, we directly injected phenylephrine, AngII, or saline into the left ventricle (*13*). In the hearts of NLC animals, phenylephrine elicited an approximate threefold increase in MAP kinase activity, whereas very little stimulation of MAP kinase activity was caused by phenylephrine in TG G_qI mice (Fig. 2C). AngII-stimulated myocardial MAP kinase activity in TG G_qI mice was also significantly reduced compared with that in NLC mice (Fig. 2C). Similar results were also obtained with endothelin I (*11*). In all agonist studies, there was no difference in basal MAP kinase activity between TG G_qI and NLC myocardial extracts (Fig. 2C). Thus, acute in vivo signaling through multiple G_q-coupled receptors is inhibited by the G_qI peptide. To demonstrate specificity, we tested MAP kinase activation elicited by the G_i-coupled receptor agonist carbachol (*14*), and responses were the same in TG G_qI and NLC mice (Fig. 2D). In addition, adenylyl cyclase activity in response to β-AR–G_s stimulation was the same in TG G_qI and NLC myocardial membrane extracts (*15*) (Table 1). Thus, the G_qI peptide is specific for inhibiting G_q-coupled receptor signaling in vivo.

To assess the in vivo physiological role of G_q-coupled receptor signaling in the development of pressure overload hypertrophy, we subjected TG G_qI and NLC mice to pressure overload by surgical transverse aortic constriction (TAC) (*16*). In this model, left ventricular hypertrophy can be seen 7 days after surgery (*17*) by using the left ventricle weight to body weight ratio (LVW/BW) as an index of myocardial mass. There was no difference in LVW/BW between sham-operated TG G_qI and NLC mice (Table 2). In the TAC group, LVW/BW in NLC animals increased by 36% compared with that in sham-operated animals. In contrast, 7 days after TAC the TG G_qI mice had a significantly smaller increase (14%) in LVW/BW compared with sham-operated TG G_qI mice ($P < 0.01$). The mean systolic pressure gradient created by TAC, an index of the load placed on the ventricle, was not different between the two groups: 66.4 ± 7.4 mm Hg for TAC in NLC animals and 62.3 ± 6.8 mm Hg for TAC in TG G_qI animals (*P*, not significant). Across a wide range of systolic pressure gradients measured, LVW/BW was lower for the TG G_qI mice compared with that in NLC mice (Fig. 3). Therefore, it appears that cardiac G_q-coupled receptors

Table 2. Physiological parameters in response to pressure overload. Data are expressed as mean ± SEM. The systolic pressure gradient (SPG) is the difference between right and left carotid arterial systolic pressure, an index of load placed on the left ventricle.

Parameter	*Sham*		*TAC*	
	NLC (n = 8)	TG G_qI (n = 8)	NLC (n = 10)	TG G_qI (n = 18)
BW (g)	20.86 ± 0.73	21.40 ± 1.56	20.37 ± 0.64	22.62 ± 0.78
LVW/BW (mg/g)	3.84 ± 0.30	3.72 ± 0.15	5.35 ± 0.21*	4.31 ± 0.12*†
SPG (mm Hg)			66.4 ± 7.4	62.3 ± 6.8

*$P < 0.05$ NLC TAC versus NLC sham; TG G_qI TAC versus TG G_qI sham (*t* test). †$P < 0.005$ TG G_qI TAC versus NLC TAC (*t* test).

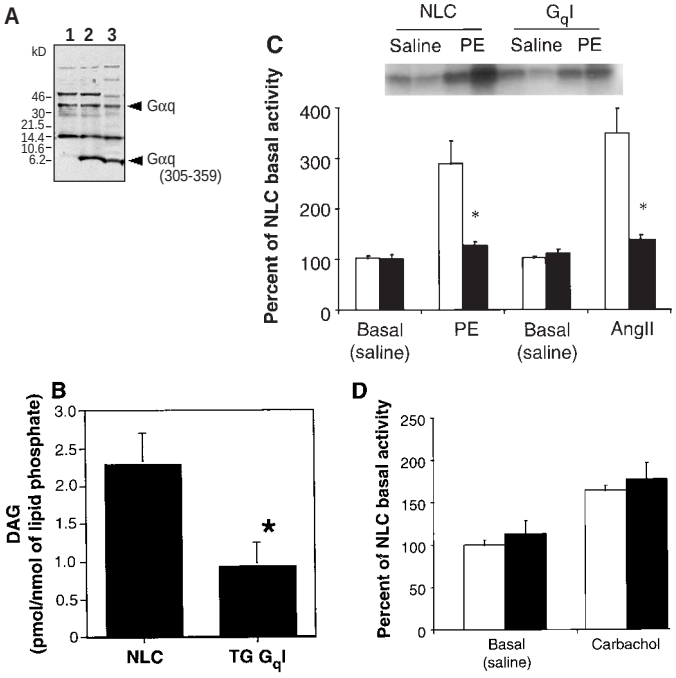

Fig. 2. Myocardial expression and in vivo inhibitory activity of the G_qI peptide. (**A**) Expression of $G\alpha_q$(305-359) was determined by protein immunoblot analysis of myocardial extracts from an NLC (lane 1) or TG G_qI (lane 2) heart, or from COS-7 cells expressing the $G\alpha_q$I(305-359) minigene product (lane 3) (*24*). (**B**) Lipid extraction was done from NLC (n = 5) and TG G_qI (n = 5) left ventricles, and basal diacylglycerol (DAG) content was quantified as described (*12*). Data shown are means ± SEM. *$P < 0.05$ versus NLC (Student's *t* test). (**C**) Left ventricle injections of the G_q-coupled receptor agonists phenylephrine (PE) (n = 6) or AngII (n = 8) (100 μM), compared with saline injections, were performed in NLC (open bars) and TG G_qI (black bars) mice, and myocardial MAP kinase activity toward myelin basic protein (MBP) was measured (*13*). Activity is expressed as the percent of NLC basal activity determined in saline-injected hearts. Data shown represent means ± SEM of phosphorylated MBP, quantified with a PhosphorImager. Also shown is a representative PE experiment done in two animals for each condition. *$P < 0.02$ versus NLC (Student's *t* test). (**D**) MAP kinase activation in response to carbachol, an agonist for G_i-coupled receptors (100 μM) (*14*) (n = 4). Carbachol-elicited responses in NLC (open bars) and TG G_qI (black bars) mice were significantly elevated ($P < 0.05$) compared with basal (saline-injected) responses (Student's *t* test).

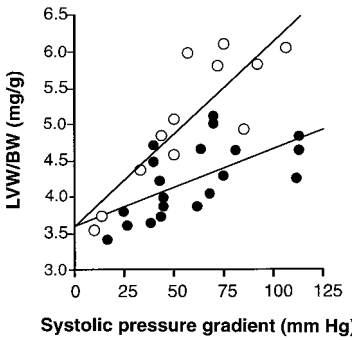

Fig. 3. Hypertrophic response to pressure overload. The index of left ventricular mass (LVW/BW) is plotted against the systolic pressure gradient produced by TAC for each NLC (n = 12) and TG G_qI (n = 20) animal (open and black circles, respectively). The slopes of the linear regressions for NLC [$y = 0.025x + 3.61$, $r = 0.85$ (r is the correlation coefficient)] and TG G_qI ($y = 0.011x + 3.61$, $r = 0.60$) animals were significantly different ($P < 0.0005$, ANOVA).

play a critical role in triggering left ventricular hypertrophy after the mechanical stimulus of hemodynamic stress.

Myocardial hypertrophy is associated with enhanced G_q signaling and accompanied by reactivation of ventricular embryonic genes including those for atrial natriuretic factor (ANF), skeletal α-actin, and β-myosin heavy chain (*1*). Similar findings have been reported in vitro after stimulation of G_q-coupled receptors, particularly α_1-ARs (*18*). We therefore measured ventricular ANF mRNA in TG G_qI and NLC mice 7 days after sham-operation or TAC. Basal ventricular ANF mRNA was nearly undetectable and not different between the two groups (Fig. 4). However, after the stimulus of pressure overload, ventricular ANF mRNA increased almost sevenfold in the NLC group but only about twofold in TG G_qI mice (Fig. 4). Furthermore, after TAC, left ventricular diacylglycerol content was increased in NLC mice (73%) but not in TG G_qI mice (*11*).

Because the depression of basal diacylglycerol content in TG G_qI mice did not affect LVW/BW ratios in the absence of pressure overload (Table 2), it appears that G_q-mediated signals do not influence the normal growth of myocytes. In fact, no phenotype is evident in these animals until stress is placed on the heart. Transgenic mice with cardiac-specific expression of a constitutively active mutant α_{1B}-AR have a hypertrophic phenotype (*4*). Transgenic mice with cardiac AngII receptor overexpression show even greater myocardial hypertrophy (*5*). Also, a transgenic mouse model with cardiac overexpression of $G\alpha_q$ itself exhibits myocardial hypertrophy (*6*).

Antagonists of AngII or endothelin I can attenuate ventricular hypertrophy and heart failure in response to pressure overload in animal models (*3, 19*). However, because these drugs act on vascular receptors to alter afterload, the direct involvement of these myocardial receptors in the hypertrophic response has not been established. Our approach was to block signaling from multiple receptors coupled to a single class of G proteins. TG G_qI mice after TAC have the opportunity to use multiple mechanisms for initiating compensatory hypertrophy except signaling through G_q-coupled receptors present on cardiomyocytes. Our results indicate that G_q is a critical molecule in the initiation of myocardial hypertrophy. Targeting the receptor–G protein interface may point the way to the development of therapies that have the potential advantage over traditional receptor antagonists of dampening an entire class of receptor signals (those coupled to a particular G protein) rather than those derived from only a single type of receptor.

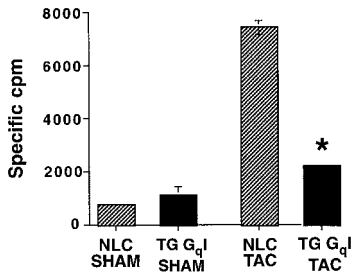

Fig. 4. Left ventricle ANF mRNA quantitation. Total RNA (15 μg) was isolated (*25*) from the left ventricles of NLC (hatched bars) and TG G_qI (black bars) hearts that underwent sham-operation or TAC. Northern blots were generated and probed with a mouse ANF cDNA followed by a glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA (*4*). The signals from the ANF blots were quantified with a PhosphorImager and normalized to the GAPDH signal. Data shown are the means ± SEM for $n = 5$ in each group. *$P < 0.05$ TG G_qI TAC versus NLC TAC (ANOVA).

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9. A 5.5-kb Sal I–Sac I fragment containing the murine αMyHC promoter was ligated into a plasmid containing the SV-40 intron polyadenylate signal (*20, 21*), and a 300–base pair fragment encoding the amino acids 305 to 359 of murine $G\alpha_q$ (G_qI) was ligated to generate pGEM-αMHC-G_qI-SV-40. The transgene was then linearized and purified before pronuclear injections done by the Duke Comprehensive Cancer Center Transgenic Facility. Offspring were screened by Southern (DNA) blot analysis with a probe to the SV-40 sequences (*4, 10, 20, 21*). Institutional Review Board approval for all mouse experiments was obtained from each institution involved.
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12. Lipid fractions were extracted from 50 mg of homogenized myocardial tissue, and ^{32}P-labeled diacylglycerol was isolated by silica gel thin-layer chromatography as described (*4*) and quantified with a PhosphorImager (Molecular Dynamics). Diacylglycerol content is expressed as picomoles of diacylglycerol per nanomole of lipid phosphate (*4*).
13. Mice were anesthetized with a mixture of ketamine (100 mg per kilogram of body weight, intraperitoneally) and xylazine (2.5 mg/kg, intraperitoneally). Direct left ventricle intracavitary injection through a left thoracotomy of 100 μl of either phosphate-buffered saline, 100 μM AngII, or 100 μM phenylephrine I was administered. Ninety seconds after injection, hearts were excised and homogenized in 2 ml of RIPA buffer [50 mM tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 0.25% deoxycholate] and then centrifuged at 40,000*g* for 30 min. We immunoprecipitated 1 mg of clarified myocardial extract in 1 ml of RIPA at 4°C for 2 hours using an antibody to MAP kinase and protein A–agarose (Santa Cruz Biotechnology). The samples were then centrifuged at 18,000*g* for 10 min, and the sedimented material was washed twice with 1 ml of RIPA and twice with 1 ml of kinase buffer [20 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 1mM dithiothreitol]. Samples were then resuspended in 40 μl of kinase buffer with MBP (0.25 mg/ml), 20 μM adenosine triphosphate (ATP), and [γ-^{32}P]ATP (20 μCi/ml) and incubated at room temperature for 30 min. The reactions were quenched with 40 μl of 2× Laemmli buffer, and 30 μl of each reaction was electrophoresed through a 4 to 20% polyacrylamide:tris-glycine gel. Phosphorylated MBP on dried gels was quantified with a PhosphorImager (Molecular Dynamics).
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16. Mice were anesthetized (*13*), and microsurgical procedures were done under a dissecting microscope (*17*). Animals were intubated, and TAC was performed as described to yield a reproducible transverse aortic constriction of 65 to 70% (*17*). Sham-operated animals underwent the same operation except for aortic constriction. After 7 days of aortic constriction mice were anesthetized (*13*), and simultaneous measurement of right and left carotid artery pressures was recorded (*17*). Hearts were excised and chambers dissected free and weighed, then snap frozen in liquid N_2.
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22. All cDNAs were subcloned by standard methods into the eukaryotic expression vector pRK5 (*8*). The cDNA fragments were obtained by using standard polymerase chain reaction conditions, and sequences were confirmed by dideoxynucleotide sequencing (*8*).
23. Transient transfection studies were performed with COS-7 cells as described (*8*). Cells were seeded in six-well tissue culture plates (10^5 cells per well) the day before transfection and then transfected by using DEAE-dextran (*8*) and a total of 1 to 2 μg of DNA per well. Assays were performed 48 hours later.
24. Expression of endogenous or transiently expressed $G\alpha_q$ or the $G\alpha_q$(305-359) and $G\alpha_q$(1-54) minigene products was determined by protein immunoblot analysis. Whole-cell RIPA buffer lysates were denatured by boiling cells in Laemmli sample buffer, then resolved on 10 to 20% polyacrylamide:tricine SDS gels (20 μg of COS-7 cell protein per lane or 50 μg of myocardial protein per lane) and transferred to nitrocellulose. Filters were probed with rabbit polyclonal antisera raised to either the $G\alpha_q$ NH_2-terminus (amino acids 13 to 29) or the $G\alpha_q$ COOH-terminus (amino acids 341 to 359) (Santa Cruz Biotechnology), and ^{125}I-labeled protein A (Amersham) or horseradish peroxidase–conjugated donkey antibody to rabbit immunoglobulin G (Jackson Laboratories) was used for detection.
25. Ventricular tissue was separated from the atria under a dissecting microscope. Total ventricular RNA was extracted with RNAzol (Biotecx) as described (*20, 21*).

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Targeting the Receptor-G_q Interface to Inhibit in Vivo Pressure Overload Myocardial Hypertrophy

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