

Overexpression of Myocardial Gs α Prevents Full Expression of Catecholamine Desensitization Despite Increased β -Adrenergic Receptor Kinase

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Abstract

Inotropic and chronotropic responses to catecholamines in young adult transgenic mice overexpressing myocardial Gs α are enhanced. One might predict that over the life of the animal, this chronically enhanced β -adrenergic receptor stimulation would result in homologous catecholamine desensitization. To test this hypothesis, old transgenic Gs α mice and age-matched controls were studied physiologically in terms of responsiveness of left ventricular function (ejection fraction) to isoproterenol in vivo and in vitro in terms of β -adrenergic receptor signaling. Old transgenic mice still responded to isoproterenol with augmented ($P < 0.05$) left ventricular ejection fraction ($+44 \pm 3\%$) compared with age-matched controls ($+24 \pm 1\%$). Although total β -adrenergic receptor density was reduced in the old transgenic mice, and G protein receptor kinase 2 (β -adrenergic receptor kinase) levels were increased, the fraction of receptors binding agonist with high affinity as well as isoproterenol- and G protein-stimulated adenylyl cyclase activities were enhanced. Thus, classical catecholamine desensitization is not effective in attenuation of persistently enhanced responses to sympathetic stimulation in mice overexpressing myocardial Gs α . To support this conclusion further, experiments were performed with chronic isoproterenol, which elicited effective desensitization in wild-type controls, but failed to elicit desensitization in overexpressed Gs α mice. The results of this study suggest that the lack of protective desensitization mechanisms may be responsible in part for the dilated cardiomyopathy which develops with chronic sympathetic stress over the life of these animals. (*J. Clin. Invest.* 1998; 101:1916–1922.) Key words: G proteins • desensitization • adenylyl cyclase • cardiomyopathy • transgenic mice

Introduction

A fundamental question concerning the regulation of myocardial function relates to the role of the β -adrenergic receptor

signaling pathway in the pathogenesis of heart failure. There are two diametrically opposed points of view. One position is that in the setting of a myocardial insult, cardiac dilation and myocardial dysfunction progress because the β -adrenergic signaling pathway desensitizes, potentially due to compensatory increases in sympathetic tone with resultant loss of inotropic input. The other view is that persistent stimulation of this pathway is deleterious and exacerbates the progression of cardiac dysfunction and heart failure. If the latter is correct, desensitization of the β -adrenergic receptor pathway could be considered protective.

We have developed and characterized a transgenic mouse model in which Gs α is overexpressed selectively in the myocardium (1–3). These animals exhibit cardiac supersensitivity to catecholamines as a result of enhanced β -adrenergic receptor coupling to various effector pathways, specifically adenylyl cyclase and L-type calcium channels (2–4). Interestingly, these animals over time develop cardiac dysfunction characterized by cardiocyte dropout, cardiac fibrosis, left ventricular (LV) dilation, depression of LV mechanical function, and an increase in sudden death. We sought to determine whether the β -adrenergic receptor signaling pathway desensitizes as these deleterious changes are occurring. Accordingly, in this study we characterized both physiologically and biochemically the function of the β -adrenergic receptor pathway in the heart of Gs α transgenic animals as they age and compared these findings with those obtained from age-matched wild-type controls. This latter point is particularly important because aging by itself can affect β -adrenergic signaling (5, 6). To further test our hypothesis, we challenged old Gs α mice with chronic isoproterenol infusions, which induce effective desensitization in normal mice (7).

We found that the transgenic mouse demonstrates incomplete desensitization. Despite decreased β -adrenergic receptor density and increased β -adrenergic receptor kinase (β ARK) activity, the older transgenic animals continue to exhibit enhanced β -adrenergic receptor signaling activity nearly identical to the young transgenic and greater than the young wild-type mice. This animal model presents a unique paradigm wherein the β -adrenergic receptor signaling pathway remains hyperresponsive despite the progressive development of myocardial dysfunction, and where enhanced sympathetic reactivity would be predicted to result in desensitization of the pathway. We hypothesize that the progressive injury that occurs in the heart of these animals over time might have been mitigated if effective desensitization of the β -adrenergic receptor signaling pathway had occurred.

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1. *Abbreviations used in this paper:* β ARK, β -adrenergic receptor kinase; GRK2, G protein receptor kinase 2; ¹²⁵I-cyp, ¹²⁵I-cyanopindolol; LV, left ventricular; LVEF, LV ejection fraction; LVID, LV internal dimension.

Methods

Physiological studies. Two groups of older mice were studied, transgenic mice (age 16 ± 0.6 mo, $n = 5$), and wild-type littermates (age 17 ± 2.3 mo, $n = 5$). Mice of either sex from the same genetic background as the transgenic mice were included in the physiological study. All mice used in this study were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. After determination of body weight, mice were anesthetized with ketamine (0.065 mg/g), acepromazine (0.002 mg/g), and xylazine (0.013 mg/g) injected intraperitoneally (2, 3, 8) and were allowed to breathe spontaneously as described previously by Hoit et al. (8). The chest was shaved, and mice were positioned prone on a warmed saline bag as support. The saline bag was attached to a warming pad (Deltaphase isothermal pad; Braintree Scientific Inc., Braintree, MA) to keep body temperature constant at 37°C. Electrocardiographic leads were attached to each limb using needle electrodes (Grass Instrument Co., Quincy, MA). Echocardiography was performed using ultrasonography (Apogee X-200; Interspec Inc., Ambler, PA). A dynamically focused 9 MHz annular array transducer was applied from below, using the saline bag as a standoff. The heart was first imaged using the two-dimensional mode in both the parasternal long axis and short axis views. The short axis views including papillary muscles were used to position the M-mode cursor perpendicular to the ventricular septum and LV posterior wall, under baseline conditions and in response to isoproterenol administered intravenously (i.v.) through a chronically implanted jugular vein catheter.

Studies were recorded on 0.5-inch videotape (S-VHS; Sony Corp., Tokyo, Japan). Freeze frames were printed on a color printer (UP-5200; Sony Corp.). The electrocardiogram was recorded simultaneously for heart rate measurement. The images were scanned into a Macintosh IICx computer and digitized at 300 pixels/in. Gray scale equalization was made using the Photoshop program (Adobe Inc., Mountain View, CA), and the images were imported into an NIH Image program for measurement. M-mode measurements of LV internal dimension (LVID) were sampled from more than three beats and averaged, using the leading edge to leading edge convention adopted by the American Society of Echocardiography (9). End-diastole (d) was measured at the time of the apparent maximal LV diastolic dimension. End-systole (s) was measured at the time of the most anterior systolic excursion of the posterior wall. LV ejection fraction (LVEF) was calculated by the cubed method as follows:

$$\text{LVEF} = [(LVIDd)^3 - (LVIDs)^3] / LVIDd^3.$$

In young G_{α} mice and their control littermates, after anesthesia with an intraperitoneal injection of ketamine (0.065 mg/g), xylazine (0.013 mg/g), and acepromazine (0.002 mg/g), chronic isoproterenol or vehicle delivery was administered using a miniosmotic pump (model 2002; Alza Corp., Palo Alto, CA) implanted subcutaneously via a small interscapular incision and using sterile surgical technique. Isoproterenol bitartrate (Sigma Chemical Co., St. Louis, MO) was prepared in 0.9% sodium chloride (pH 5.5) at a concentration calculated to deliver an average of 30 $\mu\text{g/g/d}$ during a 13-d infusion period. Control mice received pumps filled with 0.9% sodium chloride for the 13-d infusion period.

Biochemical studies. Four groups of mice were studied: two groups of young adults (i.e., 3 ± 1 mo), 38 transgenic and 28 wild-type mice, and two groups of old adults (15 ± 2 mo), 20 transgenic and 22 wild-type mice. Because of the small size of the hearts, two to three ventricles were pooled in order to assay all of the necessary studies on a single pooled sample. After cervical dislocation, hearts were immediately excised and rinsed in iced saline, blotted, and weighed. Atria were removed, and the ventricles were minced and homogenized in 1 ml of Tris buffer (100 mM Tris-HCl, 2 mM EGTA, pH 7.2), then homogenized with a Polytron S20 (Brinkman Instruments, Inc., Westbury, NY) at a setting of 6 for 15 s. The homogenate was centrifuged at 14,000 g for 20 min at 4°C. The supernatant was saved for G pro-

tein receptor kinase 2 (GRK₂) immunoblotting. The pellet was resuspended in buffer, and homogenized and centrifuged as above. The pellet was resuspended in Tris buffer with a ground glass homogenizer and frozen at -70°C .

β -Adrenergic antagonist binding studies were performed using 25 μl isoproterenol (0.1 mM) or Tris buffer, 25 μl ^{125}I -cyanopindolol (^{125}I -cyp) (0.01–0.5 nM), and 100 μl of membrane protein (10 μg). Assays were performed in triplicate, incubated at 37°C for 40 min, terminated by rapid filtration on GF/C filters (Whatman Inc., Clifton, NJ), and counted in a γ counter (Tracor Inc., Austin, TX) for 1 min. The binding data were analyzed by the interactive LIGAND program of Munson and Rodbard (10). A linear regression was performed on the amount bound versus bound to free ligand.

Agonist binding curves were performed using 100 μl membrane protein (15 $\mu\text{g/assay}$), 25 μl of isoproterenol (10^{-9} to 10^{-4} M), with 22 concentrations of isoproterenol and ^{125}I -cyp (0.07 nM). Incubation and counting were as described for antagonist binding. In the LIGAND computer analysis (10), the F test was used to compare the best fit for the data. The best fit, two-site versus one-site, was determined by the P value for the F test.

Adenylyl cyclase activity was assayed according to the method of Salomon et al. (11). Cardiac membranes (25–50 μg) were added to a solution containing 1 mM ATP ($2-3 \times 10^6$ cpm of $[\alpha\text{-}^{32}\text{P}]\text{ATP}$), 20 mM creatine phosphate, creatine phosphokinase (1 U), 1 mM cAMP (2,000–3,000 cpm of $[\text{H}]\text{cAMP}$ as an internal standard), 25 mM Tris-HCl, 5 mM MgCl_2 , 1 mM EDTA, and the test substance to measure adenylyl cyclase activity [GTP (0.1 mM), isoproterenol (0.1 mM), guanylyl-5'- γ -imidodiphosphate [Gpp(NH)p] (0.1 mM), NaF (10 mM), and forskolin (0.1 mM)]. 10 μl of stopping solution (20 mM ATP, 10 mM cAMP, 2% SDS) was added to each tube to terminate the reaction, and the tubes were heated on a dry bath at 100°C for 3 min. cAMP was separated as described previously (11, 12). Recovery of added cAMP was 50–80%.

Immunoblotting was performed with specific antibodies to G_{α} and to GRK₂ (β ARK) (13–15). G_{α} tissue extracts and GRK₂ cytosolic extracts were prepared from mouse hearts as described, and lysed in Laemmli's buffer (62.5 mM Tris-HCl, 2% SDS, 1% β -mercaptoethanol, 25% glycerol, and 0.0025% bromophenol blue) at a concentration of 1 $\mu\text{g}/\mu\text{l}$. The lysate was boiled for 5 min, and 20 μl was run on 10% SDS-PAGE. The gel was transferred to Immobilon-P membranes (Millipore Corp., Bedford, MA) using a semidry transfer apparatus (Integrated Separation Systems, Natick, MA) for 30 min at 200 mA (for two minigels) in a transfer buffer composed of 25 mM Tris-HCl, 190 mM glycine, 20% methanol, and 3% SDS at pH 8.0. The membranes were blocked with 2.5% BSA (Sigma Chemical Co.) and 2.5% nonfat dry milk (Carnation®; Nestle USA, Inc., Glendale, CA) in TBST (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.1% Tween 20) for 1 h at room temperature. G_{α} blots were probed with antisera at 1:2,000 dilution of G_{α} polyclonal antibody (Dupont-NEN, Beverly, MA), for 1 h at room temperature which demonstrated the best signal to noise ratio. GRK₂ blots were probed with antisera at 1:300 dilution of GRK₂ polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA). Unbound antibodies were eliminated by three 5-min washes in TBST followed by incubation with anti-rabbit F(ab')₂ coupled to horseradish peroxidase (Amersham Corp., Arlington Heights, IL) at 1:3,000 dilution for 20 min at room temperature. Unbound antibodies were washed as above, and horseradish peroxidase was visualized on x-ray film (Hyperfilm; Amersham Corp.) with an enhanced chemiluminescence detection kit (DuPont-NEN). Protein measurements were made according to the methods of Lowry et al. (16) and Bradford (17). The latter assay, which does not sense the protein in fibrotic tissue (18), was particularly important to use, because older mice with overexpressed G_{α} have increased cardiac collagen (2). Since the Bradford/Lowry ratio was increased in the old transgenic (1.98) compared with age-matched control (1.77), young G_{α} (1.75), and young control mice (1.75), the biochemical data in the old transgenic mice were corrected by multiplying the Lowry protein value by the Bradford/Lowry ratio.

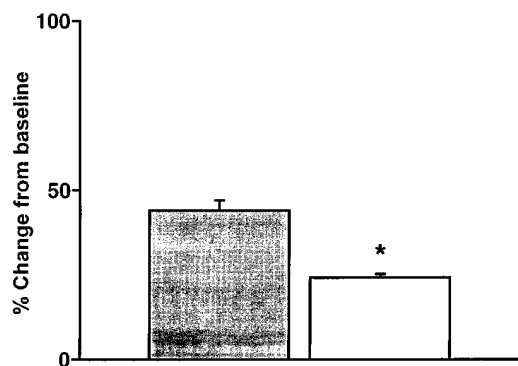


Figure 1. The increase in LVEF to isoproterenol, 0.05 $\mu\text{g/kg/min}$ i.v., was greater ($*P < 0.05$) in old G α (gray bars, $n = 5$) than old wild-type control mice (white bars, $n = 5$).

Data analysis and statistics. All data are reported as mean $\pm$ SEM. Statistical significance among young and old transgenic and control mice was calculated using a two-way factorial ANOVA. $P < 0.05$ was taken as the level of significance.

Results

Physiological studies

Old adult transgenic mice overexpressing myocardial G α exhibited enhanced responsiveness to sympathetic stimulation, as indicated by echocardiographic measurements of LVEF. For example, LVEF increased more with acute isoproterenol challenge (ΔLVEF : $44 \pm 3\%$) in older transgenic mice than in age-matched wild-type controls (ΔLVEF : $24 \pm 1\%$) (Fig. 1). Similar findings were noted previously in younger adult mice (2). Baseline heart rate was higher, $P < 0.05$, in old G α mice compared with old wild-type (365 ± 22 vs. 214 ± 15 beats per min [bpm]). With acute isoproterenol challenge, heart rates rose to higher levels, $P < 0.01$, in old G α compared with old control mice (399 ± 19 vs. 273 ± 31 bpm).

The effects of acute isoproterenol challenges were exam-

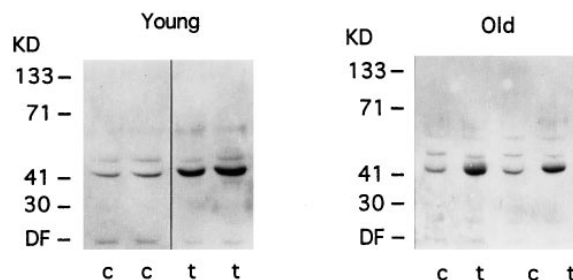


Figure 3. Immunoblotting of LV from young and old transgenic mice overexpressing myocardial G α (t). G α protein is three to five times higher in transgenic compared with littermate control mice (c). DF, Dye front.

ined on LVEF in young G α mice and young wild-type controls before and after chronic administration of isoproterenol via miniosmotic pumps (Fig. 2). Baseline values before chronic isoproterenol were not different. However, as expected, the acute isoproterenol challenges resulted in an augmented ejection fraction in the G α mice. Also, as predicted after chronic isoproterenol, baseline LV function in wild-type control mice was not different from values before chronic isoproterenol, because of the effectiveness of desensitization mechanisms. Furthermore, as predicted, the acute isoproterenol challenge failed to increase LVEF after chronic isoproterenol in the wild-type mice. Surprisingly, chronic isoproterenol did not result in desensitization in the G α mice, i.e., LV baseline ejection fraction after 2 wk of chronic isoproterenol remained at near-maximal levels ($> 90\%$ ejection fraction). Because LVEF was near maximal, it could not increase further with acute isoproterenol challenges. This confirms the hypothesis that the G α mice are resistant to catecholamine desensitization.

Biochemical studies

Western blotting. It was important to demonstrate that protein levels of G α were still increased in old transgenic mice. West-

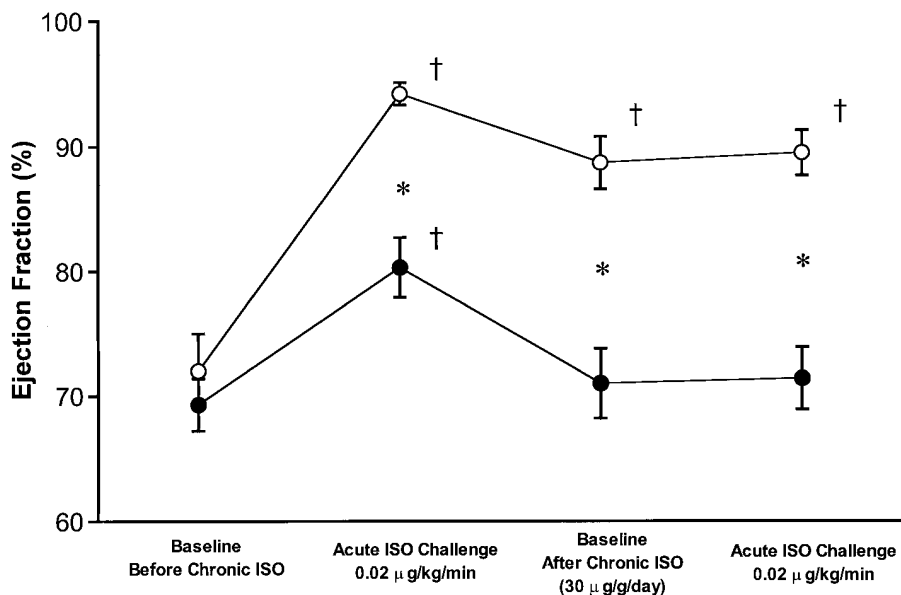


Figure 2. The effects of acute isoproterenol (0.02 $\mu\text{g/kg/min}$, i.v.; ISO) and chronic (2 wk) isoproterenol (30 $\mu\text{g/kg/day}$) are compared on LVEF in young G α (open circles) and wild-type control mice (filled circles). Acute isoproterenol induced a greater effect on LVEF in G α mice. However, when isoproterenol was administered chronically, desensitization was observed in controls, i.e., LVEF returned to baseline was then insensitive to further stimulation. In contrast, in G α mice, chronic isoproterenol did not induce desensitization, i.e., LVEF remained at near-maximal levels. An additional acute isoproterenol challenge did not elicit further effects, since LVEF was already at near-maximal levels. *Differences, $P < 0.05$, between G α -overexpressed and control mice. †Significant differences from the original baseline before chronic isoproterenol.

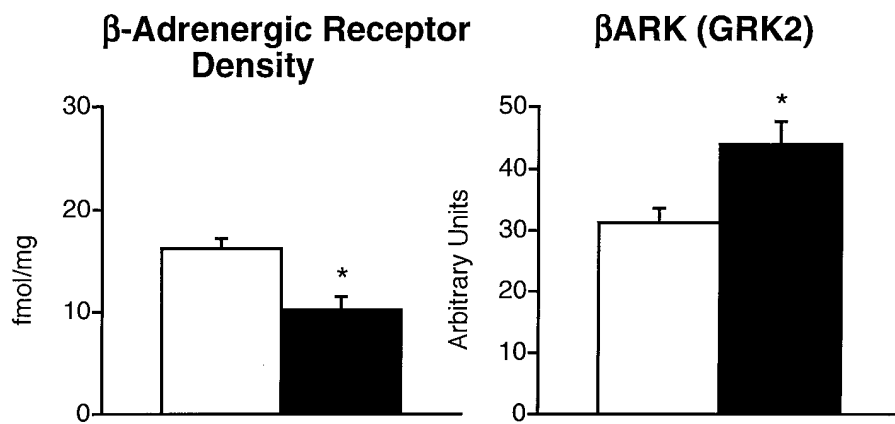


Figure 4. Total β -adrenergic receptor density (left) is reduced (control $n = 6$, Gs α $n = 5$), and GRK $_2$ (β ARK) levels (right) are increased in old Gs α ($n = 11$) compared with age-matched control mice ($n = 11$). Black bars, Old Gs α mice. White bars, Old wild-type mice. * $P < 0.05$.

ern blotting indicated that protein levels remain 3.4 ± 0.6 -fold increased in old transgenic compared with control mice (Fig. 3). There were no differences in GRK $_2$ levels in young Gs α versus age-matched control mice. However, in old Gs α mice, GRK $_2$ levels were increased $42 \pm 2\%$ ($P < 0.05$; Fig. 4).

β -Adrenergic receptor signaling. In young transgenic mice, β -adrenergic receptor antagonist binding was not changed from wild-type control levels (20 ± 1.2 vs. 21 ± 2.8 fmol/mg). However, the fraction of β -adrenergic receptors binding agonist with high affinity was increased in young transgenic compared with wild-type control mice (75 ± 5 vs. $48 \pm 10\%$). In addition, both isoproterenol- and G protein-stimulated adenylyl cyclase activity via GPP(NH)p were increased (Fig. 5, and Table I). Sodium fluoride showed a slight but nonsignificant increase in adenylyl cyclase activity.

In old transgenic mice, although β -adrenergic receptor density, assessed with 125 I-cyp, decreased (Fig. 4; $P < 0.05$, 10 ± 1.3 vs. 16 ± 1.0 fmol/mg), β -adrenergic receptors binding agonist

with high affinity (Fig. 6) remained elevated ($70 \pm 5\%$) compared with age-matched wild-type controls ($50 \pm 4\%$, $P < 0.05$). Consistent with this, isoproterenol- and G protein-stimulated adenylyl cyclase activities were also increased ($P < 0.05$) in transgenic compared with age-matched wild-type control mice (Table I, and Fig. 7). In fact, the adenylyl cyclase activity in old Gs α mice was even greater than in young wild-type controls. Consistent with this finding, the old Gs α -overexpressed mice have a significantly greater number ($P < 0.05$) of high affinity receptors and greater isoproterenol-stimulated adenylyl cyclase activity (26 ± 1.1 vs. 19 ± 1.6 pmol/mg/min) compared with young wild-type controls (Table I).

Effects of age. Except for β -adrenergic receptor density, which fell slightly in old wild-type mice and further in old transgenic mice, there were no differences due to age, in terms of Gs α protein levels or levels of adenylyl cyclase activity between old and young wild-type control mice, as well as between old and young mice overexpressing myocardial Gs α .

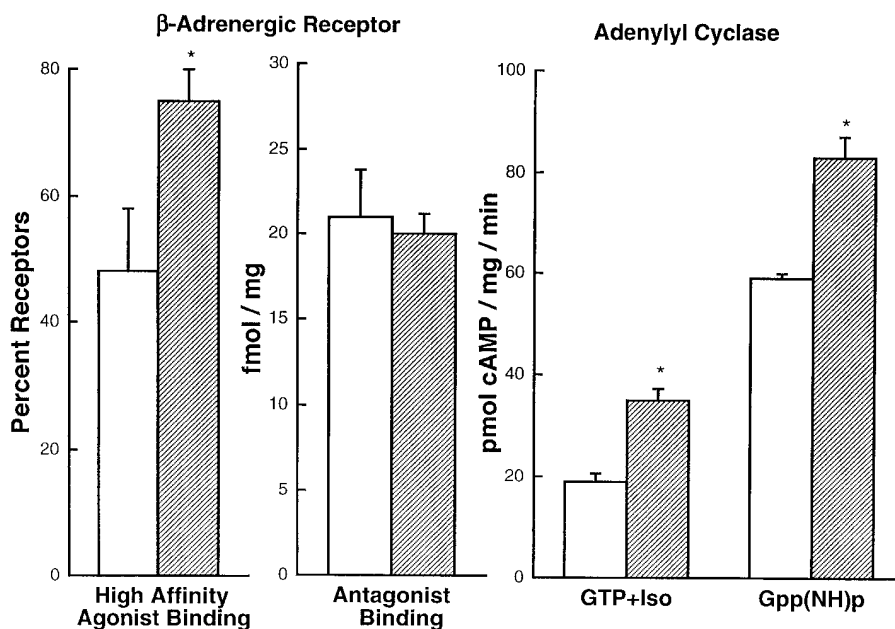


Figure 5. The fraction of β -adrenergic receptors binding agonist with high affinity (left) is increased in young transgenic mice overexpressing myocardial Gs α (shaded bars) ($n = 19$) compared with age-matched control mice (white bars) ($n = 5$), as is adenylyl cyclase activity (right) ($n = 6$). * $P < 0.01$. Iso, Isoproterenol.

Table I. Adenylyl Cyclase Activity: Mice Overexpressing Myocardial Gs α (pmol cAMP/mg/min)

	Basal	GTP	GTP + ISO	Gpp(NH)p	NaF	Forskolin
Young controls (<i>n</i> = 6)	6.6 $\pm$ 0.7	8.5 $\pm$ 0.9	19 $\pm$ 1.6	59 $\pm$ 0.9	77 $\pm$ 4.2	90 $\pm$ 6.9
Young Gs α (<i>n</i> = 5)	8.2 $\pm$ 0.6	15 $\pm$ 1.0*	35 $\pm$ 2.3*	83 $\pm$ 4.1*	81 $\pm$ 3.1	99 $\pm$ 5.9
Old controls (<i>n</i> = 6)	5.8 $\pm$ 0.4	11 $\pm$ 0.4	19 $\pm$ 0.9	45 $\pm$ 1.8	70 $\pm$ 3.5	88 $\pm$ 6.5
Old Gs α (<i>n</i> = 6)	10 $\pm$ 0.4	18 $\pm$ 0.6*	26 $\pm$ 1.1*	75 $\pm$ 2.4*	92 $\pm$ 3.6	102 $\pm$ 6.8

**P* < 0.01, different from age matched controls. ISO, Isoproterenol.

Discussion

The sympathetic nervous system plays a pivotal role in the pathogenesis of heart failure. An increase in sympathetic tone is a major compensatory mechanism in response to a variety of stresses. In the acute situation, e.g., in response to postural changes, exercise, or hemorrhage, the compensatory adjustments effected by increases in sympathetic tone are essential. Under chronic conditions, e.g., in the pathogenesis of heart failure, the extent to which these compensatory adjustments are beneficial remains controversial. In part, this is because chronically elevated sympathetic activity elicits desensitization of the β -adrenergic receptor, which in turn reduces the effectiveness of adrenergic stimulation. The conundrum that arises is whether the compensatory increases in sympathetic tone or the secondary adjustment of β -adrenergic receptor desensitization is the salutary mechanism in the pathogenesis of heart failure. This investigation was designed to address this question.

Transgenic models overexpressing key elements of the β -adrenergic receptor pathway provide a unique opportunity to address the perplexing problem of chronically enhanced sympathetic stimulation, desensitization, and development of cardiomyopathy. One of these models, the mouse with overexpressed cardiac Gs α (1–3), is a prime candidate, since this mouse model is characterized by enhanced responsiveness to sympathetic stimulation as a young adult (2). As the animal

ages, it develops hypertrophy and a picture resembling human cardiomyopathy (3). We would have predicted that chronically enhanced sympathetic stimulation would elicit desensitization, which in turn would have protected the heart from further stress, since a decrease in functionally active β -adrenergic receptors leads to a decrease in cAMP synthesis and a blunted responsiveness of the heart to β -adrenergic receptor agonists (19). The goal of this investigation was to determine if catecholamine-desensitization mechanisms were invoked in this model later in life.

The major finding of this study, which was opposite to the predicted response based on previous studies (1–3), was that biochemical catecholamine-desensitization mechanisms were invoked but were not sufficiently powerful to eliminate the enhanced physiological response to β -adrenergic receptor stimulation in the Gs α mice. For example, older Gs α mice exhibited β -adrenergic receptor downregulation and increased levels of GRK $_2$ (β ARK), two pathognomonic features of β -adrenergic receptor desensitization (19–22). GRK $_2$ was studied since it is the predominant isoform in the heart (13). However, decreased isoproterenol-stimulated high affinity agonist binding was not observed, and although adenylyl cyclase activity was decreased, it was still higher than that observed in even young wild-type control mice (Fig. 4). Interestingly, both isoproterenol- and G protein-stimulated adenylyl cyclase activity were enhanced in the old transgenic Gs α mice compared with age-matched controls, whereas forskolin responses were not significantly different. Forskolin is a direct stimulator of the catalytic unit. Although forskolin binding studies have suggested that its affinity is enhanced in the presence of Gs α -GTP, direct stimulation of the enzyme does not require that Gs α be stimulated simultaneously. Thus, the cyclase results suggest that stimulation both through the β -adrenergic receptor and the G protein are augmented in the mice overexpressing Gs α , whereas the content and/or activity of the catalytic unit of adenylyl cyclase itself remains similar to that of wild-type mice. Most importantly, an acute isoproterenol challenge still elicited enhanced responses of ejection fraction (Fig. 1) in older mice overexpressing myocardial Gs α , demonstrating accordingly that desensitization mechanisms were ineffective.

The second major finding of this study was that chronic isoproterenol administration, an intervention widely used to elicit catecholamine desensitization *in vitro* (19–22) and *in vivo* (7), did elicit desensitization in wild-type mice as it had previously in normal mice (7), but failed to elicit desensitization in Gs α mice. In the Gs α mice after chronic isoproterenol, LVEF remained at near-maximal levels, whereas in wild-type controls, LVEF had already returned to control levels and did not respond further to acute isoproterenol challenges, i.e., the classical picture of desensitization was evoked.

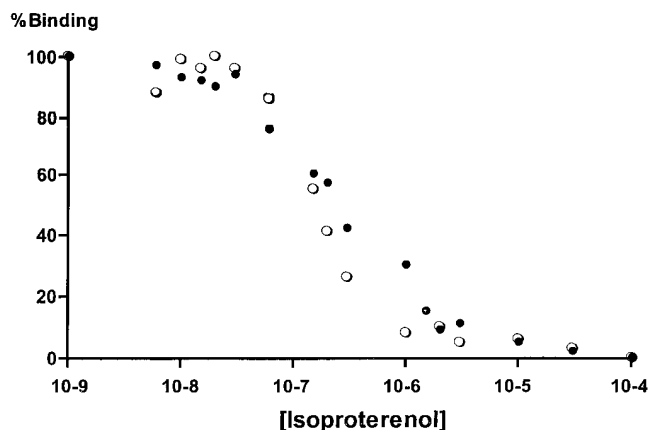


Figure 6. Representative isoproterenol agonist competition curves are compared in sarcolemmal membranes from one old Gs α mouse heart (open circles) and one wild-type control heart (filled circles). The curve from the Gs α mouse is shifted to the left, which results in increased numbers of β -adrenergic receptors binding agonist with high affinity.

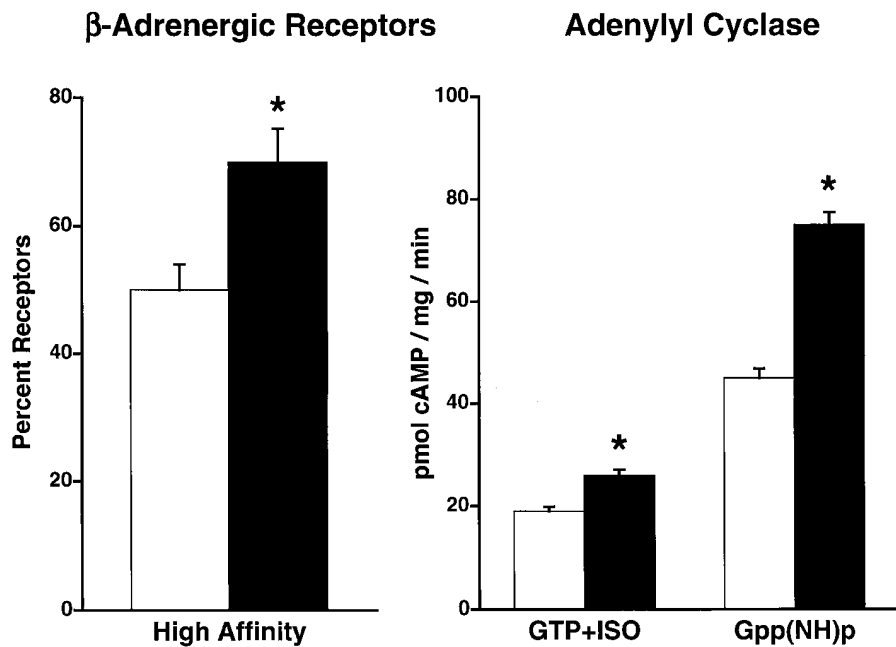


Figure 7. The fraction of β -adrenergic receptors binding agonist with high affinity (left) is increased in old transgenic mice overexpressing myocardial $G_{s\alpha}$ (black bars) ($n = 4$) compared with age-matched control mice (white bars) ($n = 5$), as is adenylyl cyclase activity (right) ($n = 6$). * $P < 0.05$. Iso, Isoproterenol.

We can only speculate as to why desensitization mechanisms are not sufficiently powerful in the mice overexpressing myocardial $G_{s\alpha}$. One hypothesis is that augmented cardiac $G_{s\alpha}$ prevents classical desensitization, potentially due to a "turning down" of sympathetic neural tone, in the presence of a myocyte effector pathway which is constitutively enhanced, i.e., cardiac output and arterial pressure are increased due to a chronically enhanced effector pathway. Sympathetic tone may be decreased reflexly, which in turn leads to reduced receptor occupancy by agonist, and consequently lack of homologous desensitization. In support of this concept, arterial pressure is higher in the $G_{s\alpha}$ mice (23). Furthermore, the results of spectral analysis of heart rate variability (23) also support the concept that sympathetic neural tone is actually lower in these mice with overexpressed $G_{s\alpha}$, leading to lower receptor occupancy and preventing desensitization (23). These findings suggest that the output of the β -adrenergic receptor signaling pathway is increased at any given level of receptor occupancy in the transgenic mice overexpressing $G_{s\alpha}$. As a result, over the life of the animals, there is less average β -adrenergic receptor occupancy, resulting in attenuated desensitization. An alternative explanation is that the augmented $G_{s\alpha}$ protein, which is still present in the older mice, continues to enhance β -adrenergic receptor coupling to cyclase, due to the enhanced stoichiometric relationship between $G_{s\alpha}$ and receptor (24). In support of this concept are the persistent findings of β -adrenergic receptors in the high affinity state. In further support of this position are the studies with chronic isoproterenol, which increased LV ejection to near-maximal levels and failed to elicit desensitization.

One concern in this study was that the increased fibrosis in the old $G_{s\alpha}$ mice might complicate the interpretation of the data. Accordingly, the Bradford (17) as well as Lowry assays (16) were studied. The Bradford assay does not detect the protein content in fibrotic tissue, because it is insensitive to collagen (18). Moreover, this factor could not explain the actual

increase in the fraction of agonist high affinity receptors and the enhanced adenylyl cyclase activity in the old transgenic mice. Indeed, when adjusting for this factor, the biochemical differences in β -adrenergic receptor signaling in the old $G_{s\alpha}$ mice were even greater than before the adjustment for fibrosis.

Another potential complicating factor is aging, which by itself will affect the cardiovascular system and induce β -adrenergic receptor desensitization (5, 6). For that reason, it was particularly important to study age-matched controls. Indeed, there were relatively modest decreases in β -adrenergic receptors with aging, but less of an effect on adenylyl cyclase activity. Importantly, the fraction of β -adrenergic receptors binding agonist with high affinity in the older $G_{s\alpha}$ mice was actually greater than in either the young or old wild-type controls (37%). Similarly, the percentage of isoproterenol-stimulated adenylyl cyclase activity in the older $G_{s\alpha}$ mice was 46% greater than young wild-type controls and 40% greater than old wild-type controls. Therefore, aging per se was not the major factor regulating β -adrenergic receptor signal transduction in these groups of mice. It is likely that the more prominent cardiovascular aspects of aging are expressed in even older, senescent animals, which were not studied because of the problem of increased premature mortality in $G_{s\alpha}$ -transgenic animals (3).

It is difficult to extrapolate from the biochemical and molecular studies in the transgenic animals to the physiological phenotype (2, 3). This may be due in part to the fact that the phenotype requires a lifetime of gene expression to become manifest. Moreover, it is difficult to extrapolate from the findings of enhanced β -adrenergic receptor signaling in $G_{s\alpha}$ mice to ineffective desensitization mechanisms. It is for these reasons that a combination of physiological and cellular techniques will be critical to derive the necessary mechanistic information on the role of altering one component of the β -adrenergic receptor pathway and the resultant physiological consequences in vivo over the life of the genetically altered animal.

The major implications of this investigation are related to understanding the pathogenesis of cardiomyopathy and heart failure. If our hypothesis, which is admittedly controversial, is correct, that the chronically enhanced β -adrenergic receptor signaling in the face of impaired catecholamine desensitization promotes the development and progression of cardiomyopathy and heart failure, then the extrapolation of these results to clinical heart failure becomes important. For example, the results of these studies in mice lend support to the concept that β -adrenergic receptor desensitization during the development of heart failure may be salutary (15, 25). In support of this concept are results from recent studies with carvedilol, which appear promising (26, 27), and prior adverse results from studies in patients with chronic β -adrenergic stimulation therapy (28–30).

Finally, however, it is important to point out that this model was not developed to mimic the phenotypic changes associated with human end-stage heart failure. For example, our argument that these results are consistent with the demonstration that beta blockers have a positive effect in the long term in patients with heart failure is again not related to phenotypic changes seen in humans. Findings in the $G\alpha_x$ mouse argue that therapies aimed at turning down the β -adrenergic receptor pathway in the heart might have a positive effect in preventing the progressive dysfunction seen in human heart failure. This is the rationale for relating our findings to those seen in studies of human heart failure, particularly with beta blockers, where the goal is to prevent continuous β -adrenergic receptor stimulation.

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