Apoptosis of Cardiac Myocytes in Gsα Transgenic Mice

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Abstract—The stimulatory GTP-binding protein Gsα transmits signals from catecholamine receptors to activate adenylyl cyclase and thereby initiate a cascade leading to cardiac chronotropy and inotropy. Transgenic mice overexpressing the Gsα subunit (Gsa) selectively in their hearts exhibit increased cardiac contractility in response to β-adrenergic receptor stimulation. However, with aging, these mice develop a cardiomyopathy. This study sought morphological and biochemical evidence that overexpression of Gsa is associated with increased myocyte apoptosis in the older animals and to determine whether such overexpression can promote apoptosis of isolated neonatal cardiac myocytes exposed to β-adrenergic receptor agonists. In the hearts of 15- to 18-month-old Gsα transgenic mice, histochemistry and electron microscopy illustrated the existence of numerous myocytes with abnormal nuclei embedded in collagen-rich connective tissue. Terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling (TUNEL, for in situ labeling of DNA breaks) demonstrated that ≈0.6% of myocyte nuclei contained fragmented DNA. Agarose gel electrophoresis provided further biochemical evidence of apoptosis by showing internucleosomal DNA fragmentation. Cultured cardiac myocytes from newborn Gsα transgenic mice showed increased TUNEL staining and internucleosomal DNA fragmentation compared with wild-type controls when treated with the β-agonist isoproterenol. Thus, enhanced activation of β-adrenergic signaling by overexpression of Gsa in the hearts of transgenic mice induces apoptosis of cardiac myocytes. This represents a potential mechanism that may contribute to the development of cardiomyopathy in this model. (Circ Res. 1999;84:34-42.)

Key Words: adenylyl cyclase ■ β-adrenergic receptor ■ catecholamine ■ programmed cell death ■ cardiomyopathy

The heterotrimeric GTP-binding proteins (G proteins), composed of α, β, and γ subunits mediate signal transduction in a broad diversity of cell types.1-3 The stimulatory G protein Gsα transmits signals from β-adrenergic receptors (BARs) to adenylyl cyclase (AC), leading to activation of this enzyme, production of cAMP, and enhancement of cardiac contractility.1,2 Alterations in this signaling pathway (BAR-Gs-AC), particularly catecholamine desensitization, are a hallmark of heart failure in both human disease and animal models.3-4 However, it remains controversial whether chronic βAR stimulation or the reverse, desensitization, is adaptive or maladaptive in the pathogenesis of heart failure. Several lines of evidence show that acutely5 or chronically6-8 enhanced stimulation with catecholamines can result in myocyte death and cardiac fibrosis. We previously established a transgenic mouse model by selectively overexpressing a Gsα transgene in the heart,7-9 which enhances inotropic and chronotropic responses of the heart to sympathetic stimulation.7 Furthermore, with aging the transgenic heart develops morphological alterations characteristic of cardiomyopathy, including myocyte hypertrophy and fibrosis,7-8 suggesting that persistent βAR activation may be deleterious over the long term. However, other studies favor the opposing view that enhancement of β-adrenergic signaling may be beneficial, eg, overexpressing a β-adrenergic signaling component may be a treatment to reverse the cardiac depression that occurs in heart failure.10,11

We hypothesize that overdriving the βAR/Gs/AC signaling pathway may induce apoptosis as well as necrosis. A key observation is that the older animals overexpressing cardiac Gsα develop significant myocyte hypertrophy but have only a modest increase in heart weight/body weight,7 suggesting the potential for myocyte deletion. Apoptosis or genetically programmed cell death plays an important role in determination of tissue cellularity during embryonic development and adult tissue turnover.12 We examined the hearts of mice overexpressing the Gsα transgene in a cardiac-selective manner for the presence of programmed myocyte death. We also tested the hypothesis that cardiac myocytes cultured in a controlled environment undergo apoptosis, when the Gs-
coupled βAR-AC pathway is stimulated vigorously for a short period in vitro. This study demonstrates that increased myocyte nuclear degeneration and DNA fragmentation occur in the hearts of transgenic mice overexpressing Gsα. Our results suggest that intrinsic, long-term overactivation of the βAR/Gs/AC pathway may accelerate apoptosis of cardiac myocytes and thereby play a role in the development and/or progression of cardiomyopathy and heart failure.

Materials and Methods

Gsα Transgenic Mice

Transgenic mice selectively overexpressing Gsα in the heart were generated by microinjection of a Gsα minigene construct into the eggs of C57BL/6J mice as previously described. Briefly, exons 1 to 12 from a canine Gsα cDNA within a 1.3-kb Multi-BamHI fragment were conjugated to a human Gsα genomic fragment containing intron 12, exon 13, and the polyadenylation signal. The chimeric minigene construct was then positioned downstream of a rat α-myosin heavy chain promoter contained within a 0.9-kb EcoRI-XbaI gene fragment. This construction was then inserted into the plasmid pGEM-7Z (Promega) for propagation, and a 3.5-kb KpnI-SacI fragment was isolated for microinjection. Positive founders carrying the transgene were bred to normal adult C57BL/6XC3H (B6C3) F(1) hybrid females to establish independent germiles. The successful establishment and expression of the transgene in the mice were confirmed by Southern blotting analysis for the transgene DNA. Northern blotting for Gsα transcripts, and Western blotting for the Gsα protein.9 There was 3- to 5-fold overexpression of the protein for Gsα in the heart.9,13

Tissue Preparation and Histochemical Examination

After deep anesthesia with an intraperitoneal injection of sodium pentobarbital, the hearts were removed from adult mice at either 4 to 7 months or 15 to 18 months of age and then fixed by immersion in 10% phosphate-buffered formalin or by in situ perfusion fixation with formalin through the left ventricular apex. The fixed tissues were dehydrated, embedded in paraffin, and sectioned at 6-μm thickness. Histological examination was carried out by staining with hematoxylin and eosin and Gomori aldehyde fuchsin trichrome. For silver staining of the glycocalyx and other matrix substances, 1.0-μm-thick sections embedded in glycolmethacrylate were prepared for staining using a microwave procedure (Acustain, Sigma). Myocytes were judged to be cut normal to their long axis by the nearly round shape of perfused capillaries in the region.

Electron Microscopy

Transmission electron microscopy was performed on a separate series of animals in which the myocardium was perfusion fixed with 2% glutaraldehyde, en bloc stained with osmium tetroxide, and embedded in Spurr epoxy resin, and thin sections were rehydrated through 100%, 95%, 75%, and 0% ethanol; and incubated in 50% ethanol. The tissue was then mixed with 1 mL of DNA extraction solution containing 20 μmol/L Tris-HCl, pH 7.4, 0.1 mol/L NaCl, 5 μmol/L EDTA, and 0.5% SDS. For isolation of DNA from cultured myocytes, 1 mL of DNA extraction buffer was directly added into the culture flask after removing the culture medium. The cell lysates were incubated with 100 μg/mL DNase-free RNase A at 37°C for 16 hours. After incubation, 1 mL of phenol/chloroform (1:1) was mixed with the enzyme-digested cell lysates and then centrifuged at 20,000g for 20 minutes; DNA in the upper (aqueous) phase was incubated with 5 μg/mL DNAse-free RNase A at 37°C for 1 hour and extracted with phenol/chloroform again. DNA was collected by precipitation with 1 mL of isopropanol and 0.1 mL of 5 mol/L NaCl at –20°C overnight. After centrifugation, the resulting DNA pellets were washed with 75% ethanol and air dried. DNA was dissolled in 10 μmol/L Tris-HCl buffer with 1 μmol/L EDTA, and its concentration was determined at 260 nm by spectrophotometry. DNA electrophoresis was carried out in 1.5% agarose gels containing 1 μg/mL ethidium bromide, and DNA bands were visualized under UV light.

Cell Viability

The viability of the isolated myocytes was determined by staining with the nucleic acid–binding fluorochromes acridine orange and ethidium bromide.14,15 At the end of incubation with isoproterenol, the chamber slides were incubated with the DNA-binding dyes, acridine orange and ethidium bromide, at 10 μg/mL each for 2 minutes on ice. Coverslips were applied to the slides, and the sections were observed under a Nikon E800 fluorescent microscope with a triple filter. Viable cells exclude ethidium bromide but not acridine orange, which stains nuclei, yielding a green fluorescence.

DNA Isolation and Electrophoresis

Hearts from Gsα transgenic or control mice were removed from deeply anesthetized animals, snap frozen, and crushed in liquid nitrogen. The tissue was then mixed with 1 mL of DNA extraction solution containing 20 μmol/L Tris-HCl, pH 7.4, 0.1 mol/L NaCl, 5 μmol/L EDTA, and 0.5% SDS. For isolation of DNA from cultured myocytes, 1 mL of DNA extraction buffer was directly added into the culture flask after removing the culture medium. The cell lysates were incubated with 100 μg/mL DNase-free RNase A at 37°C for 16 hours. After incubation, 1 mL of phenol/chloroform (1:1) was mixed with the enzyme-digested cell lysate and then centrifuged at 20,000g for 20 minutes; DNA in the upper (aqueous) phase was incubated with 5 μg/mL DNAse-free RNase A at 37°C for 1 hour and extracted with phenol/chloroform again. DNA was collected by precipitation with 1 mL of isopropanol and 0.1 mL of 5 mol/L NaCl at –20°C overnight. After centrifugation, the resulting DNA pellets were washed with 75% ethanol and air dried. DNA was dissolved in 10 μmol/L Tris-HCl buffer with 1 μmol/L EDTA, and its concentration was determined at 260 nm by spectrophotometry.

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**Immunoblotting Assay**

Total membrane proteins were extracted from cardiac cells of Gsα or wild-type mice. Protein (30 μg/lane) was loaded onto a 10% SDS-PAGE gel. After electrophoresis, protein bands were transblotted to a membrane, which was then blocked and immunostained with anti-Gsα antibody (1:5000). Anti-rabbit IgG conjugated with peroxidase was used as the second antibody. The blots were developed by enhanced chemiluminescence (Amersham).

**Statistical Analysis**

Data are reported as mean±SD. The difference between means was evaluated using Student’s t test. For statistical analysis of data from multiple groups, ANOVA was used. Significance levels were established at P<0.05.

**Results**

**Histopathological Evaluation of the Myocardium**

Histopathological evaluation confirmed our previous observations of absence of lesions in young adult (4 to 7 months old) animals and extensive multifocal areas of fibrosis in 16-month-old Gsα transgenic mice (Figure 1). In addition to the multifocal fibrosis, cellular degeneration was apparent, including pale-staining cells due to loss of myofibrils, cytoplasmic vacuolation, nuclear aberrations, including irregular size, condensed chromation, and vacuoles, and hypertrophy and atrophy of myocytes. The individual variation and increased size in myocyte cross-sectional area was shown by staining 1-μm-thick methacrylate sections with a silver stain to outline the glycocalyx surrounding each cell (Figure 2).7

**Nuclear Morphology and in Situ DNA 3’ End Labeling**

The myocytes of the transgenic hearts also displayed distinct morphological alterations in their nuclei. Light microscopy showed alterations in the nuclear morphology of transgenic myocytes characterized by prominent chromatin condensation and vacuole formation (Figure 1). By transmission electron microscopy, we observed marked irregularity of the nuclear membrane with invaginations and vacuole formation (Figure 3b). Hence, both light and electron microscopy demonstrated nuclear morphological alterations in transgenic myocytes, but no such changes were found in age-matched, wild-type controls (Figures 1a and 3a). In most cells, these nuclear morphological alterations occurred with little cytoplasmic abnormality (Figure 3b). However, in other cells, nuclear changes were accompanied by disorganization or loss of the striated myofibrils and other degenerative changes. Most of the myocytes with cytoplasmic and/or nuclear degenerative changes maintained an intact plasma membrane.

Evaluation of tissue sections stained by the TUNEL technique revealed only small numbers of TUNEL-positive cells in the control hearts. The myocytes of normal hearts were of regular shape, and counterstaining of their nuclei showed a
homogeneous blue fluorescent stain with DAPI. The transgenic 15- to 18-month-old mice had a significant increase in the number of TUNEL-positive myocyte nuclei compared with wild-type controls, (0.6±0.1% of myocytes versus 0.1±0.04% in control). The 4- to 7-month-old animals had only rare TUNEL-positive cells, and there was no significant difference between transgenic and wild-type animals (Figure 4). An example of a TUNEL-positive myocyte is shown in Figure 5. Nonmyocyte interstitial cells were also labeled by the TUNEL procedure and were more frequently present in the older transgenic mice than in wild-type animals (data not shown). Limiting the counting of total myocyte nuclei and the TUNEL-positive nuclei to areas with true cross sections of myocytes made it possible to selectively count only those nuclei that clearly were within a myocyte (Figure 5).

**Internucleosomal DNA Fragmentation in Hearts Overexpressing Gsα**

Internucleosomal DNA fragmentation biochemically characterizes apoptosis. Agarose gel electrophoresis of DNA isolated from the myocardium of 15- to 18-month-old mice

![Figure 3](image3.png)

**Figure 3.** Transmission electron micrographs of left ventricular myocytes from wild-type (a) and Gsα transgenic (b) mice. Note the irregularity of the nuclear outline, chromatin condensation, and vacuole formation in the nucleus of a transgenic myocyte (b). Bar=2 μm.

![Figure 2](image2.png)

**Figure 2.** Light photomicrographs of silver-stained 1-μm sections of left ventricular subendocardial myocardium from wild-type and Gsα transgenic 15-month-old mice. Variable increase in cross-sectional area of Gsα transgenic myocytes compared with wild type is evident in these silver-stained sections. Bar=50 μm.

![Figure 4](image4.png)

**Figure 4.** The number of TUNEL-positive myocyte nuclei per 10 000 myocyte nuclei is shown for young adult (4 to 7 months old) and old (15 to 18 months old) wild-type and Gsα transgenic mice. There was a significant increase in TUNEL-positive myocyte nuclei in the older Gsα transgenic hearts but not in the younger Gsα transgenic hearts compared with respective wild-type controls. Data represent mean±SD (n=5 or 6 for each group). *P<0.05 using ANOVA.
showed bands of DNA fragments at 180 to 200 bp or multiples with the laddering appearance typical of apoptosis (Figure 6a, lanes 1 and 2). In contrast, little or no DNA laddering was observed in the age-matched wild-type animals (Figure 6a, lanes 3 and 4).

To confirm overexpression of Gsα in the hearts exhibiting internucleosomal DNA fragmentation, we examined Gsα protein content by immunoblotting with rabbit polyclonal anti-Gsα antibody. As shown in Figure 6b, much stronger Gsα bands (short isoform encoded by the transgene) were observed in the blots prepared from the transgenic animals (lanes 1 and 2) than from wild-type controls (lanes 3 and 4), indicating the presence of overexpressed Gsα protein in the transgenic hearts with increased DNA fragmentation.

In Vitro Apoptosis of Myocytes With Overexpressed Gsα in Response to β-Adrenergic Stimulation

To determine whether apoptosis of myocytes might occur as a direct consequence of overactivation of the β-adrenergic pathway, short-term vigorous stimulation of this signaling pathway in cardiac myocytes was carried out in vitro. Cardiac myocytes isolated from the hearts of newborn Gsα transgenic or wild-type mice were exposed to isoproterenol under identical culture conditions. After 2 days in culture, no difference in cell viability (>95%) was observed between untreated Gsα and wild-type cells. After exposure for 2 days to the βAR agonist isoproterenol (5 to 20 μmol/L), many myocytes were contracted and fragmented (Figure 7c). By fluorescent microscopy, nuclear aberrations were also seen (Figure 7d).

We also analyzed nuclear DNA integrity by the TUNEL technique in neonatal myocytes after exposure to isoproterenol (5 to 20 μmol/L) for 48 hours. There was a concentration-dependent increase in the number of TUNEL-positive nuclei in the isoproterenol-treated Gsα neonatal myocyte cultures when compared with the wild-type controls (Figure 8). To determine the sizes of DNA fragments, we analyzed nuclear DNA from both control and isoproterenol-treated cells by agarose gel electrophoresis. Under the baseline condition of primary culture, no internucleosomal DNA fragmentation occurred in myocytes from the hearts of either Gsα transgenic or wild-type mice (Figure 9, lanes 4 and 5). Treatment with isoproterenol for 2 days markedly increased internucleosomal DNA fragmentation in myocytes isolated from Gsα trans-
genic hearts (Figure 9, lane 2) but not in wild-type myocytes (Figure 9, lane 3).

Discussion

In the present investigation, we have demonstrated apoptosis, a form of genetically programmed cell death, as a potential mechanism mediating myocyte death in the heart. We used a combination of techniques to obtain morphological and biochemical evidence for apoptosis, including histopathological evaluation with diverse staining techniques, electron microscopy, in situ nuclear DNA-end labeling, and DNA agarose gel electrophoresis. Although other forms of cell death might have occurred, our data suggest that apoptosis contributes to the loss of myocytes in the heart with overexpressed Gsα.

βARs, via the stimulatory G protein Gs, transduce the signal from norepinephrine to activate AC, thereby catalyzing the synthesis of cAMP. This signaling pathway is important for enhancement of cardiac contractility and heart rate, particularly when there is an increase in demand for cardiac output.1,2,18 Rapid changes in cardiac output, occurring in a time frame measured in seconds, are required of the heart to meet a varied set of demands. Thus, the βAR signaling pathway is being continuously activated and deactivated as the sympathetic nerves adjust their activity in response to the body’s greater or lesser demand for blood supply. In contrast, in pathophysiological states such as heart failure, it is thought that the sympathetic nerves are continuously activated, as the organism senses an inadequate cardiac output and attempts to correct the situation. What remains unclear is whether this state of persistent sympathetic nerve stimulation is, in fact, deleterious to the heart over the long term.

Transgenic mice with overexpression of cardiac Gsα respond to a sympathetic stimulus with enhanced contractility.7,13 The augmented response is not due to a heightened state of sympathetic nerve activity but rather to an accentuated postsynaptic response pathway. Thus, we believe that this mouse model mimics the state of heightened sympathetic nerve activity that occurs in heart failure, albeit by a different mechanism and in the absence of a preceding cardiac insult. It also offers a unique perspective on the consequences of chronically enhanced βAR stimulation, occurring over the life of the animals. Initially, these Gsα transgenic mice exhibit an enhanced cardiac response to catecholamines. As they age, these mice develop cardiac dilatation with decreased ejection fraction but do not develop myocardial desensitization to catecholamines.13 Consequently, myocyte hypertrophy, cellular dropout, and fibrosis occur, mimicking the human syndrome of cardiomyopathy.7,8

We observed that in the hearts of older Gsα transgenic mice, an increased number of cardiac cells showed changes characteristic of apoptosis. The observation that a significant difference in TUNEL staining existed between the transgenic and wild-type control hearts in the older mice (15 to 18 months old) but not in the younger animals (4 to 7 months of age) suggests an age dependency of accelerated apoptosis in the transgenic hearts. Therefore, the Gsα transgene itself appears to exert no direct cytotoxic effect on myocytes. Apoptosis in aging tissues has also been reported to mediate cell loss.19 However, the aging process itself cannot account for the myocyte degeneration and apoptosis in Gsα transgenic mice, since age-matched wild-type mice do not display the histopathological alterations found in the transgenic mice. As a consequence of Gsα overexpression and chronically enhanced β-adrenergic signaling, the hearts of the transgenic animals may express proapoptotic factors and become sensitive to apoptotic stimuli from a variety of environmental sources. Alternatively, it is possible that the apoptotic program is only initiated after cardiac dysfunction develops (ie, that environmental factors created by the cardiomyopathy in turn initiate signals that activate the apoptotic program, namely, an altered neurohormonal milieu).
We performed in vitro experiments to test the above possibilities. In particular, we examined whether apoptosis could be induced in the Gsα-overexpressing myocytes in vitro by assessing the effects of short-term potent β-adrenergic stimulation on myocyte death in culture. The results from our in vitro experiments demonstrate that stimulation with the β-adrenergic agonist and G-protein activator isoproterenol promotes myocyte apoptosis in Gsα myocytes but not in myocytes from wild-type controls. Hence, overexpression of Gsα appears to increase the apoptotic response of myocytes to isoproterenol. This finding extends a previous report by Mann et al5 demonstrating that catecholamine stimulation is deleterious and can induce death of cardiac myocytes in culture.

Results from recent studies on cAMP-mediated cell death by apoptosis are controversial. cAMP reportedly induces apoptosis of cultured Schwann cells20 and contributes to apoptosis of human thymocytes,21 but it has also been shown to prevent T cell receptor–mediated apoptosis22 and reduce apoptosis mediated by atrial natriuretic peptide in myocytes.23 Thus, the role of AC and cAMP in regulation of myocyte

![Figure 7](image1.png)

**Figure 7.** Phase-contrast and fluorescent photomicrographs of wild-type (a and b) and Gsα transgenic (c and d) neonatal myocytes exposed to isoproterenol for 48 hours. Phase-contrast photography shows cell disruption, shrinking, and detachment of isoproterenol-treated Gsα transgenic myocytes (c, arrows). Staining with acridine orange and ethidium bromide shows nuclear chromatin condensation and fragmentation in isoproterenol-treated Gsα transgenic myocytes (d, arrows) characteristic of apoptosis. Bar=50 μmol/L.

![Figure 8](image2.png)

**Figure 8.** In situ DNA 3’ end labeling (TUNEL) of neonatal myocytes isolated from Gsα and wild-type control mice were exposed to isoproterenol for 48 hours. Note a concentration-dependent increase in the number of TUNEL–positive nuclei in myocyte cultures treated with isoproterenol. Data represent mean±SD of triplicate cultures. *P<0.05.

![Figure 9](image3.png)

**Figure 9.** Agarose gel electrophoresis of DNA isolated from wild-type (WT) and Gsα transgenic myocytes with or without isoproterenol stimulation. Lane 1 indicates DNA size markers; lane 2, isoproterenol-stimulated Gsα transgenic cells; lane 3, isoproterenol-stimulated WT cells; lane 4, untreated Gsα transgenic cells; and lane 5, untreated WT cells.
apoptosis appears controversial and potentially not inconsistent with our findings of Gsα-mediated apoptosis, since isoproterenol mediates a significant increase in L-type Ca2+ channel activity and in the Ca2+ transient in Gsα-overexpressing myocytes as compared with wild-type controls (Reference 24 and S.-J. Kim et al, unpublished data, 1998).

A variety of factors have been reported to trigger cell death via apoptosis in the heart, including reperfusion injury after ischemia, myocardial infarction,5,25,26 pressure overload,27 and mechanical stretch.28 A recent report has shown that transgenic overexpression of an activated form of the G protein, Gqα, can induce cardiomyopathy and myocyte apoptosis.29 The present study provides evidence that acceleration of myocyte apoptosis also occurs secondary to overexpression of Gsα, a protein that promotes β-adrenergic signaling and thereby hyperresponsiveness to catecholamines. Our finding that mature myocytes exhibit apoptosis provides a potential explanation to the paradox of myocyte hypertrophy without an increase in heart weight that occurs in the older Gsα transgenic mice. It is thus plausible that the cellular hypertrophy was offset by a decrease in myocyte number caused by apoptosis, explaining the lack of an overall increase in heart weight in the transgenic mice, in spite of increased myocyte size. This consequence of cardiac Gsα overexpression is particularly interesting in light of our present understanding of heart failure and the factors that contribute to its progression. Two opposing views currently exist with regard to the activity of the βAR-Gs-AC signaling pathway in both the development and progression of heart failure. One holds that catecholamine stimulation may be harmful to the treatment of heart failure,10 another view suggests that chronic sympathetic nerve stimulation is disadvantageous and that inhibition of βAR activity with antagonists has beneficial effects over the long term.30,31 Our findings in the Gsα transgenic mice favor the latter possibility and support the hypothesis that chronic, heightened β-adrenergic signaling can result in apoptosis in the heart, leading to myocyte loss and potentially contributing to cardiac decompensation. Enhanced apoptosis occurs not only in this murine model of cardiomyopathy but also in human idiopathic dilated cardiomyopathy32,33 and chronic heart failure,34–33 thus implicating apoptosis in the development or progression of human cardiomyopathy and heart failure.

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24. Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P. Apoptotic and necrotic myocyte cell death via apoptosis in the heart, including reperfusion injury after ischemia, myocardial infarction,5,25,26 pressure overload,27 and mechanical stretch.28 A recent report has shown that transgenic overexpression of an activated form of the G protein, Gqα, can induce cardiomyopathy and myocyte apoptosis.29 The present study provides evidence that acceleration of myocyte apoptosis also occurs secondary to overexpression of Gsα, a protein that promotes β-adrenergic signaling and thereby hyperresponsiveness to catecholamines. Our finding that mature myocytes exhibit apoptosis provides a potential explanation to the paradox of myocyte hypertrophy without an increase in heart weight that occurs in the older Gsα transgenic mice. It is thus plausible that the cellular hypertrophy was offset by a decrease in myocyte number caused by apoptosis, explaining the lack of an overall increase in heart weight in the transgenic mice, in spite of increased myocyte size. This consequence of cardiac Gsα overexpression is particularly interesting in light of our present understanding of heart failure and the factors that contribute to its progression. Two opposing views currently exist with regard to the activity of the βAR-Gs-AC signaling pathway in both the development and progression of heart failure. One holds that catecholamine stimulation may be harmful to the treatment of heart failure, another view suggests that chronic sympathetic nerve stimulation is disadvantageous and that inhibition of βAR activity with antagonists has beneficial effects over the long term. Our findings in the Gsα transgenic mice favor the latter possibility and support the hypothesis that chronic, heightened β-adrenergic signaling can result in apoptosis in the heart, leading to myocyte loss and potentially contributing to cardiac decompensation. Enhanced apoptosis occurs not only in this murine model of cardiomyopathy but also in human idiopathic dilated cardiomyopathy and chronic heart failure, thus implicating apoptosis in the development or progression of human cardiomyopathy and heart failure.


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