# Decreased $G_{s\alpha}$ mRNA Levels Accompany the Fall in $G_s$ and Adenylyl Cyclase Activities in Compensated Left Ventricular Hypertrophy

## In Heart Failure, Only the Impairment in Adenylyl Cyclase Activation Progresses

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## Abstract

**We have previously reported that there is a global reduction in adenylyl cyclase associated with a decrement in $G_s$ functional activity in cardiac sarcolemma from animals with pressure overload-induced hypertrophy and heart failure. This study was performed to determine whether hypertrophy alone in the absence of heart failure is sufficient to promote these changes and whether the superimposition of heart failure intensified these changes. Basal and stimulated adenylyl cyclase and $G_s$ activity, as determined in the S49 cyc$^-$ reconstitution assay, were measured in sarcolemma from normal (NL), left ventricular hypertrophy (LVH) and heart failure (HF) animals. Simultaneously, we measured the mRNA level encoding for the $G_{s\alpha}$ subunit. These studies indicate that $G_s$ activity and $G_{s\alpha}$ mRNA are decreased by ~ 30% both in the failing heart and even in the heart with compensated hypertrophy before heart failure develops ($G_s$ activity, pmol cyclic AMP/10 min per μg, NL 4.2±0.4, LVH 3.0±0.2, HF 3.2±0.3; $G_{s\alpha}$ mRNA, pg/10 μg RNA, NL 131±9.0, LVH 104±7.4, HF 97.4±9.1; $P < 0.05$ as compared with NL for LVH and HF). Accompanying this decrement in $G_s$ activity is a fall in adenylyl cyclase, both basal and stimulated. However, we also identified a further decrease in adenylyl cyclase without any additional change in $G_s$ or in its alpha subunit mRNA level. This is seen only in the sarcolemma from animals with heart failure as compared with those with compensated LV hypertrophy (e.g., NaF-stimulated activity, pmol cyclic AMP/min per mg, NL 420.2±17.5, LVH 347.1±29.6, HF 244.2±27.3; $P < 0.05$ compared with NL for LVH and HF, $P < 0.05$ compared with LVH for HF). In summary, these studies indicate that both $G_s$ and adenylyl cyclase activities fall in parallel with the development of LV hypertrophy followed by a further decrement in adenylyl cyclase, independent of $G_s$, in the setting of heart failure. (*J. Clin. Invest.* 1991. 87:293–298.) Key words: catecholamines • $G_{i\alpha 2}$ mRNA • pressure overload • beta adrenergic receptor**

## Introduction

In contrast to the slower process of hypertrophy, activation of the sympathetic nerves provides a rapid mechanism for facilitating cardiac performance when the ventricle is stressed (1). For some time, however, it has been known that this process can dysfunction in the setting of heart failure (2, 3). We had previously reported that there is a loss of adenylyl cyclase responsiveness and a concomitant decrease in the functional activity of $G_s$, the heterotrimeric ($\alpha$, $\beta$, $\gamma$) GTP-binding protein which couples the $\beta$-adrenergic receptor to adenylyl cyclase, in cardiac sarcolemma prepared from animals with pressure overload-induced heart failure (4, 5). However, we had not delineated where in time, from compensated hypertrophy to decompensated heart failure, that these abnormalities occur. We therefore set out to determine whether hypertrophy itself, before the onset of heart failure, is associated with an alteration in the activity of any of these components.

The process of hypertrophy requires that the cardiocyte undergo a major change in its pattern of gene transcription (6). It is now well recognized that a variety of contractile and structural genes, principally expressed in the neonatal and fetal periods, are reactivated with a concomitant decrease in the expression of their respective adult isoforms as hypertrophy develops (7). It was also our goal, therefore, to determine whether the decrease in the functional activity of $G_s$ was associated with a parallel alteration in the mRNA levels encoding its alpha subunit, which is the component that binds GTP and activates adenylyl cyclase (8). Such a finding would suggest that the genetic changes required for a cardiocyte to hypertrophy, in some manner, also underlie the functional alterations in the cyclase transduction pathway which develops in this model of pressure overload-induced hypertrophy. In this study, we have therefore quantitated adenylyl cyclase activity, $G_s$ functional activity and $G_{s\alpha}$ mRNA levels in groups of animals with either compensated left ventricular hypertrophy (LVH)[1] or LV hypertrophy plus failure to identify where in time these abnormalities occurred.

## Methods

*Preparation of model (9–18).* LV pressure overload was induced using a canine model as described by Kleinman (9) and previously used by our laboratory (4, 5). Pressure overload LV hypertrophy was induced in 16 dogs from 3 litters by implantation of a 1-cm wide Teflon band around the ascending aorta distal to the coronary arteries in mongrel puppies 8–10 wk of age. The band was implanted through a thoracotomy in the fourth right intercostal space by use of sterile surgical technique and sodium thiamylal anesthesia (12.5 mg/kg). The band was tightened until a thrill could be palpated over the aortic arch, and the chest was closed. A sham-operated control was chosen at random from each litter. The puppies were allowed to recover from surgery and grow to maturation. The Teflon band created a fixed supravalvular aortic lesion, which became relatively more stenotic as the puppies grew. 6 dogs

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1. *Abbreviations used in this paper:* LVH, left ventricular hypertrophy.

spontaneously developed LV congestive failure. Evidence of congestive failure included LV end-diastolic pressure > 20 mmHg, exercise intolerance, and evidence of pulmonary congestion at autopsy.

In prior studies using this model of aortic banding, the dogs with compensated LV hypertrophy have been characterized by normal LV systolic and diastolic wall stresses as well as indices of LV systolic and diastolic function at baseline (15–18), whereas those dogs that develop LV failure are characterized by elevated LV systolic and diastolic wall stresses and altered LV systolic and diastolic function at baseline (17, 18).

*Implantation of instruments.* Instruments were implanted in the aortic-banded dogs and in control dogs of similar weight at 13–14 mo of age. In aortic-banded dogs anesthesia was induced with sodium thiamylal (8 mg/kg i.v.) and maintained with halothane (1 vol%). In control dogs, pentobarbital sodium (25 mg/kg) was used for anesthesia. An incision was made in the fifth left intercostal space by use of sterile surgical technique. Tygon catheters (Norton Industrial Plastics, Akron, OH) were implanted in the descending aorta, in the left atrium, and in the left ventricle. A miniature pressure gauge (Konigsberg Instruments, Pasadena, CA) was also implanted in the left ventricle. The thoracotomy incision was closed in layers, and the animals were monitored daily and allowed to recover for 2 wk before study. 16 aortic-banded dogs and 13 control animals (including the 3 sham-operated littermates) were instrumented in this fashion. Animals used in this study were monitored in accordance with the guidelines of the Committee on Animals of Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Council (DHEW publication No. [NIH] 85-23, revised 1985).

*Experimental measurements.* Control recordings were performed in a quiet, darkened laboratory with the unsedated conscious dog resting comfortably on its right side. Statham P23 ID strain gauge manometers (Gould Inc., Cleveland, OH) were calibrated against a mercury manometer. LV pressure was measured by use of the solid-state miniature pressure gauge calibrated in vitro with a mercury manometer, and in vivo by use of the LV pressure measurements from the catheters and Statham strain gauge manometers. The data were recorded on a multichannel oscillograph (Gould-Brush Inc., Cleveland, OH) and multichannel tape recorder (Honeywell Inc., Denver, CO).

*Membrane preparation.* LV myocardium was obtained immediately at the time of death. The endocardium and epicardium were removed. Two types of membrane preparations were employed. For a small group of animals, a highly enriched sarcolemmal preparation was obtained according to the method of Jones and Besch (19) as previously reported from our group. However, a less purified preparation obtained by differential centrifugation as previously described (20), was examined in all animals. Since the patterns of changes observed for the two preparations was identical both directionally as well as in the percentage of differences among the groups, the results with the latter preparation are presented in this report. In all cases, $Na^+,K^+$-ATPase was quantitated as an independent marker of sarcolemmal content (19) and was not different among the groups (normals, 2.55±0.14; LVH, 3.05±0.23; LV failure, 2.69±0.31 μmol $PO_4$/h per mg).

*Reconstitution of $G_s$ into cyc$^-$ membranes.* Using the stable reconstitution protocol devised by Sternweiss et al. (21), sarcolemma was first solubilized in 2% cholate in a buffer of 16 mM Tris, pH 8.0, 0.8 mM EDTA, 0.8 mM DTT. The cholate extract was centrifuged at 20,000 *g* for 30 min, the endogenous adenylyl cyclase was inactivated by incubation at 30°C for 10 min, and then the supernatant was diluted into a Lubrol buffer in preparation for reconstitution into 60 μg of S49 cyc$^-$ membranes, which were prepared according to the method of Ross et al. (22). Aluminum fluoride-responsive adenylyl cyclase was assessed over a range of solubilized sarcolemma that was known to produce a linear response (1.5–4.5 μg for the crude membrane preparation). The slope of this line (pmol of cyclic AMP/min vs. added solubilized sarcolemma) was used as a measure of $G_s$ functional activity (5).

*Enzyme assays.* $Na^+,K^+$-ATPase activity was measured according to the method of Jones and Besch (19). Protein concentration was determined according to the method of Lowry et al. (23). Cyclic AMP was quantitated by a modification (24) of the method of Salomon (25).

*RNA measurements.* RNA was extracted according to a modification of the method of Chomczynski and Sacchi (26) using the commercially available reagent RNAzol (Cinna/Biotecx Inc.). For each RNA preparation, a Northern blot was run in parallel with dot blot analyses to insure the integrity of the RNA preparation. RNA for $G_{s\alpha}$ or $G_{i\alpha 2}$ was quantitated using the method previously employed in our laboratory (27). In brief, a standard curve for both $G_{s\alpha}$ and $G_{i\alpha 2}$ was prepared by synthesizing the sense strand RNA from a pGEM 3 vector containing a full length cDNA for either message. 1:2 serial dilutions of RNA over the appropriate concentration range were blotted onto nitrocellulose using a HybriDot manifold (Bethesda Research Laboratories, Bethesda, MD). Canine ventricular RNA was blotted in a similar fashion. The blots were then hybridized and washed under the same conditions as for the Northern blots. In each case, the specificity of the dot blot hybridization was assured by including a Northern blot with each hybridization. For all dot blots, RNA dilutions which insured that the $^{32}P$-labeled probe was present in excess over the corresponding RNA, were employed. Autoradiography was then carried out and the x rays were scanned with a laser densitometer to quantitate the relative intensity of each of the dots. For all serial dilutions, calculations were restricted to the linear portion of the dilution curve; that is, dot blot intensity versus RNA dilution. Based on the standard curve, the mRNA concentration for $G_{s\alpha}$ and $G_{i\alpha 2}$ could be calculated. In addition, we also corrected for potential differences in RNA loading by the following method. A 30-mer oligonucleotide, which recognizes a highly conserved region in the 28S ribosomal RNA, was synthesized (28). This probe was end labeled and a parallel set of dot blots was then hybridized with this probe. The average value for a 10 μg sample was then calculated from all of the individual samples. The ratio of an individual sample to this average was then used to correct the $G_{s\alpha}$ and $G_{i\alpha 2}$ mRNA levels that had been determined as previously described.

*Statistical analysis.* Data were stored and analyzed on an IBM PC/AT computer (IBM Instruments, Inc., Danbury, CT) and are expressed as mean±SEM. The data were first tested for normality (Shapiro-Wilk test) and tested for homoscedasticity (Levene test) and then analyzed for statistical significance using the Fisher Least Significance Difference (29). We confirmed the results of the LSD test with the results from another multiple comparison technique, the Studentized-Maximum Modulus test.

## Results

### *Physiologic characterization*

A total of 29 animals were studied: 13 normals (group I), 10 with compensated hypertrophy (group II), and 6 who went on to develop frank heart failure (group III). As can be seen in Table I, the group II and group III animals had the same degree of LV hypertrophy with an increase in the mean left ventricle/body weight ratio of 55% for both groups. Thus, the onset of congestive heart failure was not accompanied by an additional increase in LV mass. Similarly, the gradient across the ascending aortic constriction (LV systolic pressure − aortic systolic pressure) was not different between the two groups. However, differences were apparent between the two groups in certain hemodynamic parameters. As would be predicted, there was an increased resting heart rate in the group III animals with heart failure as well as a significant increase in their LV end-diastolic pressure to 29.1±3.7 mmHg, as compared with 6.9±0.8 for the controls and 13.8±1.3 for the group II animals.

### *Biochemical characterization*

*Adenylyl cyclase activities.* In addition to assaying adenylyl cyclase basal activity (no stimulatory agents), its responsiveness

*Table I. Hemodynamic and Morphologic Characterization of the Model*

| | Normal (group I) ($n$ = 13) | Hypertrophy (group II) ($n$ = 10) | Heart failure (group III) ($n$ = 6) |
|---|---|---|---|
| Body weight (*kg*) | 23.6±1.5 | 21.3±2.0 | 23.4±0.9 |
| LV free wall/body weight (*g/kg*) | 4.9±0.2 | 7.6±0.3* | 7.6±0.8* |
| LV end diastolic pressure (*mm Hg*) | 6.9±0.8 | 13.8±1.3* | 29.1±3.7*‡ |
| LV systolic pressure (*mm Hg*) | 127±4.6 | 228±9.0* | 207±16.8* |
| Mean arterial pressure (*mm Hg*) | 103±4.3 | 98±2.8 | 78±5.1* |
| Systolic aortic pressure (*mm Hg*) | 124±4.2 | 113±3.4 | 102±6.8* |
| Heart rate (*beats/min*) | 95±4 | 95±5 | 151±4*‡ |

* $p < 0.05$ different from group I; ‡ $P < 0.05$ different from group II. Values are mean±SEM.

to a variety of stimuli, which act either at the level of the β-adrenergic receptor or distal to it ($G_s$ and adenylyl cyclase catalytic unit), was quantitated. As previously reported (4, 5), a generalized depression in all of these activities was seen in Group III animals (Table II). However, a significant decrease in these activities was also found in Group II animals, those with compensated LVH without congestive heart failure. However, of particular interest, the decrease in Group III animals was significantly greater than that seen in Group II. These differences reached statistical significance for both basal and stimulated activities.

*$G_s$ functional activity.* As part of the characterization of the adenylyl cyclase activation pathway, $G_s$ activity was quantitated using the S49 cyc⁻ reconstitution assay (5, 21) (Fig. 1). This assay measures the functional activity of $G_s$ by assessing the ability of solubilized sarcolemma to reconstitute fluoride-stimulated adenylyl cyclase activity in membranes prepared from the S49 lymphoma cell line cyc⁻, which is genetically deficient in this protein but contains a functional adenylyl cyclase catalytic unit. As was expected based on our previous findings (5), $G_s$ activity was decreased by ~ 30% in the sarcolemma of the animals with LVH and heart failure (Group III). However, a loss of similar magnitude also occurred in the Group II animals (Table II). Thus, the decrease in $G_s$ occurs in hypertrophy before the onset of congestive heart failure.

*Messenger RNA levels.* Northern blots were performed on all RNA samples with each of the $^{32}$P-labeled probes. Representative blots are shown in Fig. 2. In parallel, a dot blotting protocol was employed which allowed quantitation of messenger RNA levels for $G_{s\alpha}$ and $G_{i\alpha 2}$ as previously described (27) (Fig. 3). We felt that this was important so that changes in $G_s$ functional activity could be quantitatively correlated with any changes in message levels that might be detected. RNA loading accuracy was corrected by the use of a probe that hybridized to the 28S rRNA (28). As previously reported (27), we found that the steady-state $G_{s\alpha}$ mRNA levels were several-fold greater than that of $G_{i\alpha 2}$ in normal animals. However, an ~ 30% reduction in $G_{s\alpha}$ mRNA levels was found in both the Group II and Group III animals (Table II). The magnitude of this change and its pattern parallel that for $G_s$ functional activity as reported above. That is, the level fell in hypertrophy (Group II) without any further decrement in the setting of LV decompensation and heart failure (Group III). In contrast, $G_{i\alpha 2}$ mRNA levels were essentially unchanged in the ventricles of animals with hypertrophy (Group II) or heart failure (Group III).

## Discussion

Several years ago (4, 20), we chose to study a large animal model of heart failure in order to identify the cellular and molecular changes that underlie this pathophysiological state. This model involved aortic banding of young animals which then

*Table II. Adenylyl Cyclase and $G_s$ Activities and mRNA Quantitation*

| | | Normal (group I) ($n$ = 13) | Hypertrophy (group II) ($n$ = 10) | Heart failure (group III) ($n$ = 6) |
|---|---|---|---|---|
| Adenylyl cyclase cAMP (*pmol/min/mg*) | Basal | 107.5±7.7 | 90.0±10.0* | 55.7±7.8*‡ |
| | GTP | 148.6±9.3 | 125±12.0* | 77.5±7.6*‡ |
| | GTP + ISO | 271.4±15.8 | 203.9±21.3* | 119.0±15.8*‡ |
| | Gpp(NH)p | 463.8±24.6 | 386.2±34.0* | 249.7±24.1*‡ |
| | NaF | 420.2±17.5 | 347.1±29.6* | 244.2±27.3*‡ |
| | Forskolin | 756.5±52.8 | 662.0±73.7 | 442.7±56.6*‡ |
| $G_s$ reconstitution cAMP (*pmol/10 min/μg*) | | 4.2±0.4 | 3.0±0.2* | 3.2±0.3* |
| $G_{s\alpha}$ mRNA (*pg/10 μg of total RNA*) | | 131.0±9.0 | 104.1±7.4* | 97.4±9.1* |
| $G_{i\alpha 2}$ mRNA (*pg/10 μg of total RNA*) | | 18.1±0.7 | 18.8±1.1 | 18.7±2.1 |

* $P < 0.05$ different from group I; ‡ $P < 0.05$ different from group II. Values are mean±SEM.

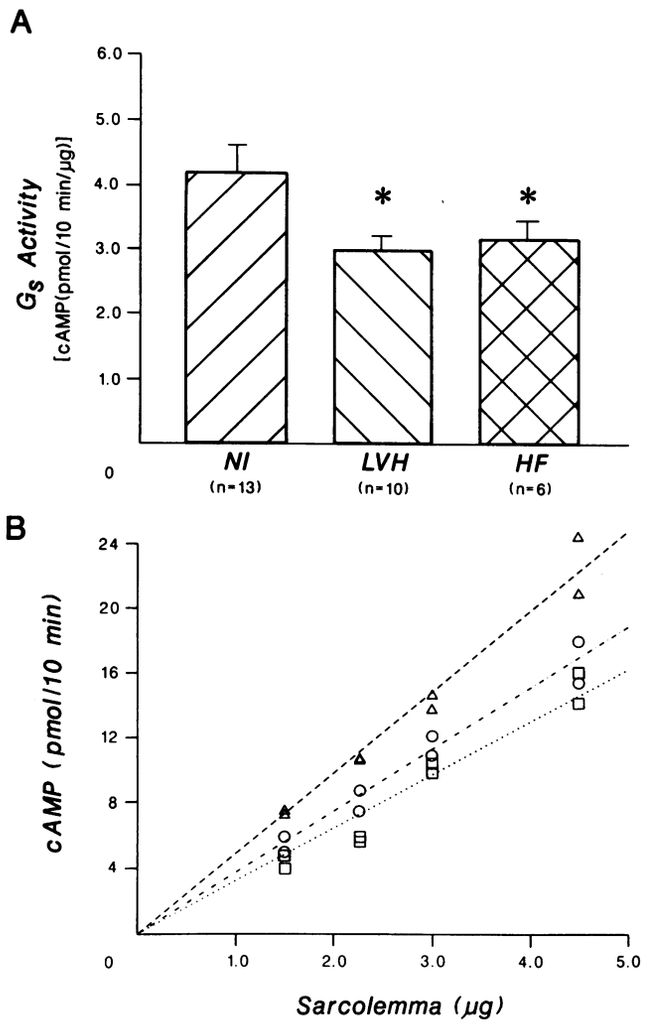


*Figure 1.* (*A*) Bar graph shows the activity of sarcolemmal $G_s$, as assayed by reconstitution, for all animals in each group. Values are mean±SEM (*$P < 0.05$). (*B*) Graph shows the data from a representative experiment in which $G_s$ activity was assayed over a range of solubilized sarcolemma. The slope of this line (pmol cyclic AMP/10 min per μg) is used as the measure of $G_s$ activity. △, normal; ○, hypertrophy; □, heart failure.

progressed to develop severe LV hypertrophy followed eventually by LV decompensation and clinical heart failure. This model is similar to pressure overload-induced hypertrophy that occurs as a common cause of human heart failure in the United States population. Long standing hypertension which leads to LVH, for example, is known to be a common cause of heart failure in the population. However, this model of pressure overload-induced hypertrophy and heart failure is likely different in terms of underlying biochemical and genetic processes than the most well characterized variety of human heart failure; that is, idiopathic dilated cardiomyopathy. Bristow and co-workers as well as others (30–32) have characterized the β-adrenergic receptor signal transduction pathway in this disease state and have determined that primary abnormalities occur at the level of the β-adrenergic receptor. These changes are at least in part consistent with a process of downregulation since there is loss of antagonist binding sites and an agonist-specific loss in adenylyl cyclase stimulation (33). However, recently other abnormalities have also been reported to occur in this variety of heart failure (34, 35). It is of interest to note that Feldman et al. (35) reported an increase in $G_{s\alpha}$ mRNA in the failing human heart.

In the model of pressure overload-induced hypertrophy and heart failure that we have studied, a reduction in β-adrenergic receptor density does not occur (4, 20). In contrast, in this model of heart failure, there is an actual increase in the number of sarcolemmal β-adrenergic receptors. Despite this increase in receptor number, there is a global reduction in adenylyl cyclase activation when the enzyme is stimulated by any of a variety of agents that either act at the level of the receptor or distal to it. In this model of heart failure, three abnormalities have been previously defined. Despite the increase in absolute receptor number, there is a loss of the high affinity or "coupled form" of the β-adrenergic receptor that forms a high affinity complex with hormone and $G_s$, the GTP-binding protein which couples the β-adrenergic receptor to adenylyl cyclase (36). We have also reported that a 30–50% reduction in the functional activity of $G_s$ itself occurs as determined with the S49 cyc$^-$ reconstitution assay (5). Finally, as noted above, there is a global loss in adenylyl cyclase activity. We have not defined whether this abnormality in cyclase stimulation is at the level of the G protein only or, in fact, involves the catalytic unit itself.

These prior studies, however, have not determined whether the loss in $G_s$ activity occurs in the setting of hypertrophy itself or whether this occurs with the development of LV decompensation and heart failure. From this study, it is clear that the loss in $G_s$ activity occurs in the setting of LVH before baseline systolic dysfunction appears. This loss in $G_s$ functional activity is associated with a reduction in the level of the messenger RNA that encodes the $G_{s\alpha}$ subunit. We have not defined whether this reduction in $G_{s\alpha}$ mRNA is due to decreased synthesis or increased turnover. Since a variety of genes are known to be either activated or repressed as cardiac hypertrophy ensues (7), it is likely that this alteration in the mRNA is occurring at the level of gene transcription itself. Nevertheless, the reduction in $G_{s\alpha}$ mRNA is of similar magnitude as that determined for $G_s$

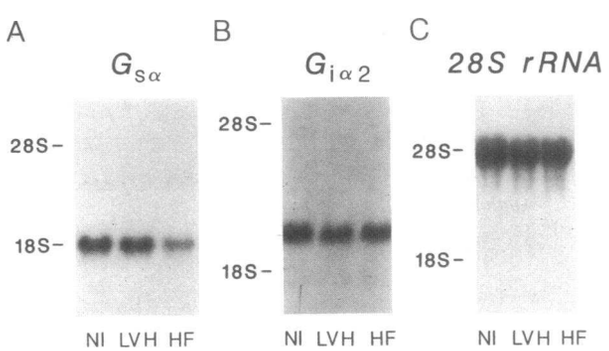


*Figure 2.* Representative Northern blots of canine heart RNA. 20 μg of total RNA were loaded per lane. (*A*) The Northern blots were hybridized with the complementary cDNA encoding for the alpha subunit of $G_s$. (*B*) The Northern blots were hybridized with the complementary cDNA encoding for the alpha subunit of $G_{i\alpha 2}$. (*C*) The Northern blots were hybridized with a 30-mer oligonucleotide that recognizes a highly conserved region in the 28S rRNA.

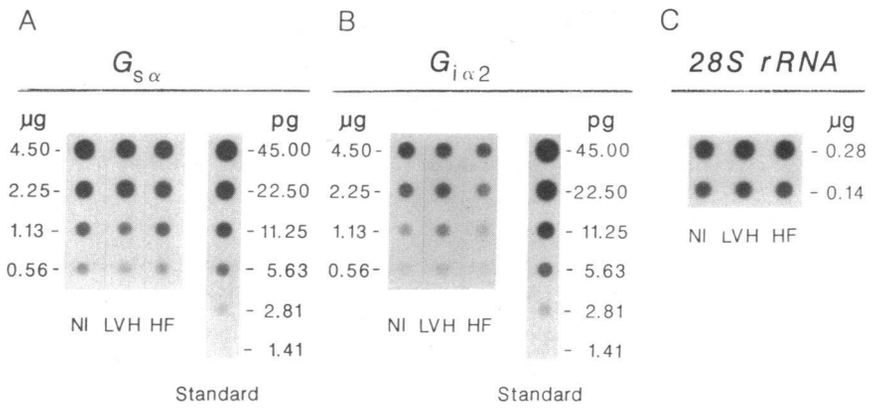


*Figure 3.* (*A* and *B*) Standard curves employing $G_{s\alpha}$ or $G_{i\alpha 2}$ mRNA prepared from pGEM templates or dilutions of total ventricular RNA were hybridized with $G_{s\alpha}$ or $G_{i\alpha 2}$ $^{32}$P-labeled probes. (*C*) Dilutions of RNA were hybridized with a 30-mer oligonucleotide that detects the 28S rRNA.

functional activity. It is also consistent with our earlier study in which a decrease in $G_{s\alpha}$ subunit was detected by cholera toxin-catalyzed [$^{32}$P]ADP ribosylation. It is reasonable therefore to suggest that the reduction in the level of message encoding the alpha subunit of $G_s$ is the responsible mechanism underlying its functional deficiency. It is also evident from these studies that no further reduction in either $G_s$ functional activity or in $G_{s\alpha}$ mRNA levels occurs as heart failure develops. Thus, the decrement in $G_s$ and in its alpha subunit mRNA appears to represent a process associated with hypertrophy per se. By the nature of these measurements, this reduction cannot reflect differences in RNA loading, quality, or yield. Moreover, the reduction in $G_{s\alpha}$ mRNA was apparent when its level was either normalized to that of 28S ribosomal RNA, the major constituent of cellular RNA, or was compared with the level of $G_{i\alpha 2}$ mRNA, a transcript that is highly homologous to $G_{s\alpha}$. The parallel fall in $G_s$ activity and $G_{s\alpha}$ mRNA is reminiscent of the findings of de le Bastie et al. (37) who detected a fall of similar magnitude in the mRNA and protein for $Ca^{++}$-ATPase in the hearts of rats with severe cardiac hypertrophy due to pressure overload. As suggested by these authors, it may be that the expression of certain genes, e.g., $G_{s\alpha}$ and $Ca^{++}$-ATPase, is not maintained as the cardiocyte enlarges.

It is also evident from these studies that there is a second process that underlies a further loss in adenylyl cyclase activity independent of any change in $G_s$. That is, comparison of Group II and Group III animals indicates that a further reduction in adenylyl cyclase activity occurs in the setting of heart failure without any further decrement in $G_s$ functional activity. In this regard, we have previously reported that there are no significant alterations in the level of $G_i$, as determined by pertussis toxin labeling (38) in the sarcolemma from animals with heart failure, nor were any alterations in $G_{i\alpha 2}$ mRNA levels detected in this study. Taken together, these data suggest that the abnormality that additionally occurs in the setting of LV decompensation is likely to be at the level of the catalytic unit of adenylyl cyclase itself. We have no evidence that bears on whether this represents a transcriptional event or rather is secondary to a posttranslational modification of the enzyme, e.g., phosphorylation (39). Since increased cardiac sympathetic nerve activity would likely be coincident with the onset of LV decompensation, this second process may be mediated through the activation of a second messenger pathway triggered by enhanced adrenergic receptor stimulation.

It is also interesting to speculate as to the mechanism underlying the loss in $G_s$ functional activity which is coincident with the decrement in $G_{s\alpha}$ mRNA levels. We have already reported that a similar quantitative reduction in $G_s$ activity occurs in the setting of long term norepinephrine exposure even in the absence of the development of frank hypertrophy (40). It may be that both in hypertrophy and with long term norepinephrine exposure, the same underlying process is activated which results in a reduction in $G_{s\alpha}$ mRNA levels. This effect could potentially be mediated through activation of the $\alpha_1$-adrenergic receptor. It has also been demonstrated that $\alpha_1$-adrenergic receptor activation plays a role in promoting hypertrophy of neonatal cardiocytes in vitro (41). Whether a similar mechanism is operative in the intact adult animal under conditions of LV pressure overload was not addressed in our studies. However, it remains intriguing that loss of $G_s$ functional activity occurs both with development of LVH and with long term administration of norepinephrine, but not with long term exposure to isoproterenol, a pure $\beta$-agonist (42). This effect might therefore be mediated through activation of a pathway that is not normally stimulated via the $\beta$-adrenergic receptor; for example, alpha-1 receptor mediated activation of phospholipase C leading to an increase in intracellular calcium and diacylglycerol with subsequent activation of protein kinase C. Such a possibility could potentially be addressed through the use of in vitro methods to determine the effects of $\alpha_1$-adrenergic receptor activation on $G_{s\alpha}$ gene expression in cardiocytes.

There is also a similar quantitative decrease in $G_s$ activity with acute myocardial ischemia (43). In view of the potential for the severely hypertrophied heart to undergo acute episodes of reduced subendocardial coronary perfusion and subsequent episodes of myocardial ischemia, these two disease states may have alterations in $G_s$ activity as a common denominator.

In summary, these results demonstrate that $G_s$ functional activity and its alpha subunit mRNA are decreased to the same extent in the hypertrophied and compensated left ventricle even before failure ensues. Associated with this change is a fall in adenylyl cyclase activity of a magnitude that parallels the decrement in $G_s$. However, these studies also indicate that a second process ensues wherein a further decrement in adenylyl cyclase activity occurs without a further change in $G_s$ functional activity or in its mRNA levels. This additional loss of adenylyl cyclase activity is seen only in the sarcolemma from animals with LV hypertrophy and heart failure as compared with those with compensated LVH. This further decrement in adenylyl cyclase activity may play a causal role in the ultimate

progression from a state of compensated hypertrophy to one of frank heart failure (44).

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