Reduced Lymphocyte Stimulatory Guanine Nucleotide Regulatory Protein and β-Adrenergic Receptors in Congestive Heart Failure and Reversal With Angiotensin Converting Enzyme Inhibitor Therapy

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Adrenergic hyposresponsiveness in congestive heart failure has been understood previously in terms of a reduction in β-adrenergic receptors. We have examined another hypothesis, one that states the stimulatory guanine nucleotide regulatory protein (Gs) that couples the β-adrenergic receptor to adenylate cyclase activity is also decreased in congestive heart failure. In addition to the 40% decrease in lymphocyte β-adrenergic receptors in patients in congestive heart failure (5.9±0.7 vs. 9.7±1.4 fmol/mg, p<0.05), we found an 80% decrease in levels of Gs compared with age- and sex-matched healthy control subjects (72.5±19 vs. 376±73 fmol/mg, p<0.05). Myocardial Gs levels correlated significantly with lymphocyte Gs levels. We also assessed the hypothesis that reductions in β-adrenergic receptors and in Gs, are reversible after successful therapy with angiotensin converting enzyme inhibitors. Treatment with either captopril or lisinopril was associated with clinical improvement, an increase in β-adrenergic receptor density (from 5.5±0.7 to 8.7±1.5 fmol/mg), and a twofold increase in Gs levels (p<0.05). Thus, the data are compatible with Gs serving as an adaptable and reversible regulator of the adrenergic response in congestive heart failure. In view of the fact that Gs is a transducing element common to all hormones that stimulate cyclic adenosine 5’-monophosphate production, the observations could extend to other abnormal neurohumoral mechanisms in congestive heart failure. (Circulation 1988;78:1373–1379)

Sympathetic activation provides inotropic and chronotropic support for both normal and failing hearts. In chronic heart failure, however, the myocardium becomes refractory to stimulation by the β-adrenergic catecholamines and shows a characteristic blunted adrenergic responsiveness.1,2 To account for diminished adrenergic function in congestive heart failure, Bristow and colleagues3,4 have shown a reduction in β-adrenergic receptor number in tissue obtained from the failing heart. Reduced β-receptor number is associated with a proportionate loss in maximal isoproterenol-stimulated adenylate cyclase activity. Similarly, decreased β-adrenergic receptor density has been shown in peripheral lymphocytes of subjects in congestive heart failure.5 For both myocardial and lymphocyte β-receptors, the decrease in β-adrenergic receptor number correlates with altered hemodynamic indexes6 and impaired left ventricular performance.22 Vatner and associates7 have demonstrated in a canine model of congestive heart failure a marked decrease in high-affinity β-adrenergic receptor binding sites, suggesting that altered β-adrenergic function in congestive heart failure may also be due to
an uncoupling of these receptors from their effector units. This observation suggests other sites in the receptor-effector complex besides the receptors themselves that could conceivably be abnormal and thereby account for diminished adrenergic responsiveness in congestive heart failure. Components not yet studied in human subjects are the guanine nucleotide regulatory proteins that serve to transmit the signal generated by the hormone-receptor unit to its effector. One such protein of particular interest with respect to adenylyl cyclase is the stimulatory guanine nucleotide binding protein \( G_{s} \). A reduction in the amount of \( G_{s} \) could account for functional uncoupling between \( \beta \)-adrenergic receptors and adenylyl cyclase and, ultimately, may contribute to the blunted responsiveness of the failing heart to catecholamines.

We have also studied the effect of treatment with angiotensin converting enzyme inhibitors captopril and lisinopril with respect to the components of the \( \beta \)-adrenergic receptor complex. Angiotensin converting enzyme inhibitors can inhibit norepinephrine release and decrease plasma norepinephrine levels in addition to their salutary clinical and hemodynamic effects. Changes in endogenous catecholamine concentrations have been associated in other settings, with concomitant changes in \( \beta \)-adrenergic receptors. We examined the possibility that one mechanism whereby converting enzyme inhibition treatment improves cardiac function may be via an increase in \( \beta \)-adrenergic receptor number or an increase in \( G_{s} \), which may augment \( \beta \)-adrenergic receptor–adenylyl cyclase coupling. To address these questions, lymphocytes were used as accessible surrogate tissue to study serial changes in the \( \beta \)-adrenergic receptors and the guanine nucleotide regulatory proteins.

**Subjects and Methods**

**Patient Population**

Twenty-two patients with severe chronic congestive heart failure (New York Heart Association Class II [2], III [14], or IV [6]), despite previous therapy with digitalis and diuretics, were admitted to the General Clinical Research Center of Columbia Presbyterian Medical Center. Patients were maintained on a 2-g sodium diet and their previous therapeutic schedules of digitalis and diuretics. One patient had to be withdrawn because of worsening renal function, despite liberalizing fluid restriction and concomitant diuretic therapy. No other vasodilators were used. A subgroup of the study population consisting of nine men had a complete set of measurements of baseline and post-therapy catecholamines, at rest and exercise, as well as lymphocyte \( \beta \)-adrenergic receptors and guanine nucleotide regulatory proteins. This smaller group of patients was indistinguishable from the larger group with respect to age, New York Heart Association classification, etiology of heart disease, radionuclide ejection fraction, and resting and exercise catecholamines. Further details of this population are found in “Results.” Informed consent was obtained; the protocol was approved by the Institutional Review Board of Columbia Presbyterian Medical Center.

**Control Subjects**

Thirteen men (average age, 45 ± 3 years), with no history of cardiac disease or hypertension and no symptoms suggestive of left ventricular dysfunction had early morning resting blood specimens obtained for lymphocyte studies. The exercise protocol was not a part of the study for the control subjects.

**Clinical Studies**

On day 1, maximum treadmill exercise time was determined with a symptom–limited, modified Naughton exercise protocol. On the morning of day 3, plasma samples were obtained for supine norepinephrine determinations. On day 4, blood was again obtained for supine norepinephrine measurements and for lymphocyte \( \beta \)-receptor studies. Patients then underwent a second exercise test during which plasma samples were obtained for upright resting and peak exercise catecholamines. There was no significant change between the first and second exercise tests, and pretreatment exercise time was determined by the average of exercise tests performed on days 1 and 4. Resting supine norepinephrine concentrations were calculated as the average of the day 3 and 4 samples. Four weeks after initiation of captopril \((n=9)\) or lisinopril \((n=13)\), exercise time on a modified Naughton protocol was again determined and plasma samples were obtained for resting supine, resting upright, and exercise catecholamine measurements and lymphocyte studies.

**Plasma Norepinephrine**

Blood was collected from an indwelling venous catheter. Resting supine samples were obtained before the patients arose in the morning. With the venous catheter in place, blood samples were also obtained before and at peak exercise. Norepinephrine levels were determined by the radioenzymatic assay of Passon and Peuler.

**Preparation of Lymphocytes**

At the same time blood was drawn for supine resting plasma catecholamines on day 4, 40 ml of heparinized blood was obtained for lymphocyte studies. Blood samples were similarly obtained from control subjects. Lymphocytes were isolated according to a modification of the methods of Boyum and as previously published by Mann et al. Ten milliliters of blood routinely yielded 1–2x10⁷ cells consisting of 70–85% lymphocytes and 15–30% monocytes. Lymphocytes were stored as pellets at -70°C and used only when post-therapy specimens were available to be assayed concurrently.

**\( \beta \)-Adrenergic Receptor Binding Assay**

\( \beta \)-Adrenergic receptors were measured in lymphocytes according to a modification of our method.
Lymphocytes were thawed, suspended in phosphate-buffered saline (pH 7.4) without calcium and magnesium, homogenized, and centrifuged at 400g. Cells were subsequently resuspended in phosphate-buffered saline at approximately 2 mg/ml. One hundred microliters of the lymphocyte suspension were incubated with 40 μl [125I]iodocyanopindolol (ICYP) for 60 minutes at 20° C in a Hanks-Tris-HCl (50 mM, pH 7.4) buffer. Subsequently, 5 vol Tris-HCl (10 mM, pH 8, at 4° C) were added as a 15-minute postincubation period to reduce nonspecific binding. After rapid filtration over Whatman GF/B glass filters and two washes with 5 ml incubation buffer, radioactivity retained by the filters was detected in a Packard Autogamma Scintillation Spectrometer. Specific binding was determined by the difference in binding with and without propanolol (10 μM) and was more than 80% of total binding. Insufficient material precluded a complete Scatchard analysis in all cases. Data for all patients are presented at the fixed ligand concentration of 100 pM because higher concentrations yielded excessive nonspecific binding.

Identification and Quantification of Regulatory Proteins

ADP-ribose is covalently linked to the α-subunit of Gs in the presence of cholera toxin. Gs was assayed by measuring the incorporation of [32P]ADP-ribose into membrane protein with [32P]NAD as the substrate by the methods of Kaslow et al and modified by us. Cholera toxin was activated by incubation with diethiothreitol (20 mM) for 10 minutes at 30° C. Identical membrane preparations were used for receptor and ADP-ribosylation studies. Twenty-five microliters of lymphocytes (35–65 μg), prepared as previously described and thawed for ADP-ribosylation, were incubated in 65 μl buffer containing 50 mM K2PO4 (pH 7.5), 10 units aprotinin, 13 mM thymidine, 3.2 mM ADP-ribose, 13 mM arginine, 0.2 mM Gpp(NH)p, [32P]NAD (18–54 Ci/mmol, 50–80 μM), and 20 μg activated cholera toxin. The complete reaction mixture was incubated for 20 minutes at 30° C and was terminated by the addition of 1 ml ice-cold 7% trichloroacetic acid (TCA) and centrifuged at 12,000g. The pellet was then resuspended in 1% TCA, recentrifuged, and subsequently solubilized with sodium dodecyl sulfate sample buffer and boiled for 2 minutes. Electrophoresis was performed on vertical slab gels at 300 V for 3 hours. Gels were then stained with Coomassie blue and analyzed by autoradiography with Kodak XRP-5 film. The concentration of Gs was calculated from the number of counts in the labeled bands of interest on the gel (42 kDa), the amount of [32P]NAD added to the incubation mixture, and the specific activity of [32P]NAD. Gel slices were corrected for background radioactivity by cutting out the comigrating band in the absence of cholera toxin. Simultaneous assay of pretherapy and post-therapy lymphocytes used the same lot of [32P]NAD, incubation medium, and cholera toxin. Under these conditions, lymphocytes showed a linear dose-response relation between the amount of cholera toxin-dependent ADP-ribosylation and lymphocyte protein concentration in the range of 20–70 μg protein. The exogenous addition of factors such as ADP ribosylation factor, ATP, and NADP, which appear to be required in other systems for the ADP-ribosylation reaction, was not necessary. Furthermore, for cholera toxin-dependent ADP-ribosylation, detergent extraction of the membrane tissue was not required.

In a related study of the correlation of human myocardial and lymphocyte Gs in severe congestive heart failure, ADP-ribosylation reactions were also performed in an identical manner. Cardiac tissue was obtained at the time of transplantation, and blood was obtained for lymphocyte isolation before cyclosporine infusion. This patient population consisted of 11 men and four women (mean age, 37 ± 4 years). Two patients had coronary artery disease, five had primary valvular or congenital heart disease, and eight had idiopathic cardiomyopathy.

Preparation of Cardiac Membrane Tissue

Left ventricular anterolateral free wall myocardial tissue was dissected free of fat, vessels, and endocardial and epicardial surfaces. Tissue was minced and polytronized (Brinkman, Westbury, New York) in 4 vol (wt:vol) buffer (0.25 M sucrose, 0.03 M, 1 mM EDTA, 0.1 mM PMSF). The resulting crude homogenate was centrifuged at 1,500g for 10 minutes at 4° C, and the resulting supernatant was immediately centrifuged at 43,000g for 20 minutes. This final pellet was resuspended in buffer at a protein concentration of approximately 2 mg/ml and stored in aliquots at −60° C.

Statistical Methods

Statistical significance of paired (before and after) and unpaired (normals vs. congestive heart failure) samples was assessed by Student’s two-tailed t test. To normalize for interassay variability with respect to measurements of guanine nucleotide regulatory proteins, logarithmic transformation functions were used. Relative changes in G proteins after angiotensin converting enzyme inhibition therapy were calculated as the Gs pre:Gs post ratio.

Results

Baseline and follow-up studies included a modified Naughton exercise protocol, resting and peak exercise catecholamine concentrations, lymphocyte isolation for β-adrenergic receptor studies, and stimulatory guanine nucleotide proteins. The average age of the nine men who had this complete evaluation was 52.8 ± 4.2 years. All patients were suffering from idiopathic cardiomyopathies. Baseline clinical status was New York Heart Association (NYHA) Class III (n = 6) and Class IV (n = 3). The average radionuclide ejection fraction for the 10 subjects was 19 ± 2.5% (normal, >45%). Resting
nepinephrine concentration was 494±74 pg/ml (normal laboratory value, 65–320 pg/ml); peak exercise norepinephrine was 1,725±439 pg/ml.

Comparison of Normal Subjects With Patients in Congestive Heart Failure

In patients with congestive heart failure, β-adrenergic receptors were reduced in circulating lymphocytes compared with control subjects. Lymphocyte ICYP binding was 40% below normal (5.5±0.7 vs. 8.7±1.4 fmol/mg, p<0.05, Figure 1A). These results confirm those previously reported in human subjects and suggest that, in this respect, our population of patients is similar to those in previous studies.

Particular attention in our study was directed at the stimulatory regulatory protein Gs. Measurements were made in identical blood samples used to detect β-adrenergic receptors. Gs levels were reduced by 80% in the lymphocytes of patients with congestive heart failure in comparison with control subjects (72.5±19 vs. 376±73 fmol/mg, p<0.05, Figure 1B). Thus, congestive heart failure was associated with reductions not only in β-receptor density but also in Gs.

Response to Converting Enzyme Inhibitor

Treatment with captopril (37.5–75 mg/day, n=5) or lisinopril (5–10 mg/day, n=5) for 4–6 weeks was associated with overall improvement in exercise tolerance in 20 of 22 patients. Within the subgroup of nine patients who had complete biochemical markers, five patients improved by one NYHA class; two patients improved by two NYHA classes; and two patients deteriorated by one NYHA class. Exercise time increased from 333±34 to 432±52 seconds (p<0.05, Figure 2). There was no difference between captopril and lisinopril in responses of patients.

After therapy, β-adrenergic receptor density increased 1.5-fold (p<0.05, Figure 3). Accompanying the increase in β-receptor density was a twofold increase in Gs levels (p<0.05, Figure 4). Figure 5 shows sample autoradiographs of sodium dodecyl sulfate–polyacrylamide gels prepared from lymphocytes of two patients before and after treatment with converting enzyme inhibitors. In both, there is an obvious increase in the amount of 32P incorporation into a band migrating as Gs.

Successful therapy was not associated with changes in norepinephrine levels either at rest (493±74 to 466±45 pmol/ml) or at peak exercise (1,725±439 to 1,723±359 pmol/ml, Figure 6); however, exercise time for the same peak catecholamine level increased, as noted, by 21%.

Although therapy was associated with an increase in β-adrenergic receptors and Gs, there was no significant correlation between the changes in β-adrenergic receptors and changes in Gs levels (r=0.17, p>0.1). In addition, there was no significant correlation between the clinical response to angiotensin converting enzyme inhibition, as assessed by changes in maximum exercise time on a modified Naughton protocol, and change in either β-adrenergic receptor density.
density \( r = -0.19 \) or levels of \( G_s \) \( r = -0.23 \). Also, changes in \( \beta \)-adrenergic receptor density and in \( G_s \) were unrelated to changes in resting supine or peak exercise plasma catecholamine levels \( r = -0.22 \) for receptors, \( r = 0.50 \) for log \( G_s \).

**Correlation of Lymphocyte and Left Ventricular \( G \) Proteins**

Previous investigators had established correlations between lymphocyte and myocardial \( \beta \)-adrenergic receptor density.\textsuperscript{23,24} We sought to address the issue of relevance of surrogate lymphocyte \( G \) proteins as a marker of central cardiac tissue \( G \) proteins. In related work with a group of patients with congestive heart failure who were to undergo cardiac transplantation, we determined \( G_s \) levels in their lymphocytes and in their cardiac tissue. There was a significant, positive correlation between lymphocyte and cardiac \( G_s \) levels \( r = +0.7, p < 0.01 \); Figure 7).

**Discussion**

The results of the present study suggest that diminished adrenergic responsiveness in congestive heart failure may be due to elements of the \( \beta \)-adrenergic receptor complex besides the \( \beta \)-receptor itself. The decrease in lymphocyte ICYP binding in congestive heart failure is consistent with previous studies.\textsuperscript{23} In addition to this established observation, however, the new observation is that the guanine nucleotide regulatory protein \( G_s \), which links receptors for \( \beta \)-adrenergic catecholamines to adenylyl cyclase activity, is also decreased in the lymphocytes of patients with congestive heart failure. Altered \( G_s \) levels, as a postreceptor site of \( \beta \)-adrenergic receptor modulation, could explain the observations of Vatner et al.\textsuperscript{7} in which congestive heart failure was associated with a decrease in the high-affinity state of the \( \beta \)-adrenergic receptor, an index of receptor linkage to \( G_s \). The data may also relate to reduced responsiveness of glucagon in congestive heart failure because glucagon is similarly mediated via the \( G_s \)-adenylate cyclase complex. This study has focused attention on the expression of \( G_s \) levels as monitored by cholera toxin–dependent ADP-ribosylation. Functional assays for \( G_s \) are required to substantiate that reductions in cholera toxin substrate are similarly associated with reductions in \( G_s \) activity. However, it is noteworthy that in pseudohypoparathyroidism, a genetic disease of blunted parathyroid hormone responsiveness, reduced cholera toxin substrate correlates strongly with functional assays for \( G_s \).\textsuperscript{26} We do not yet know whether the message for \( G_s \) at the transcriptional level is also affected by congestive heart failure in humans, although work by Homcy and colleagues in the canine aortic-banding hypertrophy model of heart failure does suggest that there are significant changes at this level (C.J. Homcy, personal communication).

Do changes in \( G_s \) levels in the circulating, peripheral lymphocyte reflect similar changes in cardiac tissue itself? Certainly, there are precedents for using the peripheral blood element as a biologically relevant surrogate marker for \( \beta \)-adrenergic receptors,\textsuperscript{23,24} \( \alpha \)-receptors,\textsuperscript{27} and insulin receptors\textsuperscript{28} when target tissue is difficult to obtain. With respect

![Figure 4](http://circ.ahajournals.org/)

**FIGURE 4.** *Plot of changes in the stimulatory guanine nucleotide binding proteins \( (G_s) \) after therapy with lisinopril or captopril. Individual and mean±SEM data are shown.*

![Figure 5](http://circ.ahajournals.org/)

**FIGURE 5.** *Representative autoradiographs demonstrating lymphocyte stimulatory guanine nucleotide regulatory protein \( (G_s) \) preparations of two patients before and after therapy. There is an increase in the amount of \([32P]ADP-ribose \) incorporated into the 42-kDa band, which migrates as \( G_s \) ("Subjects and Methods").*
to the heart as a target organ, both lymphocyte β-adrenergic receptors and cardiac β-receptors are reduced in congestive heart failure.2,3 For Gs, results in the dog subjected to heart failure by aortic banding have shown similar reductions in cardiac Gs levels by ADP-ribosylation and by cyclo-oxygenase reconstitution assays.29 Our preliminary studies show a significant positive correlation in congestive heart failure between human left ventricular tissue and lymphocyte Gs levels (Figure 7) and thus suggest that lymphocyte Gs measurements may reflect more central cardiac tissue.21

The second part of our study focused on the adaptive capability of the β-adrenergic receptor complex after therapy with angiotensin converting enzyme inhibitors. Treatment with either captopril or lisinopril favorably modulated both components of the β-adrenergic receptor complex, the β-adrenergic receptor itself, and Gs. Treatment with converting enzyme inhibitors was also associated with subjective and objective clinical improvement, as assessed by an increase in exercise time duration.

Figure 6. Plot of changes in supine resting plasma norepinephrine levels before and 4 weeks after angiotensin converting enzyme inhibitor therapy.

Figure 7. Plot of comparison of human left ventricle and lymphocyte stimulatory guanine nucleotide regulatory protein (Gs). With a log transformation function, left ventricular Gs is compared with lymphocyte Gs obtained from patients who underwent cardiac transplantation (r = +0.7, p < 0.01).

“Normalization” of β-receptors and Gs after treatment was not associated with significant changes in resting supine or peak exercise plasma norepinephrine levels, raising the distinct possibility that the changes in lymphocyte ICYP binding do not simply reflect “down regulation” of the β-adrenergic receptor by physiological agonist with disease and subsequent “resensitization” with therapy due to a decrease in agonist stimulation.30 Moreover, as yet, there is no evidence that Gs levels are similarly regulated. We also note that the plasma catecholamine levels of our patients were only minimally elevated and did not change significantly with therapy although both β-receptors and Gs levels increased with therapy. Thus, changes mediated by plasma catecholamine levels cannot completely explain our findings. Possibly, the relatively normal resting supine and upright catecholamine levels may have been due to a selection bias of our entrance criteria—the ability to perform on a treadmill test and the fact that our protocol called for the measurement of catecholamine levels only after the patients were hospitalized, placed on bed rest, and initiated on a strict 2-g sodium diet. Evidence also suggests that nearly normal circulating catecholamines do not rule out the possibility that local myocardial levels may have been elevated in the setting of congestive heart failure. Thus, although the data with respect to β-receptors do not totally exclude down regulation, it is likely that other mechanisms are involved to control the level of Gs.

The increase in levels of β-adrenergic receptors and Gs may or may not be a special feature of angiotensin converting enzyme inhibition in congestive heart failure. Specifically, these drugs are associated with inhibition of the sympathetic nervous system9 and decreased norepinephrine release at the nerve terminal.10 Alternatively, the favorable modulation of the β-adrenergic receptor complex might be due to an overall improvement in cardiac function and hemodynamic status and thus be independent of the specific therapy used. In this regard, our preliminary findings in another study suggest that higher Gs:Gs ratios are associated with improved left ventricular hemodynamic indexes (lower pulmonary capillary wedge pressure and higher stroke volume index).31 Further studies are in progress to assess whether the restoration of these changes are specific to converting enzyme inhibition and to determine what the hemodynamic and/or neurohumoral stimulus is for changes in the various components of the β-adrenergic receptor complex. Although we cannot exclude a decrease in central myocardial catecholamine levels to explain partial restoration of β-adrenergic receptor components, classic up-regulation has not been demonstrated in this study.

The reduction in both β-receptors and Gs in congestive heart failure and their subsequent return toward normal with this therapy does not provide insight about the chronology of both events in relation to each other. The fact that changes in these two variables do not significantly correlate with each other may indicate, in fact, that the two elements are subject to independent regulatory control. Another important consideration is the possibility that one element (Gs or the β-adrenergic receptor) may be directing the presence and/or functional availability of the other. Finally, we can
not yet conclude that a reduction in $G_s$—or for that matter, β-receptor levels—may be a primary abnormality in the pathophysiology of blunted adrenergic responsiveness in congestive heart failure.

The data indicate that in congestive heart failure the heart shows adaptation by reducing its complement of a pivotal regulator of the adrenergic response. To the extent that many other hormones also use $G_s$ as a transducing element, these observations could extend to other abnormal neurohumoral mechanisms in congestive heart failure.

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References


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