Increased Messenger RNA Level of the 
Inhibitory G Protein α Subunit Giα-2 
in Human End-Stage Heart Failure

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In human heart failure the positive inotropic and cAMP-elevating effects of both β-adrenoceptor agonists and phosphodiesterase inhibitors are diminished. This has been explained at least in part by an increase in the inhibitory signal–transducing G protein (Gi) and unchanged stimulatory G protein (Gs). In the present study we determined the mRNA expression pattern of the α subunits of Gt1α, Gi2α, Gi3α, and Gs in myocardial tissue samples of patients undergoing heart transplantation. Northern blot analysis of total RNA extracted from left ventricles with 32P-labeled cDNAs demonstrated expression of Giα-2, Giα-3, and Gsα mRNA. In contrast, Gisα mRNA was not detectable. To investigate whether the increased ratio of Gi/Gs might be due to altered gene expression, we compared mRNA levels of Giα-2, Giα-3, and Gs in left ventricular myocardium from failing hearts with idiopathic dilated cardiomyopathy (n=8) and ischemic cardiomyopathy (n=6) and from nonfailing hearts from transplant donors (n=8). Compared with nonfailing control hearts, the Giα-2 mRNA was increased by 75±26% (p<0.05) in idiopathic dilated cardiomyopathy hearts and 90±26% (p<0.05) in ischemic cardiomyopathy hearts. Giα-1, and Gsα mRNA levels were similar in the three groups. The results suggest that as in other mammalian species, Giα-2, and Giα-3 mRNA are the predominant Gsα mRNA subtypes in human ventricular myocardium. An upregulation of Giα-2 but not of Giα-3 mRNA probably underlies the increase in Giα protein and might thus be involved in the pathophysiological process leading to reduced responsiveness to cAMP-increasing agents in end-stage heart failure. (Circulation Research 1992;70:688–696)

KEY WORDS • heart failure • G proteins • mRNA expression

In studies of ventricular preparations from patients undergoing heart transplantation, it has been shown that the positive inotropic and cAMP-elevating effects of both β-adrenoceptor agonists and phosphodiesterase inhibitors are diminished in the failing heart. In contrast, drugs bypassing the β-adrenoceptor/adenylate cyclase signaling pathway, such as dibutyryl-cAMP, dihydrouabain, and Ca2+, exhibit an unchanged positive inotropic effect. These results demonstrate the ability of failing myocardium to exert a maximal contractile response and suggest a defective adenylate cyclase stimulation with deficient CAMP production as an important cause of decreased myocardial contractility.

It is well established that β-adrenoceptor downregulation occurs in heart failure and might partly explain the diminished inotropic and cAMP response to β-adrenoceptor agonists. However, there is increasing evidence for an important additional role of Gi proteins in regulating adenylate cyclase activity and hence inotropy in human end-stage heart failure. G proteins are heterotrimeric proteins composed of α, β, and γ subunits, which occupy a key regulatory role as transducers of many pathways: G, couples β1 and β2-adrenoceptors; H receptors and prostaglandin E2 receptors couple to the adenylate cyclase in a stimulatory way; G, couples α-adrenoceptors, M5-cholinoceptors, and A1-adenosine receptors to the adenylate cyclase in an inhibitory way. Evidence for an additional direct regulation of cardiac Ca2+ and Na+ channels or K+ channels by α subunits of G, or G, respectively, further underlines the importance of G proteins in various signal-transducing pathways.

Recently, a 35–40% increase in the 40-kd α subunit of Gi, in failing human hearts with idiopathic dilated cardiomyopathy (IDC) has been demonstrated by pertussis toxin–catalyzed ADP-ribosylation. Studies with cholera toxin–catalyzed labeling revealed unchanged Gαi levels. Furthermore, reconstitution experiments in cyc+ cells showed that the functional activity of Gαi is also unchanged in the failing human heart. Alterations in the G protein amount and/or function could account for the discrepancies between changes in β-adrenoceptor density and adenylate cy-

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Supported by the Deutsche Forschungsgemeinschaft.

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Received July 24, 1990; accepted November 21, 1991.
class–stimulating and positive inotropic effects of β-adrenoceptor agonists in humans as well as in animal models. Furthermore, they might explain the reduced efficacy of receptor-independent phosphodiesterase inhibitors in heart failure.

By now, more than 12 different G protein α subunits have been identified; at least seven of these (four G_{αi}, G_{αo}, and G_{αs}) are thought to be involved in the adenylate cyclase signaling pathway. There is no clear evidence so far for functional differences between the four G_α subunits or the three G_α subunits; equipotent effects on the adenylate cyclase and Ca^{2+} channels (G_{βγ}) or on atrial K^+ channels (G_{β}) have been shown. However, tissue-specific and developmental differences in the expression of the three inhibitory G protein α subunits suggest that differences in the coupling to receptors or effectors might exist.

Because pertussis toxin–catalyzed ADP-ribosylation does not discriminate between different G_{α} subunits, it is currently not known which of the three G_{α} subunits is responsible for the measured increase of the 40-kd signal in heart failure. With the use of cDNA probes the present study focused on the following main questions: 1) Is the increase in G_{α} protein accompanied by an increase in G_{α} mRNA levels in the failing heart? 2) Are G_{α} mRNA levels changed in the failing heart? 3) Are there differences in G_{α} mRNA levels between IDC and ischemic cardiomyopathy (ICM)? Therefore, we characterized the G_{α} mRNA expression pattern qualitatively in the human heart with cDNA probes encoding G_{αi1}, G_{αi2}, G_{αo3}, and G_{αs} and quantified G_{αo2}, G_{αo3}, and G_{αs} mRNA levels in left ventricular myocardium from patients with IDC and ICM compared with nonfailing human myocardium.

**Materials and Methods**

**Cardiac Tissue**

Failing human hearts were obtained from patients undergoing heart transplantation because of end-stage heart failure caused by IDC (n=8) or ICM (n=6). The diagnosis of ischemic heart disease was confirmed by left heart catheterization and coronary angiography. Patients with other forms of cardiomyopathy, such as muscular dystrophy or acute myocarditis, were excluded from the study. All of the patients were classified as New York Heart Association (NYHA) class IV with markedly abnormal pretransplant hemodynamics (Table 1). There were no significant differences between the two groups in mean age, mean cardiac index, mean pulmonary capillary wedge pressure, or mean left ventricular ejection fraction. Before cardiectomy all patients received conventional medical treatment including cardiac glycosides, organic nitrates, diuretics, angiotensin converting enzyme inhibitors, and other vasodilators in varying combinations. No patient received catecholamines, α- or β-adrenoceptor antagonists, or phosphodiesterase inhibitors. Nonfailing control hearts (n=8) were obtained from prospective multiple organ donors whose hearts could not be transplanted for surgical reasons or because of blood group incompatibility. Aortic and pulmonary valves were excised and later used for valve replacements. Some of these patients had a history of hypertension or had arteriosclerosis of great vessels on surgical inspection (Table 2). However, none had a history of heart failure, and they were thus classified as nonfailing donors. The mean age of organ donors did not differ significantly from heart recipients but did tend to be lower. Before explantation, seven of eight patients received low doses of dopamine to maintain renal function; one of them also received dexamethasone. Another patient received a single dose of an α-adrenoceptor antagonist. Written informed consent was obtained from all patients or the family of prospective heart donors before cardiectomy.

**Tissue samples** from the free walls of the left ventricles were obtained at the time of explantation and rapidly frozen in liquid N\(_2\). Care was taken not to store samples, to section heart tissue, to endocardium, epicardium, or great vessels. Tissue samples were stored at \(-80^\circ\mathrm{C}\) until use.

**Total RNA Preparation**

Preparation of total RNA was performed according to the method of Auffray and Rougeon (procedure C)\(^{21}\) with modifications that have been previously described in detail.\(^{22}\) Samples (1–1.5 g) of frozen tissue were minced to fine powder in liquid N\(_2\) with a mortar, homogenized in 4 M LiCl/8 M urea with a polytron homogenizer (PT 10-35, Kinematica GmbH, Lucerne, Switzerland) for 15 seconds at setting 5, and incubated overnight at 4°C. Total cellular RNA was precipitated by centrifugation at 17,000g for 30 minutes at 0°C. After a proteinase K (Boehringer Mannheim, Mannheim, FRG) digestion in 0.01 M Tris and 0.5% sodium dode-
Table 2. Clinical and Anamnestic Data From Nonfailing Heart Donors and Steady-State Levels of G_{\infty} \text{mRNA}

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>Others</th>
<th>Drugs at HTX</th>
<th>G_{\infty2}</th>
<th>G_{\infty3}</th>
<th>G_{\infty tot}</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>43</td>
<td>CB</td>
<td>Fatty liver, obesity*</td>
<td>DA 50 mg/hr</td>
<td>2.37</td>
<td>6.53</td>
<td>2.74</td>
</tr>
<tr>
<td>M</td>
<td>44</td>
<td>SA bleeding</td>
<td>Fatty liver*</td>
<td>DA 6 mg/hr</td>
<td>3.05</td>
<td>3.68</td>
<td>6.21</td>
</tr>
<tr>
<td>M</td>
<td>53</td>
<td>Cerebral venous thrombosis, cerebral edema</td>
<td>Suspicion of cerebral cancer*</td>
<td>DA 50 mg/hr Dexamethasone 20 mg bolus</td>
<td>2.39</td>
<td>8.96</td>
<td>3.27</td>
</tr>
<tr>
<td>M</td>
<td>56</td>
<td>Polytrauma, CB</td>
<td>Arteriosclerosis</td>
<td>DA 50 mg/hr</td>
<td>2.25</td>
<td>14.1</td>
<td>2.58</td>
</tr>
<tr>
<td>F</td>
<td>46</td>
<td>CA, CB</td>
<td>Obesity, hypertension, mild arteriosclerosis</td>
<td>DA 6 mg/hr</td>
<td>3.89</td>
<td>13.1</td>
<td>4.64</td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>CA, CB</td>
<td>Arteriosclerosis, nicotine abuse</td>
<td>DA 6 mg/hr</td>
<td>3.64</td>
<td>6.66</td>
<td>4.82</td>
</tr>
<tr>
<td>M</td>
<td>41</td>
<td>CB</td>
<td>Wernicke encephalopathy*</td>
<td>DA 6 mg/hr</td>
<td>1.81</td>
<td>15.6</td>
<td>3.41</td>
</tr>
<tr>
<td>F</td>
<td>27</td>
<td>CB</td>
<td>Drug addiction†</td>
<td>PB 1.5 mg/kg bolus</td>
<td>2.94</td>
<td>7.43</td>
<td>2.92</td>
</tr>
</tbody>
</table>

Mean: 2.79 9.5 3.8
±SEM 0.25 1.5 0.45

Given are the diagnoses leading to clinical death, other diagnoses in patients' history or from pathological examination, medication at time of heart transplantation, and steady-state levels of G_{\infty2}, G_{\infty3}, and G_{\infty} mRNA per amount of total RNA in arbitrary units as described in "Materials and Methods." HTX, heart transplantation; CB, intracerebral bleeding; SA bleeding, subarachnoidal bleeding; CA, aneurysm of cerebral artery; DA, dopamine; PB, phenoxylbenzamine.

*Patient's history stated that there were no clues for any disease of the heart or circulatory system.

†Drugs used were alcohol, diaminophosphate, and codeine.

cDNA Probes

Plasmids (pGEM-2) with cDNA inserts for rat G_{\infty1}, G_{\infty2}, G_{\infty3}, and G_{\infty} were gifts from Dr. R.R. Reed. A plasmid (bluebriscue) with the cDNA insert for human G_{\infty3} was supplied by Dr. E.J. Neer. The plasmids were transformed into Escherichia coli, and positive clones were picked and grown in rich medium. Large-scale preparation was performed according to the alkaline lysis method with cesium chloride (Paesel GmbH, Frankfurt, FRG) density gradient centrifugation. Inserts were cut with EcoRI (Boehringer Mannheim) and gel-purified. Insert sizes and purity of the cDNA were determined in 1% agarose gels with a DNA molecular weight standard purified from Boehringer Mannheim. Sizes were as follows: 1,950 base pairs (bp) (G_{\infty1}), 1,750 bp (G_{\infty2}), 3,070 bp (G_{\infty3}), 620 ± 1,120 bp (EcoRI fragments of G_{\infty3}), and 2,200 bp (human G_{\infty3}). The full-length cDNA of G_{\infty2} and the 1,120-bp cDNA fragment of G_{\infty3} were used for probing.

Because of a 94% nucleotide homology in the coding regions between rat G_{\infty1} and G_{\infty3}, we used a 600-bp Xba I/EcoRI fragment of the 3' noncoding end of G_{\infty3} and a 625-bp EcoRV/EcoRI fragment of the 3' noncoding end of rat G_{\infty3} for hybridization analysis. Comparative probing with the full-length cDNA of both G_{\infty1} and G_{\infty3} revealed identical results. Because the rat G_{\infty3} probe did not hybridize with total RNA extracted from human heart, all hybridizations with human hearts were performed with an EcoRI/HindIII (550-bp coding region) restriction fragment of the human G_{\infty3} probe. Analysis with an Xba I/EcoRI fragment (1,000-bp 3' noncoding end) revealed identical results (not shown).

The cDNA probes were 32P labeled (nick translation kit, Amersham Buchler, Braunschweig, FRG) with [32P]dCTP (3,000 Ci/mmol, New England Nuclear-Dupont, Bad Homburg, FRG) to a specific activity of 3.2–8.0 × 10^6 dpm/μg. Unbound radioactivity was separated by gel filtration with Sephadex G-50 DNA grade (Pharmacia Fine Chemicals, Uppsala, Sweden).

Hybridization Procedure

Blot membranes were prehybridized at 42°C overnight in a solution containing 50% formamide, 5×
Denhardt (1 mg/ml Ficoll, polyvinylpyrrolidone, and bovine serum albumin), 0.9 M NaCl, 0.06 M NaH_{2}PO_{4}, 0.006 M EDTA, 0.1% SDS, and 200 μg/ml tRNA from yeast. Radiolabeled probes were added to fresh hybridization solution (prehybridization solution) in a concentration of 1–2×10^{6} dpm/ml. Hybridization was performed in 50 μl/cm^{2} at 42°C for 48 hours. After hybridization, blot membranes were washed briefly with 2× SSC and 0.1% SDS, followed by 10 minutes of incubation in 2× SSC and 0.1% SDS at room temperature and three 20-minute washes in 0.2× SSC and 0.1% SDS at 65°C. Wet blot membranes were sealed in plastic wrap and exposed to medium-sensitive medical x-ray film (R2, 3M, Italy) for 1–20 days at −80°C by using intensifier screens. For subsequent hybridizations with other radiolabeled cDNAs, the former probe was removed by pouring boiling 0.1% SDS over the blot membrane and allowing it to cool down to room temperature. Filters were exposed to highly sensitive x-ray film (X-OMAT AR, Kodak) for 48–96 hours to verify complete removal of the probe. It has been shown in preliminary experiments that this time of exposure was sufficient to control removal of the former probe.

Quantification of Specific mRNA

Hybridization intensity of autoradiographic signals on slot blots was measured quantitatively by two-dimensional densitometry (TLC II, CAMAG, Berlin, FRG). All determinations were done in triplicate. Total RNA (5 μg each) was applied for determination of G_{Sa} and G_{Sa} mRNA levels; 10 μg total RNA was used for G_{Sa} mRNA since lower signal intensities were expected. Slot blots were hybridized sequentially two or three times. Control hybridizations with the same probe had been performed before and revealed reproducibility of signal ratios on one blot up to the third or fourth hybridization.

The intensity of autoradiographic signals of identical RNA samples on different blots varied between films because of minor differences in amount and specific activity of the labeled cDNA probe and exposure time of the films. Therefore, blots were standardized by a total RNA standard (prepared in milligram quantities from failing human hearts). Autoradiographic density of hybridization signals was plotted versus the applied amount of total RNA standard (Figure 1). A curve-fitting by a second-order polynomial was performed (using SIGMAPLOT [Jandel Scientific, Corte Madera, Calif.]) to determine the relative amount of specific mRNA for all other RNA samples on this blot in arbitrary units (an x axis value of 1 [1 μg RNA standard] was defined as 1 arbitrary unit).

Nonspecific binding was determined by parallel probing of tRNA (Pharmacia) on each blot in triplicate. Nonspecific binding was seen only with G_{Sa} cDNA. Hybridization signals amounted to maximally 15% of sample values and were subtracted from all other signals on the blot.

To correct measurements for minor differences in the amount of total RNA applied on the blot membrane and for possible differences in the amount of intact poly(A) RNA in the RNA samples, all blots were reprobed with 32P-labeled oligo(dT)_{12-18} (Pharmacia). Oligo(dT)_{12-18} was radiolabeled with [32P]dTTP to a specific activity of 5×10^{6} dpm/μg by using terminal deoxyribonucleotidyl transferase (Pharmacia) according to Höppner et al.26 Hybridization was performed under the same conditions as with G_{Sa} cDNA except for the lack of formamide in the hybridization solution. Washing of the blots was performed at a final stringency of 0.2× SSC and 0.1% SDS at 42°C for 1 hour. Blots were exposed on medium-sensitive film for 5–16 hours, and autoradiographic signals were quantified as described for G_{Sa} mRNA measurements. There was no correlation between G_{Sa} mRNA or poly(A) RNA levels and patient age or sex or the duration of storage of the frozen tissue (not shown).

Statistics

Values presented for steady-state levels of specific mRNAs are arithmetic mean±SEM. Statistical significance was estimated using Student’s t test for unpaired observations. A value of p<0.05 was considered significant.

Results

Northern Blot Analysis

Northern blots of 20 μg total RNA extracted from human ventricular myocardium and, for comparison, from rat ventricular myocardium or rat brain are shown in Figure 2. In human and rat myocardium, a nick-translation 32P-labeled cDNA probe encoding rat G_{sa} hybridized to a predominant band at 2.4 kilobases (kb). The rat G_{Sa} cDNA hybridized to a 3.5 kb mRNA in total RNA extracted from rat ventricular myocardium and rat brain but did not detect any specific signal in total RNA from human myocardium (not shown). In contrast, hybridization with two different restriction fragments of a human G_{Sa} cDNA resulted in a predominant band at 2.8 kb and two faint bands at about 4.0 and 1.7 kb in human ventricular myocardium (Figure 2 shows hybridization with the HindIII/EcoRI fragment). There was...
only weak hybridization to a faint band at about 3.5 kb in rat heart total RNA. \(G_{i\alpha1}\) mRNA was not detectable in human or rat ventricular myocardium even after a long exposure time of 10 days (Figure 2). There was, however, hybridization of the \(G_{i\alpha1}\) cDNA to a single band at about 3.5 kb in total RNA from rat brain serving as a positive control (Figure 2). The \(G_{i\alpha1}\) cDNA detected one predominant band at 1.9 kb and a small but clearly distinguishable band at about 1.8 kb in human myocardium. Interestingly, hybridization of total RNA from rat myocardium demonstrated the smaller \(G_{i\alpha1}\) mRNA band at 1.8 kb to be the predominant message. In both human and rat tissues a further signal at about 4.5 kb could be seen after long exposure time (not distinguishable in Figure 2), which is in accordance with a detailed analysis of \(G_{i\alpha1}\) mRNA subtype expression in human and dog myocardium.\(^{25}\) There were no qualitative differences between failing and nonfailing myocardium (not shown). Moreover, probing of total RNA extracted from human failing and nonfailing atria revealed an expression pattern similar to that in ventricular myocardium (not shown).

**Slot Blot Quantification of Specific mRNA Levels**

Relative concentrations of \(G_{i\alpha2}\), \(G_{i\alpha3}\), and \(G_{i\alpha1}\) mRNAs in myocardium from failing and nonfailing hearts (Figures 3–5) were measured on slot blots. In failing hearts, the steady-state level of \(G_{i\alpha2}\) mRNA (Figure 3A) was increased by 75±26% in IDC (\(n=8, \text{4.95±0.65}\) arbitrary units) and increased by 90±26% in ICM (\(n=5, \text{5.31±0.72}\) arbitrary units) compared with nonfailing hearts (\(n=8, \text{2.79±0.25}\) arbitrary units). In contrast, steady-state levels of \(G_{i\alpha3}\) and \(G_{i\alpha1}\) mRNAs were not significantly different within the three groups studied (Figures 4A and 5A).

To correct for minor differences in the applied amount of intact mRNA per sample, individual values obtained from hybridization with \(G_{i\alpha2}\), \(G_{i\alpha3}\), and \(G_{i\alpha1}\) mRNA were referred to autoradiographic density values from hybridization with \(^{32}\)P-labeled oligo[d(T)]. These experiments revealed almost identical results.

**Discussion**

**\(G_{i\alpha}\) mRNA Expression Pattern**

The present study demonstrates expression of mRNA encoding \(\alpha\) subunits of the inhibitory \(G\) proteins \(G_{i\alpha2}\) and \(G_{i\alpha3}\) and of the stimulatory \(G\) protein \(G_{i\alpha1}\) in human ventricular myocardium. The results suggest that \(G_{i\alpha2}\) and \(G_{i\alpha3}\) are the predominant \(G_{i\alpha}\) mRNA subtypes in quantity, whereas \(G_{i\alpha1}\) mRNA was not detectable. \(G_{i\alpha1}\) cDNA detected one predominant band at 1.9 kb and a small but clearly distinguishable band at about 1.8 kb in the human heart. In contrast, hybridization with total RNA from rat heart revealed the smaller \(G_{i\alpha1}\) mRNA band at 1.85 kb to be the predominant message. The two bands in human myocardium probably reflect different splicing products of the unique \(G_{i\alpha}\) gene, four of which are known so far: \(G_{i\alpha1+2}\) corresponding to \(M\), 52,000

**Figure 2.** Northern analysis of 20 \(\mu\)g total cellular RNA from failing human left ventricular myocardium (HH) with idiopathic dilated cardiomyopathy, rat ventricular myocardium (RH), and rat brain (RB). Hybridization was performed with \(^{32}\)P-radiolabeled cDNA encoding rat \(G_{i\alpha1}\) (600-bp XbaI fragment), rat \(G_{i\alpha2}\) (full-length), human \(G_{i\alpha3}\) (550-bp HindIII fragment), and \(G_{i\alpha1}\) (1,100-bp EcoRI fragment) under high stringency conditions. Autoradiographic exposure time was 10 days (\(G_{i\alpha1}\)), 2 days (\(G_{i\alpha2}\)), 5 days (\(G_{i\alpha3}\)), and 1 day (\(G_{i\alpha1}\)). The positions of the 28S and 18S rRNA and of the RNA size ladder in kilobases (kb) are indicated.

**Figure 3.** Graph showing steady-state levels of \(G_{i\alpha2}\) mRNA in failing human hearts with idiopathic dilated cardiomyopathy (IDC) and ischemic cardiomyopathy (ICM) and in nonfailing hearts (NF). Panel A: \(G_{i\alpha2}\) mRNA level per total RNA. Panel B: \(G_{i\alpha2}\) mRNA level per amount of poly(A) RNA, which was determined by reprobing slot blots with \(^{32}\)P-labeled oligo[d(T)]. The ordinates give mRNA levels in arbitrary units and in percent of the \(G_{i\alpha2}\) mRNA level in nonfailing hearts. Arbitrary units were calculated by referring individual autoradiographic densities on slot blots to an RNA standard curve as depicted in Figure 1 and described in "Materials and Methods." Values are mean±SEM. The number of hearts is indicated in the columns.
protein and G\textsubscript{ia3-4}, corresponding to M, 45,000 protein.\textsuperscript{27} In both human and rat myocardium, a further signal at about 4.5 kb could be seen after long exposure time (not shown), which has previously been described in total RNA from human ventricles.\textsuperscript{27} The identity of the 4.5-kb message is currently not known.

The G\textsubscript{a} mRNA expression pattern in human and rat ventricular myocardium (as well as the expression pattern in human atria [not shown]) and the base length of the different mRNAs reported here are consistent with reports of rat, hamster, and canine myocardium.\textsuperscript{20,24,26,29} In these animal species, G\textsubscript{ia2} has been reported to be the predominant G\textsubscript{ia} subtype in the heart, and G\textsubscript{ia3} has been consistently found at low expression levels. The lack of G\textsubscript{ia3} is consistent with the reported mRNA expression pattern in adult human\textsuperscript{30} and rat\textsuperscript{20,24} myocardium.

There are, however, discrepancies concerning the expression level and the message size of G\textsubscript{ia3} mRNA in the human heart. Feldman et al\textsuperscript{30} reported G\textsubscript{ia3} mRNA to be the predominant message of G\textsubscript{a} proteins in human left ventricles. In this study, a rat G\textsubscript{ia3} cDNA was used for Northern analysis. The same probe did not result in any hybridization with human total RNA in our experiments, irrespective of the cDNA fragment or the tissue (ventricles, atria, liver, kidney) studied (not shown). Without giving exact information on the size of the cDNA (fragment) used, the authors described an unusual, short G\textsubscript{ia3} mRNA at 1.85 kb. In other studies, a larger G\textsubscript{ia3} mRNA has been reported. The group that cloned the human G\textsubscript{ia3} cDNA and screened different human tissues described low levels of G\textsubscript{ia3} mRNA in fetal atrium and determined a message size of 2.8 kb.\textsuperscript{25} In rat, hamster, and canine heart, G\textsubscript{ia3} mRNA was consistently reported to be 3.5 kb in base length.\textsuperscript{24,26,29} In accordance with these studies, we determined a predominant G\textsubscript{ia3} message size of 2.8 kb in human myocardium and one of about 3.5 kb in rat myocardium. The nature of the two additional faint bands in human myocardium at about 4.0 and 1.7 kb is not known but might result from cross-hybridization to other members of the family of highly conserved G protein and subunits. Because of the use of rat cDNA for hybridization analysis of G\textsubscript{ia2} and human cDNA in the case of G\textsubscript{ia3}, the intensity of hybridization signals cannot be compared directly with each other. Thus the present study does not allow a clear statement of whether G\textsubscript{ia2} or G\textsubscript{ia3} mRNA is the major G\textsubscript{a} subtype in human myocardium. However, approximately similar signal intensities of
Gm and Gm hybridization were seen in spite of the nonhomologous hybridization and a shorter exposure time for Gm (Figure 2). Therefore, quantitative predominance of Gm mRNA may be suggested in human myocardium. This would be in agreement with antibody studies of bovine and human myocardium, which described at the protein level the majority of Gm protein to be of the Gm subtype.

**Gm mRNA Levels: Failing Versus Nonfailing Human Hearts**

The main result of the present study is that steady-state levels of Gm mRNA were increased in failing human hearts compared with nonfailing hearts. This suggests that the increase in Gm protein in failing heart membranes might be due to increased Gm mRNA levels and thereby due to enhanced de novo synthesis. Gm mRNA levels were similarly increased in different causes of heart failure as IDC and ICM. This suggests that alterations in the expression of Gm protein might be a secondary phenomenon, independent from the pathogenesis of heart failure.

Our finding that Gm mRNA levels are increased both in IDC and ICM was surprising, since results obtained from pertussis toxin labeling of failing human heart membranes revealed an increased Gm mRNA level in IDC and an unchanged Gm protein level in ICM compared with nonfailing controls. This discrepancy cannot readily be explained. However, it has been reported in invertebrates (sea urchin) that mRNA can be stored in an inactive form temporarily, indicating that mRNA levels do not necessarily directly correlate to translational activity. In addition, there is some evidence for a physiological role of endogenous ADP-ribosylation and/or phosphorylation of Gs subunits. Tanuma et al purified an endogenous ADP-ribosyltransferase C, which ADP-ribosylates a cysteine residue in Gs as does pertussis toxin. Watanabe et al reported that phosphorylation of Gs by the cAMP-dependent protein kinase A resulted in a decrease in the ADP-ribosylation by pertussis toxin. These results indicate that the accessibility of Gs for pertussis toxin can be influenced by posttranslational modifications and hence pertussis toxin--catalyzed ADP-ribosylation does not necessarily reflect the true protein level of Gs. This is probably the main reason for the discrepancies between mRNA and pertussis toxin measurements since recent preliminary results, obtained by using antibodies for quantification of Gs proteins, showed increased Gm in both IDC and ICM.

What are the possible mechanisms for upregulation of Gm mRNA levels? One of the common features in end-stage heart failure is an elevated plasma catecholamine level. It is noteworthy in this context that a consensus sequence of a "cAMP response element" has been described in the promoter region of the Gm gene and is lacking in the Gm gene. Thus, it seems reasonable to assume that in severe heart failure an increased adrenergic drive via the cAMP cascade might cause an increased transcription rate of the Gm gene and hence increased Gm mRNA levels. Such a mechanism would also explain the lack of an increase in Gm mRNA demonstrated in the present study. Support for this hypothesis comes from a recent study in which chronic β-adrenergic stimulation under in vivo conditions caused a selective increase in myocardial Gm and Gm mRNA and unchanged Gm mRNA steady-state levels in rats.

In light of these results, it was surprising that in the present study Gm mRNA levels were not increased in parallel with Gm. Furthermore, this finding is in apparent contrast to the study of Feldman et al who reported an increase of Gm mRNA in failing human hearts. In addition to the methodological problems discussed above, it is interesting that in the study of Feldman et al all Gm mRNA levels investigated were significantly increased by 60–90% (Gm and Gm) or tended to be higher (Gm, 36%). In this study normalization of dot blots was performed by reprobing the blots with a β-actin probe, although it is not known if alterations in the expression of β-actin occur in human heart failure. Indeed, regulation of β-actin mRNA levels is suggested by isofrom shifts in the actin and myosin filament family in experimentally induced cardiac hypertrophy and/or failure and by developmental decreases in the expression of cardiac β-actin.

Thus, discrepancies between studies may result from the general problem of unavailability of appropriate standards for quantification. At present, this problem is not resolved satisfactorily. We used standardization of blots with oligo(d(T)) and yielded nearly identical results as by referring individual mRNA values to the amount of total RNA. Our finding of an unchanged Gm mRNA suggests that Gm is either not regulated at all or that mechanisms other than cAMP-mediated increases in gene transcription are involved in the regulation of Gm subunits in human heart. Distinct regulation of Gm and Gm mRNA levels like those shown here suggests different functions of the two Gm subunits in the pathogenesis of heart failure. It would be important in this context to investigate with subtype-specific antibodies the proportion of Gm and Gm protein in the upregulation of Gm in heart failure. This needs further elucidation.

It might be argued that the results of the present study were compromised by an inhomogenous control group containing patients with a history of hypertension and patients who received drugs at time of transplantation (Table 2). In addition, the influence of cardiovascular drugs such as cardiac glycosides and angiotensin converting enzyme inhibitors on Gm mRNA levels in the IDC and ICM groups cannot be excluded. This is certainly true. However, as seen in Table 2 there was no correlation between any anamnestic data and Gm mRNA levels in the control group. Furthermore, neither now nor in the future will biochemical studies be possible on hearts from untreated patients. Moreover, because of an efficient transplantation program in Europe, the availability of nonfailing control hearts will always be extremely limited for research without any objections against its use as a donor organ.

In conclusion, the present study demonstrates a significant increase of mRNA levels of Gm in human end-stage heart failure caused by IDC and ICM without changes in Gm and Gm mRNA levels. The increase in Gm mRNA very likely underlies the increase in Gm protein in end-stage heart failure, which at least in part explains deficient cAMP production by the adenylate cyclase and the diminished positive inotropic effect of
β-adrenoceptor agonists and phosphodiesterase inhibitors. Because the G_{m2} gene is under cAMP-dependent control, it is suggested that the increased adrenergic drive might increase G_{m2} gene expression via cAMP. Upregulation of G_{m2} might thus play an important pathophysiological role as a basic mechanism of adaptation of the myocardial cell to increased adrenergic stimulation in severe heart failure.

Acknowledgments

We are grateful to Dr. R.R. Reed (Baltimore, Md.) and Dr. E.J. Neer (Boston, Mass.) for providing the rat and human G_{m} cDNAs, respectively, and to Dr. H.H. Arnold (Hamburg, FRG) for his methodical advice. We also thank Ms. A. Düssel and Ms. A. Harneit for their generous and excellent help in various technical aspects of the work.

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Circ Res. 1992;70:688-696
doi: 10.1161/01.RES.70.4.688

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