Enhanced G\textsubscript{i} Signaling Selectively Negates \(\beta_2\)-Adrenergic Receptor (AR)– but Not \(\beta_1\)-AR–Mediated Positive Inotropic Effect in Myocytes From Failing Rat Hearts

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**Background**—Myocardial contractile response to \(\beta_1\)- and \(\beta_2\)-adrenergic receptor (AR) stimulation is severely impaired in chronic heart failure, in which G\textsubscript{i} signaling and the ratio of \(\beta_2/\beta_1\) are often increased. Because \(\beta_2\)-AR but not \(\beta_1\)-AR couples to G\textsubscript{i} and G\textsubscript{s} with the G\textsubscript{i} coupling negating the G\textsubscript{s}-mediated contractile response, we determined whether the heart failure–associated augmentation of G\textsubscript{i} signaling contributes differentially to the defects of these \(\beta\)-AR subtypes and, if so, whether inhibition of G\textsubscript{i} or selective activation of \(\beta_2\)-AR/G\textsubscript{i} by ligands restores \(\beta_2\)-AR contractile response in the failing heart.

**Methods and Results**—Cardiomyocytes were isolated from 18- to 24-month-old failing spontaneously hypertensive (SHR) or age-matched Wistar-Kyoto (WKY) rat hearts. In SHR cardiomyocytes, either \(\beta_2\)-AR subtype–mediated inotropic effect was markedly diminished, whereas G\textsubscript{i} proteins and the \(\beta_2/\beta_1\) ratio were increased. Disruption of G\textsubscript{i} signaling by pertussis toxin (PTX) enabled \(\beta_2\)- but not \(\beta_1\)-AR to induce a full positive inotropic response in SHR myocytes. Furthermore, screening of a panel of \(\beta_2\)-AR ligands revealed that the contractile response mediated by most \(\beta_2\)-AR agonists, including zinterol, salbutamol, and procaterol, was potentiated by PTX, indicating concurrent G\textsubscript{i} and G\textsubscript{s} activation. In contrast, fenoterol, another \(\beta_2\)-AR agonist, induced a full positive inotropic effect in SHR myocytes even in the absence of PTX.

**Conclusions**—We conclude that enhanced G\textsubscript{i} signaling is selectively involved in the dysfunction of \(\beta_2\)- but not \(\beta_1\)-AR in failing SHR hearts and that disruption of G\textsubscript{i} signaling by PTX or selective activation of \(\beta_2\)-AR/G\textsubscript{i} signaling by fenoterol restores the blunted \(\beta_2\)-AR contractile response in the failing heart. (Circulation. 2003;108:1633-1639.)

**Key Words:** receptors, adrenergic, beta | heart failure | proteins | contractility
stimulation and, if so, to determine whether β2-AR/G, selective activation by certain agonists is able to restore the contractile support by β-AR stimulation in the failing heart. Specifically, we (1) determined the abundance and function of both β-AR subtypes (β-AR and β2-AR) and Ga proteins (Gα and Gβ) in cardiomyocytes from spontaneously hypertensive rats (SHR) and age-matched Wistar-Kyoto rats (WKY), (2) investigated the potential role of Gα signaling in the defects of β-AR subtype-mediated contractile response in failing SHR cardiomyocytes, (3) and screened a number of β2-AR ligands to identify “β2-AR/Gα-signaling–selective” agonists and tested the agonists in cardiomyocytes from failing SHR hearts.

Methods

Echocardiography and Hemodynamic Studies

Transsthoracic echocardiographic studies were performed in rats as previously described. Briefly, a 2D short-axis view of the left ventricle (LV) was obtained at the level of the papillary muscles. M-mode tracings were recorded through the anterior and posterior LV walls. The percentage shortening of the endocardium was calculated as (LVDD–LVSD)/LVDD×100, where LVDD is LV internal diastolic dimension and LVSD is LV internal systolic dimension. The heart rates, blood pressures, and respiratory rates of all animals were monitored at monthly intervals. Blood pressure was measured by the tail-cuff method.

Measurement of Cardiac Myocyte Contraction

Myocytes were isolated from 18- to 24-month-old SHR or WKY rats by use of standard enzymatic techniques. Cells were perfused with a HEPES buffer containing (in mmol/L): 137 NaCl, 5.4 KCl, 1.2 MgCl2, 1 NaH2PO4, 1 CaCl2, 20 glucose and 20 HEPES (pH 7.4) and electrically stimulated at 0.5 Hz at 23 °C. Cell length was monitored by an optical edge-tracking method as previously described. In a subset of experiments, aliquots of cells were incubated with PTX (1.5 μg/mL at 37 °C for 3 hours). An individual myocyte was exposed to only 1 dose of 1 agonist, and measurements were obtained under steady-state conditions after a 10-minute exposure to the designated agonist.

Receptor Radioligand Binding

β-AR radioligand binding studies were performed in membranes from LVs by use of the nonselective β-AR antagonist [125I]-labeled cyanopindolol ([125I]-CYP), as described previously. Maximal binding (Bmax) was measured by use of a saturating amount of [125I]-CYP on 25 μg of membrane protein from LVs. Inclusion of 10 μmol/L propranolol defined nonspecific binding. For competition isotherms, membranes (25 μg total protein) were incubated with 50 pmol/L [125I]-CYP and increasing dilutions of ICI118,551 (ICI), a selective β2-AR antagonist, as described previously. The percentage of β2-AR was calculated by use of GraphPad Prism.

TABLE 1. Hemodynamic and Echocardiographic Parameters, Heart Weight/Body Weight Ratios, and Resting Cell Length of 18- to 24-Month-Old WKYs and SHR

<table>
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<th>Hemodynamic Parameters</th>
<th>Echocardiographic Parameters</th>
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<td><strong>Rats, n</strong></td>
<td><strong>Hemodynamic Parameters</strong></td>
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<tr>
<td><strong>Heart Rate, beats/min</strong></td>
<td><strong>Systolic BP, mm Hg</strong></td>
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<td>WKY (18–24 mo)</td>
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<tr>
<td>SHR (18–24 mo)</td>
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Data are mean±SD. *P<0.05 vs age- and sex-matched controls.

Western Analysis of Gα and Gβ Proteins

Cardiac membranes from myocytes were used to determine Gα and Gβ protein amounts by Western analysis. The antibodies recognizing the αi2, Gαi2, and Gβi2 were obtained from Santa Cruz Biotechnology.

Statistical Analysis

Results are expressed as mean±SEM. Significance was determined by use of Student’s t test, with a value of P<0.05 considered to be statistically significant.

Results

Chronic Heart Failure Model: SHR

SHRs develop hypertension at ≈6 to 8 weeks of age. By the age of 18 to 24 months, ≈50% of these rats develop severe dilated chronic heart failure, many aspects of which have been characterized. At the time of study (ie, 18 to 24 months old), all SHRs used in this study had severe physical signs of heart failure (eg, resting tachycardia, tachypnea, pleural and/or pericardial effusions, and ascites). Cardiac dilation in the SHR group was evidenced by echocardiographic measurements (Table 1). Cardiac hypertrophy was confirmed by significant increases in the heart weight/body weight ratio, by echocardiographic measurements of LV posterior wall thickness, and by increased resting cell length (Table 1).

Alterations in β-AR Density and G Protein Abundance in Failing SHR Hearts

We first examined total β-AR density, β1- and β2-AR subpopulations, and the abundance of Gα and Gβ proteins in myocytes from 18- to 24-month-old SHR and age-matched WKY cardiac myocytes. There was a significant decrease (24%) in the Bmax in SHR relative to WKY hearts, without alterations in the binding affinity (Table 2). To define the relative abundance of β1-AR and β2-AR, competition isotherms were performed by use of the selective β2-AR antagonist ICI. The β2/β1-AR ratio in control animals is 44/56, which is similar to the ratio previously reported. The β2/β1-AR ratio was significantly increased in SHR relative to WKY hearts (Table 2). This result indicates that the reduction in total β-AR density is largely accounted for by the loss of β1-AR. Thus, a selective downregulation of β1-AR leads to a relative increase in the β2-AR density in the failing SHR heart, a situation similar to what has been reported in failing human heart.

In addition, both Gαi2 and Gβi2 but not Gαi3 were elevated by 2-fold in SHR compared with WKY hearts (Figure 1),
proximal failure with respect to the major changes that occur in the SHR heart failure model resembles human heart failure with respect to the major changes that occur in the proximal β-AR signaling system.

Disruption of Gi, Signaling by PTX Selectively Restores β2-AR– but Not β1-AR–Mediated Positive Inotropic Effect in Cardiomyocytes From Failing SHR Hearts

Although the baseline contraction amplitude in myocytes from failing SHR hearts was not significantly different from that of control animals (see Figure 2 legend), the positive inotropic effect of either β-AR subtype stimulation was markedly attenuated. Figure 2A shows that myocyte contractile response to β1-AR stimulation by norepinephrine (NE) in the presence of α1-AR and β2-AR blockade by prazosin (10⁻⁶ mol/L) and ICI (10⁻⁷ mol/L), respectively, was markedly blunted in SHR myocytes compared with that in WKY cells, consistent with the downregulation of the receptor density. Figure 2B illustrates that β2-AR stimulation by zinterol had only a minor positive inotropic effect in SHR ventricular myocytes, whereas it enhanced the contraction amplitude by >2-fold in control cardiomyocytes from age-matched WKY's. The dose-response curve of zinterol to increase contraction amplitude was severely shifted downward in SHR compared with WKY. Thus, myocyte contractile response to either β1-AR or β2-AR was overtly attenuated in SHR cardiomyocytes relative to WKY myocytes, even though only β2-AR density was selectively downregulated, with little change in β1-AR density in SHR hearts (Table 2).

To test the hypothesis that exaggerated Gi signaling might be differentially involved in the impairment of β2-AR versus that of β1-AR in the failing heart, we treated myocytes with PTX to disrupt Gi function. Remarkably, PTX not only enhanced the contractile response to β2-AR stimulation in both failing SHR and control myocytes but also abolished the difference between the 2 groups (Figure 2B). In contrast, PTX did not affect the whole dose-response of β2-AR stimulation by NE in either WKY or SHR myocytes (Figure 2A). These results indicate that enhanced Gi signaling is specifically involved in the defect of β2-AR contractile response but not in the dysfunction of β1-AR signaling in the SHR heart failure model. These results also suggest that β2-AR–coupled Gi signaling remains largely intact in the failing heart.

Screening for Ligands Bypassing β2-AR/Gi Pathway

To determine whether disruption or avoidance of the β2-AR–coupled Gi signaling pathway is able to restore β2-AR–mediated contractile support in the failing SHR heart, we searched for ligands that activate β2-AR–coupled G, signaling but not the G, pathway. Six known β2-AR–selective agonists and 2 nonselective β-AR agonists were tested in the presence of the β2-AR antagonist CGP20712A (CGP; 3×10⁻⁷ mol/L). Zinterol had been well characterized in previous studies as a selective β2-AR agonist.6–9,10–13 The specificity of other tested agonists, including salbutamol, proterol, and fenoterol, for β2-AR stimulation was verified by the fact that the highly selective β2-AR antagonist CGP (3×10⁻⁷ mol/L) did not block their contractile responses, whereas it fully abolished the effect induced by the β2-AR agonist NE (10⁻⁷ mol/L) (Figure 3). Cell contractile response to β-AR stimulation and its PTX sensitivity were then measured to represent the overall readout of G, (in PTX-treated cells) or combined G,-G, signaling (in non–PTX-treated cells).

Most ligands tested, including salbutamol, proterol, and zinterol, enhanced the contraction amplitude of WKY myocytes in a dose-dependent manner, with a maximal increase of

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**Figure 1.** Gi and Gs protein abundance in SHR and WKY cardiac myocytes. A, Representative Western blots probed with antibodies reacting with α-subunits of Gs, G13, or G12, as indicated. B, Average data of Gs, G13, and G12 levels in SHR and WKY cardiomyocyte membranes (P<0.01 vs WKY, n=6 experiments in cells from 12 hearts).
PTX was observed for partial blockade; B, dose-response of CGP (0.14% (n = 28 cells from 6 to 10 cells) of resting cell length for SHR and WKY cells, respectively; n = 5 to 6 cells from 6 to 10 hearts). Data are expressed as % of control (n = 0.33% (n = 126 cells from 10 hearts). PTX does not alter basal contraction (4.88 ± 0.14% and 5.52 ± 0.34% of resting cell length for SHR and WKY cells, respectively; n = 28 cells from 6 hearts for each group).

Figure 2. PTX selectively restores β2- but not β1-AR-mediated positive inotropic effect in SHR cardiomyocytes. A, Dose-response of β2-AR stimulation by NE (plus α1-AR and β2-AR blockade); B, dose-response of β2-AR stimulation by zinterol to increase myocyte contraction in presence or absence of PTX. Baseline contraction amplitudes are 5.60 ± 0.23% (n = 126 cells from 10 hearts) and 4.93 ± 0.33% (n = 76 cells from at least 10 hearts) of resting cell length for WKY and SHR, respectively. Data are expressed as % of control (n = 6 to 10 cells from 6 to 10 hearts). PTX does not alter basal contraction (4.88 ± 0.14% and 5.52 ± 0.34% of resting cell length for SHR and WKY cells, respectively; n = 28 cells from 6 hearts for each group).

Figure 3. Specificity of β2-AR agonists fenoterol (FEN), propranolol (PROC), and salbutamol (SALB). β2-AR selective antagonist CGP (3 × 10⁻⁷ mol/L) blocks contractile response induced by NE (10⁻⁷ mol/L) plus α1-AR and β2-AR blockade but not equipotent positive inotropic effects induced by fenoterol, propranolol, or salbutamol (n = 6 to 8 cells from 6 hearts for each group, *P < 0.001 vs NE (–CGP)).

Discussion

Enhanced G1 Signaling Selectively Contributes to the Diminution of β2-AR– but Not β1-AR–Mediated Contractile Response in Myocytes From Failing SHR Hearts

Although previous studies have demonstrated that PTX treatment partially restores the diminished contractile response to mixed β-AR stimulation in a rat myocardial infarction heart failure model and in myocytes from failing human hearts, the present study provides the first documentation that enhanced G1 signaling is, in fact, selectively involved in heart failure–associated attenuation in β2-AR– but not β1-AR–mediated increase in myocyte contractility. This is because inhibition of β2-AR/G1 signaling by PTX treatment enables β2-AR stimulation to induce a full contractile response, thus abolishing the difference between the failing and normal hearts, whereas it does affect β1-AR–induced inotropic effect. These findings are consistent with our previous notion that β1-AR but not β2-AR dually couples to G1 and G6, and that the G6 coupling exhibits an inhibitory effect on the G1 signaling.

Although the heart failure–associated defect of β2-AR contractile response is largely attributable to enhanced β2-AR/G1 signaling, multiple mechanisms might be involved in β2-AR dysfunction, including receptor downregulation (Table 2) and receptor desensitization resulting from upregulated G protein–coupled receptor kinases. In this regard, it has been shown that the level and enzymatic activity of β2-AR kinase 1 (βARK1), a prototypic G protein–coupled receptor kinase, are significantly elevated in human and SHR.
failing hearts. The increased βARK1 abundance/activity may contribute to the reduced β2-AR signaling in chronically failing hearts. Further studies are needed to determine the relative contributions of enhanced G\textsubscript{i} function and βARK1 to subtype-specific defects of β-AR in the context of heart failure.

**Selective Downregulation of β1-AR May Serve as a Protective Mechanism in Heart Failure**

It has been highly controversial as to whether enhancing β-AR signaling is beneficial or deleterious for the failing heart. The prevalent view is that chronically increasing nonselective β-AR stimulation is toxic to the heart, because there is an inverse relationship between the plasma level of catecholamines and survival in heart failure patients and because β-AR blockade reduces both the morbidity and mortality in heart failure patients. More recent studies have demonstrated that β1-AR induces apoptosis in cultured rodent cardiomyocytes. Similarly, cardiac transgenic overexpression of β1-AR in mice leads to marked myocyte hypertrophy, apoptosis, and heart failure. Thus, chronic β2-AR stimulation is detrimental to the myocardium.

In contrast, selective enhancement of β2-AR signaling might be beneficial for the failing heart. This hypothesis is supported by the fact that β2-AR stimulation protects cardiac myocytes against a wide array of apoptotic insults. In addition, overexpression of cardiac β2-AR by 100- to 200-fold does not induce hypertrophy or heart failure at least up to the age of 1 year. In fact, crossing transgenic mice overexpressing cardiac β2-AR at appropriate levels (30-fold) with transgenic mice overexpressing G\textsubscript{q} not only improves cardiac performance but also prevents hypertrophy in the G\textsubscript{q} overexpression heart failure model, although extremely high levels of β2-AR overexpression induce pathological phenotypes and fail to rescue the genetic mouse heart failure model. Furthermore, the beneficial effect of β2-AR stimulation in the context of heart failure is clearly supported by the analysis of polymorphisms of β2-AR in chronic heart failure patients. The prognosis of heart failure patients with Ile164 polymorphism (a Thr-to-Ile switch at amino acid 164)

![Image of graphs showing contractile response to various agonists](attachment:image.png)

**Figure 4.** Effects of PTX treatment on contractile responses to β2-AR agonists. Dose-response of contraction amplitude in myocytes subjected to salbutamol (A), procaterol (B), zinterol (C), and fenoterol (D) in presence and absence of PTX. Data are expressed as % of control (% C; n=6 to 8 cells from 6 hearts for each data point). Baseline contractions are 5.29±0.13% (n=146 cells from 24 hearts) and 5.57±0.13% (n=166 cells from 24 hearts) of resting cell length for PTX-treated and non-treated group, respectively.

**Figure 5.** Fenoterol restores contractile response of failing SHR myocytes to β2-AR stimulation. A, Examples of cell contractile response to zinterol (ZINT, 10^{-5} mol/L) and fenoterol (FEN, 10^{-6} mol/L) in representative SHR myocytes. B, Dose-response of fenoterol in myocytes from failing SHR and non-failing WKY hearts. See legend of Fig. 2 for baseline contraction. Data are presented as % of control (% C; n=10 to 18 cells from 5 hearts for each data point).
with reduced β2-AR signaling) relative to patients without the β2-AR variant is much worse, and they are significantly more likely to receive earlier aggressive interventions or cardiac transplantation.

In the present study, we found that in failing SHR hearts, β1-AR is selectively downregulated with little or no loss of β2-AR, similar to the situation of chronic human heart failure. In light of evidence for the opposing effects of β1-AR and β2-AR stimulation on cardiac myocyte apoptosis and the distinct phenotypes of transgenic overexpression of β1-AR versus β2-AR, the selective downregulation of β2-AR may represent a cardiac protective mechanism to slow the progression of cardiomyopathy and contractile dysfunction.

Selective Activation of β2-AR/Gi Signaling in Failing SHR Hearts

Because heart failure in many instances is related to a selective loss of β1-AR and because chronic stimulation of β2-AR exhibits no known detrimental effects, as discussed above, β2-AR would appear to be a potentially important therapeutic target for the treatment of chronic heart failure. However, most of the commonly used nonselective β-AR agonists (eg, isoproterenol) cannot discriminate β2-AR from β1-AR. In addition, most β2-AR agonists cannot discriminate β2-AR-coupled Gs signaling from Gi signaling. The situation is even worse in chronically failing hearts with elevated Gi signaling, such that β2-AR stimulation by zinterol elicits only a minor contractile response (Figure 2). To unmask the β2-AR/Gi signaling from the inhibition by Gi, we identified fenoterol as an apparent β2-AR/Gi-selective agonist, as evidenced by the lack of PTX sensitivity of its positive inotropic effect (Figure 3). In cardiomyocytes from failing SHR hearts, fenoterol elicits a contractile response that is comparable to that in cells from nonfailing WKY heart cells (Figure 4). It is also noteworthy that there may be some advantages to use of fenoterol instead of β1-AR agonists to provide contractile support in the failing heart. Our preliminary experiments have shown that, unlike β1-AR stimulation, β2-AR stimulation by fenoterol does not induce cardiac myocyte apoptosis (Zhu et al, unpublished data), although it increases cAMP/PKA signaling, perhaps because of distinct compartmentation of cAMP signal in response to different β-AR subtype activation.

Regarding receptor theory, the Gi-specific β2-AR stimulation, as demonstrated here for native β2-AR in intact cardiomyocytes, provides new evidence to support the idea that there are multiple active conformational states for a given receptor and that ligands may lead the receptor to a subset of downstream signaling pathways (ligand-directed receptor trafficking). In contrast to fenoterol, a well-characterized β2-AR inverse agonist, ICI, can direct β2-AR to a Gi-coupled form and result in a negative inotropic effect in cardiomyocytes from failing human hearts or rabbit and mouse myocytes in which β2-AR and/or Gi proteins are overexpressed.

In summary, the present study reveals 3 major findings. First, enhanced Gi signaling is selectively involved in the dysfunction of β2-AR but not of β1-AR. Inhibition of Gi signaling with PTX enables β2-AR stimulation to induce a full contractile response in myocytes from failing SHR hearts without affecting β1-AR contractile response. Second, β2-AR-coupled Gi and Gi pathways appear to be activated in an agonist-dependent manner. Most agonists tested stimulate both pathways, whereas fenoterol predominantly activates Gi signaling, as manifested by the lack of PTX sensitivity of its positive inotropic effect. Third, the apparent β2-AR/Gi-selective agonist fenoterol fully restores β2-AR contractile response in failing SHR hearts. Therefore, we conclude that enhanced Gi signaling is specifically involved in the dysfunction of β2-AR but not of β1-AR in regulating myocyte contractility in the failing heart and that an apparent selective activation of β2-AR-coupled Gi, bypassing the Gi pathway, provides a novel means to restore the diminished β2-AR responsiveness in the SHR heart failure model. Thus, signaling-selective receptor stimulation by agonists may offer a wide range of novel therapeutic opportunities by enhancing the desired effects while avoiding the adverse side effects of therapeutic agents.

Acknowledgments

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References


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