Enhanced $G_i$ 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 $G_i$ signaling and the ratio of $\beta_2/\beta_1$ are often increased. Because $\beta_2$-AR but not $\beta_1$-AR couples to $G_i$ and $G_s$ with the $G_i$ coupling negating the $G_s$-mediated contractile response, we determined whether the heart failure–associated augmentation of $G_i$ signaling contributes differentially to the defects of these $\beta$-AR subtypes and, if so, whether inhibition of $G_i$ or selective activation of $\beta_2$-AR 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 $\beta$-AR subtype–mediated inotropic effect was markedly diminished, whereas $G_i$ proteins and the $\beta_2/\beta_1$ ratio were increased. Disruption of $G_i$ 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 $G_i$ and $G_s$ 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 $G_i$ signaling is selectively involved in the dysfunction of $\beta_2$- but not $\beta_1$-AR in failing SHR hearts and that disruption of $G_i$ signaling by PTX or selective activation of $\beta_2$-AR/G$_{i/0}$, by ligands restores $\beta_2$-AR contractile response in the failing heart. (Circulation. 2003;108:1633-1639.)

**Key Words:** receptors, adrenergic, beta ■ heart failure ■ proteins ■ contractility

Stimulation of $\beta$-adrenergic receptors ($\beta$-ARs) by catecholamines plays a pivotal role in regulating cardiac function. At least 2 $\beta$-AR subtypes, $\beta_1$-AR and $\beta_2$-AR, are expressed in cardiac myocytes. Chronic heart failure in animal models and in humans is characterized by a severely diminished contractile response to both $\beta$-AR subtypes. The heart failure–associated defect of $\beta$-AR contractile response is accompanied by a selective downregulation of $\beta_1$-AR (resulting in a higher $\beta_2/\beta_1$ ratio) and an increase in the amount or activity of pertussis toxin (PTX)–sensitive inhibitory $G$ proteins ($G_i$) without any abnormality in stimulatory $G$ proteins ($G_s$).

Although $\beta_1$-AR and $\beta_2$-AR are closely related $G$ protein–coupled receptors, these $\beta$-AR subtypes couple to different $G$ proteins and exhibit different effects on phosphorylation of protein kinase A (PKA) target proteins, cardiac excitation-contraction coupling, and cardiac cell survival and cell death. Most importantly, $\beta_1$-AR stimulation activates the $G_i$-cAMP-PKA signaling cascade, whereas the $\beta_2$-AR couples to PTX-sensitive $G_s$ proteins, $G_\alpha$ and $G_\beta\gamma$, in addition to $G_i$. The coupling of $\beta_2$-AR to $G_i$ protein functionally confines the $\beta_2$-AR–$G_i$–stimulated cAMP/PKA signaling to a subsarcolemmal microdomain via the $G_\gamma$-$\gamma$-phosphatidylinositol 3-kinase pathway and negates the $G_i$-mediated protein phosphorylation and contractile effect in ventricular myocytes of many mammalian species (for review, see Reference 22).

The overall goal of the present study was to determine whether $G_i$ signaling is selectively involved in the heart failure–associated defective contractile response to $\beta_2$-AR.
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 (β1-AR and β2-AR) and G proteins (G1 and Gq) in cardiomyocytes from spontaneously hypertensive rats (SHR) and age-matched Wistar-Kyoto rats (WKY), (2) investigated the potential role of Gq, 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/Gq-signalizing–selective” agonists and tested the agonists in cardiomyocytes from failing SHR hearts.

Methods

**Echocardiography and Hemodynamic Studies**

Transthoracic 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 LVDS 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.

**Western Analysis of G, and Gq Proteins**

Cardiac membranes from myocytes were used to determine G and Gq protein amounts by Western analysis. The antibodies recognizing the α-subunits of Gq, Gq, and Gq 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 Gq and Gq 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 Gq and Gq, but not Gq, were elevated by ∼2-fold in SHR compared with WKY hearts (Figure 1),

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

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Data are mean±SD. *P<0.05 vs age- and sex-matched controls.
resulting in an increase in the Gs/Gi ratio, as is the case in human heart failure.5 Taken together, these results suggest that 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 G i 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 β2-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 WKYs. 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 β2-AR density in SHR hearts (Table 2).

To test the hypothesis that exaggerated G i 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 G i 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 β1-AR stimulation by NE in either WKY or SHR myocytes (Figure 2A). These results indicate that enhanced G i 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 G i signaling remains largely intact in the failing heart.

Screening for Ligands Bypassing β2-AR/G i Pathway

To determine whether disruption or avoidance of the β2-AR–coupled G i 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 i signaling but not the G s pathway. Six known β2-AR-selective agonists and 2 nonselective β-AR agonists were tested in the presence of the β1-AR antagonist CYP20712A (5 × 10^-7 mol/L). Zinterol had been well characterized in previous studies as a selective β2-AR agonist.6–9,10–15 The specificity of other tested agonists, including salbutamol, procaterol, and fenoterol, for β2-AR stimulation was verified by the fact that the highly selective β2-AR antagonist CYP (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 β2-AR stimulation and its PTX sensitivity were then measured to represent the overall readout of G i, Gi signaling (in non–PTX-treated cells) or combined Gs/Gi signaling (in PTX-treated cells).

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

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<th>Table 2. β-AR Radioligand Binding Properties and Competition of [125I]ICYP Binding With β-AR Antagonist ICI in LV Membranes From 18- to 24-Month-Old SHRs and Age- and Sex-Matched WKYS</th>
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*P<0.05, †P<0.01 vs WKY.
PTX selectively restores $\beta_2$-AR but not $\beta_1$-AR-mediated positive inotropic effect in SHR cardiomyocytes. A, Dose-response of $\beta_2$-AR stimulation by NE (plus $\alpha_1$-AR and $\beta_2$-AR blockade); B, dose-response of $\beta_2$-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).

$\sim$2-fold (Figure 4A through 4C). Inhibition of G$i$ function by PTX enabled the $\beta_2$-AR agonists to induce a full contractile response, with the maximal response being a 3-fold increase (Figure 4A through 4C). In other words, with respect to their inotropic effects, inhibition of G$i$, signaling converted these partial $\beta_2$-AR agonists to full agonists. These results are consistent with previous findings that $\beta_2$-AR can simultaneously activate G$s$ and G$i$.6–9 A similar potentiating effect of PTX was observed for $\beta_2$-AR stimulation induced by other known $\beta_2$-AR agonists such as terbutaline and celenbuterol, or by nonselective agonists, epinephrine and isoprotanol, plus the $\beta_1$-AR blocker CGP (data not shown).

Interestingly, another selective $\beta_2$-AR agonist, fenoterol,25 evoked a full contractile response that was insensitive to PTX treatment (Figure 4D). It appears that fenoterol selectively stimulates the $\beta_2$-AR/G$i$, signaling pathway, whereas other $\beta_2$-AR agonists activate both G$s$ and G$i$ proteins in terms of their effects on cardiac myocyte contractility.

Rescue of the Depressed $\beta_2$-AR Contractile Response by Fenoterol in Myocytes From Failing SHR Hearts

We next determined whether the apparent $\beta_2$-AR/G$i$, signaling–specific agonist fenoterol could rescue the diminished contractile response to $\beta_2$-AR stimulation in myocytes from failing SHR hearts. Figure 5A shows typical continuous recordings of myocyte contractile responses to zinterol or fenoterol. Fenoterol induced a robust increase in contraction amplitude in a representative SHR cell. This effect was completely reversed by the $\beta_2$-AR antagonist ICI ($10^{-7}$ mol/L), indicating that the positive inotropic effect of fenoterol is mediated by selective $\beta_2$-AR stimulation. In contrast, zinterol had a very minor effect in another cell from the same heart. Figure 5B shows the average dose-response of contraction amplitude to fenoterol in cardiomyocytes from SHR and age-matched WKY rats. The 2 dose-response curves virtually overlapped each other, in sharp contrast to the markedly suppressed contractile response to zinterol in failing SHR heart cells (Figure 2B). Thus, fenoterol is able to restore a full contractile response to $\beta_2$-AR stimulation in SHR myocytes even in the absence of PTX, thereby abolishing the difference between SHR and WKY.

Discussion

Enhanced G$i$ Signaling Selectively Contributes to the Diminution of $\beta_2$-AR– but Not $\beta_1$-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 $\beta$-AR stimulation in a rat myocardial infarction heart failure model26 and in myocytes from failing human hearts,27 the present study provides the first documentation that enhanced G$i$, signaling is, in fact, selectively involved in heart failure–associated attenuation in $\beta_2$-AR– but not $\beta_1$-AR–mediated increase in myocyte contractility. This is because inhibition of $\beta_2$-AR/G$i$, signaling by PTX treatment enables $\beta_2$-AR stimulation to induce a full contractile response, thus abolishing the difference between the failing and normal hearts, whereas it does affect $\beta_1$-AR–induced inotropic effect. These findings are consistent with our previous notion that $\beta_2$-AR but not $\beta_1$-AR dually couples to G$s$ and G$i$, and that the G$i$, coupling exhibits an inhibitory effect on the G$s$, signaling.6–9

Although the heart failure–associated defect of $\beta_2$-AR contractile response is largely attributable to enhanced $\beta_2$-AR/G$i$, signaling, multiple mechanisms might be involved in $\beta_2$-AR dysfunction, including receptor downregulation1–3 (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 $\beta$-AR kinase 1 ($\beta$ARK1), a prototypic G protein–coupled receptor kinase,28 are significantly elevated in human29 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 Gα 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 β2-AR induces apoptosis in cultured rodent cardiomyocytes. Similarly, cardiac transgenic overexpression of β2-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 Gq not only improves cardiac performance but also prevents hypertrophy in the Gq 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 of the β2-AR) is better than that of patients with the Val164 polymorphism.
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.39

In the present study, we found that in failing SHR hearts, β2-AR is selectively downregulated with little or no loss of β2-AR, similar to the situation of chronic human heart failure.1–3 In light of evidence for the opposing effects of β2-AR and β2-AR stimulation on cardiac myocyte apoptosis16–19 and the distinct phenotypes of transgenic overexpression of β1-AR versus β2-AR,33–35 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/Gs Signaling in Failing SHR Hearts**

Because heart failure in many instances is related to a selective loss of β2-AR and because chronic stimulation of β2-AR exhibits no known detrimental effects, as discussed above,35 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 β2-AR agonists (eg, isoproterenol) cannot discriminate β2-AR from β2-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/Gs signaling from the inhibition by Gi, we identified fenoterol as an apparent β2-AR/Gs–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 β2-AR agonists to provide contractile support in the failing heart. Our preliminary experiments have shown that, unlike β2-AR stimulation, β2-2 stimulation by fenoterol does not induce cardiac myocyte apoptosis (Zhu et al, unpublished data), although it increases cAMP/PKA signaling, perhaps because of distinct compartmentalization of cAMP signal in response to different β-AR subtype activation.11–13,40,41

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 receptor42–45 and that ligands may lead the receptor to a subset of downstream signaling pathways (ligand-directed receptor trafficking).45 In contrast to fenoterol, a well-characterized β2-AR inverse agonist, ICI, can direct β2-2 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.44

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 β2-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 β2-AR contractile response. Second, β2-AR–coupled Gi and Gs 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/Gs–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 β2-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.

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**References**


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