Upregulation of $\beta_3$-Adrenoceptors and Altered Contractile Response to Inotropic Amines in Human Failing Myocardium

Stéphane Moniotte, MD; Lester Kobzik, MD; Olivier Feron, PhD; Jean-Noël Trochu, MD; Chantal Gauthier, PhD; Jean-Luc Balligand, MD, PhD

Background—Contrary to $\beta_1$- and $\beta_2$-adrenoceptors, $\beta_3$-adrenoceptors mediate a negative inotropic effect in human ventricular muscle. To assess their functional role in heart failure, our purpose was to compare the expression and contractile effect of $\beta_3$-adrenoceptors in nonfailing and failing human hearts.

Methods and Results—We analyzed left ventricular samples from 29 failing (16 ischemic and 13 dilated cardiomyopathic) hearts (ejection fraction 18.6±2%) and 25 nonfailing (including 12 innervated) explanted hearts (ejection fraction 64.2±3%). $\beta_3$-Adrenoceptor proteins were identified by immunohistochemistry in ventricular cardiomyocytes from nonfailing and failing hearts. Contrary to $\beta_3$-adrenoceptor mRNA, Western blot analysis of $\beta_3$-adrenoceptor proteins showed a 2- to 3-fold increase in failing compared with nonfailing hearts. A similar increase was observed for $\text{G}_{i2}$ proteins that couple $\beta_3$-adrenoceptors to their negative inotropic effect. Contractile tension was measured in electrically stimulated myocardial samples ex vivo. In failing hearts, the positive inotropic effect of the nonspecific amine isoprenaline was reduced by 75% compared with that observed in nonfailing hearts. By contrast, the negative inotropic effect of $\beta_3$-preferential agonists was only mildly reduced.

Conclusions—Opposite changes occur in $\beta_1$- and $\beta_3$-adrenoceptor abundance in the failing left ventricle, with an imbalance between their inotropic influences that may underlie the functional degradation of the human failing heart. (Circulation. 2001;103:1649-1655.)

Key Words: receptors, adrenergic, beta ■ heart failure ■ catecholamines ■ contractility ■ nitric oxide synthase

Cardiomyocyte $\beta_1$- and $\beta_3$-adrenoceptors mediate the classic increase in cardiac inotropic state after stimulation by endogenous catecholamines. Activation of $\beta$-adrenergic signaling is limited in time by rapid homologous desensitization, mediated by receptor phosphorylation, including through G-protein–coupled receptor kinases (ie, GRK2 or $\beta$-adrenoceptor kinase, $\beta$ARK$^1$) and in the longer term by agonist-elicted decreases in receptor density. In the chronically failing heart, the inotropic response is diminished in the face of elevated circulating catecholamines, in part through decreased $\beta_3$-adrenoceptor mRNA and proteins, increased abundance and activity of $\beta$ARK, and increased abundance of the inhibitory G protein, $\text{G}_i$, which couples receptor stimulation to decreases in intracellular production of cAMP, a key mediator for the contractile effects of $\beta$-adrenoceptor stimulation.

Transgenic models or gene transfer experiments in failing cardiomyocytes highlighted the importance of these signaling molecules in the development of contractile dysfunction and opened new therapeutic avenues for heart failure. Cardiac-specific overexpression of $\beta_3$-adrenoceptor or $\text{G}_{i2}$ transgenes that positively couple to cAMP formation resulted in enhanced cardiac contraction and responsiveness to catecholamines, at least up to 12 weeks. Adenovirus-mediated transfection of $\beta_3$-adrenoceptors or a $\beta$ARK inhibitor in cardiomyocytes from chronically paced failing rabbit hearts restored cAMP production in these cells after agonist stimulation. However, transgenic mice overexpressing $\beta_3$-adrenoceptors that displayed early enhanced contractility developed cardiac hypertrophy, fibrosis, and heart failure when studied at a later age (beyond 35 weeks). Therefore, short-term benefits of enhancing $\beta_3$-adrenoceptor signaling must be distinguished from potential toxic effects of adrenergic stimulation over longer periods, at least in these animal models. How some or all of these paradigms apply to human heart failure, however, is unknown. In particular,
the clinical experience that chronic treatment of heart failure patients with β-adrenergic blockers slows the progression of the disease and improves outcomes suggests a more complex regulation of myocardial inotropism by catecholamines. After molecular cloning of a third β-adrenoceptor subtype, the β₃-adrenoceptor, and its detection in human adipocytes, evidence was provided for its functional role in human ventricular myocardium from nonfailing, denervated hearts. In contrast to β₁- and β₂-subtypes, stimulation of β₃-adrenoceptors with β₁-preferential agonists or norepinephrine (in the presence of full blockade with α₁-, β₁-, and β₂-adrenoceptor antagonists) inhibited cardiac contractility. This effect implicated the inhibitory G protein Go, and more recently was shown to involve the production of nitric oxide (NO) by the endothelial isoform of NO synthase expressed within the ventricular myocardium, or eNOS. The regulation of the β₁-adrenoceptor pathway in the human failing heart, however, is unknown. Here, we first demonstrate the expression of β₁-adrenoceptors in ventricular myocytes from nonfailing human hearts and show that contrary to β₁-adrenoceptors, the abundance of β₁-adrenoceptors increases in ischemic or dilated cardiomyopathic hearts. This is paralleled by increased abundance of Gox₂ coupling proteins in the same hearts but decreased expression of eNOS. In addition, we show that these opposite changes in abundance of β₁-adrenoceptor subtypes result in striking impairment of the positive inotropic effect of the nonspecific β₁-adrenoceptor agonist isoprenaline but less attenuation of the negative inotropic effect of the β₁-preferential adrenoceptor agonist BRL 37344. These findings substantiate the hypothesis that overstimulation of the relatively desensitization-resistant β₁-adrenoceptor pathway in the human failing heart may further decrease cardiac inotropy and open the perspective for correcting the disordered adrenergic regulation of the failing heart with specific antagonists of the human cardiac β₁-adrenoceptor.

Methods

Reverse Transcription–Polymerase Chain Reaction Amplification

Because cDNA synthesis is a critical step for reverse transcription–polymerase chain reaction (RT-PCR) quantitation experiments, efforts have been made to optimize reverse transcription for a reproducible maximum efficiency. Total RNA isolated from frozen left ventricular (LV) specimens was reverse transcribed with SuperScript II (Gibco BRL) at 45°C for 50 minutes. β₁- and β₂-adrenoceptor cDNA was then amplified by PCR (35 cycles) at the following annealing temperatures: 54°C (β₁-AR), 55.5°C (β₂-AR), or 60°C (36B4) (see sequences of primers in Table 1). The absence of contamination by genomic DNA was ascertained in parallel amplifications of RT(−) samples, which always remained negative.

The density of ethidium-stained bands, analyzed on 3% agarose gels, was quantified with image-analysis software (ImageJ, NIMH), and data were normalized to the level of transcripts for the housekeeping gene 36B4 (acidic ribosomal phosphoprotein PO).

Western Blotting Experiments

Denatured proteins from human ventricular tissues were separated on 10% SDS-PAGE gels (β₂-AR) or 12% 6 mol/L urea gels (Gox₂) and transferred on nitrocellulose. Membranes were incubated for 4 hours with the primary antibody (ie, anti-eNOS, Transduction Labs; anti-β₁-adrenoceptors, generously provided by Dr J. Arch, SmithKline-Beecham Pharmaceuticals, Harlow, UK; or anti-Gox₂, a gift from Dr M. Böhm, Universität zu Köln, Germany), washed in TBST (Tris-buffered saline containing 0.1% Tween 20), incubated with the secondary antibody at 1:10 000, and revealed by chemiluminescence. Densitometric values for each sample were expressed as a percentage of the mean value obtained for the corresponding control (nonfailing) samples analyzed on the same gel.

Immunohistochemistry

Immunohistochemical localization of the β₁-AR was performed on cryostat sections of human endomyocardial biopsy specimens from failing and nonfailing hearts. The same rat monoclonal anti-β₁-adrenoceptor primary antibody was used, followed by detection with an avidin-biotin complex immunoperoxidase method and diaminobenzidine as chromogen. Control experiments were run in parallel with normal rat IgG as primary antibody.

Contractility Measurements

Ventricular samples were electrically stimulated (0.6 Hz) in a tissue chamber perfused with oxygenated Tyrode’s solution (2.7 mmol/L CaCl₂; 37±0.5°C) and tensions recorded at steady state by a mecanoelectric force transducer (Akers, AE 801; SensoNor), as described previously. The cumulative concentration-response curves of isoprenaline and the β₁-preferential agonist BRL 37344 were determined by superfusion with increasing concentrations of the drugs. Changes in tension were expressed as percent increase (or decrease) of baseline (each sample being its own control). To determine agonist potencies from the concentration-response curves, the concentrations producing 50% of maximum effect (EC50) were determined by fitting curves with the Bolzmann equation. pD2 values were calculated according to the equation pD2 = -log(EC50).

Drugs

Isoprenaline was obtained from Sigma Chemical Co and BRL 37344 (4-[2-hydroxy-(3-chlorophenyl)ethyl-amino]propyl-phenoxyacetate) from Research Biochemicals Int.

Statistical Analysis

Data are presented as mean±SEM. Statistical significance of the drug effect was assessed by 1-way ANOVA followed by a Dunnett test. Comparison of the concentration-response curves was performed by 2-way ANOVA. Statistically significant differences between groups in terms of mRNA or protein expression were calculated by a Student’s t test for unpaired data.

Results

Patient Characteristics

Nonfailing heart tissues were obtained from 3 sources, with the approval of local ethics committees. Thirteen samples of denervated hearts were collected from endomyocardial biopsy specimens taken in parallel.

<table>
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<th>mRNA</th>
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<td>R: ATATGGGAGAGCTTTCCAG</td>
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SmithKline-Beecham Pharmaceuticals, Harlow, UK; or anti-Gox₂, a gift from Dr M. Böhm, Universität zu Köln, Germany, washed in TBST (Tris-buffered saline containing 0.1% Tween 20), incubated with the secondary antibody at 1:10 000, and revealed by chemiluminescence. Densitometric values for each sample were expressed as a percentage of the mean value obtained for the corresponding control (nonfailing) samples analyzed on the same gel.
All received immunosuppressive therapy, including azathioprine, prednisolone, and cyclosporin, but were free of any cardiovascular-specific medications. Eight samples of normal, innervated myocardium were obtained from donor hearts that were not transplanted for technical reasons (mean ejection fraction 64.1±2%). Six of 8 donors had received synthetic inotropic amines in the 48 hours preceding explantation. Four additional nonfailing hearts were explanted from patients with cystic fibrosis.

Failing myocardial tissue was obtained from explanted hearts of 29 recipients at transplantation (mean ejection fraction 18.6±2%). Sixteen patients had ischemic cardiomyopathy and 13 had dilated cardiomyopathy. Details regarding their drug regimens are shown in Table 2.

Expression of β-Adrenoceptor Subtypes in Normal and Failing Hearts
Cardiomyocytes are the predominant cell type within normal human ventricle to express β1- and, to a much lesser extent, β2-adrenoceptors. The abundance of these 2 subtypes is known to be altered in the failing heart, with a reduction of β1-adrenoceptor proteins, whereas β2-adrenoceptors are primarily unchanged. Accordingly, we found that the abundance of mRNAs for β1-adrenoceptors was reduced by 48.1±8.5% in failing hearts (n=14 patients; 9 ischemic and 5 dilated cardiomyopathies) compared with levels measured in extracts from nonfailing patients (1 denervated and 4 nondenervated hearts; P<0.05). In the same extracts, the abundance of β2-adrenoceptor mRNAs was unchanged (P=0.08).

Using a monoclonal antibody specific for the human β3-adrenoceptor in extracts of nonfailing hearts, we found a single immunodetected band of a size compatible with a glycosylated form of the receptor, as observed in other human tissues. Of note, the abundance of β3-adrenoceptor proteins was higher in nonfailing, nondenervated cardiac tissues than in tissues from nonfailing but denervated transplanted hearts (Figure 1B). Moreover, compared with levels observed in nonfailing, nondenervated hearts, β3-adrenoceptors were significantly increased in failing cardiac tissues from either ischemic or dilated cardiomyopathic hearts (Figure 1A and 1B). Subsequent analysis of extracts from epicardial, midmyocardial, and subendocardial layers from both ventricles showed no transmural difference in β3-adrenoceptor abundance (not shown).

Immunolocalization of β3-Adrenoceptors in the Human Ventricle
To identify the specific cell type(s) expressing β3-adrenoceptors within the human ventricle, the same antibody was used in immunohistochemical experiments on similar tissue samples (Figure 2). No staining was detectable above background when the primary antibody was omitted or nonspecific rat IgG was used. However, with the specific antibody, a strong signal was detected in cardiac myocytes from nonfailing, nondenervated transplanted tissue (Figure 2A). A light staining was also present in vessels. Consistent with immunoblotted signals as described above, the intensity of cardiomyocyte labeling appeared to be higher in sections

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Abundance of β3-adrenoceptor, Ga12, and eNOS proteins in nonfailing and failing human hearts. A, Representative immunoblotted β3-adrenoceptor signals in whole-heart extracts from 5 patients with normal hearts and 4 with overt heart failure. B, Mean β3-adrenoceptor protein densitometric data in nonfailing nondenervated (Normal) or denervated hearts and failing ischemic (ICM) or dilated (DCM) cardiomyopathic hearts. Data are expressed as percentage of mean level in nondenervated, normal hearts. *P<0.05, **P<0.01, ***P<0.001 vs normal hearts. C and D, Mean densitometric data and representative eNOS (C) or Ga12 (D) immunoblotted signals in nonfailing (Normal) and failing ICM or DCM hearts. Data are expressed as percentage of mean levels in nonfailing hearts; *P<0.05, **P<0.001 vs nonfailing hearts.

| TABLE 2. Clinical Characteristics of Patients |
|---|---|---|---|---|---|---|---|---|---|
| Sex Ratio, M/F | Age, y | EF, % | β-Blocker, % | Digitalis, % | ACE Inhibitor, % | Diuretic, % | CA, % |
| Nonfailing | 1.2 | 34.1±6.6 | 64.2±3 | 0 | 0 | 0 | 0 |
| Failing | 3.8 | 50.8±2.7 | 18.6±2 | 44 | 28 | 76 | 88 |

EF indicates ejection fraction; CA, calcium antagonists.
of ventricular tissue from dilated or ischemic cardiomyopathic hearts (Figure 2C and 2E).

**Changes in Abundance of G\(\alpha_i-2\) and eNOS Proteins**

Our previous studies\(^{21}\) had demonstrated that the contractile effect of \(\beta_3\)-adrenoceptor agonists is mediated, at least in part, through a pertussis toxin–sensitive stimulation of NO production. To assess potential changes in the expression of these signaling molecules in the failing heart, we compared the abundance of G\(\alpha_i-2\) proteins, the main G\(\alpha_i\) isoform in human ventricle,\(^{11}\) and eNOS, the NO synthase constitutively expressed in human cardiomyocytes,\(^{21}\) in failing and nonfailing hearts (Figure 1C and 1D). In agreement with previous studies,\(^{9–11}\) we found that the level of immunoblotted G\(\alpha_i-2\) proteins was increased in failing myocardium from ischemic (\(n=11\) patients) and dilated (\(n=8\) patients) cardiomyopathic hearts compared with nonfailing hearts (\(n=5\) patients: 3 nondenervated, 2 denervated) (Figure 1D). By contrast, the abundance of eNOS proteins was decreased in ischemic (\(n=16\) patients) and dilated (\(n=8\) patients) cardiomyopathic hearts compared with nonfailing hearts (\(n=10\) patients: 6 nondenervated, 4 denervated) (Figure 1C). Again, no transmural difference in eNOS abundance was observed between the various layers of myocardium from both ventricles (not shown).

**Contractile Responsiveness to \(\beta\)-Adrenergic Agonists**

To assess the functional consequences of these expressional changes in \(\beta\)-adrenergic signaling molecules, we analyzed the contractile response of human ventricular biopsy specimens both to isoprenaline, a nonspecific \(\beta\)-adrenergic agonist, and to BRL 37344, a \(\beta_3\)-preferential adrenergic agonist, ex vivo. Basal tension values in nondenervated, nonfailing hearts were 537±204.3 μN in dissected trabeculae (\(n=4\)) and 6.8±21.1 μN in biopsy specimens (\(n=6\)), and 675±157.6 μN and 847±139.4 μN in ischemic (\(n=15\)) and dilated (\(n=16\)) cardiomyopathic trabeculae, respectively. Variations between trabeculae and biopsy specimens are explained by anatomic differences in sarcomere orientation\(^{24}\). In LV samples from nonfailing, nondenervated hearts, isoprenaline produced an increase in absolute contractile tension with a pD\(_2\) of 7.52±0.06 and an effect at 1 μmol/L amounting to 370±69% over basal contractile tone (Figure 3A), and BRL 37344 produced a decrease in absolute tension with a pD\(_2\) of 9.08±0.21 and a maximum reduction of 44±4% below basal contractile tension at 1 μmol/L (Figure 3B). This negative inotropic effect was attenuated by the specific antagonist of the human \(\beta_3\)-adrenoceptor, L-748,337 (with 0.1 μmol/L: \(-23±6.0\%\) \([n=6]\); without L-748,337: \(-50.3±1.8\%\) \([n=6]\) of basal tone; \(P<0.01\)). In trabeculae from failing hearts, however, the positive inotropic effect of 1 μmol/L isoprenaline was greatly reduced to 94±24% and 12±14% over basal tension in ischemic and dilated cardiomyopathic hearts, respectively (Figure 3A) (pD\(_2\) 7.34±0.21 and 7.64±0.15), and the negative inotropic effect of BRL 37344 was attenuated to 32±8% and 18±6% below basal tension, respectively (Figure 3B) (pD\(_2\) 8.3±0.20 and 8.3±0.23). Therefore, the failing hearts exhibited a loss of
responsive to both agonists that was more prominent for isoproterenol than for the β₂-adrenergic agonist BRL 37344 (Figure 3C).

Discussion
We demonstrated that in addition to β₁- and β₂-adrenoceptors, β₃-adrenoceptors are expressed in human nonfailing ventricular myocardium. This substantiates our previous observation of a functional response to catecholamines in the human heart through a β-adrenergic receptor that is distinct from the β₁- and β₂-subtypes. Indeed, contrary to β₁- and β₂-adrenoceptor pathways, activation of this receptor by norepinephrine in the presence of α₁- and β₁-β₂-antagonists, as well as by β₃-preferential adrenoceptor agonists such as BRL 37344 and CGP 12177 (which also combines antagonistic properties for β₁-β₂-adrenoceptors), decreases contractile force. Of note, the order of potency of these agonists differs for their lipolytic and inotropic effects, a phenomenon likely due to differences in their specificity for the β₂-receptor across species and even tissues (for a review, see Gauthier et al27). In addition to specific transcripts,20 we now identified β₃-adrenoceptor proteins in human ventricular muscle as observed in other human tissues with the same antibody (Figure 1A). The specificity of the antibody for the β₁-subtype, as opposed to human β₁- and β₂-adrenoceptors, was previously ascertained against recombinant human β₁-, β₂-β₃-subtypes heterologously expressed in Chinese hamster ovary (CHO) cells, with no cross-reactivity to β₁-β₂-subtypes.25 Of interest, we found a robust expression of the β₃-adrenoceptor in nonfailing hearts from normal donors, further extending the potential physiological role of this pathway in the innervated myocardium as opposed to denervated, transplanted hearts. In the latter, we also found a downregulation of β₁-adrenoceptor mRNA (data not shown), although this may be at variance with previous functional results.28 Moreover, we showed that the β₁-adrenoceptor is expressed predominantly in human cardiomyocytes (Figure 2), where downstream signaling molecules such as Gα₂ and eNOS are colocalized, suggesting a direct rather than paracrine regulation of cardiomyocyte contractility by β₁-adrenoceptor stimulation. The functional significance of the fainter staining observed in vessels awaits the results of additional studies of human vascular reactivity.

A downregulation of β₁-adrenoceptors is well established in the failing human heart,3 whereas β₂-adrenoceptors are primarily unchanged.3 Accordingly, we found that the abundance of β₁- (but not β₂-) adrenoceptor mRNAs was significantly decreased in failing myocardium. Aside from this downregulation, the desensitization of failing myocardium to the positive inotropic effect of catecholamines has been attributed in part to the upregulation of G proteins of the αi isoform that couple to inhibition of adenyl cyclase.10 In the present study, as in previous studies,9-11 the abundance of Gαi₂ proteins was increased in failing ventricular myocardium (Figure 1D). Because β₁-adrenoceptors are the predominant positively inotropic β-adrenoceptors in cardiac muscle, their downregulation is thought to account for a significant

Figure 3. Contractile tension of human ventricular biopsy specimens in response to inotropic amines ex vivo. A, Concentration-dependent positive inotropic effect of nonspecific β-adrenoceptor agonist, isoproterenol, in nondenervated nonfailing (n = 4 patients) and failing ischemic (*; n = 6 patients) and dilated (Φ; n = 7 patients) cardiomyopathic heart tissues. P<0.001 for ischemic and dilated cardiomyopathic hearts vs nonfailing hearts by 2-way ANOVA. B, Concentration-dependent negative inotropic effect of β₁-preferential agonist, BRL 37344, in nondenervated nonfailing (n = 6 patients) and failing ischemic (*; n = 9 patients) and dilated (Φ; n = 9 patients) cardiomyopathic heart tissues. P<0.05 for dilated and ischemic hearts vs nonfailing hearts by 2-way ANOVA. C, Attenuation of inotropic responses to 1 μmol/L isoprenaline (upper bars) and 1 μmol/L BRL 37344 (lower bars) in failing ischemic (ICM) and dilated (DCM) cardiomyopathic heart tissues compared with responses in nonfailing hearts (Normal). Results are normalized to response obtained with 1 μmol/L of either agonist in nonfailing hearts, expressed as 100%. Attenuation of isoprenaline responses was significantly more pronounced than that of BRL 37344. P=0.0001 by 2-way ANOVA.

Figure 4. Postulated changes in β-AR signaling in cardiomyocytes from nonfailing to failing myocardium.

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part of the loss of inotropic response to catecholamines in the failing heart. Accordingly, we observed >75% attenuation of the positive inotropic effect of isoproterenol in ventricular biopsy specimens from ischemic and dilated cardiomyopathic hearts. Moreover, we now show that contrary to β₁-adrenoceptors, the abundance of the negatively inotropic β₂-adrenoceptor increases in the failing myocardium. This was apparent both by immunohistochemistry and Western blotting in whole-muscle extracts across the different ventricular layers (Figures 1 and 2). That these increased β₂-adrenoceptors are functional was ascertained from the observation that stimulation of failing heart tissue with β₂-preferential agonists did produce a negative inotropic effect ex vivo (Figure 3).

However, changes in contractile response did not strictly parallel those in β₁-receptor abundance. Despite increased β₂-adrenoceptors, the negative inotropic effect was blunted in cardiomyopathic tissue compared with responses observed in nonfailing cardiac tissue. This may be explained by concurrent alterations in postreceptor coupling mechanisms in the failing heart. Although intracellular pathways coupling β₁-adrenoceptor stimulation are still incompletely characterized, we had shown that they involved the production of NO in the human ventricle.²¹ Our observation of decreased eNOS expression in failing hearts provides one explanation for the attenuated β₁-response. However, when compared with responses measured in nonfailing heart, the negative inotropic effect of β₂-adrenoceptor agonists was more preserved in failing hearts than the β₁-β₂ positive inotropic effect of isoproterenol, thereby potentially producing an imbalance between these inotropically opposed pathways (Figure 3C). Aside from the increased receptor abundance, the relative persistence of the β₂-adrenoceptor response may be related to the resistance of this subtype to homologous desensitization because it lacks the consensus sequences for phosphorylation by GRKs,²² or to the parallel upregulation of Goα₂, proteins, because the coupling of β₂-adrenoceptors to their functional effect was shown to be sensitive to pertussis toxin.²⁰ Therefore, the attenuated contractile effect of isoproterenol, a nonspecific β₁-β₂-β₂-agonist, in failing hearts may integrate a persistent β₂-adrenoceptor, negatively inotropic effect superimposed on a sharply downregulated β₁ response. A similar imbalance is even more likely to occur in vivo in the face of increased local and circulating catecholamines, eg, norepinephrine, which has a relatively high affinity for the β₂-adrenoceptor (unlike the β₁-subtype).¹⁷ Nevertheless, because they were mostly based on mechanical experiments in vitro, these paradigms will need further rigorous validation in patients.

The concurrent activation of inverse inotropic pathways, mediated by distinct β-adrenoceptor subtypes, helps to rationalize previous observations on the regulation of catecholamine responsiveness of the heart in vivo. After our demonstration of the countervailing, negatively inotropic influence of eNOS on the β-adrenergic response in isolated cardiomyocytes,²⁰ others observed that inhibition of cardiac NOS or eNOS gene disruption results in a potentiation of the positive inotropic effect of isoprenaline or dobutamine in dogs,³⁰ humans,³¹ and eNOS-deficient mice.³² Of note, this potentiation was only apparent in patients with heart failure,³³ where we would anticipate a prevailing influence of the β₁/NOS pathway over β₁/β₂-signaling, according to our present results. Likewise, a recent study showed that the potentiation of the positive inotropic effect of isoprenaline by NOS inhibition is abolished in mice homozygously deficient for the β₁-adrenoceptor,³⁴ confirming the involvement of this subtype in the NO-dependent regulation of catecholamine responsiveness in vivo. The β₂-adrenoceptor pathway may subservice different physiological functions in the heart (Figure 4). In the normal heart, it may exert a negative counterregulation against excessive positive inotropic stimulation, thereby moderating oxygen consumption, preventing calcium overload and ultimately cardiomyocyte toxicity, as exemplified by the phenotype of β₁-adrenoceptor–overexpressing mice.¹⁵ At early stages of cardiac dysfunction, endogenous NO production may, in addition to attenuating β₁-β₂-adrenergic inotropic responses, improve diastolic relaxation,³⁵ thereby compensating systolic dysfunction by increasing diastolic reserve. In terminally failing hearts, the residual negative inotropic effect may become maladaptive and aggravate systolic dysfunction (Figure 4, right). More insights into these potential roles and their impact on the clinical use of β-blockers should be gained from linear studies of animal models and patients with progressive heart failure as new specific antagonists for the human β₂-adrenoceptor become available.

Acknowledgments

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References


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