Endogenous β3-adrenoreceptor activation contributes to left ventricular and cardiomyocyte dysfunction in heart failure

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Morimoto, Atsushi, Hiroshi Hasegawa, Heng-Jie Cheng, William C. Little, and Che-Ping Cheng. Endogenous β3-adrenoreceptor activation contributes to left ventricular and cardiomyocyte dysfunction in heart failure. Am J Physiol Heart Circ Physiol 286: H2425–H2433, 2004.—The objective of the present study was to test the hypothesis that endogenous β3-adrenoreceptor (AR) activation contributes to left ventricular (LV) and cardiomyocyte dysfunction in heart failure (CHF). Stimulation of the β3-AR inhibits cardiac contraction. In the failing myocardium, β3-ARs are upregulated, suggesting that stimulation of β3-ARs may contribute to depressed cardiac performance in CHF. We assessed the functional significance of endogenous β3-AR activation in 10 conscious dogs before and after pacing-induced CHF. Under normal conditions, L-748,337, a specific β3-AR antagonist, produced a mild increase in LV contractile performance assessed by the slope (Emax) of the LV pressure-volume relation (18%, 6.2 ± 0.9 vs. 7.3 ± 1.2 mmHg/ml, P < 0.05) and the improved LV relaxation time constant (τ; 28.4 ± 1.9 vs. 26.8 ± 1.0 ms, P < 0.05). After CHF, the plasma norepinephrine concentration increased eightfold, and L-748,337 produced a larger increase in Emax (34%, 3.8 ± 0.7 vs. 5.1 ± 0.8 mmHg/ml, P < 0.05) and a greater decrease in τ (46.4 ± 4.2 vs. 41.0 ± 3.9 ms, P < 0.05). Similar responses were observed in isolated myocytes harvested from LV biopsies before and after CHF. In the normal myocyte, L-748,337 did not cause significant changes in contraction or relengthening. In contrast, in CHF myocytes, L-748,337 produced significant increases in contraction (5.8 ± 0.9 vs. 6.8 ± 0.9%, P < 0.05) and relengthening (33.5 ± 4.2 vs. 39.7 ± 4.0 μm/s, P < 0.05). The L-748,337-induced myocyte response was associated with improved intracellular Ca²⁺ concentration regulation. In CHF myocytes, norepinephrine caused a decrease in contraction and relengthening, and adding isoproterenol to norepinephrine caused a further depression of myocyte function. Stimulation of β3-AR by endogenous catecholamine contributes to the depression of LV contraction and relaxation in CHF.

endogenous catecholamine; ventricular and myocyte function; Ca²⁺ regulation

UNDER NORMAL CONDITIONS, β-adrenorenergic stimulation of the heart results in increased force and frequency of myocardial contraction and the rate of relaxation. This effect is mediated through stimulation of β1- and β2-adrenergic receptors (ARs) and coupled through Gs proteins (2, 22, 24). β2-ARs are also able to activate nonclassical signaling pathways, suggesting a function distinct from the β1-AR subtype (45, 46). A third β-AR, β3-AR, was initially identified in fat tissue (19) and some vascular tissues (11). Recently, β3-ARs have been identified in normal myocardial tissue in several species, including humans (8, 11, 12, 18, 25). In contrast to β1- and β2-ARs, β3-ARs are linked to Gi proteins, and stimulation of β3-ARs inhibits cardiac contraction and relaxation (8, 11, 12, 18, 25). Tavernier et al. (43) found that cardiac overexpression of human β3-AR in mice reproduces ex vivo the negative inotropic effects. Although the precise physiological and pathophysiological roles of β3-ARs remain uncertain, recent reports suggest that in the normal heart, β3-ARs participate in nitric oxide (NO)-mediated negative feedback control over contractility. Stimulation of β3-ARs with a specific agonist [BRL-37,344 (BRL)] resulted in a negative inotropic effect in human donor hearts through altered NO signaling (12) and β3-AR deficiency blocked NO-dependent inhibition of myocardial contractility in transgenic mice (44).

In heart failure (CHF), the sympathetic nervous system (SNS) is activated, cardiac β1-ARs are downregulated, and β1- and β2-ARs are desensitized and uncoupled from Gs protein. The β2-AR-coupled Gi pathway is enhanced (45), thus reducing the increment in myocardial contraction produced by adrenergic stimulation (22). In contrast, β3-ARs are upregulated in failing human hearts (25), in canine myocardium after the development of CHF produced by rapid pacing (8), and in diabetic rat hearts (9). β3-ARs are activated at higher catecholamine concentrations than β1- and β2-ARs (21) and are relatively resistant to chronic, agonist-induced desensitization processes (20). In addition, Gi proteins, which are involved in β3-AR signaling, are elevated in failing myocardium (32, 37, 47).

Thus we hypothesized that in severe CHF, as β1- and β2-AR pathways become less responsive, the inhibitory effects of the β3-AR pathway stimulated by elevated endogenous catecholamine may contribute to the detrimental effects of adrenergic activation in CHF. Accordingly, this study was undertaken to assess the effects of stimulation of β3-ARs by endogenous catecholamines on left ventricular (LV) contractile performance in conscious dogs before and after pacing-induced CHF. In addition, we studied the effects of stimulation of β3-ARs in myocytes harvested from the LV obtained by serial biopsies before and after CHF. Our results provide new insights concerning the significance of endogenous β3-AR activation in CHF, adding another dimension to the current framework of disordered cardiac adrenergic regulation in CHF.

MATERIALS AND METHODS

This investigation was approved by the Institutional Animal Care and Use Committee. The investigation conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1985). Ten adult, heartworm-negative, mongrel dogs (weight 25–35 kg) were instrumented to measure LV and left atrial (LA) pressures with micromanometers and the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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three orthogonal LV dimensions with sonomicrometers. Hydraulic occluder cuffs were placed around the venae cavae. A pacemaker was implanted as we previously described (5, 27).

Studies were performed after full recovery from instrumentation with the animals lying quietly and unsedated in a sling. Variably loaded pressure-volume (P-V) loops were generated by transient caval occlusions (5, 27). The signals were digitized at 200 Hz. Each data acquisition lasted for 15–20 s, spanning several respiratory cycles.

To evaluate the functional role of endogenous β3-AR activation, we used L-748,337, a highly selective β3-AR antagonist (β3-ANT) (3, 24), or nadolol, a potent β1- and β2-ANT, which does not inhibit β3-ARs (14, 39).

The dose of L-748,337 (50 μg/kg iv) has been demonstrated to selectively block β3-ARs (3, 24, 25). To assess the adequacy of β3-AR blockade, we measured the response to the infusion of a selective β3-AR agonist, BRL (0.4 nM·kg⁻¹·min⁻¹ iv) with and without pretreatment with L-748,337. To confirm the β3-AR selectivity of L-748,337, we administered a nonselective β-AR agonist, dobutamine (6 μg·kg⁻¹·min⁻¹ iv) or epinephrine (0.2 μg·kg⁻¹·min⁻¹ iv).

LV Functional Responses

The effects of L-748,337 (50 μg/kg iv) were determined under normal resting conditions with reflexes intact and with the heart rate (HR) kept constant by right atrial pacing at 140 beats/min and also after autonomic blockade with metoprolol (0.5 mg/kg iv) and atropine (0.1 mg/kg iv).

After the completion of the baseline studies, as previously described (6), rapid RV pacing (at 220–240 beats/min) was initiated using our pacing protocol. After pacing for 4–5 wk, when the LV end-diastolic pressure during the nonpaced period had increased by >20 mmHg over the prepac ing control level, CHF data were obtained. This level of CHF was chosen because the animals had begun to show clinical evidence of CHF (anorexia, mild ascites, and pulmonary congestion) but able to remain at a stable degree of CHF during the whole study period (~2 wk) to minimize the day-to-day variations during data collections.

Control data were recorded and blood samples were obtained after the pacemaker had been turned off and the animal had stabilized for at least 30 min. The above protocols were then repeated.

LV volume, rate of LV relaxation (τ), LV end-systolic pressure-end-systolic volume relations and its slope (Eₜₛₛ), and stroke work (SW)-end-diastolic volume relations and its slope (Mₛₑₑ) were analyzed (23, 24). LV arterial coupling was quantitated as the rate of arterial elastance (Eₐ) determined as end-systolic pressure/stroke volume. The LV P-V area (PVA; which represents the total mechanical energy) was determined as the area under the end-systolic P-V curve and systolic P-V trajectory above the end-diastolic P-V curve. The efficiency of conversion of mechanical energy to external work of the heart was calculated as SW/PVA (27).

Determination of Cardiomyocyte Functional Responses

Myocyte isolation. As we reported previously (5, 8), myocytes were isolated from LV myocardial biopsies obtained from 10 dogs before and after CHF. Briefly, myocardial biopsies were obtained at the time of surgery and after 4–5 wk of pacing before euthanization of the animals. The tissue specimens were incubated in cold (4°C) 2.3%-butanediene monoxide (BDM; Sigma) protective solution (pH 7.3, osmolality 285 mosm/l) consisting of basal solution HBS-1, which contained the following (in mM): 130 NaCl, 5.4 KCl, 2.0 NaHCO₃, 15.0 glucose, and 10.0 HEPES, and added 1.2 mM MgSO₄·7H₂O plus 30 mM BDM and 10 μl insulin (0.01 μl/ml). The tissue specimens were minced into 2- to 3-mm³ cubes within the protective solution at 4°C, then transferred to a sterilized centrifuge tube, and maintained in this continuous oxygen-equilibrated protective solution for 30 min at room temperature. The enzymatic digestion was initiated by adding 6 ml of prewarmed 0.2% trypsin solution made of 6 ml of basal solution HBS-1 plus 12 mg of trypsin type XI (DPCC treated, 0.2%, Sigma), and the specimens were gently agitated in a water bath for 5 min at 37°C. Collagenase (0.05%) was then prepared from the basal solution of HBS-1 (100 ml) plus 50 mg of collagenase (Worthington No. M 9b2717) and 200 mg of BSA (fraction V, fatty acid free, Sigma) and prewarmed to 37°C. The supernatant with broken cells was removed after specimens were gently agitated in a water bath, and fresh collagenase solution was added every 5 min. After 20 min, the dispersed myocytes were checked under a microscope. The isolated myocytes were then collected using transfer pipettes in sterilized centrifuge tubes on ice containing 5 ml of cold protective solution. The myocytes were washed twice by gentle centrifugation at 500 rpm for 1 min. The cell pellet was then resuspended in HBS-1 solution. After each settling, the HBS-1 solution was changed stepwise to increase calcium concentrations (i.e., 250, 500, and 1,000 μM). Finally, the cells were suspended in HBS-1 with 1.8 mM CaCl₂ and stored at room temperature until ready for use.

The numbers of viable cells were counted at ×100 magnification using a hemocytometer (Cambridge Instruments). The yield of viable myocytes from the LV biopsies was similar (84 ± 5.8%) both before and after CHF. At room temperature (22°C), the isolated myocytes maintained a rod-shaped morphology for ~21–32 h. An overall viability of 70% is indicative of a good-quality isolation (38) and is consistent with previous studies (5, 8).

Myocyte function. As we have described previously (6, 41), the measurements of myocyte dimensions and functional responses were made in 60–80 randomly selected, rod-shaped cells from each experiment. After stabilization, steady-state data were recorded. The myocytes were then randomly exposed to L-748,337 (10⁻⁷ M), which blocked the contractile response to BRL (10⁻⁸ M) but had no effect on the response to epinephrine (10⁻⁷ M) and zinterol (10⁻⁶ M). Data were acquired after drug exposure and after drug washout. Preliminary studies showed that myocyte baseline contraction and relaxation as well as myocyte contractile response to L-748,337 (10⁻⁷ M) measured at 22 and 37°C were similar. Thus data were reported only for myocytes studied at 22°C from 10 animals.

In the second group, myocytes were exposed to isoproterenol (10⁻⁹–10⁻⁶ M), and data were acquired after 3–5 min of drug exposure and 5–15 min after drug washout. We found that 10⁻⁸ M, a physiologically relevant concentration of isoproterenol (17), was well tolerated by both normal and CHF myocytes and caused nearly maximum response in the percent shortening. Higher concentrations of isoproterenol were frequently associated with spontaneous contractions in CHF myocytes.

Myocytes were perfused with nadolol (10⁻⁵ M) (8), and then isoproterenol (10⁻⁸ M) was added. In a subgroup, this protocol was followed by superfusion of L-748,337 (10⁻⁷ M).

Statistical Analysis

Data are summarized as means ± SD. Multiple comparisons were performed by ANOVA. When a significant overall effect was present, intergroup comparisons were performed by using a Bonferroni correction for multiple comparisons. In each animal, the measurements of myocyte contraction and intracellular Ca²⁺ concentration ([Ca²⁺]ι) transient were averaged from myocytes studied. The mean differences in cell dimensions, cell dynamics, and indo-1 AM fluorescence ratios of the normal and CHF myocytes obtained from the animals before and after CHF were obtained. Significance was established as P < 0.05.

Drugs

(5S)-N-[4-[2-{{3-[3-(acetamidomethyl)phenox y]-2-hydroxypropyl]- amino}ethyl]phenyl]benzenesulfonamide (L-748,337) was a gift from Merck Research Laboratories (Rahway, NJ). BRL was obtained from Tocris (Ballwin, MO). Zinterol was provided by Squibb Pharmaceutical Research Institute (Princeton, NJ). Nadolol, isopro-
terenol, dobutamine, and epinephrine were obtained from Sigma (St. Louis, MO).

RESULTS

Evaluation of L-748,337 as a $\beta_2$-ANT

Before CHF, BRL decreased LV end-systolic pressure (19 ± 4 mmHg), increased HR (29 ± 10 beats/min), and decreased dP/dt max ($P < 0.05$) consistent with our previous report (7). BRL caused rightward shifts of three LV P-V relations with significant decreases in $E_{es}$ (20%), 6.3 ± 1.8 vs. 7.9 ± 1.9 mmHg/ml; $dE/dt_{max}$ (18%), 99.8 ± 10.3 vs. 121.3 ± 11.6 mmHg·s⁻¹·ml⁻¹, and $M_{SW}$ (12%), 70.1 ± 2.5 vs. 80.2 ± 29.9 mmHg/ml), indicating that in the normal intact LV, BRL had a negative inotropic effect. After pretreatment with L-748,337 (50 µg/kg iv), BRL failed to decrease dP/dt max and LV-P-V relations, demonstrating an adequacy of $\beta_3$-AR blockade produced by L-748,337.

Dobutamine (6 µg·kg⁻¹·min⁻¹ iv) and epinephrine (0.2 µg·kg⁻¹·min⁻¹ iv) produced a 36% and 45% increase in $E_{es}$, respectively. After pretreatment with L-748,337 (50 µg·kg⁻¹·min⁻¹ iv), these responses persisted. However, the addition of nadolol, a $\beta_1$- and $\beta_2$-ANT, prevented these dopbutamine- and epinephrine-induced positive inotropic responses, demonstrating that L-748,337 blocks $\beta_3$-ARs but not $\beta_1$- and $\beta_2$-ARs.

Effects of Pacing-Induced CHF

In dogs with pacing-induced CHF, plasma norepinephrine levels increased from 377 ± 112 to 2,908 ± 781 pg/ml. Consistent with our previous reports (4, 5, 6, 28, 29, 40) after 4–5 wk of rapid pacing, end-diastolic volume (43.2 ± 8.9 vs. 57.9 ± 9.8 ml) and end-diastolic pressure (7.2 ± 1.8 vs. 33.2 ± 3.6 mmHg) significantly increased, whereas ejection fraction (36.8 ± 3.1% vs. 18.9 ± 2.3%) was significantly reduced due to decreased stroke volume (15.9 ± 2.7 vs. 11.0 ± 2.0 ml). There was a progressive LV spherical dilation. The length of the myocyte was increased (from 118 ± 7 to 151 ± 9 µm) and the length-width ratio was greater (51 ± 7.3%) than the normal cells. After CHF, there was a 39% decrease in LV contractility as measured by $E_{es}$ (3.8 ± 0.7 vs. 6.2 ± 0.9 mmHg/ml) and a significantly slower rate of relaxation as measured by $\tau$ (46.4 ± 4.2 vs. 28.4 ± 1.9 ms), which correlated with 46% and 58% reductions of isolated myocyte contraction [systolic amplitude (SA) 5.8 ± 0.9 vs. 10.8 ± 1.5%] and relaxation ($dR/dt_{max}$, 33.5 ± 4.2 vs. 79.3 ± 10.6 µm/s), respectively (Tables 1 and 2 and Figs. 1 and 2).

Effect of Endogenous $\beta_3$-AR Stimulation on LV Performance

Before CHF, blockade of $\beta_3$-ARs with L-748,337 had no effect on HR or LV end-systolic pressure or end-diastolic volume, but resulted in a leftward shift of the LV end-systolic pressure-end-systolic volume relation with significantly increased $E_{es}$ (18%, 7.3 ± 1.2 vs. 6.2 ± 0.9 mmHg/ml), which was accompanied with similar significant increases in the slopes and leftward shifts ($dE/dt_{max}$, 15%, 59.5 ± 6.0 vs. 51.7 ± 4.4 mmHg·s⁻¹·ml⁻¹; $M_{SW}$, 11%, 78.6 ± 2.7 vs. 70.8 ± 2.8 mmHg; $P < 0.05$). L-748,337 also decreased $\tau$ (Table 1 and Fig. 1). Similar findings were also observed after autonomic blockade and at a matched HR by right atrial pacing (Fig. 3). These effects were augmented after CHF. After CHF, in response to L-748,337, HR, end-systolic pressure, and end-diastolic volume remained relatively unchanged. However, L-748,337 produced significantly greater rel-

| Table 1. Effects of L-748,337 on steady-state hemodynamics |
|---|---|---|---|
| | Control | L-748,337 | After CHF |
| Heart rate, beats/min | 107 ± 11 | 109 ± 10 | 128 ± 12* |
| Peak +dP/dt, mmHg/s | 2.627 ± 284 | 2.769 ± 295† | 1.520 ± 323* |
| Peak –dP/dt, mmHg/s | -2.240 ± 206 | -2.399 ± 153† | -1.519 ± 243† |
| LV end-diastolic pressure, mmHg | 7.2 ± 1.8 | 6.4 ± 1.9 | 33.2 ± 3.6* |
| LV end-systolic pressure, mmHg | 109.8 ± 7.3 | 109.4 ± 5.8 | 99.2 ± 5.7 |
| Minimum LV pressure, mmHg | 1.6 ± 1.9 | 1.2 ± 1.8 | 20.4 ± 1.7† |
| Mean LA pressure, mmHg | 6.3 ± 1.9 | 5.5 ± 2.0 | 26.9 ± 4.2* |
| LV end-diastolic volume, ml | 43.2 ± 8.9 | 42.8 ± 8.7 | 57.9 ± 9.8* |
| LV end-systolic volume, ml | 27.3 ± 7.3 | 26.5 ± 7.6† | 46.9 ± 6.6* |
| Stroke volume, ml | 15.9 ± 2.7 | 16.2 ± 2.9 | 11.0 ± 2.0* |
| $E_{es}$, mmHg/ml | 6.9 ± 1.0 | 6.7 ± 1.9 | 9.0 ± 1.0* |
| $\tau$, ms | 28.4 ± 1.9 | 26.8 ± 1.0† | 46.4 ± 4.2* |
| $E_{es}/E_{es}$ | 6.2 ± 0.9 | 7.3 ± 1.2† | 3.8 ± 0.7* |
| $E_{es}/E_{es}$ | 0.90 ± 0.12 | 1.10 ± 0.20 | 0.42 ± 0.09* |
| SW/PV | 0.64 ± 0.05 | 0.69 ± 0.02† | 0.41 ± 0.09* |

Values are means ± SD; n = 10. CHF, heart failure; $dP/dt$, first derivative of left ventricular (LV) pressure; LA, left atrial; $E_{es}$, arterial elastance; $\tau$, time constant of LV relaxation; $E_{es}$, slope of linear end-systolic pressure-end-systolic volume relation (values are means ± SE); SW, stroke work; PVA, pressure-volume area; *$P < 0.05$, CHF control vs. normal control; †$P < 0.05$, L-748,337 vs. corresponding control value; ‡$P < 0.05$, L-748,337-induced percent changes after CHF vs. before CHF.

| Table 2. Effect of L-748,337 on myocyte contractile performance and [Ca²⁺], transient |
|---|---|---|---|
| Before CHF | L-748,337 | After CHF |
| Resting length, µm | 118.1 ± 7.4 | 117.9 ± 7.5 | 151.4 ± 8.6* |
| Percent shortening | 10.8 ± 1.5 | 11.2 ± 1.9 | 5.8 ± 0.9* |
| Velocity of shortening, µm/s | 102.4 ± 8.1 | 103.2 ± 9.2 | 56.3 ± 8.5* |
| Velocity of shortening, µm/s | 79.3 ± 10.6 | 80.9 ± 10.6 | 33.5 ± 4.2* |
| Amplitude of [Ca²⁺], transient | 0.31 ± 0.03 | 0.31 ± 0.04 | 0.25 ± 0.01* |

Values are mean ± SD; n = 10. [Ca²⁺], intracellular Ca²⁺ concentration. *$P < 0.05$, CHF control vs. normal control; †$P < 0.05$, L-748,337 vs. corresponding control value.
active increases in $E_{\infty}$ (34%, 5.1 ± 0.8 vs. 3.8 ± 0.7 mmHg/ml), $dE/dt_{\max}$ (39%, 44.9 ± 7.8 vs. 32.3 ± 5.4 mmHg·s$^{-1}$·ml$^{-1}$), and $M_{SW}$ (32%, 66.3 ± 5.4 vs. 50.2 ± 6.4 mmHg) and had a greater reduction in $\tau$ (Table 1 and Fig. 1). After CHF, L-748,337 caused decreases in end-diastolic pressure and mean LA pressure with an associated downward shift of the early diastolic position of the LV P-V loop (Fig. 1). In addition, LV arterial coupling and the efficiency of conversion of mechanical energy to external work of the heart were significantly improved by L-748,337 after CHF (Table 1).

Effect of $\beta_3$-AR Activation on Myocyte Contractile and $[Ca^{2+}]$, Transient Responses

Effect of $\beta_3$-ANT. As summarized in Table 2 and displayed in Figs. 2 and 4, L-748,337 had no effect on normal myocytes. However, in CHF myocytes, L-748,337 produced significant increases in percent shortening (SA), velocity of shortening ($dL/dt_{\max}$), velocity of relengthening ($dR/dt_{\max}$), and $[Ca^{2+}]$, transients.

Effect of isoproterenol. Isoproterenol (10$^{-8}$ M) in normal myocytes resulted in significant increases in SA (69%, 17.9 ± 1.1 vs. 10.6 ± 1.9%), $dL/dt_{\max}$ (206.5 ± 31.0 vs. 96.9 ± 24.0 μm/s), $dR/dt_{\max}$ (129.6 ± 33.0 vs. 66.9 ± 14.5 μm/s), and $[Ca^{2+}]$, transients (0.32 ± 0.01 vs. 0.28 ± 0.01%). In contrast, in CHF myocytes, the isoproterenol-induced increases in SA (20%, 7.1 ± 1.9 vs. 5.9 ± 2.1%), $dL/dt_{\max}$, $dR/dt_{\max}$, and $[Ca^{2+}]$, transients (0.26 ± 0.02 vs. 0.24 ± 0.01%) were significantly reduced.

Effect of $\beta_3$- and $\beta_3$-ANT with and without isoproterenol. In normal myocytes, superfusion of nadolol alone or in combination with isoproterenol produced no significant change in cell contraction, relaxation, or $[Ca^{2+}]$, transients. In contrast, in CHF myocytes, superfusion of nadolol significantly decreased SA (5.3 ± 1.8 vs. 6.1 ± 1.4%), $dL/dt_{\max}$, $dR/dt_{\max}$, and the peak $[Ca^{2+}]$, transient. The addition of isoproterenol caused further reductions in SA (4.7 ± 1.6 vs. 5.3 ± 1.8%), $dL/dt_{\max}$, and $dR/dt_{\max}$. The peak $[Ca^{2+}]$, transient also significantly decreased in CHF myocytes (Figs. 4 and 5). These responses were abolished by adding L-748,337 to the bath.

DISCUSSION

We investigated the effects of endogenous $\beta_3$-AR activation in a model of CHF that mimics many of the functional and neurohormonal changes of clinical CHF (2, 5, 6). After CHF, blocking $\beta_3$-AR improved LV contraction and relaxation. Thus stimulation of $\beta_3$-AR by the high levels of endogenous catecholamines in CHF contributes to the impaired LV contraction and relaxation.

$\beta_3$-ARs were initially found to be widely expressed in adipose tissue (19). Stimulation of $\beta_3$-ARs in adipose tissue increases lipolysis and has been studied as a potential target for antiobesity and antidiabetes drugs (1). Recently, $\beta_3$-ARs have been identified in the heart, where they produce a negative inotropic effect (8, 12, 13, 18, 25). These effects are mediated through G$i$ proteins and involve NO-dependent and -independent effects (8, 13). In the normal heart, $\beta_3$-ARs may exert a counterregulation against excessive positive inotropy from adrenergic stimulation. $\beta_3$-ARs have been found to be upregulated in patients with CHF (25) and in dogs with pacing-induced CHF (8). Although upregulation of cardiac $\beta_3$-ARs in
CHF has been demonstrated, its functional significance in CHF has not previously been determined. Our present findings demonstrate the functional significance of the increased β3-AR gene expression previously reported in the failing human and canine hearts (8, 25).

We found that in conscious animals, blocking β3-AR with L-748,337 improved LV contraction and relaxation both before and after CHF. These effects are independent of the changes of HR, reflexes, and loading conditions. The effects were more marked after CHF. This is probably the result of much higher levels of endogenous catecholamines and upregulation of β3-ARs in CHF. In CHF, there is sustained SNS activation. This leads to desensitization of cardiac β1- and β2-ARs, which causes a decrease in the contractile response to β-AR agonists (22). In contrast, β3-ARs are activated at higher catecholamine concentrations than β1- and β2-ARs and are relatively resistant to chronic, agonist-induced desensitization (20, 21, 24, 30). In failing hearts, β3-AR abundance is increased (8, 13, 24). In
addition, Gi proteins, implicated in β3-AR signaling, are also elevated in failing myocardium (11). All of these mechanisms may contribute to the increased β3-AR-mediated cardiac depression we observed in CHF. β3-AR stimulation has been reported to produce vasodilatation primarily in the skin (35). We found that the β3-AR agonist BRL exerted a vasodilating action, decreasing end-systolic pressure, end-diastolic volume, and total systolic re-

Fig. 4. Examples of the effect of nadolol (Nad; a β1- and β2-AR antagonist) with and without the presence of isoproterenol (Iso; β1-, β2-, and β3-AR agonist) on myocyte contractile and [Ca2+]i transient responses in myocytes from the LV of an animal before and after CHF. In the normal myocytes, Nad had no effect on cell contraction or relaxation. In contrast, after CHF, Nad decreased percent shortening (SA), dL/dtmax, and dR/dtmax. The peak systolic [Ca2+]i transient was also reduced. The addition of Iso to Nad caused further decreases in SA, dL/dtmax, dR/dtmax, and [Ca2+]i transient in the CHF myocyte, suggesting the functional significance of β3-ARs in CHF.

Fig. 5. Group means (±SD) of β3-antagonist or Nad-induced changes in myocyte contraction and [Ca2+]i transients before and after CHF. Compared with control, in the normal myocytes, superfusion of L-748,337 caused no significant changes in SA and [Ca2+]i transients; with the blockade β1- and β2-ARs with Nad, both SA and [Ca2+]i transients tended to decrease. However, these changes failed to reach statistical significance. In contrast, in CHF myocytes, L-748,337 significantly increased SA and [Ca2+]i transients. In contrast, superfusion of Nad significantly decreased SA and the peak [Ca2+]i transient. The addition of Iso to the superfusion caused further reductions in SA and [Ca2+]i transients. These responses were abolished by adding L-748,337 in the superfusion.
tance and subsequently producing a tachycardia through baroreflex activation. However, blocking endogenous β3-AR with L-748,337 failed to produce vasodilating action, and the HR was unchanged. These findings are consistent with the observations made in beagle dogs by Pelat et al. (30) and suggest that endogenous β3-AR activation does not have much effect on HR or blood pressure in CHF.

Our findings are comparable with the past reports on the effects of exogenous β3-AR agonists (7, 18, 25) but contrary to the findings of Tavernier et al. (42), who reported no response to β3-AR agonists in the normal dog heart.

We also studied the effects of β3-AR stimulation on cardiac myocytes isolated from the LV before and after CHF. These studies removed the effects of extracardiac factors that may influence contractility. We found that the β3-ANT increased the contraction and relaxation of CHF cardiac myocytes. This occurred even though no catecholamines had been added to the bathing solution. This effect may have been due to reversal of β3-AR stimulation by retained catecholamines because the CHF animals had very high circulating catecholamine levels. Alternatively, they might be due to some other effect of the β3-ANT, such as xanthine oxidase (XO) inhibition. L-748,337 is a selective β3-ANT with a high affinity for human β3-ARs. However, the structure of L-748,337 has not been thoroughly investigated. As yet, the specific portion of the molecule responsible for this activity is unclear (3). It is unknown whether L-748,337 may have other properties such as XO inhibition. Ekelund et al. (10) recently demonstrated a fourfold increase in myocardial XO in dogs with pacing-induced CHF. We observed that L-748,337 had no effect on normal myocytes from animals with much lower catecholamine levels. Additionally, we found that adding isoproterenol (10⁻⁸ M) to the bath in the presence of a β1- and β2-ANT (nadolol) impaired contraction and relaxation of the CHF myocytes. These responses were abolished after the addition of the β3-ANT. It is clear that further studies on the properties of L-748,337 are warranted.

Exogenous β3-AR stimulation has negative inotropic effects in both normal and CHF LV myocytes (11, 12, 18, 25). However, in the present study, β3-ANTs only produced a slight increase in the normal LV contraction and produced no apparent inotropic effect in the normal myocytes. In normal myocytes, adding a physiologically relevant concentration of isoproterenol (10⁻⁸ M) to the superfusion in the presence of nadolol also failed to cause significant alterations in cell contractile performance. This may be due to the high isoproterenol doses that are required to activate β3-ARs in normal myocytes.

Potential Limitations of Methods

Several methodological issues should be considered in interpreting our data.

First, we used endocardial diameter gauges to measure LV volume. This technique has been extensively validated in past studies and accurately reflects relative LV volume under a wide variety of normal and pathological conditions. We have further evaluated the effect of shape changes by assessing the constancy of calculated LV volume during isovolumic relaxation, when the actual LV volume is constant but the LV shape changes. The accurate measurement of LV volume using endocardial diameter gauges depends on proper alignment of the crystals, and some crystals may not be precisely placed at the endocardium, leading to errors in the estimation of absolute ventricular volume. This might explain the relatively low ejection fractions also reported in normal dogs by Rankin et al. (33), Miyazaki et al. (23), and Morita et al. (26). Moreover, the instrumentation may produce some LV damage, thus potentially depressing LV performance. However, after recovery from the operation, the animals had good exercise tolerance, and the HR, LV pressure, peak dP/dt, and cardiac output were all within the normal range.

Second, we studied an animal model of CHF (pacing tachycardia) that reproduces many of the functional and neurohormonal features of clinical CHF, but we cannot be certain that our results apply to CHF of other causes. In addition, this study was performed in animals with a stable CHF. If the animals had been in a more extreme degree of CHF, blocking β3-ARs might show more marked improvement on LV and myocyte functional performance.

Third, we used enzymatic dissociation to isolate myocyte from biopsy canine heart tissue before and after CHF. Because not all cells recover after enzymatic dissociation, there is a potential sampling bias toward those cells that survived. To avoid the potential sampling bias, myocyte function evaluation was performed by three different people. In addition, after each drug superfusion, data were again collected after washout of the drug to verify the true drug effect. This bias could be further complicated by the possibility that a different subpopulation of cells survived from the CHF heart tissue. However, we and others previously reported (5, 8, 36) that the individual myocyte isolated by this technique in a pacing-induced canine model retained the morphological and contractile properties similar to those observed in intact muscle. Over the years, we have consistently obtained a high yield of viable myocytes using this preparation from both normal and CHF heart tissues. Furthermore, evidence of CHF-induced changes was clearly demonstrated by the alterations in the morphology, contraction, and relengthening of myocytes isolated after CHF. Thus our observation of altered response to β3-AR in CHF myocytes is unlikely to be due to sampling bias or artifacts introduced by the enzymatic isolation process.

Chronic therapy of CHF patients with a relatively selective β1-AR (metoprolol and bisoprolol) or nonselective β1- and β2-AR blockers (carvedilol) improves survival and LV contractile performance (15, 22, 31). However, acute β-AR blockade in CHF leads to a depression in LV performance. Recent studies indicate that all these β-AR blockers bind to β1-ARs. Differential binding properties to β3-AR might imply different responses (22, 34). Metoprolol appears to leave β3-ARs relatively unblocked (8). Recent evidence indicates that carvedilol also has a very high affinity for β3-ARs (3, 16, 34). Our observations suggest that some of the acute cardiac depression seen with initiating therapy with metoprolol may be partly due to leaving the depressive effects of β3-AR unopposed. It is possible that the addition of β3-AR blockade could improve the acute tolerability and benefit of β1- and β2-AR blockade.

Our findings have several other potential clinical implications. First, β3-AR blockade may provide a pharmacological method of inotropic support for the failing heart. This might not be accompanied by the adverse effects of β1- and β2-AR stimulation. Secondly, it is possible that chronic β3-AR block-
ade might have favorable effects in CHF, but this was not addressed by our acute studies. Finally, our findings also indicate that using β3-AR agonists for the treatment of obesity and diabetes (1) may have cardiac side effects, especially in CHF patients.

In conclusion, this study demonstrates that in pacing-induced CHF, β3-AR activation by endogenous catecholamine exacerbates LV and cardiomyocyte systolic and diastolic dysfunction.

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