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Cardiac dilatation and pump dysfunction without intrinsic myocardial systolic failure following chronic β-adrenoreceptor activation

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Osadchii OE, Norton GR, McKechnie R, Deftereos D, Woodiwiss AJ. Cardiac dilatation and pump dysfunction without intrinsic myocardial systolic failure following chronic β-adrenoreceptor activation. *Am J Physiol Heart Circ Physiol* 292: H1898–H1905, 2007. First published December 8, 2006; doi:10.1152/ajpheart.00740.2006.—There is no direct evidence to indicate that pump dysfunction in a dilated chamber reflects the impact of chamber dilatation rather than the degree of intrinsic systolic failure resulting from myocardial damage. In the present study, we explored the relative roles of intrinsic myocardial systolic dysfunction and chamber dilatation as mediators of left ventricular (LV) pump dysfunction. Administration of isoproterenol, a β-adrenoreceptor agonist, for 3 mo to rats (0.1 $mg \cdot kg^{-1} \cdot day^{-1}$) resulted in LV pump dysfunction as evidenced by a reduced LV endocardial fractional shortening (echocardiography) and a decrease in the slope of the LV systolic pressure-volume relation (isolated heart preparations). Although chronic β-adrenoreceptor activation induced cardiomyocyte damage (deoxynucleotidyl transferase-mediated dUTP nick-end labeling) as well as β_1- and β_2-adrenoreceptor inotropic downregulation (attenuated contractile responses to dobutamine and salbutamol), these changes failed to translate into alterations in intrinsic myocardial contractility. Indeed, LV midwall fractional shortening (echocardiography) and the slope of the LV systolic stress-strain relation (isolated heart preparations) were unchanged. A normal intrinsic myocardial systolic function, despite the presence of cardiomyocyte damage and β-adrenoreceptor inotropic downregulation, was ascribed to marked increases in myocardial norepinephrine release, to upregulation of α-adrenoreceptor-mediated contractile effects as determined by phenylephrine responsiveness, and to compensatory LV hypertrophy. LV pump failure was attributed to LV dilatation, as evidenced by increased LV internal dimensions (echocardiography), and a right shift and increased volume intercept of the LV diastolic pressure-volume relation. In conclusion, chronic sympathetic stimulation, despite reducing β-adrenoreceptor-mediated inotropic responses and promoting myocyte apoptosis, may nevertheless induce pump dysfunction primarily through LV dilatation, rather than intrinsic myocardial systolic failure.

isoproterenol; cardiac remodeling; contractility; inotropic responses

THERE IS A STRONG RELATIONSHIP between increased cardiac chamber dimensions (cardiac dilatation) and poor clinical outcomes. In otherwise healthy people, cardiac chamber enlargement is associated with an increased morbidity and mortality (20, 27) and the development of heart failure (34). Moreover, chamber dimensions predict clinical outcomes in patients with either mild or severe heart failure (19, 21, 32, 37). The contemporary explanation for the relationship between chamber dimensions and clinical outcomes is that chamber enlargement reflects a compensatory response of the failing myocardium to restore stroke volume (the Frank-Starling effect). Any potential benefits of chamber enlargement are, however, offset by increases in ventricular wall stress (Laplace's law) and further decreases in pump function. However, as cardiac chamber dilatation usually coexists with myocardial damage and dysfunction, the extent to which pump dysfunction in a dilated chamber reflects the impact of chamber dilatation or the degree of underlying myocardial systolic failure is uncertain. Recent evidence obtained by our group suggests that the presence of cardiac dilatation and pump dysfunction may be independent of the degree of underlying intrinsic myocardial systolic dysfunction (3, 23, 35). Nevertheless, these studies were conducted in pressure-overload states where, even without pump failure, intrinsic myocardial systolic dysfunction may still occur (23). Moreover, in these studies, we were unable to explain a preserved intrinsic myocardial systolic function following chronic β-adrenoreceptor (β-AR) activation, despite the likelihood that β-AR inotropic downregulation (7, 14) or cardiac myocyte apoptosis (25, 28) may have occurred. The primary aim of the present study was therefore to clarify whether cardiac dilatation can occur independent of intrinsic myocardial systolic failure and consequently drive pump dysfunction. For this purpose, we assessed the impact of chronic β-AR activation on cardiac geometry and function in the absence of pressure-overload hypertrophy using a model previously described (38). Moreover, in this same model, we aimed to identify the potential mechanisms of a preserved intrinsic myocardial systolic function despite the possibility of simultaneous β-AR inotropic downregulation and cardiac myocyte damage/apoptosis.

METHODS

Animal model. The present study complies with the *Guide to the Care and Use of Experimental Animals* and was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance number 2003/77/3). Male Sprague-Dawley rats weighing 350–500 g were used in the present study. Chronic adrenergic activation was achieved by daily administration of isoproterenol (Iso), a nonselective β-AR agonist, at a dose of 0.1 mg/kg given intraperitoneally daily for 3 mo. On the basis of our findings that 1 mo of Iso administration produces no impact on pump function (24) and that 6 mo of Iso administration produces advanced pump dysfunction with marked chamber dilatation (38), we selected an interim period (3 mo) for the present study to study that period in which the transition to pump failure occurs. We used a low dose of Iso to avoid the

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development of marked myocyte necrosis, a finding usually observed at higher concentrations of this agent (4, 8, 31). Control rats received daily injections of the saline vehicle (≈0.2 ml).

Echocardiography and systolic blood pressures. To determine left ventricular (LV) chamber function, intrinsic myocardial systolic function, and LV dimensions in vivo, echocardiography was performed 24 h after the last dose of Iso or vehicle, using a Hewlett-Packard Sonos 2000 sector scanner and a 7.5-MHz transducer, as previously outlined (23, 38). All measurements were obtained 15 min after an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (15 mg/kg). LV internal dimensions were measured according to the American Society for Echocardiography's leading edge method (26). Measurements were made from three consecutive beats, and endocardial (FS_{end}) and midwall (FS_{mid}) fractional shortening were determined as previously described (23, 24). LV FS_{end} and FS_{mid} were used as load-dependent indexes of LV chamber and intrinsic myocardial function, respectively. Systolic blood pressure was measured using a tail-cuff technique as previously described (35).

Isolated, perfused heart preparations. Load-independent measures of LV systolic chamber and intrinsic myocardial function as well as contractile responses to inotropic agents were determined ex vivo under controlled conditions as previously outlined (23, 24, 38). Briefly, rats were anesthetized with intraperitoneal injections of ketamine and xylazine (75 and 15 mg/kg, respectively) ~24 h after the last dose of Iso or vehicle. The chest was opened, and hearts were immediately excised and mounted on an isolated, perfused heart apparatus. The hearts were retrogradely perfused via the aorta at a constant flow with carefully filtered, warmed (37°C) physiological saline solution saturated with 95% O_2-5% CO_2. The solution contained (in mM) 118.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, and 10.0 glucose and had a pH of 7.4. The coronary flow rate was measured by collecting coronary effluent and was adjusted to achieve a rate of 10 ml/min per gram of heart weight. The mean coronary perfusion pressures were 79.8 ± 1.7 and 77.3 ± 1.3 mmHg for the control and Iso-treated groups, respectively. To exclude rate-dependent variations in contractility, the cardiac preparations were paced at a constant frequency of 330 beats/min via platinum wire electrodes attached to the right atrium and the apex of the LV with the voltage 10% above threshold. In our hands, this heart rate is lower than that which induces ischemic-induced increases in filling pressures in crystalloid-perfused rat hearts. To record LV developed pressure, the left atrium was trimmed and a water-filled balloon-tipped cannula coupled to a Statham P23 pressure transducer was introduced through the atrioventricular orifice into the LV cavity. LV developed pressure, recorded on a Hellige polygraph, was calculated as the difference between end-systolic and end-diastolic pressure. The maximum rate of LV pressure development ($+dP/dt$) was obtained using a differentiator (model 13-4616-71; Gould Instrument Systems, Valley View, OH) with a high-frequency cutoff set at 300 Hz.

Baseline LV systolic function and chamber dimensions ex vivo. Baseline LV systolic function and chamber dimensions were assessed by reconstructing LV systolic and diastolic pressure-volume relations from recordings obtained at the beginning of experiments before pharmacological interventions. With the use of a micromanipulator, the LV balloon volume was gradually increased by increments of 0.01 ml starting from 0.16 ml, and LV end-diastolic, developed, and peak systolic pressures were determined at as many multiple small increments in volume as were practically possible. Load-independent measures of systolic chamber function were determined from the slope of the linear portion (defined as those points that achieved $r^2 > 0.98$ on linear regression analysis) of the LV peak systolic pressure-volume relation. Load-independent measures of intrinsic myocardial systolic function were assessed by constructing LV developed systolic stress-strain relations and comparing the slopes of these relationships (23, 24). LV developed systolic stress and strain values were calculated by using previously described equations (36). The LV volume intercept (V_0) of LV diastolic pressure-volume relationship was used as an index of LV chamber dilatation (23, 38).

Pharmacological responses. To evaluate whether Iso administered at the doses employed in this study altered the contractile responsiveness to β-AR or α-AR agonists, isolated, perfused heart preparations were allowed to stabilize for 15 min before assessment of inotropic responses to pharmacological agents. Before the pharmacological assessments, LV volumes were increased to 0.18–0.19 ml to elicit physiologically relevant (80–100 mmHg) LV developed pressures normally recorded in the midportion of LV systolic pressure-volume relation. The values determined in the absence of pharmacological agents are indicated as "baseline" LV developed pressure. The pharmacological agents used in this study were dissolved in the same physiological saline solution used to perfuse the hearts and were infused just proximal to the aortic cannula by means of a Harvard infusion pump (model 22M T/W). The rate of infusion was 0.3 ml/min, and the total duration of infusion of each concentration of each agent was 60 s. Concentration-response relations were constructed by assessing contractile responses to incremental concentrations of pharmacological agents used. Intervals of at least 5 min were allowed between infusions for LV developed pressures to return to baseline values after a preceding pharmacological exposure. Before infusions of active agents, we ascertained that the vehicle produced no LV contractile response. Concentration-contractile response curves were constructed for norepinephrine, a β- and α-AR agonist; dobutamine, a β_1-AR agonist; salbutamol, a β_2-AR agonist; and phenylephrine, an α-AR agonist, to compare the efficacy (maximal inotropic response) and potency (EC_{50}) in control and Iso-treated rats. *dl*-Isoproterenol hydrochloride, *dl*-arterenol hydrochloride (norepinephrine), dobutamine hydrochloride, salbutamol hemisulfate, and *l*-phenylephrine hydrochloride were purchased from Sigma Chemicals (St. Louis, MO).

Myocardial norepinephrine release. To determine whether reduced β-AR-mediated inotropic responsiveness may be compensated for by an increased myocardial norepinephrine release, myocardial norepinephrine was measured in the coronary effluent of isolated, perfused hearts. Samples of coronary effluent were collected for 1 min from hearts of control and Iso-treated rats while perfused at a constant flow rate. Since norepinephrine release decreases when measured at incremental LV filling volumes (35), all measurements were performed at filling volumes of 0.19 ml. For the collection of coronary effluent samples, preparations were paced at the same rate as described in *Isolated, perfused heart preparations* (330 beats/min). The coronary effluent was stabilized by adding Na_2EDTA and $HClO_4$ to 10 ml of coronary effluent to achieve a final concentration of 0.01 mol/l and 0.025%, respectively. Norepinephrine was immediately extracted from 1 ml of coronary effluent by using alumina (Sigma) adsorption with a Tris buffer at pH 8.6 eluted with 0.1 M $HClO_4$ and then stored at −70°C. Norepinephrine concentrations were measured by using reversed phase, ion-exchange high-performance liquid chromatography with electrochemical detection (12). Since all hearts were perfused at the same flow rate per gram of tissue, myocardial norepinephrine release was expressed as the concentration of norepinephrine in the effluent (35).

Myocyte apoptosis. To determine whether Iso administered at the doses employed in this study was able to produce myocardial damage/apoptosis, Iso was administered to rats at 0.1 mg/kg per day for 5 days before cardiac tissue was obtained within a half hour of the last Iso dose. A longitudinal slice of the LV from the apex to the base through the LV free wall was obtained from all rats for histology. LV tissue was stored, prepared, and sectioned as previously described (35, 38). The degree of apoptosis was quantified on 5-μm-thick tissue sections. DNA fragments in the tissue sections were detected by using a nonradioactive in situ apoptotic cell death detection kit [DeadEnd Colorimetric deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) system; Promega, Madison, WI], where TdT is incorporated in the biotinylated nucleotide at the 3′-OH DNA

ends. Horseradish-peroxidase-labeled streptavidin binds to biotinylated nucleotides, which subsequently stains dark brown in response to hydrogen peroxide and diaminobenzidine (1). Both positive (DNase treated) and negative (no addition of TdT) control tissue sections were incorporated in each assay. The number of apoptotic cardiomyocyte nuclei and the total number of cardiomyocyte nuclei (hematoxylin and eosin stain) in each slide were counted on 10 evenly spaced fields from the apex to the base using a computer-based image acquisition and analysis system at ×400 magnification (Axiovision 3, Carl Zeiss, Gottingen, Germany). Apoptotic nuclei were expressed as a percentage of the total number of nuclei.

The degree of tissue fibrosis was also determined on 5-μm-thick tissue sections as previously described (35). After sections were stained with van Gieson's stain, a grade was assigned, where 0 indicates no evidence of patchy fibrosis; 1 and 2 indicate patchy fibrosis in less than or more than 20% of the field, respectively; 3 and 4 indicate diffuse contiguous subendocardial fibrosis in less than or more than 50% of the field, respectively; and 5 and 6 indicate full thickness fibrosis in less than or more than 50% of the field, respectively.

Data analysis. Results are expressed as means ± SE. The magnitude of inotropic responses elicited by pharmacological agents was expressed as a percent increase in LV developed pressure. The concentrations of substances that produced 50% of the maximal contractile response (EC_{50}) were determined from regression analysis using logistic sigmoid function curves (log concentration vs. effect) and presented as pEC_{50} values ($pEC_{50} = -\log_{10} EC_{50}$). The slopes of LV systolic and diastolic pressure-volume and stress-strain relations in Iso-treated and control rats were determined by linear regression analysis. The impact of a range of concentrations of norepinephrine, dobutamine, salbutamol, and phenylephrine on LV developed pressure was assessed by a repeated-measures ANOVA, followed by a Tukey-Kramer post hoc test. Comparisons of all variables between control and Iso-treated rats were performed by an unpaired Student's *t*-test. Probability values <0.05 were considered to be significant.

Table 1. *Impact of chronic Iso administration on cardiac weight in rats*

	Control	Iso Treated
n	29	34
Body weight, g	576±12	596±9
Heart weight, g	1.69±0.03	2.14±0.04*
LV weight, g	1.24±0.02	1.61±0.02*
Right ventricular weight, g	0.39±0.01	0.42±0.02
Heart weight/body weight, g/kg	2.96±0.04	3.60±0.06*
LV weight/body weight, g/kg	2.16±0.03	2.70±0.04*

Values are means ± SE for *n* rats. Iso, isoproterenol; LV, left ventricular. *$P < 0.0001$ vs. control.

RESULTS

Cardiac weights. Chronic Iso administration increased absolute heart and LV weight, as well as heart weight and LV weight-to-body weight ratios (Table 1). Absolute heart and LV weights were increased by ~30% (Table 1).

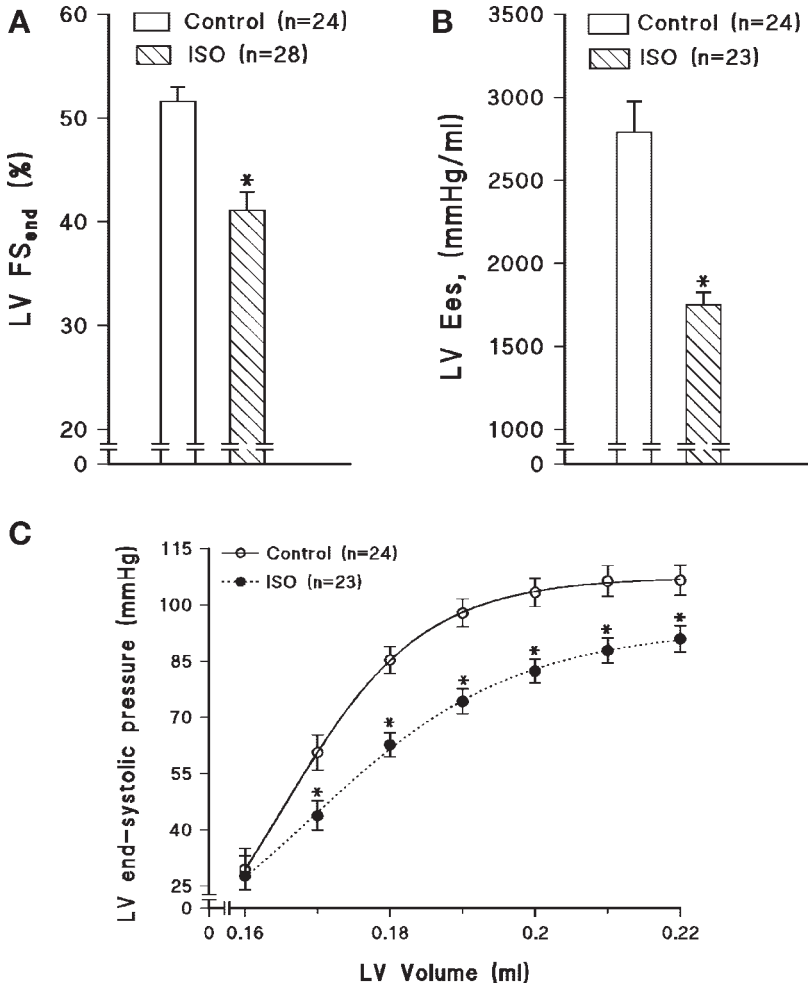

Fig. 1. Impact of chronic isoproterenol (Iso) administration on left ventricular (LV) chamber pump function in rats. LV endocardial fractional shortening (FS_{end}) (*A*) as determined by echocardiography and LV systolic pressure-volume relations (*C*) as determined from isolated, perfused heart preparations are shown. *B* shows the slopes (E_{es}) of LV systolic pressure-volume relations derived from the linear portion of the relations presented in *C*. *$P < 0.01$ vs. control.

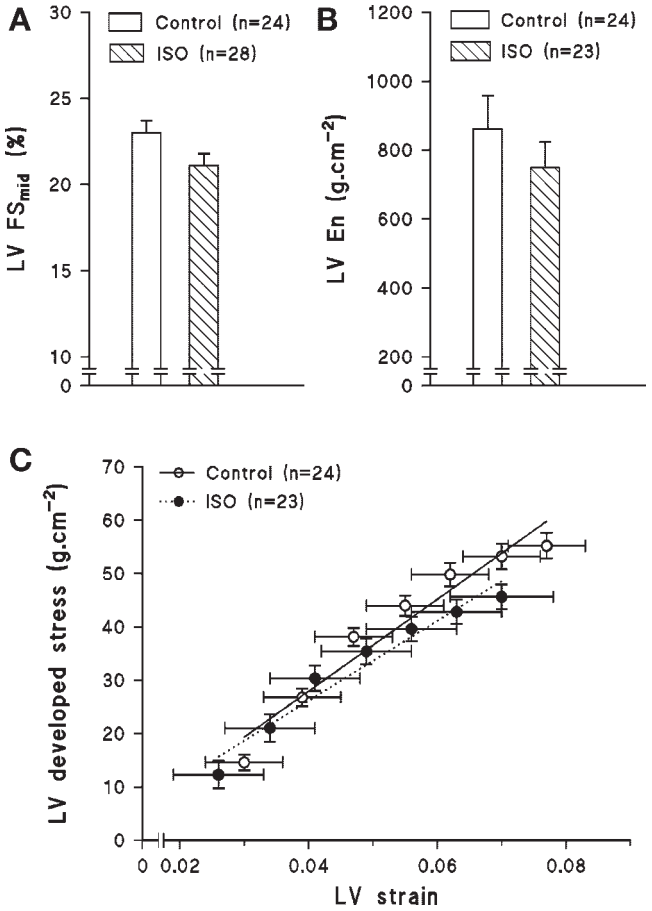

Fig. 2. Impact of chronic Iso administration on LV intrinsic myocardial systolic function in rats. LV midwall fractional shortening (FS_{mid}) (*A*) as determined by echocardiography and LV systolic stress-strain relations (*C*) as determined from isolated, perfused heart preparations are shown. *B* shows the slopes (E_n) of LV systolic stress-strain relations derived from the relations presented in *C*.

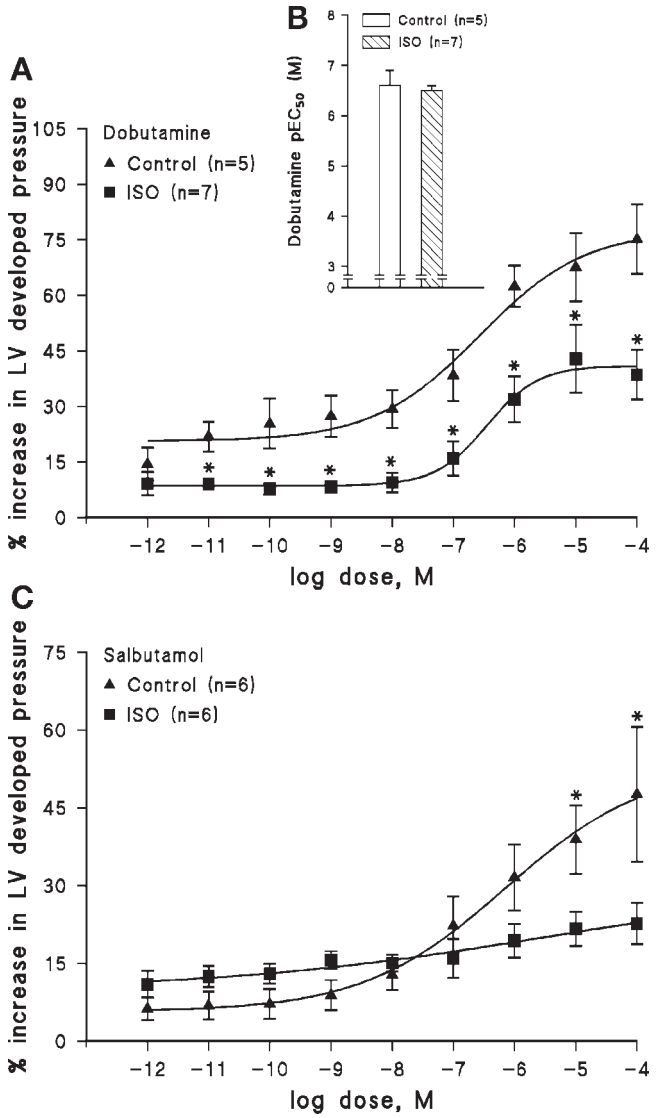

Fig. 3. Impact of chronic Iso administration on LV inotropic responses (percent increase in developed pressure) to dobutamine (*A* and *B*) and salbutamol (*C*) in rats is shown. The abscissa in *A* and *C* represent the log of molar concentrations of agents used. *B* shows dobutamine pEC_{50} values derived from the dose-response relations presented in *A*. Salbutamol pEC_{50} values were not calculated because uniform dose-response relations in Iso-treated rats were noted. *$P < 0.05$ vs. control rats.

LV systolic chamber function. When LV function and contractile responses to pharmacological agents were assessed, coronary flow was adjusted to comparable values in heart preparations taken from control and Iso-treated rats (9.5 ± 0.1 and 9.3 ± 0.2 ml/min per gram of heart weight, respectively; $P = 0.4$).

Chronic Iso administration decreased LV FS_{end} as determined using echocardiography (Fig. 1*A*) and produced a right shift (Fig. 1*C*) and decrease in the slope (end-systolic elastance, E_{es}) of the LV peak systolic pressure-volume relationship (Fig. 1*B*). In intact rats, neither heart rate (echocardiography: control, 289 ± 8 beats/min; Iso treated, 280 ± 7 beats/min) nor systolic blood pressure (tail-cuff assessments: control, 132 ± 5 mmHg; Iso treated, 125 ± 7 mmHg) differed between the groups. In isolated perfused hearts, +dP/d*t* was decreased in the Iso-treated group at all LV filling volumes above 0.17 ml [data at 0.18 ml: control, 1,885 ± 69 ($n = 24$); Iso treated, 1,503 ± 88 ($n = 23$); $P = 0.0015$].

Table 2. *Baseline LV developed pressures and ±dP/dt in control and Iso-treated rats*

	LV Developed Pressure, mmHg		LV ±dP/d*t*, mmHg/s	
	Control	Iso Treated	Control	Iso Treated
Norepinephrine	85±5 (12)	80±5 (15)	1,810±63 (12)	1,740±90 (15)
Dobutamine	86±3 (5)	80±5 (7)	1,824±59 (5)	1,752±84 (7)
Salbutamol	84±4 (6)	79±5 (6)	1,682±97 (6)	1,772±100 (6)
Phenylephrine	83±4 (6)	78±5 (6)	1,769±93 (6)	1,617±64 (6)

Values are means ± SE. Number of animals is given in parentheses. No differences were noted between the groups. ±dP/d*t*, maximum rate of LV pressure development.

Intrinsic myocardial systolic function. In contrast to changes in LV systolic chamber function induced by β-AR activation (Fig. 1), chronic Iso administration failed to modify either LV FS_{mid} as determined using echocardiography (Fig. 2*A*) or alter the slope of the developed systolic stress-strain (E_n) relationship (Fig. 2, *B* and *C*). Thus chronic β-AR activation promoted

LV chamber (Fig. 1) but not intrinsic myocardial systolic (Fig. 2) dysfunction.

β_1- and β_2-AR-induced inotropic responses. Similar baseline LV developed pressures and +dP/d*t* values were noted in control and Iso-treated rats when comparing groups in which hearts were exposed to different adrenergic agonists (Table 2).

Despite a normal intrinsic myocardial systolic function following β-AR activation (Fig. 2), chronic Iso administration produced a marked decrease in inotropic responses to the β_1- and β_2-AR selective agonists dobutamine (Fig. 3*A*) and salbutamol (Fig. 3*C*). Reduced contractile responses to dobutamine were noted over a wide range of concentrations (10^{-11}–10^{-4} M) (Fig. 3*A*). The inotropic efficacy of dobutamine, as assessed by the magnitude of the maximal contractile response achieved at a dose of 10^{-4} M, was reduced following chronic β-AR activation [control ($n = 5$): 75 ± 9%; Iso treated ($n = 7$): 39 ± 7%, $P = 0.02$]. The contractile potency of dobutamine, as determined from pEC_{50} values, was nevertheless unchanged following chronic β-AR activation (Fig. 3*B*). With respect to salbutamol, a uniform concentration-response curve was noted in Iso-treated rats (Fig. 3*C*), thus preventing us from calculating pEC_{50} values in this group. Nevertheless, contractile responses to salbutamol were reduced at the two highest concentrations evaluated (10^{-5} and 10^{-4} M) in Iso-treated as compared with control rats (Fig. 3*C*).

β-AR-induced apoptosis and patchy fibrosis. Although chronic β-AR activation failed to promote a reduction in intrinsic myocardial systolic function (Fig. 2), clear evidence of β-AR-induced apoptosis was noted. After 5 days of Iso administration, a marked increase in the percentage of cardiomyocyte nuclei that were apoptotic was observed [control ($n = 9$): 0.85 ± 0.26%; Iso treated ($n = 10$): 5.02 ± 0.87%, $P = 0.001$] (average number of apoptotic cardiomyocyte nuclei per field: control, 0.38 ± 0.10; Iso treated, 2.12 ± 0.30; average number of cardiomyocyte nuclei per field: control, 49.00 ± 3.44; Iso treated, 45.70 ± 4.48). However, there was no histological evidence of patchy fibrosis after 5 days of Iso administration (fibrotic score: control, 0.33 ± 0.17; Iso treated, 0.71 ± 0.18, $P = 0.15$).

Nonselective α- and β-AR- and selective α-AR-induced inotropic responses. In keeping with a normal intrinsic myocardial function (Fig. 2), although inotropic responses to β-AR agonists were markedly attenuated (Fig. 3), norepinephrine-mediated contractile responses were unchanged following chronic Iso administration (Fig. 4, *A* and *B*). The ability to sustain normal norepinephrine-induced contractile responses despite reduced β-AR-mediated inotropic effects was attributed to upregulation of α-AR-mediated contractile effects. Indeed, phenylephrine-induced increases in LV systolic function following chronic Iso administration were considerably

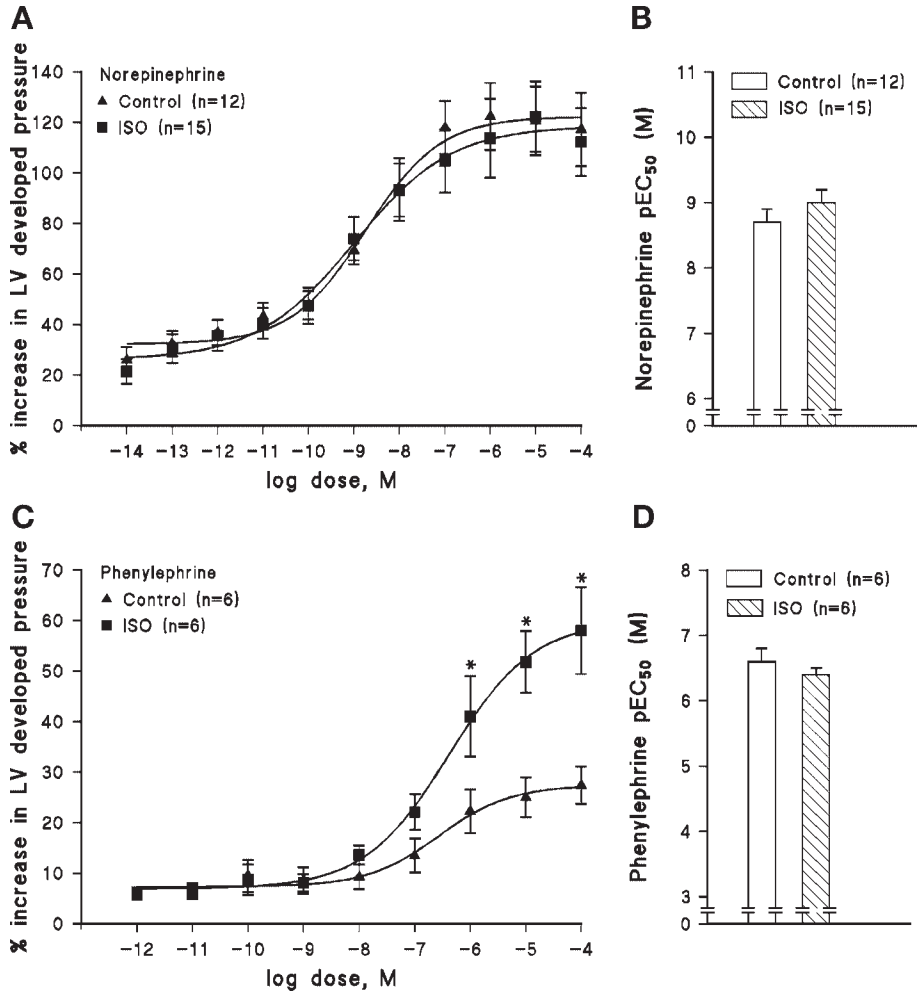

Fig. 4. Impact of chronic Iso administration on LV inotropic responses (percent increase in developed pressure) to norepinephrine (*A* and *B*) and phenylephrine (*C* and *D*) in rats. The abscissa in *A* and *C* represent the log of molar concentrations of agents used. Norepinephrine (*B*) and phenylephrine (*D*) pEC_{50} values were derived from the dose-response relations presented in panels *A* and *C*, respectively. *$P < 0.05$ vs. control rats.

enhanced (Fig. 4*C*). An increased magnitude of phenylephrine-induced contractile responses was noted over the concentration range of 10^{-6}–10^{-4} M in Iso-treated as compared with control rats (Fig. 4*C*). The maximal inotropic response elicited by phenylephrine at a dose of 10^{-4} M was double that of control rats [control ($n = 6$): 27 ± 4%; Iso treated ($n = 6$): 58 ± 9%, $P = 0.01$]. However, chronic β-AR activation did not affect the contractile potency of phenylephrine as evidenced by pEC_{50} values (Fig. 4*D*).

Myocardial norepinephrine release. As coronary flow was similar between rat hearts, myocardial norepinephrine release was determined as norepinephrine concentrations in the coronary effluent. In keeping with a normal intrinsic myocardial systolic function (Fig. 2), despite the presence of marked cardiomyocyte cell loss and downregulation of β-AR-mediated inotropic responses (Fig. 3), markedly higher coronary effluent norepinephrine concentrations were found in Iso-treated as compared with control rats [control ($n = 15$): 0.1 ± 0.01 nM; Iso treated ($n = 15$): 3.1 ± 0.09 nM, $P = 0.003$].

LV chamber dilatation. In the present study, reductions in LV pump function following chronic Iso administration (Fig. 1) were largely attributed to LV dilatation. Indeed, both LV end-systolic and LV end-diastolic dimensions were increased in Iso-treated rats in vivo as determined by echocardiography (Fig. 5*A*). These differences in LV chamber dimensions were not attributed to a reduced intrinsic myocardial function with subsequent increases in filling volumes, but rather to geometric LV remodeling. Indeed, LV diastolic pressure-volume relations were right shifted (Fig. 5*C*), and the volume intercept of the LV diastolic pressure-volume relation (LV V_0) was increased (Fig. 5*B*).

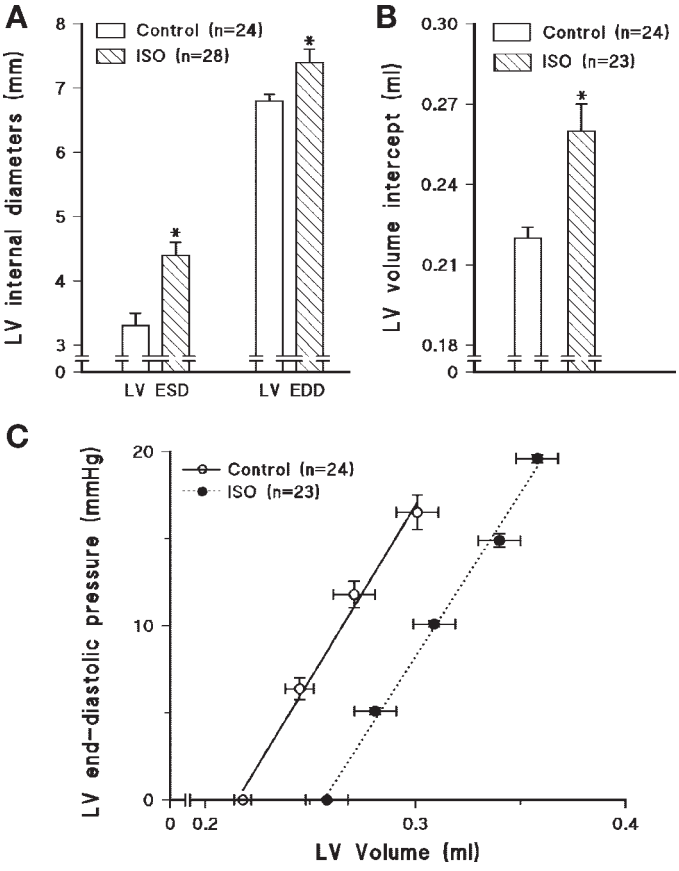

Fig. 5. Impact of chronic Iso administration on LV internal dimensions in rats. LV end-systolic diameter (ESD) and end-diastolic diameter (EDD) as determined by echocardiography (*A*) as well as the LV end-diastolic volume intercept LV (*B*) of the LV diastolic pressure-volume relations (*C*) as determined in isolated, perfused heart preparations are shown. *$P < 0.01$ vs. control.

DISCUSSION

The main findings of the present study are as follows. Chronic β-AR activation promoted reductions in LV systolic chamber function, an alteration that was nevertheless associated with normal overall intrinsic myocardial systolic function. Overall intrinsic myocardial systolic function remained intact despite marked β-AR-induced apoptosis and downregulation of β_1- and β_2-AR-mediated contractile responses. The ability to maintain intrinsic myocardial systolic function despite the presence of β-AR-induced apoptosis and downregulation of β-AR-mediated contractile responses could be explained by a considerably enhanced myocardial norepinephrine release, upregulation of α-AR-mediated contractile responses, as well as compensatory cardiomyocyte hypertrophy. Because intrinsic myocardial systolic function was maintained, LV pump dysfunction following chronic β-AR activation was principally attributed to the presence of geometric chamber remodeling as evidenced both by increases in LV internal dimensions and a right shift in the LV diastolic pressure-volume relation.

The results of the present study are consistent with the notion recently put forward that detrimental LV remodeling (LV dilatation) can, in certain circumstances, be relatively more important than intrinsic myocardial contractile disturbances in promoting pump dysfunction in cardiac disease (23). Prior studies have demonstrated the relatively more important role of LV dilatation in animal models of myocardial infarction (2), pressure-overload hypertrophy (23), and pressure-overload hypertrophy together with chronic adrenergic overstimulation (3, 35). However, this is the first study to show that pump dysfunction induced by persistent adrenergic activation in otherwise normal hearts may occur primarily as a consequence of LV dilatation. Moreover, this is the first study to provide an explanation that embodies all prior theories of the mechanisms of sympathetic-induced pump dysfunction. In this regard, we concur with the notion that chronic β-AR activation promotes both myocardial damage (apoptosis) and downregulation of β-AR-mediated inotropic responses. However, we have also provided potential reasons to explain why myocardial damage (apoptosis) and downregulation of β-AR-mediated inotropic responses do not translate into a reduction in overall intrinsic myocardial systolic function. First, myocardial norepinephrine release is considerably enhanced following chronic Iso administration, thus providing additional sympathetic drive. Second, α-AR-induced inotropic drive is markedly increased as evidenced by upregulation of contractile responses to phenylephrine, an α-AR agonist. Third, although apoptosis occurs, compensatory hypertrophy is likely to maintain or even increase the available amount of overall myofilament apparatus available for contraction.

Myocardial contractile dysfunction has previously been reported after administration of a single dose of Iso of about 3 orders of magnitude higher than that utilized in the present study (13, 30). LV contractile dysfunction in these studies was

largely related to advanced myocardial necrosis (8, 31). In contrast, in our present study, the Iso dose employed has previously been shown by us to produce minimal cardiac myocyte necrosis (35, 38), data supported by findings in the present study. The dose employed by us is nevertheless sufficient to promote deleterious interstitial changes (qualitative rather than quantitative changes) upon chronic administration (3, 35, 38) and hence to induce LV dilatation.

In the present study, a 30-fold increase in coronary effluent norepinephrine concentrations was noted in Iso-treated as compared with control rat hearts. This change could result from enhanced myocardial norepinephrine release due to either a storage defect following chronic β-AR activation (9, 22) or a reduced neuronal norepinephrine reuptake due to changes in myocardial noradrenergic nerve function (10, 15) or both. Importantly, a sustained elevation of myocardial interstitial norepinephrine concentrations has been shown to negatively correlate with reduced sarcolemmal β-AR density after chronic β-AR stimulation (10). This could account for the profound downregulation of β-AR-mediated inotropic responses noted in the present study.

In the present study, preserved inotropic responses to norepinephrine occurred in association with markedly reduced contractile effects of Iso. These changes concur with those reported on in isolated papillary muscles from failing human hearts (5). One potential explanation for this apparent paradox is that an impaired neuronal reuptake may result in higher interstitial myocardial norepinephrine concentrations following an infusion of exogenous norepinephrine, thus maintaining a normal contractile potency of norepinephrine despite β-AR inotropic downregulation (5). Alternatively, as suggested from the present study, α-AR-mediated inotropic upregulation may counterbalance β-AR-mediated inotropic downregulation, resulting in normal contractile effects of nonselective α- and β-AR agonists, such as norepinephrine. Previous studies have demonstrated that, despite myocardial β-AR downregulation following chronic Iso administration, α-AR-induced inotropic upregulation may occur (6, 24). These changes may be mediated by an increased density of myocardial α_1-ARs (17, 18, 29, 33).

In the present study, a relatively short-term period of Iso administration (daily for 5 days) resulted in a sixfold increase in apoptosis as determined using a TUNEL technique, a finding consistent with the actions of β-AR activation (25, 28). Despite evidence of marked apoptosis, intrinsic myocardial contractile function was preserved. There are three potential explanations for this apparent paradox. First, the technique used to assess apoptosis not only labels apoptotic but also oncotic cells and cells undergoing DNA repair (16). Thus, although the technique reveals cell damage, it is not an accurate assessment of the number of cells that undergo cell death. Second, although cardiomyocyte cell death may occur, increases in norepinephrine release and upregulation of α-AR-induced inotropic responses may compensate for a decrease in available number of cells to contract. Third, despite cell death, heart weight still increases, a consequence of mainly cardiomyocyte hypertrophy. Thus compensatory hypertrophy is likely to maintain or even increase the available amount of overall myofilament apparatus available for contraction.

Caution should be exercised in overinterpretation of the data from the present study. First, the results of the present study do not suggest that myocardial contractile disturbances mediated by either apoptosis or β-AR downregulation are not important in the progression of heart failure. Both apoptosis and β-AR downregulation are both thought to play a key role in the progression of heart failure. The present study, rather, highlights the mechanisms that may preserve overall intrinsic myocardial systolic function despite marked β-AR downregulation and apoptosis. In contrast, however, adverse structural remodeling with cardiac dilatation is not counteracted by opposing changes that maintain pump function. Consequently, following chronic β-AR activation, cardiac dilatation can precede contractile disturbances and explain the initial decreases in pump function. Second, caution should be exercised in translating the present data to the effects of sympathetic overactivation in human heart failure. The rat myocardium may exhibit a greater α-AR-induced inotropic responsiveness than does the human myocardium. Thus α-AR-mediated inotropic upregulation may not contribute to maintaining intrinsic myocardial function in human heart failure. Last, the present study was conducted in male rats only. Whether chamber dilatation plays as great a role in female rats after chronic sympathetic stimulation requires further study.

In conclusion, LV pump failure following chronic β-AR activation may be associated with LV dilatation in the absence of intrinsic myocardial systolic failure. Normal total contractile function occurs largely because of increases in myocardial norepinephrine release and α-AR-mediated inotropic upregulation counterbalancing the negative inotropic actions of apoptosis and β-AR downregulation. These data provide the first direct evidence to support the view that pump dysfunction in a dilated chamber reflects the impact of cardiac dilatation and not necessarily the extent of the underlying myocardial failure.

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