Reversible effects of isoproterenol-induced hypertrophy on in situ left ventricular function in rat hearts

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Isoproterenol (Iso) infusion of Iso in the rat results in marked cardiac hypertrophy, the various mechanisms for this remodeling are not fully understood (1, 16, 18, 19, 25, 26). Furthermore, we have previously validated the accuracy of the measurement of LV volume in situ normal rat hearts by comparing the stroke volume (SV) measured with a conductance catheter (SV_c) with that measured by electromagnetic flowmetry (SV_em) (11). However, we have not evaluated yet the accuracy of this LV volumetry in hypertrophic hearts.

METHODS

Animals and drug infusion. Male Wistar rats (n = 34) weighing 272–410 g (8–10 wk old) were used in the present experiments. Delivery of drug was achieved by implanting an osmotic minipump (model 1003D, Alzet, Cupertino, CA) subcutaneously in the neck under pentobarbital (50 mg/kg ip) anesthesia. Either Iso (1.2 or 2.4 mg·kg$^{-1}$·day$^{-1}$ for 3 days; Iso group) or vehicle (saline 24 μl·day$^{-1}$ for 3 days; Sa group) was infused by subcutaneous implantation of an osmotic minipump. After verifying the development of cardiac hypertrophy, we recorded continuous LV pressure-volume (P-V) loops of in situ ejecting hypertrophied rat hearts. The curved LV end-systolic P-V relation (ESPVR) and systolic P-V area (PVA) were obtained from a series of LV P-V loops in the Sa and Iso groups 1 h or 2 days after the removal of the osmotic minipump. PVA at midrange LV volume (PVA_{mLVV}) was taken as a good index for LV work capability (13, 15, 20, 21). However, in rat hearts during remodeling, whether PVA_{mLVV} is a good index for LV work capability has not been determined yet. In the present study, in contrast to unchanged end-systolic pressure at midrange LV volume, PVA_{mLVV} was significantly decreased by isoproterenol treatment relative to saline; however, these measurements were the same 2 days after pump removal. Simultaneous treatment with a β$_1$-blocker, metoprolol (24 mg·kg$^{-1}$·day$^{-1}$), blocked the formation of cardiac hypertrophy and thus PVA_{mLVV} did not decrease. The reversible changes in PVA_{mLVV} reflect precisely the changes in LV work capability in isoproterenol-induced hypertrophied rat hearts mediated by β$_1$-receptors. These results indicate that the present approach may be an appropriate strategy for evaluating the effects of antihypertrophic and antifibrotic modalities.

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Kohden; Tokyo, Japan) was placed around the ascending aorta. LV conductance volume and electromagnetic aortic flow data were simultaneously obtained by varying the preload with gradual inferior vena cava occlusion. We compared SVem against SVcc. SVem was calculated by beat-to-beat integration of the aortic flow signal, and SVcc was obtained as the minimal conductance volume subtracted from the maximal conductance volume in each beat.

Histological analysis. The LV was fixed with 3.7% paraformaldehyde in PBS, embedded in paraffin, and cut into 6-μm slices, which were stained with hematoxylin-eosin for morphological analysis or with Masson’s trichrome staining for the detection of fibrosis. For morphometrical analysis, photographs of six LV sections from the Sa (n = 3), Iso 1.2 (n = 3), Iso 2.4 (n = 3), Meto + Iso 1.2 (n = 3), and Iso(−) (n = 3) groups were taken at ×400 magnification, and cross-sectional images of cardiac myocytes were digitized by digital microscope (FUJIX Digital Camera HC-2500). LV cardiac cell size and collagen volume fraction were determined by counting computerized pixels in digital image of myocyte and collagen area stained by Masson’s trichrome stain.

Polyacrylamide gel electrophoresis and Western blots for sarco(endo)plasmic reticulum Ca2+-ATPase. Membrane proteins from the LV myocardium of each heart were isolated as described previously (20, 30). The frozen hearts were homogenized and centrifuged at 1,000 g for 10 min. The supernatants were centrifuged at 100,000 g for 60 min at 4°C. The 100,000-g pellets were cellular membrane fractions and used for immunoblotting of sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA2).

Membrane proteins (20 μg/lane) were separated on SDS-polyacrylamide gels (10% for SERCA2) in a minigel apparatus (MiniPROTEAN II, Bio-Rad Laboratories) and transferred to polyvinylidene difluoride membranes. The membranes were blocked (4% Block Ace, Dainippon Pharmaceutical; Osaka, Japan) and then incubated with anti-SERCA2 antibody (1:1,000 dilution, Affinity Bio Reagents). The antigens were detected by the enhanced chemiluminescence method (ECL Western blotting detection kit, Amersham) with peroxidase-linked anti-mouse IgG (1:1,000 dilution). After immunoblotting, the film was scanned with a scanner, and the intensity of the bands was calculated by NIH Image analysis.

Measurements of LV volume and pressure. One hour or 2 days after the removal of the minipump, we evaluated LV function in hypertrophied rat hearts by simultaneous measurements of LVV and LVP. The rat was anesthetized with ethylcarbamate (0.7 g/kg ip) and −chloralose (60 mg/kg ip). The trachea was intubated, and rat was ventilated with room air. The chest was opened, and a conductance catheter (1.5 Fr) was introduced into the LV through an apical stab, as described above. A 3.0-Fr catheter-tip micromanometer was also inserted through the apex into the LV to obtain reliable LVP data.

The principle of conductance catheter method of measuring LVV has been described in detail (3, 4, 11). When hemodynamics was

Fig. 1. A: protocols for treatment of each rat in the vehicle-treated group (0.1% ascorbic acid in saline, 2.4 μl/day for 3 days; Sa group); isoproterenol (Iso)-treated groups [1.2 or 2.4 mg·kg−1·day−1 for 3 days; Iso 1.2 and Iso 2.4 groups, respectively; and 2 days after the removal of the minipump in the Iso 1.2 group; Iso(−) group]; and metoprolol (Meto) and Iso-treated group (24 mg·kg−1·day−1 for 3 days + Iso 1.2; Meto + Iso 1.2 group). LV, left ventricular. B: parameters used in the evaluation of pressure-volume (P-V) loops and the end-systolic P-V relation (ESPVR). Top, end-systolic pressure (ESP) at end-systolic volume (ESPesv); stroke volume (SV); end-diastolic volume (EDV); end-systolic volume (ESV); and effective arterial elastance (Ea), defined as ESPesv/SV. Bottom, volume intercept of ESPVR (V0); systolic P-V area at midrange LV volume (mLVV) at end systole (PVA mLVV); ESP at mLVV (ESP mLVV).
stable, a series of LV P-V loops was obtained during increasing afterload by a gradual occlusion of the ascending aorta. The occlusion was performed for 1–2 s until end-diastolic volume (EDV) slightly increased. Occlusion was limited so as not to evoke any arrhythmia. This intervention was repeated six times at 10-min intervals. The respirator was stopped during data acquisition to avoid respiratory fluctuation influences on cardiac signals.

In the final part of each experiment, parallel conductance and thus constant offset volume were measured by injecting hypertonic saline (10% NaCl solution, 0.025 ml) into the pulmonary artery to change transiently the resistivity of the blood in the LV (13, 15). The calculated constant offset volume was subtracted from the measured LV conductance volume to obtain LV absolute blood volume, i.e., absolute LVV. LVP and the three individual segmental conductance volume signals were digitized and stored at 12-bit accuracy at a sampling frequency of 500 Hz for later analyses. At the end of each experiment, a lethal dose of pentobarbital sodium was injected into the rat. The LV including the interventricular septum, and the right ventricle (RV) was excised and weighed, respectively.

Data analysis. In the in situ rat LV, a curvilinear ESPVR is obtained by drawing an upper enveloping curve on a series of P-V loops in a similar manner to previously reported methods (13, 15). The LV end-systolic P-V data on the upper left shoulder of all P-V loops were plotted and fitted by the method of least squares using the following equation: 

\[ \text{LVP} = A (1 - \exp[-B(LVV - V_0)]) \]

where A and B are fitted parameters and V_0 is systolic unstressed volume. We obtained the best-fit ESPVR curve in each heart in the Sa, Iso 1.2, Iso 2.4, Meto + Iso 1.2, and Iso(−) groups. V_0 has been previously measured in postmortem isolated rat normal LVs. The V_0 values are determined to be 0.02 ± 0.005 ml/g (n = 7)(27). In the present study, the mean V_0 value of hearts in the Sa group obtained as volume intercepts of the best-fit ESPVRs was 0.025 ± 0.014 ml/g (n = 7)(see Table 2). Therefore, we judged the V_0 values obtained by curve fit are reliable.

LV PVA is a measure of the total mechanical energy generated by a LV contraction. The PVA of an isovolumic contraction represents the maximal capability of external mechanical work of the LV at a given preload (24). In the present study, PVA was defined as the area enclosed by the ESPVR curve, the volume axis [instead of given preload (24)]. In the present study, PVA was defined as the area enclosed by the ESPVR curve, the volume axis [instead of given preload (24)]. In the present study, PVA was defined as the area enclosed by the ESPVR curve, the volume axis [instead of given preload (24)]. In the present study, PVA was defined as the area enclosed by the ESPVR curve, the volume axis [instead of given preload (24)]. In the present study, PVA was defined as the area enclosed by the ESPVR curve, the volume axis [instead of given preload (24)]. In the present study, PVA was defined as the area enclosed by the ESPVR curve, the volume axis [instead of given preload (24)].

The PVA as a function of LVV was obtained by integrating the above exponential function from the extrapolated V_0 along the volume axis: 

\[ \text{PVA} = A(LVV - V_0) - A(1 - \exp[-B(LVV - V_0)])/B \]

We had proposed that, in situ hearts, PVA at an appropriate LVV on the curvilinear PVA-volume relationship is valuable to evaluate LV mechanoenergetics (13, 15). In the present study, we calculated mLVV that was the value of \[ \text{V}_0 + \left( \text{maximum ESV} - \text{minimum ESV} \right) \] on the ESPVR × 1/2] from each P-V loop. Each example of end-systolic pressure (ESP) and PVA at midrange LVV (ESP_{mLVV} and PVA_{mLVV}) is shown in Fig. 1B, bottom. ESPVR is very close to the volume axis, as shown in Fig. 4, and thus the effect of excluding the ESPVR measure would be small. Effective arterial elastance (E_a) is defined as the ratio of ESP_{EWS} to SV of the LV under stable hemodynamics. SV was obtained by the formula (EDV – ESV) (Fig. 1B, top). The ejection fraction (EF) was obtained by the formula \[ \text{SV} = \text{EDV} - \text{ESV} \].

**RESULTS**

**Cardiac weights.** LV wet weight (WW)-to-body weight (BW) ratio was 1.94 ± 0.10 mg/g in the Sa group and significantly increased by 32% in the Iso 1.2 group and by 50% in the Iso 2.4 group (P < 0.05 vs. the Sa group; Table 1). RV WW/BW was 0.541 ± 0.040 mg/g in the Sa group and significantly increased by 22% in the Iso 1.2 group and by 42% in the Iso 2.4 group (P < 0.05 vs. the Sa group). LV dry weight (DW)/BW was 0.488 ± 0.037 mg/g in the Sa group and significantly increased by 22% in the Iso 1.2 group and by 32% in the Iso 2.4 group (P < 0.05 vs. the Sa group). RV DW/BW was not significantly increased in the Iso 1.2 group but was significantly increased in the Iso 2.4 group (P < 0.05 vs. the Sa group). In the Iso(−) group, LV WW/BW was still significantly larger (P < 0.05 vs. the Sa group), but other LV DW/BW, RV WW/BW, and RV DW/BW values were not different from those in the Sa group. In the Meto + Iso 1.2 group, none of the LV WW/BW, RV DW/BW, and RV DW/BW values were different from those in the Sa group.

**Histological and Western blot analyses.** Microscopic examination showed cardiomyocyte hypertrophy as evidenced by increased cell size and fibrosis in the Iso 1.2 and Iso 2.4 groups (Fig. 2, A and B). Intraluminal LVVs in the Iso 1.2 and Iso 2.4 groups were distinctly smaller than that in the Sa group. The collagen area in the Iso 1.2 and Iso 2.4 groups was significantly larger than that in the Sa group (P < 0.05), and the area in the Iso(−) group returned to the almost the same level as that in the Sa group (Fig. 2C). Size of LV cardiac cells was significantly larger in the Iso 1.2 (156 ± 73% of Sa, n = 90) and Iso 2.4 groups (160 ± 66% of Sa; n = 90) than in the Sa group (P < 0.05) and significantly returned to 128 ± 48% of Sa (n = 90) in the Iso(−) group. The amount of expression of SERCA2a was significantly depressed in the Iso 1.2 and Iso 2.4 groups (P < 0.05) from that in the Sa group and returned to the

**Table 1. Comparison of cardiac wet weights and dry weights among Sa, Iso 1.2, Iso 2.4, Iso(−), and Meto + Iso 1.2 groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW, g</th>
<th>LV WW/BW, mg/g</th>
<th>RV WW/BW, mg/g</th>
<th>LV DW/BW, mg/g</th>
<th>RV DW/BW, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sa</td>
<td>7</td>
<td>341±38</td>
<td>1.94±0.10</td>
<td>0.541±0.040</td>
<td>0.488±0.037</td>
<td>0.127±0.014</td>
</tr>
<tr>
<td>Iso 1.2</td>
<td>7</td>
<td>346±13</td>
<td>2.56±0.10*†‡</td>
<td>0.662±0.051*</td>
<td>0.593±0.098*</td>
<td>0.139±0.007</td>
</tr>
<tr>
<td>Iso 2.4</td>
<td>7</td>
<td>341±33</td>
<td>2.91±0.28*†‡</td>
<td>0.769±0.44*†‡</td>
<td>0.643±0.052*†</td>
<td>0.169±0.013*†</td>
</tr>
<tr>
<td>Iso(−)</td>
<td>7</td>
<td>344±20</td>
<td>2.76±0.10*</td>
<td>0.611±0.065*</td>
<td>0.562±0.051*</td>
<td>0.139±0.023</td>
</tr>
<tr>
<td>Meto + Iso 1.2</td>
<td>6</td>
<td>346±22</td>
<td>2.09±0.15</td>
<td>0.615±0.075</td>
<td>0.489±0.029</td>
<td>0.143±0.016</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Sal, saline (24 μl/day for 3 days)-infused group; Iso 1.2 and Iso 2.4: isoproterenol (1.2 or 2.4 mg·kg⁻¹·day⁻¹ for 3 days)-infused group; Iso(−): 2 days after the removal of the osmotic minipump in the Iso 1.2-infused group; Meto + Iso 1.2: metoprolol (24 mg·kg⁻¹·day⁻¹ for 3 days) and Iso 1.2-infused group; BW: body weight; LV WW, left ventricular (LV) wet weight; RV DW, LV dry weight; RV WW, right ventricular (RV) wet weight; RV DW, RV dry weight; *P < 0.05 vs. the Sa group; †P < 0.05 vs. the Iso(−) group; ‡P < 0.05 vs. the Meto + Iso 1.2 group.
almost the same level in the Iso(−) group as in the Sa group (Fig. 2D).

Comparison of SV_em and SV_cc. Figure 3, A and B, shows representative scattergrams of SV_em and SV_cc for a typical heart from the Sa and Iso 2.4 (hypertrophied) groups. The correlations between SV_em and SV_cc were high and linear (correlation coefficients: r = 0.988 and 0.995, respectively) in both heart groups, although the regression line slightly shifted downward in the hypertrophied rat heart. Pooled data from the Sa and Iso 2.4 groups also showed linear correlations (r = 0.909, n = 3, and r = 0.853, n = 6, respectively). The intercepts of both regression lines of the pooled data were close to zero, and their slopes were close to 1.0, although the correlation coefficient in hypertrophied remodeling hearts (Fig. 3D) was lower than normal-shaped hearts (Fig. 3C).

Comparison of P-V loops and ESPVRs among the Sa, Iso 1.2, Iso 2.4, and Iso(−) groups. Representative sets of P-V loops for a typical heart from the Sa, Iso 1.2, Iso 2.4, and Iso(−) groups are shown in Fig. 4. The shapes of ESPVRs hardly differed among the four groups, but working ranges of LVV were markedly different in hypertrophied hearts in both the Iso 1.2 and Iso 2.4 groups (Fig. 4, B and C). The Iso(−) heart showed almost similar P-V loops, and the ESPVR was similar to that in a heart from the Sa group (Fig. 4, D vs. A).

Mean mLV V, ESP_mLV V, and PVA_mLV V. Mean mLVVs in the Iso 1.2 (45 ± 10% of Sa) and Iso 2.4 groups (42 ± 11% of Sa) were significantly smaller than that in the Sa group (0.123 ± 0.0322 ml/g, P < 0.05), but there were no significant differences between the Iso 1.2 and Iso 2.4 groups (Fig. 5A). In contrast, mean ESP_mLV V was unchanged in all groups (Fig. 5B). Mean PVA_mLV V in the Iso 1.2 (51 ± 16% of Sa) and Iso 2.4 groups (47 ± 14% of Sa) was significantly smaller than that in the Sa group (7.16 ± 2.72 mmHg·ml·beat⁻¹·g⁻¹, P < 0.05; Fig. 5C), indicating that the LV systolic function, i.e., mechanical work capability, was impaired in hypertrophied rat hearts. Indeed, cardiac output (CO) in the Iso 1.2 (62 ± 14% of Sa) and Iso 2.4 groups (63 ± 9.8% of Sa) was also significantly smaller than that in the Sa group (37.3 ± 5.7 ml/min, P < 0.05; Fig. 5D). None of mean mLV V, PVA_mLV V, and VO values differed between the Iso 1.2 and Iso 2.4 groups. Mean PVA_mLV V and CO in the Iso(−) group showed the almost the same levels as those in the Sa group, although the mean mLV V still remained significantly smaller than that in the Sa group (P < 0.05; Fig. 5A, C, and D).

A typical heart from the Meto + Iso 1.2 group showed almost similar P-V loops and ESPVR to those in a typical heart from the Sa group (data not shown). Mean mLV V in the Meto + Iso 1.2 group was significantly larger than that in the Iso 1.2 group (Fig. 5A). Mean ESP_mLV V in the Meto + Iso 1.2 group was unchanged (Fig. 5B). Mean PVA_mLV V in the Meto + Iso 1.2 group was significantly larger than that in the Iso 1.2 group (P < 0.05; Fig. 5C), indicating that the impairment of mechanical work capability in hypertrophied rat hearts was blocked by simultaneous treatment with Meto.
Fig. 3. Representative scattergrams of SV from electromagnetic flowmetry (SV_em) and from the conductance catheter system (SV_cc) for typical hearts (A and B) from the Sa and Iso 2.4 groups and those of pooled data for 3 hearts from the Sa group (C) and for 6 hearts from the Iso 2.4 group (D).

Fig. 4. Representative sets of P-V loops and ESPVR for typical hearts from the Sa (A), Iso 1.2 (B), Iso 2.4 (C), and Iso(-) groups (D).
Indeed, CO in the Meto/Iso 1.2 group was also significantly larger than that in the Iso 1.2 group ($P < 0.05$; Fig. 5D). None of the mean mLV, PVA mLV, and CO values differed between the Sa and Meto/Iso 1.2 groups, indicating that simultaneous treatment with Meto almost completely blocked the formation of hypertrophy and thus the impairment of LV systolic function.

Effects of the β1-blocker Meto on Iso-induced hypertrophied hearts. Microscopic examination identified marked inhibition of hypertrophy by Meto in the Meto/Iso 1.2 group, accompanied with inhibition of distinct fibrosis (Fig. 6, A and B). The collagen area in the Meto/Iso 1.2 group was significantly smaller than that in the Iso 1.2 group and was the almost the same level as that in the Sa group (Fig. 6C). The sizes of cardiac cells in the Meto/Iso 1.2 group (106 ± 47% of Sa, $n = 90$) were significantly smaller than those in the Iso 1.2 group and were the almost the same levels as those in the Sa group. The amount of expression of SERCA2a in the Meto/Iso 1.2 group was significantly larger than in the Iso 1.2 group and showed almost the same level as in the Sa group (Fig. 6D).

Other hemodynamic parameters. Mean ESV, EDV, and SV values in the Iso 1.2 and Iso 2.4 groups were significantly smaller than those in the Sa group ($P < 0.05$), but mean EF and mean ESPSV values were unchanged in all groups (Table 2). Mean $E_a$ was significantly larger in the Iso 1.2 (175 ± 38% of Sa) and Iso 2.4 groups (197 ± 49% of Sa) than that in the Sa group (482 ± 128 mmHg/ml⁻¹·g⁻¹) due to significantly smaller SV ($P < 0.05$). None of the mean ESV, EDV, SV, and $E_a$ values differed significantly between the Iso 1.2 and Iso 2.4 groups.

None of the mean EDV, SV, and $E_a$ values in the Iso(−) group differed from those in the Sa group, although mean ESV differed from that in the Sa group. None of the mean ESV, EDV, SV, and $E_a$ values in the Meto/Iso 1.2 group differed from those in the Sa group, indicating changes in mean ESV, EDV, SV, and $E_a$ in the Iso 1.2 group were blocked by simultaneous treatment with Meto.

Although the mean heart rate was significantly smaller in the Iso 1.2 and Iso 2.4 groups ($P < 0.05$), we have previously reported the curved ESPVR is independent of heart rate in situ rat hearts within 250–320 beats/min (15) and in excised rat hearts within 250–300 beats/min (21).

**DISCUSSION**

The accuracy of LVV measurement by the conductance catheter system has been previously well established in canine and human hearts (3, 4). We instituted the measurement of LVV in in situ rat hearts by a conductance catheter method (11, 13, 15, 27). Ito et al. (11) previously confirmed the accuracy of the LVV measurement by the conductance catheter system in in situ normal rat hearts. They showed a high and linear correlation between SVcc and SV em in normal rat hearts. Furthermore, they exhibited the linear relationships between conductance volume and absolute LVV (measured in postmortem hearts) (9), comparable with those reported in canine postmortem hearts (2, 8).

For the first time, we have validated the accuracy of LVV measurement by the conductance catheter method in hypertrophied rat hearts by comparing SVcc with SV em during inferior vena caval occlusion. Consequently, we obtained a high and linear correlation between SVcc and SV em in a hypertrophied rat heart from the Iso 2.4 group as well as in a heart from the Sa group. Both SVcc and SV em values pooled from individual

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**Fig. 5.** Mean mLV (A), ESP mLV (B), PVA mLV (C), and cardiac output (D) in the Sa, Iso 1.2, Iso 2.4, Iso(−), and Meto + Iso 1.2 groups. *$P < 0.05$ vs. the Sa group; #P < 0.05.
nase (PKA). PKA has several pathways in cardiomyocytes that
in intracellular cAMP activates cAMP-dependent protein ki-

Although many studies on the mechanisms underlying Iso-
induced cardiac hypertrophy have been reported (1, 14, 16, 18,
19, 25, 26), the conclusive mechanisms have not yet been
determined.

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**Table 2. Hemodynamics based on in situ analysis of pressure-volume loops**

<table>
<thead>
<tr>
<th></th>
<th>Sa</th>
<th>Iso 1.2</th>
<th>Iso 2.4</th>
<th>Iso(−)</th>
<th>Meto + Iso 1.2</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Va, ml/g</td>
<td>0.025±0.014</td>
<td>0.008±0.005*</td>
<td>0.009±0.009*</td>
<td>0.010±0.006*</td>
<td>0.010±0.006*</td>
</tr>
<tr>
<td>ESV, ml/g</td>
<td>0.068±0.018</td>
<td>0.028±0.012*‡</td>
<td>0.027±0.011*‡</td>
<td>0.042±0.010*</td>
<td>0.055±0.020</td>
</tr>
<tr>
<td>EDV, ml/g</td>
<td>0.227±0.048</td>
<td>0.114±0.016*‡‡</td>
<td>0.106±0.020*‡‡</td>
<td>0.178±0.034</td>
<td>0.194±0.022</td>
</tr>
<tr>
<td>SV, ml/g</td>
<td>0.158±0.032</td>
<td>0.086±0.018*††</td>
<td>0.079±0.016*††</td>
<td>0.136±0.031</td>
<td>0.139±0.015</td>
</tr>
<tr>
<td>EF, %</td>
<td>70.6±4.1</td>
<td>75.7±9.9</td>
<td>75.1±1.9</td>
<td>76.1±1.5</td>
<td>72.2±8.3</td>
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<td>ESV, mmHg</td>
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<td>70±5</td>
<td>72±9</td>
<td>78±7</td>
<td>78±8</td>
</tr>
<tr>
<td>Ees, mmHg/mL/g</td>
<td>482±128</td>
<td>843±181*</td>
<td>948±237*†‡</td>
<td>507±169</td>
<td>566±97</td>
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<tr>
<td>HR, beats/min</td>
<td>354±28</td>
<td>293±16*</td>
<td>299±19*</td>
<td>329±54</td>
<td>318±44</td>
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Values are means ± SE; n, no. of animals; Va, systolic unstressed volume; ESV, end-systolic volume; EDV, end-diastolic volume; SV, stroke volume; EF, ejection fraction; ESV, end-diastolic pressure at ESV; Ees, effective arterial elastance; HR, heart rate. *P < 0.05 vs. the Sa group; †P < 0.05 vs. the Iso(−) group; ‡P < 0.05 vs. the Meto + Iso 1.2 group.

Fig. 6. Histology and Western blot of each heart in the Sa, Iso 1.2, and Meto + Iso 1.2 groups. A: HE staining. B: MTC staining. C: LV collagen area. D: Western blot of SERCA2a. *P < 0.05.
ary responsible for all events related with cardiac hypertrophy induced by Iso infusion. Meto prevented the molecular changes related with cardiac hypertrophy by blocking the β1-AR-mediated signal transduction pathway at an early stage.

This is in agreement with a previous report by Morisco et al. (18). Iso increased LV weight-to-BW ratio and atrial natriuretic factor transcription in the adult rat in vivo, which was inhibited by a β1-antagonist but not by a β2-antagonist. These results indicate that hypertrophy is mediated by the β1-subtype (18).

Roles of the renin-angiotensin system on Iso-induced hypertrophy are controversial (16, 19). Cardiac tissue ANG II regulates myocyte growth in Iso-induced LV hypertrophy, and the reduction of ANG II partly explains the prevention of cardiac hypertrophy by the converting enzyme inhibitor (19). In contrast, other investigators asserted that neither the circulatory nor cardiac renin-angiotensin system plays a major role in the cardiac trophic responses to β-AR stimulation (16).

The amount of expression of SERCA2a was dose dependently decreased in the Iso 1.2 and Iso 2.4 groups as described previously (23). The β1-antagonist Meto completely antagonized the downregulation of SERCA2a expression in hearts from the Meto + Iso 1.2 group, indicating that the amount of SERCA2a expression was decreased mediated by β1-ARs. We predicted diastolic dysfunction due to the impaired Ca2+ uptake from the sarcoplasmic reticulum resulting from the downregulation of SERCA2a expression. The resultant upward shift of ESPVR, however, was not observed. PVA mLVV has been proposed a good mechnoenergetic index to evaluate LV function in in situ normal rat hearts (13, 15, 27), and PVA has been shown to linearly relate to myocardial oxygen consumption including energy requirements for Ca2+ handling in excitation-contraction coupling and basal metabolism (20, 21, 28). The major component of the energy requirements for Ca2+ handling is utilized by SERCA2a (20, 21, 28). The major component of the energy requirements for Ca2+ handling is utilized by SERCA2a (20, 21, 28), suggesting that the downregulation of SERCA2a expression may decrease the energy requirements for Ca2+ handling in the present hypertrophied rat hearts as in hypothyroid rats (20). On the other hand, myocardial oxygen consumption for basal metabolism per LV WW decreased in LV myocardial slices of the same type of hypertrophied rat hearts (29), where the collagen contents significantly increased. It seems likely that collagen does not consume any energy. Therefore, the total energy demand must decrease in the hypertrophied rats hearts. Our recent unpublished observations have noted the unchanged creatine phosphate-to-ATP ratio in LV myocardial slices of the same type of hypertrophied rat hearts (n = 6), indicating an unchanged energy balance between demand and supply. The suppression of energy production may occur responding to the decreased energy demand, resulting in an unchanged energy balance between demand and supply in the present hypertrophied rat hearts. Alternatively, the decreased energy demand may occur responding to the suppression of energy production, i.e., the downregulation of SERCA2a expression may be compensatory changes against the suppressed energy production.

The present cardiac hypertrophy is reversible, because 2 days after the removal of Iso, all changes in hypertrophy (collagen contents, SERCA2a, mLVV, PVA mLVV, and CO) returned to the almost the same level as those in the Sa group. This reversibility could give us the possibility that treatment with an appropriate agent or gene transfer might cure the cardiac hypertrophy associated with collagen production (fibrosis). For example, a histone deacetylase inhibitor has been reported to prevent hypertrophy induced by an infusion of Iso (14). The identification and further elucidation of antihypertrophic transcriptional pathways will offer novel therapeutic targets for drugs for the treatment of congestive heart failure. We suggested that this Iso-induced hypertrophied rat heart is an appropriate model for exploring novel therapeutic (antihypertrophic and antifibrotic) agents or genes.

Simultaneous measurement of LV P-V signals and thus recording P-V loops revealed that LVP parameters, such as ESPESV, were unchanged, but LVV parameters, such as ESV, EDV, SV, and mLVV, were significantly smaller in the hypertrophied heart than those in the normal heart. These volume changes were due to a decrease in EDV (−0.12 ml/g) rather than a decrease in ESV (−0.04 ml/g) (see Table 2). The decreased EDV indicates that the LV lumens size is reduced by cardiac myocyte hypertrophy and collagen production. The increase in Ed was attributable to a decrease in SV and no increase in ESPESV but not due to elevated total peripheral resistance.

LV ESPVR obtained by gradual increase in afterload appeared to be similar between normal and hypertrophied hearts, but PVA mLVV, which depicts mechanical work capability at mLVV (13, 15, 27), was markedly decreased in the hypertrophied heart, indicating that the LV function was impaired in hypertrophied rat hearts, although ESP mLVV did not change.

Taken together with these results, the simultaneous measurement of LV P-V signals and analysis using the framework of ESPVR-PVA, especially PVA mLVV, are substantially needed to evaluate LV function in the heart without changes in LVP such as in the hypertrophied rat heart.

We concluded that the changes in PVA mLVV precisely reflects the changes in LV work capability even in reversible Iso-induced hypertrophied rat hearts. These results indicate that the present approach might be an appropriate strategy for evaluating the effects of antihypertrophic and antifibrotic agents and gene targeting therapy.

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REFERENCES


