Isoproterenol-Induced Hypertrophied Rat Hearts: Does Short-Term Treatment Correspond to Long-Term Treatment?

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Abstract: In consideration of clinical implications, it is often complained that short-term experimental diseased heart models do not mimic long-term diseased hearts that are often clinically encountered. The aim of the present study was (i) to compare the left ventricular function between rat cardiac hypertrophy models treated with isoproterenol for 3 days (Iso 3d) and 7 days (Iso 7d) by pressure-volume measurements with a catheter method, and (ii) to follow up the left ventricular function in the same model treated with Iso up to 16 weeks with a less-invasive echocardiography. An infusion of either Iso ($1.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 3 days–16 weeks) or vehicle (saline $24 \mu\text{l} \cdot \text{day}^{-1}$ for 3 days–16 weeks; Sa group) was performed by subcutaneously implanting an osmotic minipump. There were no significant differences

in the systolic pressure-volume area at midrange left ventricular volume (PVA_{mLVV} ; a mechanical work capability index) between Iso 3d and 7d groups, though PVA_{mLVV} in both groups was significantly reduced from that in the Sa group. From echocardiography, the left ventricular function of the hypertrophy models at 3 days, 1 week, and 2 weeks was unchanged, but the model at a term longer than 4 weeks resulted in prolonged systolic failure. The results indicated that (i) no marked differences in the left ventricular mechanical work capability were found between the Iso 3d and 7d groups, and that (ii) only a 3-day Iso infusion induced the hypertrophy model similar in shape and function to that induced by a 2-week Iso infusion. We concluded that the 3-day model was sufficient.

Key words: conductance catheter, echocardiography, left ventricular volumetry, systolic pressure-volume area.

It is known that chronic infusions of a α , β -stimulant, norepinephrine, and a β -stimulant, isoproterenol (Iso), induce cardiac hypertrophy accompanied with enhanced fibrosis among cardiac interstitial cells [1–3]. Many studies on mechanisms underlying Iso-induced cardiac hypertrophy have been reported [4–13]. Cardiac oxidative stress contributes to this type of cardiac remodeling, especially in respect to wall stiffness, based on fibrogenesis in chronically Iso-infused rats ($3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 10 days), whereas cardiac phosphorylated MAP (mitogen-activated protein) kinases (extracellular signal related kinase; ERK1/2, c-JUN NH₂-terminal kinase; JNK, p38) returned to normal [4]. The AT1 (type 1 angiotensin II) receptor plays a crucial role in the development of cardiac hypertrophy and oxidative stress in chronically Iso-infused

mice ($15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 11 days) [5]. Furthermore, we have recently found that a novel histone deacetylase (HDAC) inhibitor has no effects of antihypertrophic modalities on the Iso-induced rat heart ($1.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 3 days) [6]. Nevertheless, the conclusive mechanisms underlying Iso-induced cardiac hypertrophy have not yet been determined [4–13].

We have previously succeeded in inducing cardiac hypertrophy by stimulating the β_1 -receptor for only 3 days and have validated the accuracy of left ventricular volumetry in the hypertrophied heart with a conductance catheter system [14]. In consideration of clinical implications, it is often complained that short-term experimental diseased heart models do not mimic long-term diseased hearts that we often clinically encounter. The aim of the

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Abbreviations: $\beta_1\text{ARs}$, β_1 -adrenergic receptors; Ea, effective arterial resistance: the ratio of end-systolic pressure at end-systolic volume to stroke volume of the left ventricle under stable hemodynamics; ESP_{ESV} , end-systolic pressure at end-systolic volume; ESP_{mLVV} , end-systolic pressure at midrange left ventricular volume; Iso 3d and Iso 7d, isoproterenol $1.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ infusion for 3 days and 7 days; IVSTs and IVSTd, interventricular septum thickness during systole and diastole; LVIDs and LVIDd, left ventricular internal dimensions during systole and diastole; LVPWTs and LVPWTd, left ventricular posterior wall thickness during systole and diastole; NCX1, $\text{Na}^+/\text{Ca}^{2+}$ exchanger; PKA, cAMP-dependent protein kinase; PLB, phospholamban; p-PLB, phospho-Ser¹⁶PLB; PVA_{mLVV} , systolic pressure-volume area at midrange left ventricular volume; RyR2, ryanodine receptor; Sa, Saline infusion $24 \mu\text{l} \cdot \text{day}^{-1}$ for 3 days–16 weeks; SERCA2, sarcoplasmic reticulum Ca^{2+} -ATPase; V_0 , systolic unstressed volume.

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present study was to compare specifically left ventricular function in rat hearts between 3- and 7-day hypertrophy models and to follow up on these models from the same 3-day treatment to the long-term 16-week treatment by infusing either Iso $1.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (Iso group) or vehicle (saline $24 \mu\text{l} \cdot \text{day}^{-1}$, Sa group), though by different methods.

Previous studies have revealed that the left ventricular end-systolic pressure-volume relation of isolated cross-circulated and *in situ* ejecting adult rat hearts have upward convex curves, even within the physiological range [15–17]. This convex curvature of end-systolic pressure-volume relation has also been described in mice hearts [18, 19]. The systolic pressure-volume area (PVA) obtained from the curved end-systolic pressure-volume relation corresponds to a total mechanical energy per beat and linearly correlates with myocardial oxygen consumption per beat in excised rat hearts [20–22]. Especially, we have proposed PVA at midrange left ventricular volume (PVA_{mLVV}) as a good mechanical work capability index to evaluate the left ventricular function in *in situ* normal rat hearts [15–17]. Using this index, we found no differences in the reduction of mechanical work capability between 3-

and 7-day hypertrophy models. Following up with echocardiography, we also found that the only 3-day hypertrophy model was similar in shape and function to that induced by 2-week Iso infusion. The results presented here suggested that the 3-day short-term hypertrophy model sufficiently represents the effects of 2-week Iso infusion.

METHODS

Animals and drug infusion. Experimental procedures followed the regulations of and were approved by the animal care and use committee of Nara Medical University. Male Wistar rats ($n = 21$) weighing 303 to 366 g (8 to 10 weeks) were used in the present experiments. The delivery of drugs was achieved by implanting an osmotic minipump (model 1003D, Alzet, Durect Corp, Cupertino, CA) subcutaneously in the neck under pentobarbital (50 mg/kg i.p.) anesthesia. Either Iso ($1.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 3 days and 7 days: Iso 3d and Iso 7d, $n = 7$ each), or a vehicle (0.1% ascorbic acid in saline $2.4 \mu\text{l}/\text{day}$ for 3 days: Sa, $n = 7$) was infused subcutaneously [1]. Osmotic minipumps were removed from the neck 3 or 7 days after

Fig. 1. A: Protocols for the short-term treatment of each rat in (I) Sa, (II) Iso3d, Iso3d(-), and (III) Iso7d, Iso7d(-) groups, and the long-term treatment of each rat in (I) Sa and (II) Iso groups. LV, left ventricle. **B:** The parameters evaluating for pressure-volume (P-V) loop and end-systolic pressure-volume relation (ESPVR). Upper panel: ESP_{ESV} , end-systolic pressure at end-

systolic volume; SV, stroke volume; EDV, end-diastolic volume; $\text{ESP}_{\text{ESV}}/\text{SV}$, effective arterial elastance defined as $\text{ESP}_{\text{ESV}}/\text{SV}$. Lower panel: V_0 , volume-intercept of ESPVR; PVA_{mLVV} , systolic pressure-volume area at mLVV (midrange LVV at end-systole); ESP_{mLVV} , end-systolic pressure at mLVV.

the implantation under pentobarbital anesthesia. One hour after removal of the minipump in Sa, Iso 3d, and Iso 7d groups and two days after removal of the minipump in Iso 3d and Iso 7d groups (Iso 3d[–] and Iso 7d[–], $n = 7$ each), the left ventricular function was evaluated (see protocol shown in Fig. 1A).

Polyacrylamide gel electrophoresis and Western blots for sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2), phospholamban (PLB), phospho-Ser¹⁶PLB (p-PLB), and $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX1). Membrane proteins from the left ventricular myocardium of each heart were isolated as described previously [20, 23]. The frozen hearts were homogenized and centrifuged at $1,000 \times g$ for 10 min. The supernatants were centrifuged at $100,000 \times g$ for 60 min at 4°C . The $100,000 \times g$ pellets were cellular membrane fractions and used for immunoblotting of SERCA2, PLB, p-PLB, and NCX1.

The same amount of membrane proteins ($20 \mu\text{g}/\text{lane}$) were separated on SDS–polyacrylamide gels (10% for SERCA2, 15% for PLB and p-PLB, and 7.5% for NCX1) in a minigel apparatus (Mini-PROTEAN II, Bio-Rad) and transferred to polyvinylidene difluoride membranes. The membranes were blocked (4% Block Ace, Dainippon Pharmaceutical Co., Osaka) and then incubated with anti-SERCA2 antibody (1:1,000 dilution, Affinity Bio Reagents), anti-PLB antibody (1:2,000 dilution, Upstate Biotechnology), antiphospho-PLB (Ser¹⁶) antibody (1:1,000 dilution, Upstate Biotechnology), or anti-NCX1 antibody (1:200 dilution, a generous gift from Dr. Takahiro Iwamoto, Fukuoka University). The antigens were detected by the luminescence method (ECL Western blotting detection kit, Amersham) with peroxidase-linked antimouse IgG (1:1,000 dilution). After BEING immunoblotted, the film was scanned, and the intensity of the bands was calculated by NIH image analysis.

Measurements of left ventricular volume and pressure.

One hour or two days after the removal of the minipump, we evaluated the left ventricular function in hypertrophied rat hearts by simultaneous measurements of left ventricular volume and left ventricular pressure. The rat was anesthetized with ethylcarbamate ($0.7 \text{ g}/\text{kg}$, i.p.) and α -chloralose ($60 \text{ mg}/\text{kg}$, i.p.). The trachea was intubated, and the rat was ventilated with room air. The chest was opened and a conductance catheter (1.5 F) was introduced into the left ventricular through an apical stab. A 3.0 F catheter-tip micromanometer was also inserted through the apex into the left ventricle to obtain a reliable left ventricular pressure data.

The principle of the conductance catheter method of measuring left ventricular volume has been described in detail [24–26]. When hemodynamics was stable, a series of left ventricular pressure-volume 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 slightly increased (almost un-

changed), and it was limited so as not to evoke any arrhythmia. Therefore preload recruitable stroke work is not available. 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 transiently change the resistivity of the blood in the left ventricle [15, 16]. The calculated constant offset volume was subtracted from the measured left ventricular conductance volume to obtain left ventricular absolute blood volume, i.e., absolute left ventricular volume. Left ventricular pressure and the three individual segmental conductance volume signals were digitized and stored in a 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 left ventricle, including the interventricular septum, and the right ventricle were excised and weighed, respectively.

Data analysis. In the *in situ* rat left ventricle, a curvilinear end-systolic pressure-volume relation is obtained by drawing an upper enveloping curve on a series of pressure-volume loops in a manner similar to previously reported methods [15, 16]. The left ventricular end-systolic pressure-volume data on the left-upper shoulder of all pressure-volume loops were plotted and fitted by the method of least-squares using the following equation, left ventricular pressure = $A \{1 - \exp[-B(\text{left ventricular volume} - V_0)]\}$, where A , B , and V_0 are fitted parameters. We obtained the best-fit end-systolic pressure-volume relation curve in each heart in Sa, Iso 3d, Iso 7d, Iso 3d(–), and Iso 7d(–) groups. V_0 (systolic unstressed volume) has been previously measured in postmortem isolated rat normal left ventricle. The V_0 values are determined to be $0.02 \pm 0.005 \text{ ml}/\text{g}$ ($n = 7$) [17]. In the present study, the mean V_0 values of hearts in the Sa group obtained as volume-intercepts of the best-fit end-systolic pressure-volume relations was $0.025 \pm 0.014 \text{ ml}/\text{g}$ ($n = 7$) [14]. Therefore we judged that the V_0 values obtained by curve-fit are reliable.

Left ventricular PVA is a measure of the total mechanical energy generated by a left ventricular contraction. The PVA of an isovolumic contraction represents the maximal capability of external mechanical work of the left ventricle at a given preload [27]. In the present study, PVA was defined as the area surrounded by the best-fit end-systolic pressure-volume relation curve, the volume axis (instead of negligibly small end-diastolic pressure-volume relationship curve), and the vertical isovolumic pressure-volume line at any preloaded left ventricular volume, such as midrange left ventricular volume (mLVV) (Fig. 1C).

The PVA as a function of the left ventricular volume

was obtained by integrating the above exponential function from the extrapolated V_0 along the volume axis:

$$\text{PVA} = A (\text{left ventricular volume} - V_0) - A \{1 - \exp [-B (\text{left ventricular volume} - V_0)]\} / B.$$

We had proposed that in *in situ* hearts, PVA at an appropriate left ventricular volume on the curvilinear PVA-volume relationship is valuable to evaluate left ventricular mechanoenergetics [15, 16]. In the present study, we calculated midrange left ventricular volume that was the value of $[V_0 + (\text{maximum end-systolic volume} - \text{minimum end-systolic volume}) \text{ on the end-systolic pressure-volume relation} \times 1/2]$ from each pressure-volume loop. Each example of end-systolic pressure and PVA at midrange left ventricular volume (ESP_{mLVV} and PVA_{mLVV}) is shown in the lower panel of Fig. 1C. End-diastolic pressure-volume relations are very close to the volume axis, as shown in Fig. 1, B and C, and thus the effect of excluding the measurement of end-diastolic pressure-volume relation would be small. Ea (effective arterial elastance) is defined as the ratio of end-systolic pressure at end-systolic volume (ESP_{ESV}) to stroke volume of the left ventricle under stable hemodynamics. Stroke volume was obtained by the formula (end-diastolic volume – end-systolic volume) (Fig. 1B). Ejection fraction was obtained by the formula [stroke volume/(end-diastolic volume – V_0)].

Echocardiography. For the less-invasive study to follow up the heart function in the same rats (ejection fraction and fractional shortening), the measurements of left ventricular internal dimensions during systole and diastole (LVIDs and LVIDd) and wall thickness (interventricular septum thickness during systole and diastole, IVSTs and IVSTd; left ventricular posterior wall thickness during systole and diastole, LVPWTs and LVPWTd) by tran-

sthoracic echocardiography was performed at 0 and 3 days, and 1, 2, 4, 6, 8, 10, 12, 14, and 16 weeks in the Iso ($n = 3-8$) and Sa groups ($n = 3$). Two hours after the removal of an osmotic minipump including Iso or Sa under ethyl ether anesthesia, the same rats were again anesthetized with ethyl ether. An imaging of the heart was done using a 12-MHz ultrasound probe (PLT-1202S) and an echograph, Aplio SSA-700A (Toshiba, Tokyo, Japan). M-mode recordings were performed at the level of papillary muscles. Left ventricular fractional shortening [$100 \times (\text{LVIDd} - \text{LVIDs})/\text{LVIDd}$] and ejection fraction ($100 \times \text{stroke volume}/\text{end-diastolic volume}$) were analyzed using an installed software program in the echograph. After the recording, an osmotic minipump including Iso or Sa was again implanted. This procedure was repeated throughout the entire 16 weeks.

Statistical analysis. A comparison of paired and unpaired individual values was performed by paired and unpaired *t*-tests, respectively. Multiple comparisons were performed by one-way or repeated measured analysis of variance (ANOVA) with a post hoc Bonferroni's test or the Fisher's PLSD method. A value of $P < 0.05$ was considered statistically significant. All data are expressed as the mean $\pm$ SD.

RESULTS

Cardiac weights (Fig. 2)

Left ventricular wet weight/body weight was 1.94 ± 0.10 mg/g in Sa and significantly increased by 35% in the Iso 3d group and by 29% in the Iso 7d group ($P < 0.05$ vs. Sa). The right ventricular wet weight/body weight was 0.541 ± 0.040 mg/g in Sa and significantly increased by 22% in the Iso 3d group and 26% in the Iso 7d group ($P <$

Fig. 2. Comparison of cardiac wet weights and dry weights among Sa, Iso 3d, Iso 7d, Iso 3d(-), and Iso 7d(-) groups. Sa, saline (24 μ l/day for 3 days) infused group; Iso 3d or Iso 7d, isoproterenol ($1.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 3 days and 7 days) infused group; Iso 3d(-) or iso 7d(-): 2 days after removal of osmotic minipump in isoproterenol ($1.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 3 days and 7 days) infused group; BW, body weight; WW, wet weight; DW, dry weight; LV, left ventricle; RV, right ventricle; * $P < 0.05$ vs. Sa, # $P < 0.05$ vs. Iso 3d, * $P < 0.05$ vs. Iso 7d.

0.05 vs. Sa). Left ventricular dry weight/body weight was 0.488 ± 0.037 mg/g in Sa and significantly increased by 19% in the Iso 3d group and by 12% in the Iso 7d group ($P < 0.05$ vs. Sa). Right ventricular dry weight/body weight was not significantly increased in the Iso 3d group, but it increased by 24% in the Iso 7d group ($P < 0.05$ vs. Sa). There were no significant differences in all wet and dry weights/body weight between the Iso 3d and Iso 7d groups and between the Iso 3d(–) and Iso 7d(–) groups.

Western blot analyses

Each amount of expression of SERCA2 and PLB was significantly decreased in the Iso 3d group ($P < 0.05$) from that in the Sa group and returned to almost the same level in the Iso 7d group as in the Sa group (Fig. 3). Consequently, the ratio of expression of SERCA2/PLB was unchanged in all three groups. However, each amount of expression of phospho-Ser¹⁶ PLB in the Iso3d and Iso7d groups was significantly smaller than that in the Sa group. Furthermore, the amount of expression of NCX1 was also unchanged in all three groups (Fig. 3). The amount of expression of SERCA2 and PLB and the ratio of expression of SERCA2 versus PLB in the Iso 3d(–) and Iso 7d(–) groups was almost at the same level as those in the Sa group (data not shown).

Comparison of pressure-volume loops and end-systolic pressure-volume relations among Sa, Iso 3d, Iso 7d, Iso 3d(–), and Iso 7d(–) groups

Representative sets of pressure-volume loops for a typical heart from all five groups (Fig. 4). The shapes of end-systolic pressure-volume relations hardly differed among Sa, Iso 3d, and Iso 7d groups. The working ranges of left ventricular volume were markedly different in hypertro-

phied hearts in both the Iso 3d and Iso 7d groups from those in the Sa group (Fig. 4, B and C, vs. A). Respective hearts from Iso 3d(–) and Iso 7d(–) groups showed pressure-volume loops and end-systolic pressure-volume relations almost similar to those in a heart from the Sa group (Fig. 4, D and E, vs. A).

Mean midrange left ventricular volume (mLVV), ESP_{mLVV} , and PVA_{mLVV}

Mean mLVV in the Iso 3d (45% of Sa) and the Iso 7d group (61% of Sa) was significantly smaller than in the Sa group (0.123 ± 0.032 ml/g) ($P < 0.05$), but mean mLVV in the Iso 3d(–) and Iso 7d(–) groups was not significantly different from that in the Sa group. There were no significant differences in mean mLVV between the Iso 3d and Iso 7d groups or between the Iso 3d(–) and Iso 7d(–) groups (Fig. 5A).

Mean ESP_{mLVV} in the Iso 3d and Iso 7d groups was not significantly different from that in Sa, and there were no significant differences between the Iso 3d and Iso 7d groups or the Iso 3d(–) and Iso 7d(–) groups (Fig. 5B).

However, mean PVA_{mLVV} in the Iso 3d (49% of Sa) and the Iso 7d group (49% of Sa) was significantly smaller than that in the Sa group (7.16 ± 2.72 mmHg·ml·beat^{–1}·g^{–1}) ($P < 0.05$), although there were no significant differences in PVA_{mLVV} between the Iso 3d and Iso 7d groups (Fig. 5C). Mean PVA_{mLVV} in the Iso 3d(–) and Iso 7d(–) groups was not significantly different from that in the Sa group, and there were no significant differences between the Iso 3d(–) and Iso 7d(–) groups (Fig. 5C). The impairment of the left ventricular mechanical work capability in the Iso 3d and Iso 7d groups and its reversibility by the removal of Iso seem to be similar.

Fig. 3. Comparison of Western blots of SERCA2, PLB, SERCA2/PLB, phospho-Ser¹⁶PLB, NCX1 in each heart among Sa, Iso3d, and Iso7d groups. * $P < 0.05$ vs. Sa group.

Fig. 4. Each representative set of P-V loops and ESPVR in Sa (A), Iso 3d (B), Iso 7d (C), Iso 3d(-) (D), and Iso 7d(-) (E).

Fig. 5. Mean mLVV (midrange LVV at end-systole) (A), ESP_{mLVV} (B), and PVA_{mLVV} (C) in Sa, Iso 3d, Iso 7d, Iso 3d(-), and Iso 7d(-) groups. * $P < 0.05$ vs. Sa. + $P < 0.05$ vs. Iso 7d.

Other hemodynamic parameters

Mean end-diastolic volume, stroke volume, and cardiac output in the Iso 3d and Iso 7d groups were significantly smaller than those in the Sa group ($P < 0.05$) (Fig. 6), but mean ejection fraction and mean ESP_{ESV} were unchanged in all groups (Table 1). Mean Ea was significantly larger in Iso 3d (175% of Sa) because of smaller stroke volume, but not in the other three groups (Table 1). Mean end-systolic volume, end-diastolic volume, stroke volume, cardiac output, ejection fraction, ESP_{ESV}, and Ea in the Iso 3d(-) and Iso 7d(-) groups did not differ from those in the Sa group. All hemodynamic parameters were not significantly different between the Iso 3d and Iso 7d

groups or between the Iso 3d(-) and Iso 7d(-) groups (Fig. 6 and Table 1). However, in ESV and EDV, there were significant differences between the Iso 7d and Iso 7d(-) groups, whereas there were no significant differences between the Iso 3d and Iso 3d(-) groups (Fig. 6). Although it appeared that the reversibility from Iso 7d by the removal of Iso was better than that from Iso 3d by its removal, this may be due to marked increases and fluctuations in V_0 values in the Iso 7d(-) group (Table 1). In fact, the reversibility of SV and CO in Iso 3d(-) was significant, but not in Iso 7d(-), as predicted (Fig. 6).

Although mean heart rate was significantly smaller in the Iso 3d group ($P < 0.05$), in other groups it did not differ

Fig. 6. Mean ESV (end-systolic volume) (A), EDV (end-diastolic volume) (B), SV (stroke volume) (C), and CO (cardiac output) (D) in Sa, Iso 3d, Iso 7d, Iso 3d(-), and Iso 7d(-) groups. * $P < 0.05$ vs. Sa, # $P < 0.05$ vs. Iso 3d, + $P < 0.05$ vs. Iso 7d.

Table 1. Hemodynamics based on in situ analysis of pressure-volume loops.

	Sa	Iso 3d	Iso 7d	Iso 3d(-)	Iso 7d(-)
<i>n</i>	(7)	(7)	(7)	(7)	(7)
V_0 (ml/g)	0.025 ± 0.014	$0.008 \pm 0.005^*$	0.014 ± 0.012	$0.010 \pm 0.006^*$	0.030 ± 0.036
EF (%)	70.6 ± 4.1	75.7 ± 9.9	66.2 ± 9.7	76.1 ± 1.5	60.0 ± 7.9
ESP _{ESV} (mmHg)	71 ± 7	70 ± 5	66 ± 2	78 ± 7	72 ± 5
Ea (mmHg·ml ⁻¹ ·g)	482 ± 128	$843 \pm 181^*$	769 ± 222	607 ± 169	618 ± 153
HR (beats/min)	354 ± 28	$293 \pm 16^*$	308 ± 26	329 ± 54	327 ± 30

V_0 , systolic unstressed volume; ESP_{ESV}, end-systolic pressure at ESV; ESP_{mLVV}, end-systolic pressure at mLVV (midrange LVV); Ea, Effective arterial elastance; HR, heart rate. * $P < 0.05$ vs. Sa.

from that in the Sa group (Table 1). We have previously reported that the curved end-systolic pressure-volume relation is independent of heart rate in *in situ* rat hearts within 250–320 bpm [16] and in excised rat hearts within 250–300 bpm [21]. In the present study, the mean heart rate distributed from 293–354 bpm, indicating that the curved end-systolic pressure-volume relation will not be affected by heart rate.

Echocardiographic evaluation of heart function and wall thickness

At 3 days and at 1 and 2 weeks, ejection fraction and fractional shortening in the Iso-infused rat group was not significantly decreased from those in the Sa-infused group. At 4 weeks, the ejection fraction was decreased by 12%, and this was accompanied by a 25% decrease in fractional shortening from those at day 0 in the Iso-infused rat group. These values at 4 weeks were significantly ($P < 0.05$) smaller than the respective data in the Sa-in-

fused rat group (Fig. 7A). In the 6–14-week Iso-infused rat group, both ejection fraction and fractional shortening did not further decrease, though both decreases remained significant ($P < 0.05$) compared with the respective data in the Sa-infused rat group (Fig. 7A).

At 4 weeks, left ventricular internal dimensions during systole (LVIDs) were increased by 43% from those at day 0 in the Iso-infused rat group, and this increased value was significantly ($P < 0.05$) larger and remained significant ($P < 0.05$) through the 16 weeks compared with respective data in the Sa-infused rat group (Fig. 7B). In contrast, left ventricular internal dimensions during diastole (LVIDd) in the Iso-infused rat group was not significantly larger than those in the Sa-infused rat group throughout the entire 16 weeks (Fig. 7B).

At 3 days and 1 week, interventricular septum thickness during diastole (IVSTd) as well as left ventricular posterior wall thickness during diastole (LVPWTd) were significantly ($P < 0.05$) increased by 20%–34% from

those at day 0 in Iso-infused rat hearts, and their increases remained significant ($P < 0.05$) throughout the 16 weeks, compared with the respective data in the Sa-infused rat group (Fig. 7C).

Compared with echocardiographic images of Sa-infused rat hearts at 2 and 4 weeks, there was a marked increase in LVIDs at 4 weeks accompanied with already increased LVPWTd and IVSTd at 3 days in Iso-infused rat hearts (Fig. 8), indicating LV systolic dysfunction with hypertrophy.

DISCUSSION

Although many studies on mechanisms underlying Iso-induced cardiac hypertrophy have been reported [4–13], the conclusive mechanisms have not yet been determined. However, we have revealed the most upstream mechanism: the β_1 -AR-mediated signal transduction pathway is primarily responsible for all events related to cardiac hypertrophy induced by Iso infusion [14].

The β_1 -adrenergic receptors (β_1 ARs), which bind circulating catecholamines, activate adenylate cyclase by coupling through the stimulatory G-protein, G α_s . The resulting increase in intracellular cAMP activates cAMP-dependent protein kinase (PKA). PKA has several pathways in cardiomyocytes that influence contractility in response to activated β_1 AR signaling; these include the L-type Ca^{2+} channel in the sarcolemma, the ryanodine re-

ceptor (RyR2), and phospholamban (PLB) in the sarcoplasmic reticulum (SR). The phosphorylation of PLB leads to an increase of SR Ca^{2+} ATPase (SERCA) activity and consequent accelerated Ca^{2+} accumulation in the SR. PKA hyperphosphorylation of the RyR2 is observed in

Fig. 7. Echocardiographic parameters of heart function at 3 days (D) and 1–16 weeks (WK) in isoproterenol (Iso)-infused rats and saline (Sa)-infused rats. **A:** EF (ejection fraction) and FS (fractional shortening). **B:** LVIDs (left ventricular internal dimensions during systole) and LVIDd (left ventricular internal dimensions during diastole). **C:** IVSTs (interventricular septum thickness during systole) and IVSTd (interventricular septum thickness during diastole). * $P < 0.05$ vs. Sa, # $P < 0.05$ vs. 0 WK.

Fig. 8. Echocardiographic images of hearts from saline (Sa)- and isoproterenol (Iso)-infused rats at 2 and 4 weeks. LVIDs, left ventricular internal dimensions during systole; LVIDd, left ventricular internal dimensions during diastole; LVPWTd, left ventricular posterior wall thickness during diastole. ☆, large LVIDs indicated systolic failure.

failing human hearts [28], suggesting a link between PKA signaling and negative inotropism associated with long-term β_1 AR signaling. In the present Iso group at longer than 4 weeks, ejection fraction and fractional shortening actually decreased, accompanied by increased LVIDs, IVSTd, and LVPWTd, indicating systolic failure with hypertrophy (see Figs. 7 and 8).

On the other hand, each amount of expression of phospho-Ser¹⁶PLB in the Iso 3d and Iso 7d groups with short-term β_1 AR signaling was significantly smaller than that in the Sa group (see Fig. 3). Therefore we predicted diastolic dysfunction because of the impaired Ca^{2+} uptake function into SR in both groups under the unchanged expression of NCX1 protein presented in Fig. 3. However, we did not observe a marked upward shift of end-diastolic pressure-volume relation corresponding to diastolic dysfunction in the present study, though we found in vitro a left ventricular lusitropic dysfunction in the same hypertrophy model [29]. This might be a limitation of the present pressure-volume measurement system.

Present results suggested that short-term β_1 AR signaling elicited some remodeling of Ca^{2+} handling in cardiac excitation-contraction coupling, such as the impairment of the Ca^{2+} uptake function into SR with simultaneous hypertrophy development. Unlike the present hypertrophy model, in the spontaneously hypertensive rats the high afterload may induce some remodeling of Ca^{2+} handling in cardiac excitation-contraction coupling prior to the development of hypertrophy [30]. The contribution of the remodeling of Ca^{2+} handling in cardiac excitation-contraction coupling for developing cardiac hypertrophy may not be uniform in respective hypertrophy models.

The simultaneous measurement of left ventricular pressure-volume signals and analysis using the framework of the systolic pressure-volume area (PVA), especially PVA_{mLVV} are substantially needed to evaluate the left ventricular function in the heart, because PVA_{mLVV} precisely reflects the changes in left ventricular mechanical work capability at mLVV [15–17]. Considering similar decreases in PVA_{mLVV} of Iso 3d and 7d groups, we judged that the left ventricular mechanical work capability was impaired in a similar degree in both groups, though no decreases in ejection fraction or fractional shortening were detected.

At 3 days and 1 week in Iso-infused rat data, the wall thickness parameters such as IVSTd and LVPWTd were significantly increased from those at 0 days. This result is in accordance with the significant increase in LV weights and the significant decrease in stroke volume at 3 days and 1 week in Iso-induced rat data (see Figs. 2 and 6). These increases in IVSTd (and LVPWTd) remained significant compared with their respective Sa-infused rat data for up to 16 weeks (see Fig. 7C), indicating that the left ventricular wall thickness never decreased throughout that time.

Although the mean ejection fraction values calculated

from a pressure-volume loop were smaller than those calculated by echocardiography, there were no significant differences in mean ejection fraction among the Sa, Iso 3d, and Iso 7d groups (see Table 1). Furthermore, fractional shortening and ejection fraction in Iso-infused rats at 3 days and at 1 and 2 weeks calculated by echocardiography were not significantly decreased from the respective data in Sa-infused rats. These results suggested that after 3d-, 7d-, and 2-week Iso infusions, marked hemodynamic changes evaluated by echocardiography did not occur. At 4–16 weeks, the fractional shortening and ejection fraction were significantly decreased, accompanied by significantly increased LVIDs compared to those in Sa-infused rats (see Figs. 7 and 8). Taken together, it was suggested that Iso treatment for a term longer than 4 weeks induced systolic dysfunction in a thick-walled heart (hypertrophy), but not the dilated cardiomyopathy in a thin-walled heart, for up to 16 weeks.

The present result indicated that the hypertrophy model was not so markedly changed in function and shape between 3-day and 2-week Iso infusions. During the same term of Iso infusion, expressions of immediate early genes, *GATA-4* and *Nkx-2.5*, *Egr-1*, *c-jun*, and *c-fos* were persistently increased, though after a much higher dose of Iso infusion ($30 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) than the present one ($1.2 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) [13]. We concluded that even the short-term Iso-infused model sufficiently represents the effects of up to a 2-week Iso infusion; it is plausible that the 3-day and 7-day models are corresponding to the 2-week hypertrophy model.

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