NHE-1 participates in isoproterenol-induced downregulation of SERCA2a and development of cardiac remodeling in rat hearts

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Shibata M, Takeshita D, Obata K, Mitsuyama S, Ito H, Zhang G, Takaki M. NHE-1 participates in isoproterenol-induced downregulation of SERCA2a and development of cardiac remodeling in rat hearts. Am J Physiol Heart Circ Physiol 301: H2154–H2160, 2011. First published August 19, 2011; doi:10.1152/ajpheart.00483.2011.—Impaired Ca2+ handling is one of the main characteristics in heart failure patients. Recently, we reported abnormal expressions of Ca2+-handling proteins in isoproterenol (ISO)-induced hypertrophied rat hearts. On the other hand, Na+/H+ exchanger (NHE)-1 inhibitor has been demonstrated to exert beneficial effects in ischemic-reperfusion injury and in the development of cardiac remodeling. The aims of the present study are to investigate the role of NHE-1 on Ca2+ handling and development of cardiac hypertrophy in ISO-infused rats. Male Wistar rats were randomly divided into vehicle [control (CTL)] and ISO groups without or with pretreatment with a selective NHE-1 inhibitor, BIIB-723. ISO infusion for 1 wk significantly increased the ratios of heart to body weight and left ventricle (LV) to body weight and collagen accumulation. All of these increases were antagonized by coadministration with BIIB-723. The ISO-induced significant increase in LV wall thickness was suppressed significantly (P < 0.05) by BIIB-723. ISO-induced decreases in cardiac stroke volume and a total mechanical energy per beat index, systolic pressure-volume area, and diastolic function at midrange LV volume, were normalized by BIIB-723. The markedly higher expression of NHE-1 protein in the ISO group than that in CTL group was suppressed (P < 0.05) by BIIB-723. Surprisingly, ISO-induced downregulation of the important Ca2+-handling protein sarcolema and sarcoplasmic reticulum Ca2+-ATPase 2a, the expression of which was also normalized by BIIB-723 without changes in phosphorylated phosphohemoglobin (PLB)/PLB expression. We conclude that NHE-1 contributes to ISO-induced abnormal Ca2+ handling associated with cardiac hypertrophy. Inhibition of NHE-1 ameliorates cardiac Ca2+ handling impairment and prevents the development of cardiac dysfunction in ISO-infused rats.

hypertrophy; pressure-volume area; sodium ion/hydrogen ion exchanger; sarcoplasmic reticulum calcium ion-adenosinetriphosphatase

INCREASED ACTIVITY OF THE sympathetic nervous system is one of the main pathological features of patients with heart failure. Catecholamine plays an important role in compensatory myocardial hypertrophy (3, 8, 20, 15). Cardiac hypertrophy is a critical step for the heart in transition from the adaptive to maladaptive state. Previously, we have demonstrated that chronic stimulation with a β-adrenergic agonist, isoproterenol (ISO), induces cardiac hypertrophy accompanied by enhanced fibrosis (14, 18, 21, 28, 29). Recently, we observed that chronic ISO stimulation induces left ventricular diastolic dysfunction associated with downregulation of Ca2+-handling proteins. However, lowering heart rate could compensate the diastolic dysfunction (18). On the other hand, a growing body of evidence demonstrates that impaired Ca2+ handling plays an essential role in the development of heart failure (9, 16, 27). Both pharmacological and genetic approaches have been applied in experimental models of heart failure to restore Ca2+ handling (4, 13, 17). However, specific research focused on the improvement of Ca2+ handling on the onset of cardiac hypertrophy in response to β-adrenergic stimulation is still insufficient.

Na+/H+ exchanger (NHE) is an integral membrane glycoprotein and plays a key role in maintaining intracellular pH and Na+ concentration and cellular volume. Cardiac cells express primarily the NHE-1 isof orm. NHE-1 has been demonstrated to be involved in ischemic-reperfusion injury and cardiac remodeling (2, 7, 11). The pharmacological evidence supporting the beneficial effects of NHE-1 inhibition on ischemia-reperfusion injury was confirmed by the result showing that genetic deletion of NHE-1 exhibited enhanced resistance to ischemia-reperfusion injury (25).

Recently, the link between NHE-1 activity and myocardial hypertrophy has been clearly established in elevated sympathetnic nerve activity models. In β-adrenergic receptor transgenic mice, NHE-1 inhibition prevented the development of cardiac hypertrophy and fibrosis and normalized the mRNA expression of the exchanger (5). Similarly, long-term ISO-induced cardiac hypertrophy was prevented by the inhibition of NHE-1 in rats (6). Furthermore, the development of heart failure and disturbances of cellular Na+ homeostasis and cytoplasmic and sarcoplasmic reticular Ca2+ handling in a heart failure rabbit model induced by a combined volume and pressure overloading was prevented by chronic NHE-1 inhibition (1). However, no previous studies have investigated the short-term effects of NHE-1 inhibition on the compensatory phase of cardiac hypertrophy and impaired Ca2+ handling. The present study, therefore, is designed to explore the possible roles of NHE-1 in the development of cardiac hypertrophy and intracellular Ca2+ handling impairment in response to chronic ISO infusion.

METHODS

Animals and drug administration. Male Wistar rats (8–12 wk) used in the present experiments were randomly divided into four groups, i.e., the vehicle [control (CTL)] group, a group infused with the selective NHE-1 inhibitor BIIB-723 (NHEI), and ISO-infused groups without (ISO) or with (NHEI + ISO) pretreatment with BIIB-723. Delivery of drug was achieved by implanting an osmotic minipump (Alzet; Durect, Cupertino, CA) subcutaneously in the neck under...
pentobarbital sodium (50 mg/kg ip) anesthesia. Either ISO (1.2 mg·kg⁻¹·day⁻¹ for 7 days) or vehicle (0.1% ascorbic acid in saline, 24 μl/day for 7 days) was infused subcutaneously (14, 18). Rats were received NHE-1 inhibitor BIIB-723 (3.0 mg·kg⁻¹·day⁻¹; Boehringer-Ingelheim Pharmaceuticals, Ridgefield, CT) in drinking water 3 days before the start of ISO infusion. The osmotic minipump was removed from the neck 7 days after the implantation under ethyl ether or pentobarbital sodium anesthesia for the following experiments: echocardiography, measurements of LV stroke volume and pressure volume area, heart extraction for histological analysis, and Western blot for NHE-1 and Ca²⁺-handling proteins. All surgical and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85–23, revised 1996) and were reviewed and approved by the animal care committee of Nara Medical University.

Echocardiography. Two hours after the removal of the osmotic minipump, left ventricular (LV) internal dimensions during systole and diastole were measured by 12-MHz ultrasound system. Results were expressed as mean ± SE. Differences were assessed by Student’s t-test.

Fig. 1. Role of Na⁺/H⁺ exchanger (NHE)-1 on isoproterenol (ISO)-induced cardiac hypertrophy. A: hematoxylin-eosin (HE) staining. B: left, the ratio of heart weight (HW) to body weight (BW). Right, the ratio of left ventricular weight (LVW) to BW. CTL (n = 8 hearts), NHEI (n = 8), ISO (n = 10), and ISO + NHEI (n = 10) represent control, BIIB-723, isoproterenol, and isoproterenol plus BIIB-723 groups, respectively. *P < 0.05 vs. CTL. †P < 0.05 vs. ISO.

Fig. 2. Role of NHE-1 on ISO-induced cardiac fibrosis in CTL (n = 4), NHEI (n = 4), ISO (n = 4), and ISO + NHEI (n = 4) groups. A: HE staining. B: Masson’s trichrome staining. C: left ventricular collagen area. *P < 0.05 vs. CTL. †P < 0.05 vs. ISO.
and diastole (LVIDd and LVIDs, respectively) and wall thickness [interventricular septum thickness during systole and diastole (IVSTd); LV posterior wall thickness during systole and diastole (LVPWTd)] were measured by 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 as previously reported (24). LV fractional shortening (\(\text{FS} = \frac{100 \times (\text{LVIDd} - \text{LVIDs})}{\text{LVIDd}}\)) and ejection fraction (EF) (\(100 \times \text{stroke volume/end-diastolic volume}\)) were analyzed by an installed software program in the echograph.

Measurements of LV stroke volume and pressure-volume area. Two hours after removal of the osmotic minipump, LV volume (LVV) and pressure (LVP) were simultaneously measured under constant pentobarbital sodium anesthesia. The trachea was intubated, and the rat was ventilated with room air. The chest was opened, and a conductance catheter (1.5 Fr) (10) was introduced in the LV through an apical stab to obtain reliable LVV signal. A 1.5-Fr pressure catheter was also inserted through the apex in the LV to obtain reliable LVP signal (Fig. 4A). The detailed methods of conductance catheter for measuring LV stroke volume \([= \text{end-diastolic LVV} - \text{end-systolic volume (SV)}]\) and pressure-volume area (an appropriate index for evaluating total cardiac mechanical energy/beat; it is tightly related to myocardial oxygen consumption/beat) at midrange LVV (PVA<sub>midLVV</sub>) calculated from the end-systolic pressure-volume relation (ESPVR) curve during increasing afterload by aortic gradual occlusion have been described in previous reports (14, 22–24). Therefore, PVA<sub>midLVV</sub> is a better cardiac functional index in terms of cardiac mechanoenergetics. In the present study, we calculated midrange LVV (mLVV) that was the value of \(V_0\) + maximum ESV - minimum ESV on the ESPVR \(\times 1/2\) (where \(V_0\), conductance LVV intercept and ESV is end-systolic volume) from all pressure-volume loops in each group.

**Histological analysis.** Rats were killed, and hearts were sampled and 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 detection of fibrosis (18).

**Western blotting for NHE-1 and Ca<sup>2+</sup>-handling proteins.** Membrane proteins were isolated from the LV wall of each frozen heart. 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 NHE-1, sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA2a), phospholamban (PLB), phospho-Ser<sup>16</sup> PLB (p-PLB), phospholemman (PLM), Na<sup>+</sup>-K<sup>+</sup>-ATPase-1 (NKA), and Na<sup>+</sup>/Ca<sup>2+</sup>-exchanger (NCX1) (18). The same amounts of membrane proteins (10 μg/lane) were separated on SDS-polyacrylamide gels (10% for NHE-1, SERCA2a, and NKA; 5% for PLB, p-PLB, and PLM; 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, Osaka, Japan) and then incubated with anti-NHE-1 (Cell Signaling Tech), anti-SERCA2a antibody (1:1,000 dilution; Affinity Bio Reagents), anti-PLB antibody (1:2,000 dilution; Upstate Biotechnology), anti-p-PLB antibody (p-Ser<sup>16</sup>) (1:1,000 dilution; Upstate Biotechnology), anti-PLM antibody (1:100 dilution; ABGENT), or anti-NKA antibody (1:10,000 dilution; Upstate Biotechnology) and anti-NCX1 antibody (1:200 dilution, a generous gift from Dr. Iwamoto, Fukuoka University). The antigens were detected by the enhanced chemiluminescence method (ECL Western blotting detection kit; Amersham) with peroxidase-linked anti-mouse IgG (1:2,000 dilution) or peroxidase-linked anti-rabbit IgG (1:2,000 or 1:5,000 dilution). After immunoblotting, the film was scanned with a scanner, and the intensity of the bands was calculated by NIH image analysis.

### Table 1. Echocardiography parameters in CTL, NHEI, ISO, and NHEI + ISO groups

<table>
<thead>
<tr>
<th>Group</th>
<th>IVSTd, mm</th>
<th>LVIDd, mm</th>
<th>LVPPWtd, mm</th>
<th>IVSTS, mm</th>
<th>LVIDs, mm</th>
<th>EF, %</th>
<th>FS, %</th>
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<tbody>
<tr>
<td>CTL ((n = 6))</td>
<td>1.545 ± 0.060</td>
<td>8.075 ± 0.602</td>
<td>1.547 ± 0.034</td>
<td>3.463 ± 0.086</td>
<td>3.307 ± 0.149</td>
<td>92.92</td>
<td>58.88 ± 3.526</td>
</tr>
<tr>
<td>NHEI ((n = 5))</td>
<td>1.516 ± 0.038</td>
<td>8.004 ± 0.114</td>
<td>1.534 ± 0.038</td>
<td>3.342 ± 0.089</td>
<td>3.198 ± 0.265</td>
<td>93.56 ± 1.493</td>
<td>60.06 ± 3.032</td>
</tr>
<tr>
<td>ISO ((n = 9))</td>
<td>1.913 ± 0.091*</td>
<td>7.991 ± 0.573</td>
<td>1.890 ± 0.112*</td>
<td>3.532 ± 0.310</td>
<td>3.978 ± 0.489*</td>
<td>86.51 ± 4.855*</td>
<td>50.13 ± 5.836*</td>
</tr>
<tr>
<td>NHEI + ISO ((n = 13))</td>
<td>1.681 ± 0.112*</td>
<td>8.252 ± 0.542</td>
<td>1.681 ± 0.105*</td>
<td>3.320 ± 0.206</td>
<td>4.151 ± 0.358*</td>
<td>86.65 ± 4.672*</td>
<td>49.48 ± 5.646*</td>
</tr>
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</table>

Values are means ± SD; \(n\), no. of hearts. CTL, control; NHEI, BIIB-723 infusion; ISO, isoproterenol infusion; NHEI + ISO, isoproterenol infusion with BIIB-723 pretreatment; IVSTd, interventricular septal thickness during diastole; LVIDd, left ventricular internal dimension during diastole; LVPPWtd, left ventricular posterior wall thickness during diastole; ISO, isoproterenol infusion; FS, fractional shortening. *\(P < 0.05\) vs. CTL. †\(P < 0.05\) vs. ISO.
Role of NHE-1 on the ISO-induced development of cardiac hypertrophy. Chronic ISO infusion induced eccentric cardiac hypertrophy; a selective NHE-1 inhibitor, BIIB-723, reduced the ISO-induced eccentric cardiac hypertrophy (NHEI/ISO; Fig. 1A). ISO increased the ratios of heart weight to body weight (3.533 ± 0.337 vs. 2.681 ± 0.093, P < 0.05) and LV weight (LVW) to body weight (2.832 ± 0.307 vs. 2.063 ± 0.110, P < 0.05) compared with the CTL group. BIIB-723 alone did not affect these ratios (NHEI group; heart weight to body weight, 2.592 ± 0.085; LVW to body weight, 2.031 ± 0.060) compared with the CTL group. BIIB-723 inhibited the ISO-induced increases of these ratios (NHEI/ISO group; heart weight to body weight, 3.092 ± 0.190; LVW to body weight, 2.448 ± 0.144, P < 0.05) compared with the ISO group (Fig. 1B). Histological analysis also confirmed that ISO infusion increased the cross-sectional area of cardiac cells, which was partially reduced by BIIB-723 treatment (Fig. 2A). Collagen accumulation was also found in ISO-infused rat hearts; the percentage of collagen area to whole tissue area was increased significantly (P < 0.05) compared with the CTL group (7.91 ± 2.01 vs. 4.39 ± 1.61). These increases were markedly reduced by BIIB-723; the percentage of collagen area to whole tissue area was reduced to 5.91 ± 2.49 (P < 0.05 vs. ISO) (Fig. 2, B and C). Echocardiographic data also demonstrated that ISO infusion increased cardiac IVSTd, LVPWTd, and LVIDs. The increases in IVSTd and LVPWTd were suppressed by BIIB-723 (NHEI/ISO group; Fig. 3 and Table 1), supporting the role of NHE-1 in the ISO-induced development of cardiac hypertrophy. However, the increase in LVIDs and the decrease in EF and FS were not normalized by BIIB-723 (NHEI+ISO group; Fig. 3 and Table 1). Furthermore, there existed no differences in LVIDd among all groups (Table 1). It
Induced decrease of SV compared with the ISO group (0.117 vs. 0.095 mmHg·ml·beat⁻¹·g⁻¹). BIIB-723 alone did not change the expression of all Ca²⁺-handling proteins (Fig. 7). ISO infusion resulted in downregulation of the Ca²⁺-handling proteins SERCA2a, PLB, p-PLB, PLM, and NKA to 0.655 ± 0.085-, 0.465 ± 0.091-, 0.541 ± 0.158-, 0.608 ± 0.166-, and 0.688 ± 0.096-fold of CTL (P < 0.05 vs. CTL group) with unchanged p-PLB/PLB and without changing the levels of NCX-1 protein expression (1.135 ± 0.215-fold). BIIB-723 treatment did not affect ISO-induced downregulation of PLB, p-PLB, PLM, and NKA (0.465 ± 0.099-, 0.541 ± 0.247-, 0.730 ± 0.088-, and 0.738 ± 0.123-fold, respectively). However, BIIB-723 reversed ISO-induced downregulation of only SERCA2a protein to 1.140 ± 0.185-fold of CTL (P < 0.05 vs. the ISO group) with unchanged p-PLB/PLB. This indicates that NHE-1 at least play a role in ISO-induced disturbance of Ca²⁺ handling.

Role of NHE-1 on the ISO-induced cardiac dysfunction. Basic hemodynamic data were shown in Table 2. There were no differences in LV maximal pressure among the groups. Each shape of ESPVR curves obtained by increasing afterload seemed likely from these echocardiographic data that ISO-induced cardiac dysfunction is not normalized by BIIB-723.

Role of NHE-1 on the ISO-induced cardiac dysfunction. Basic hemodynamic data were shown in Table 2. There were no differences in LV maximal pressure among the groups. Each shape of ESPVR curves obtained by increasing afterload seemed to be not different among the groups (Fig. 4). mLLV (ml/g) in each group was 0.0975 in CTL, 0.0853 in NHEI, 0.0559 in ISO, and 0.0712 in the NHEI + ISO groups. A good cardiac function index, PVA mLLV (14, 21, 22), demonstrated that ISO infusion significantly reduced PVA mLLV compared with the CTL group (3.945 ± 0.547 vs. 9.174 ± 1.467 mmHg·ml·beat⁻¹·g⁻¹, P < 0.05). BIIB-723 antagonized the ISO-induced decrease in PVA mLLV compared with the ISO group (5.977 ± 1.086 vs. 3.945 ± 0.547 mmHg·ml·beat⁻¹·g⁻¹, P < 0.05) (Fig. 5A). Furthermore, ISO infusion markedly reduced SV obtained from each LV pressure-volume loop compared with the CTL group (0.095 ± 0.016 vs. 0.140 ± 0.015 ml/g, P < 0.05). BIIB-723 alone did not affect SV (data not shown), but it antagonized the ISO-induced decrease of SV compared with the ISO group (0.117 ± 0.011 vs. 0.095 ± 0.016 ml/g, P < 0.05) (Fig. 5B). These data clearly demonstrated that NHE-1 is involved in the ISO-induced cardiac dysfunction.

Effects of an NHE inhibitor, BIIB-723, on the ISO-induced increase of NHE-1 expression. Chronic ISO infusion for 1 wk increased NHE-1 expression to 1.168 ± 0.225-fold of CTL (P < 0.05 vs. ISO) (Fig. 6).

Role of NHE-1 on the ISO-induced downregulation of Ca²⁺-handling proteins. BIIB-723 alone did not change the expression of all Ca²⁺-handling proteins (Fig. 7). ISO infusion resulted in downregulation of the Ca²⁺-handling proteins SERCA2a, PLB, p-PLB, PLM, and NKA to 0.655 ± 0.085-, 0.465 ± 0.091-, 0.541 ± 0.158-, 0.608 ± 0.166-, and 0.688 ± 0.096-fold of CTL (P < 0.05 vs. CTL group) with unchanged p-PLB/PLB and without changing the levels of NCX-1 protein expression (1.135 ± 0.215-fold). BIIB-723 treatment did not affect ISO-induced downregulation of PLB, p-PLB, PLM, and NKA (0.465 ± 0.099-, 0.541 ± 0.247-, 0.730 ± 0.088-, and 0.738 ± 0.123-fold, respectively). However, BIIB-723 reversed ISO-induced downregulation of only SERCA2a protein to 1.140 ± 0.185-fold of CTL (P < 0.05 vs. the ISO group) with unchanged p-PLB/PLB. This indicates that NHE-1 at least play a role in ISO-induced disturbance of Ca²⁺ handling.

DISCUSSION

Recent basic and clinical studies have demonstrated that disturbance of ion homeostasis plays an important role in the initiation and development of heart failure (9, 17, 27). Impaired Ca²⁺ handling is one of the common phenomena in heart failure models (9). Long-term sympathetic nerve activation resulted in heart failure accompanied by impaired Ca²⁺ handling (3, 8, 15, 20). Recently, we demonstrated that short-term stimulation with a β-adrenergic agonist, ISO, induced cardiac hypertrophy accompanied by enhanced fibrosis and downregulation of Ca²⁺-handling proteins (18). Our present data clearly demonstrated that NHE-1 played a role in the development of cardiac hypertrophy and downregulation of an important Ca²⁺- handling protein, SERCA2a, in ISO-induced hypertrophied rat hearts. NHE-1 inhibitor effectively improved cardiac dysfunction in this model, suggesting NHE-1 is involved in the ISO-induced cardiac dysfunction.

It is reported that potential mechanisms underlying for ISO-induced cardiac hypertrophy involve multiple factors. We previously reported that oxidative stress and activation of ANG
II type 1 receptor played a role in the development of cardiac hypertrophy in response to ISO stimulation (28, 29). It was also reported that chronic ISO stimulation induced an increase of NHE-1 activity, which resulted in activation of Ca$^{2+}$-dependent prohypertrophy signaling pathways (19). The role of NHE-1 in myocardial hypertrophy and remodeling was well reviewed recently by Karmazyn et al. (12). Our present observations showing that ISO stimulation increases the NHE-1 expression and cardiac hypertrophy and that an NHE-1 selective inhibitor, BIIB-723, effectively suppresses the ISO-induced increase of NHE-1 expression and cardiac hypertrophy are in concordance with the previous observations (12, 19).

Although the underlying mechanisms involved in ISO-induced NHE-1 activation were still unknown, solid evidence supported that ISO stimulation increased NHE-1 expression and its activity (1, 6). Increased NHE-1 activity could induce gene expression leading to cardiac hypertrophy and Ca$^{2+}$-dependent prohypertrophy signaling pathways (19, 26). However, it was unknown how NHE-1 affects cytosolic Ca$^{2+}$ handling. The present study first demonstrated that the NHE-1 inhibitor reversed the ISO-induced downregulation of an important Ca$^{2+}$-handling protein, SERCA2a, without changes in p-PLB/PLB expression, suggesting NHE-1 has a possible role in downregulation of SERCA2a and the resultant cytosolic Ca$^{2+}$-handling impairment in response to ISO stimulation. Although how NHE-1 could regulate SERCA2a expression is still unknown, we have speculated that increases of cytosolic Na$^+$ by NHE-1 upregulation may increase cytosolic Ca$^{2+}$ concentration by reverse-mode NCX, which may negatively regulate SERCA2a expression. To verify this speculation, we investigated whether the ISO-induced increased NCX current (21) was suppressed by NHE1. However, the increased NCX current was unaffected by NHE1 (unpublished observation). Further studies are still necessary to clarify the possible mechanisms involved in downregulation of SERCA2a in response to the activation of NHE-1.

In the present study, we could find cardiac dysfunction in echocardiography data such as EF and FS, although this cardiac dysfunction was not attenuated by NHEI. From hemodynamic data, LV maximal pressure and LV end-diastolic pressure were not different among all groups, but heart rate in ISO was decreased significantly. In the same hypertrophy model, we previously could detect that there was diastolic dysfunction indicated by elongated tau under constant pacing conditions (18) because tau is dependent on heart rate. Therefore, this index is inappropriate to evaluate cardiac diastolic dysfunction in the present in situ hearts.

Our in situ heart LV pressure-volume data revealed that ISO infusion reduced PVA$_{mlLVV}$, a more precise index for cardiac function, especially mechanical work, which has been proposed by previous reports (22–24), suggesting echocardiography, although a noninvasive measurement, may be less sufficient for monitoring cardiac function than the direct LV pressure-volume measurements. Herein, we propose again to use PVA$_{mlLVV}$ as a routine parameter to evaluate cardiac function, especially in basic research of early cardiac dysfunction models.

In conclusion, our present study demonstrated that NHE-1 contributes to ISO-induced abnormal Ca$^{2+}$ handling associated with cardiac hypertrophy. Inhibition of NHE-1 ameliorates cardiac Ca$^{2+}$-handling impairment and mitigates the development of cardiac remodeling in ISO-infused rats. The present
study may provide basic evidence for clinical treatments for early hypertensive patients with cardiac dysfunction.

GRANTS

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DISCLOSURES

Conflict of Interest: none declared.

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