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Repeated sauna therapy attenuates ventricular remodeling after myocardial infarction in rats by increasing coronary vascularity of noninfarcted myocardium

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Sobajima M, Nozawa T, Shida T, Ohori T, Suzuki T, Matsuki A, Inoue H. Repeated sauna therapy attenuates ventricular remodeling after myocardial infarction in rats by increasing coronary vascularity of noninfarcted myocardium. *Am J Physiol Heart Circ Physiol* 301: H548–H554, 2011. First published May 27, 2011; doi:10.1152/ajpheart.00103.2011.—Repeated sauna therapy (ST) increases endothelial nitric oxide synthase (eNOS) activity and improves cardiac function in heart failure as well as peripheral blood flow in ischemic limbs. The present study investigates whether ST can increase coronary vascularity and thus attenuate cardiac remodeling after myocardial infarction (MI). We induced MI by ligating the left coronary artery of Wistar rats. The rats were placed in a far-infrared dry sauna at 41°C for 15 min and then at 34°C for 20 min once daily for 4 wk. Cardiac hemodynamic, histopathological, and gene analyses were performed. Despite the similar sizes of MI between the ST and non-ST groups (51.4 ± 0.3 vs. 51.1 ± 0.2%), ST reduced left ventricular (LV) end-diastolic (9.7 ± 0.4 vs. 10.7 ± 0.5 mm, $P < 0.01$) and end-systolic (8.6 ± 0.5 vs. 9.6 ± 0.6 mm, $P < 0.01$) dimensions and attenuated MI-induced increases in LV end-diastolic pressure. Cross-sectional areas of cardiomyocytes were smaller in ST rats and associated with a significant reduction in myocardial atrial natriuretic peptide mRNA levels. Vascular density was reduced in the noninfarcted myocardium of non-ST rats, and the density of cells positive for CD31 and for α-smooth muscle actin was decreased. These decreases were attenuated in ST rats compared with non-ST rats and associated with increases in myocardial eNOS and vascular endothelial growth factor mRNA levels. In conclusion, ST attenuates cardiac remodeling after MI, at least in part, through improving coronary vascularity in the noninfarcted myocardium. Repeated ST might serve as a novel noninvasive therapy for patients with MI.

cardiovascular diseases; endothelial nitric oxide synthase; angiogenesis

PHARMACOLOGICAL AND NONPHARMACOLOGICAL therapies for cardiac anti-remodeling such as angiotensin-converting enzyme inhibitors and β-blockers have reduced hospitalization for heart failure and mortality after myocardial infarction (MI) (6, 27, 34). Percutaneous coronary intervention at the acute phase of MI improves the survival (38) but is not enough for preventing progression of heart failure or cardiac remodeling (13, 40). Left ventricular (LV) remodeling after MI has a powerful influence on subsequent cardiac events (4, 42), and, therefore, inhibiting LV remodeling after MI might be an important therapeutic target.

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Repeated sauna therapy (ST) increases endothelial nitric oxide synthase (eNOS) expression and improves vascular endothelial function as well as cardiac function in patients with chronic heart failure (19). However, the effects of repeated ST on LV remodeling after MI remain unknown. Nitric oxide (NO) has an anti-remodeling effect through inhibiting myocytic hypertrophy and by improving myocardial perfusion in association with angiogenesis (25, 30). Accordingly, the present study investigates whether repeated ST increases myocardial eNOS expression and vascularity in the noninfarcted myocardium and thus prevents LV remodeling after MI in rats.

MATERIALS AND METHODS

The present study was undertaken in accordance with the guidelines for animal experimentation at the University of Toyama, and the present experiment was approved by the Animal Experiment Committee of the University of Toyama.

Experimental animals. We induced MI in male Wistar rats (12 wk old, $n = 92$) as described (15). Briefly, the heart was exposed through a left thoracotomy achieved under pentobarbital sodium anesthesia (30 mg/kg ip). The left coronary artery was ligated 2~3 mm from its origin using a 5–0 Proline suture (Ethicon, Somerville, NJ). The heart was returned to the original position, and the chest was closed. Sham-operated rats ($n = 8$) underwent the same surgical procedure but without the coronary artery ligation. Four days after inducing MI, the rats were randomly assigned to ST ($n = 20$) and non-ST ($n = 23$) groups.

Sauna therapy. The rats received ST at 41°C for 15 min followed by 34°C for 20 min to increase the rectal temperature by ~1°C, as described (1) using an experimental far infrared-ray dry sauna system (Metos, Tokyo, Japan). This increase in core temperature induced by ST is similar to that of clinical studies of heart failure and peripheral artery disease (18, 19, 36, 37). Animals were given food and water ad libitum and maintained under controlled environmental conditions (24°C, 12:12-h light-dark cycles). The ST group underwent daily ST for 4 wk starting from *day 4* after MI.

Echocardiographic and hemodynamic studies. The rats were examined by transthoracic echocardiography using an ultrasound system equipped with a 7.5-MHz transducer (SONOLAYER SSA-260A; Toshiba, Tokyo, Japan) at the end of the study. Left ventricular end-diastolic (LVDd) and end-systolic (LVDs) diameters were measured under light anesthesia with pentobarbital sodium (15 mg/kg ip). After the echocardiographic study, a 2-Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was introduced through the right carotid artery to determine LV systolic and end-diastolic (LVEDP) pressure as well as maximal and minimal rates of pressure changes.

Quantitative real-time reverse transcriptase-polymerase chain reaction. Total RNA extracted from 100 mg of LV tissue using Isogen (Nippon Gene, Tokyo, Japan) was digested with DNase (Takara Bio, Shiga, Japan) to eliminate genomic DNA contamination. Samples of

RNA were reverse transcribed with oligo(dT) primers using an RNA PCR kit (Takara Bio). Quantitative real-time reverse transcriptase-polymerase chain reaction (QPCR) analysis proceeded using a sequence detector (Mx3000P; Agilent Technologies, Santa Clara, CA) in a total volume of 20 μl containing 1 μl of cDNA, 10 μl of reagent (Brilliant II Fast QPCR MasterMix; Agilent Technologies), 8 μl of diethylpyrocarbonate-treated water, and 1 μl of primer and TaqMan probe sets (Applied Biosystems) specific for cDNAs encoding glyceraldehyde-3-phosphate dehydrogenase (assay ID Rn99999916_s1), vascular endothelial growth factor (VEGF) A (assay ID Rn01511605_m1), natriuretic peptide precursor type A (ANP, assay ID Rn00561661_m1), and nitric oxide synthase 3, endothelial cell (eNOS, assay ID Rn02132634_s1). The PCR program comprised 40 cycles of denaturation at 95°C for 1 min, primer annealing at 40°C for 5 s, and extension at 60°C for 20 s.

Immunoblotting. Proteins of noninfarcted myocardium were immunoblotted to determine levels of rat eNOS protein. Tissue samples (20 μg of protein) were resolved by SDS-PAGE on 5–15% gels (Ready Gel J; Bio-Rad Laboratories, Tokyo, Japan), and then separated proteins were transferred to a polyvinylidene difluoride membrane [Immun-Blot PVDF Membrane for Protein Blotting (0.2 μm); Bio-Rad]. The membrane was incubated at room temperature first for 1 h with 5% skim milk and 0.1% Tween 20 in Tris-buffered saline, and then overnight with rabbit polyclonal antibodies to eNOS (eNOS antibody; Cell Signaling Technologies, Beverly, MA) at a 1:1,000 dilution in the same solution. The membrane was washed and incubated for 1 h with a 1:1,000 dilution of horseradish peroxidase-conjugated goat antibodies to rabbit IgG (anti-rabbit IgG horseradish peroxidase-linked antibody; Cell Signaling Technologies). The intensity of eNOS bands was quantified by densitometry (LAS-4000; Fujifilm, Tokyo, Japan).

Histology. The LV was cut into four transverse slices, fixed in 10% formaldehyde, embedded in paraffin, and cut into 5-μm-thick sections for staining with hematoxylin and eosin to determine myocyte size and with Masson's trichrome to determine infarct size as described (8). Rats with <30% infarcts were excluded from the analysis. With the use of an image analyzer (VM30; OLYMPUS, Tokyo, Japan), myocyte hypertrophy in the viable LV wall, remote of the infracted area, was measured as the cross-sectional area of transversally cut myocytes showing a nucleus. A total of 40 myocytes were randomly chosen from four slices at ×400 magnification. Myocyte hypertrophy was determined by an investigator who was unaware of the experimental groups.

Immunohistochemical analysis. Portions of transversely cut LV specimens were immunohistochemically examined to determine coronary arteriolar and capillary densities. Vascular smooth muscle and endothelial cells were detected by overnight incubation with antibodies to α-smooth muscle actin (α-SMA, M0851; Dako, Tokyo, Japan) and CD31 (platelet endothelial cell adhesion molecule-1; Santa Cruz Biotechnology, Santa Cruz, CA), respectively. The specimens were then incubated with biotinylated anti-rabbit IgG. Arteriolar densities were determined from the number of α-SMA-positive microvessels with an internal diameter of <50 μm and ≥1 layer of smooth muscle cells at a magnification of ×400. Capillary density was similarly determined from the number of CD31-positive vessels with an internal diameter of <10 μm at a magnification of ×800. Sixteen fields in the noninfracted area of the LV wall were randomly selected from each section and counted by an investigator who was unaware of the experimental groups.

Statistics. Data are expressed as means ± SD. Variables between ST and non-ST groups were compared using an unpaired *t*-test. Groups were compared using an ANOVA followed by a Bonferroni test to identify differences among groups. Arteriolar and capillary densities were compared using the Kruskall-Wallis test followed by the Mann-Whitney *U*-test. The survival rate between ST and non-ST group was compared using Chi-square analysis. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of ST on hemodynamics. None of the rats with MI died during or immediately after ST throughout the study period. The 4-wk survival rates of the ST and non-ST groups did not differ significantly (70 vs. 52%, respectively, $P = 0.55$). We excluded 17 rats (ST group, $n = 6$; non-ST group, $n = 11$) from later analyses because the MI was too small. We finally analyzed 14, 12, and 8 rats from the ST, non-ST, and sham groups, respectively. Infarct size did not differ significantly between the ST and non-ST groups. The rats with MI had greater right ventricular (RV) and lung weights than the sham rats, but ST attenuated the increase in RV weight (Table 1). An increase in LVEDP induced by MI was also attenuated in the ST group, although both ST and non-ST groups had higher LVEDP than the sham group. Figure 1 shows greater LVDd and LVDs in the MI than in the sham rats, but LV dilation was attenuated in ST rats.

Effects of ST on myocyte hypertrophy. The cross-sectional area of myocytes in the noninfarcted myocardium was greater in the ST and non-ST groups than in the sham group. However, ST inhibited the development of myocyte hypertrophy (Fig. 2, *A* and *B*). In agreement with the histological results, MI-induced upregulation in the gene expression of ANP was attenuated in the ST group (Fig. 2*C*).

Effects of ST on vascularity and eNOS expression. The density of both arterioles and capillaries in the noninfarcted myocardium was significantly lower in non-ST than in sham rats. However, ST increased the numbers of these vessels, and these vessel densities were not different from those of control

Table 1. *Infarct size, weight, and hemodynamics in study groups*

	Sham ($n = 8$)	Non-ST ($n = 12$)	ST ($n = 14$)
MI size, %		51.4 ± 0.3	51.1 ± 0.2
Weight			
Body wt pre, g	313 ± 11	314 ± 11	305 ± 12
Body wt post, g	379 ± 14	335 ± 29†	313 ± 23†
Heart wt, mg	950 ± 57	1,149 ± 188†	931 ± 90#
Heart wt/body wt post, mg/g	2.50 ± 0.13	3.45 ± 0.63†	2.98 ± 0.30†#
Right ventricular wt, mg	197 ± 27	362 ± 65†	289 ± 59†#
Right ventricular wt/body wt post, mg/g	0.50 ± 0.05	1.09 ± 0.23†	0.93 ± 0.21†
Lung wt, mg	1,453 ± 82	3,519 ± 683†	2,946 ± 628†
Lung wt/body wt post, mg/g	3.84 ± 0.30	10.47 ± 1.56†	9.47 ± 2.16†
Hemodynamics			
HR, beats/min	399 ± 38	384 ± 38	389 ± 37
sAoP, mmHg	127 ± 20	116 ± 19	109 ± 13
dAoP, mmHg	96 ± 21	94 ± 17	88 ± 12
LVEDP, mmHg	0.4 ± 0.5	22.3 ± 7.8†	14.5 ± 11†#
LV dp/dt_{max}, mmHg/s	1,1496 ± 3,160	5,574 ± 1,579†	6,153 ± 901†
LV dp/dt_{min}, mmHg/s	−8,755 ± 2,349	−3,300 ± 819†	−3,834 ± 647†

Values are means ± SD. ST, sauna therapy; MI, myocardial infarction; body wt pre, body wt before MI induction; body wt post, body wt at 4 wk after MI; HR, heart rate; sAoP or dAoP, systolic or diastolic aortic pressure, respectively; LVEDP, left ventricular end-diastolic pressure; LV dP/dt_{max}, peak rate of LV pressure rise; LV dP/dt_{min}, peak rate of LV pressure fall. $P < 0.05$ vs. sham-operated group (†) and vs. non-ST group (#).

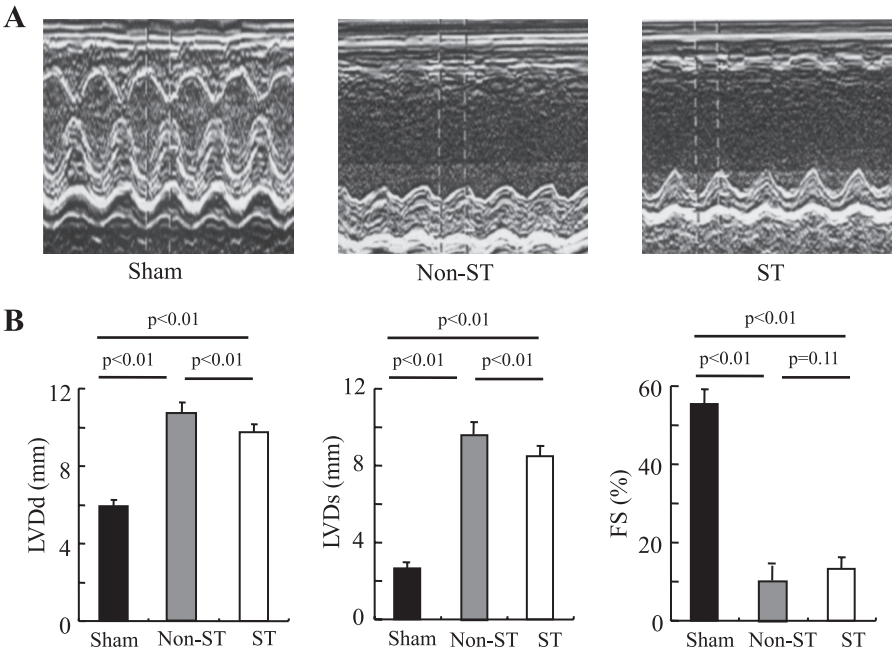

Fig. 1. Effects of sauna therapy (ST) on echocardiographic parameters at 28 days after onset of myocardial infarction (MI). *A*: representative M-mode recordings in sham, non-ST, and ST rats. *B*: comparison of left ventricular end-diastolic diameter (LVDd) (*left*), left ventricular end-systolic diameter (LVDs) (*middle*), and left ventricular fractional shortening (FS) (*right*) in sham (n = 8), non-ST (n = 12), and ST (n = 14) groups. Values are means ± SD.

animals (Figs. 3 and 4). The expression of VEGF in myocardium was also increased in ST rats compared with non-ST rats (Fig. 5), and ST upregulated eNOS mRNA and protein levels in myocardium compared with the non-ST group (Fig. 5).

DISCUSSION

The major findings of the present study are as follows. First, repeated ST inhibited ventricular remodeling after MI in rats together with the attenuation of myocyte hypertrophy. Second, the density of both arterioles and capillaries in the noninfarcted myocardium of MI rats was reduced, but ST attenuated these reductions. Finally, ST-induced attenuation of vascular densities was associated with the upregulation of eNOS and VEGF expression in the noninfarcted myocardium. These results suggest that ST-induced inhibition of LV remodeling after MI might result, at least in part, from improved coronary vascularity in the noninfarcted myocardium. To the best of our knowledge, we are the first to show that repeated ST has cardioprotective effects after MI.

ST has several benefits for heart failure (19, 20, 24), including reduced cardiac preload and afterload, improved vascular endothelial function, and the normalization of neurohormonal systems. We found that LVEDP was lower in ST than in non-ST rats, although systolic blood pressure did not signifi-

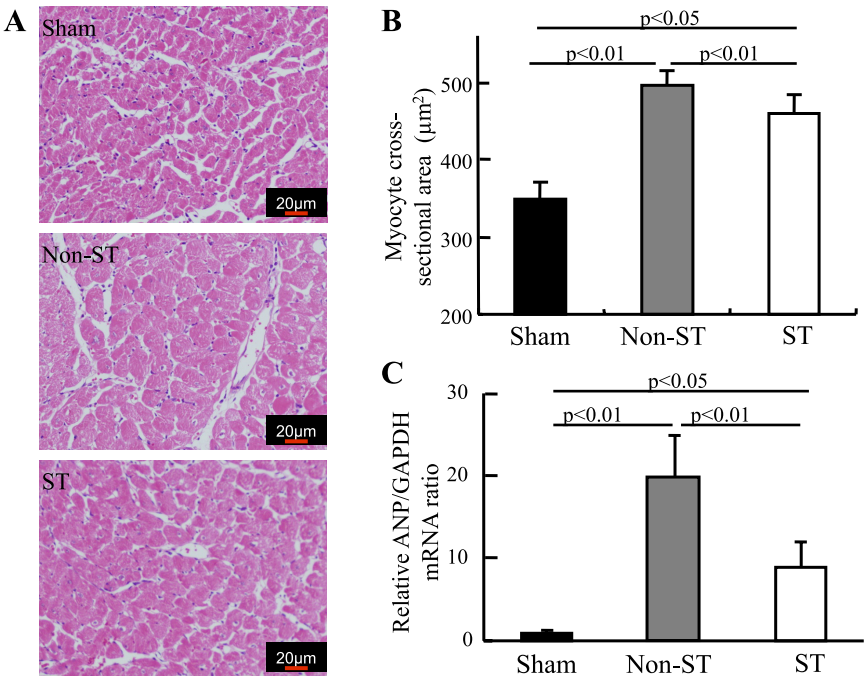

Fig. 2. Effects of ST on myocyte hypertrophy and atrial natriuretic peptide (ANP) mRNA expression in noninfarcted myocardium at 28 days after MI induction. *A*: representative photomicrographs of cardiomyocytes stained with hematoxylin and eosin (×400). *B*: quantitative morphometric analysis of cardiomyocyte cross-sectional areas. *C*: ANP mRNA expression is normalized to amounts of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Levels in sham rats were arbitrarily assigned a value of 1.0. The no. of rats in *B* and *C* were 4, 6, and 7 in sham, non-ST, and ST groups, respectively. Values are means ± SD.

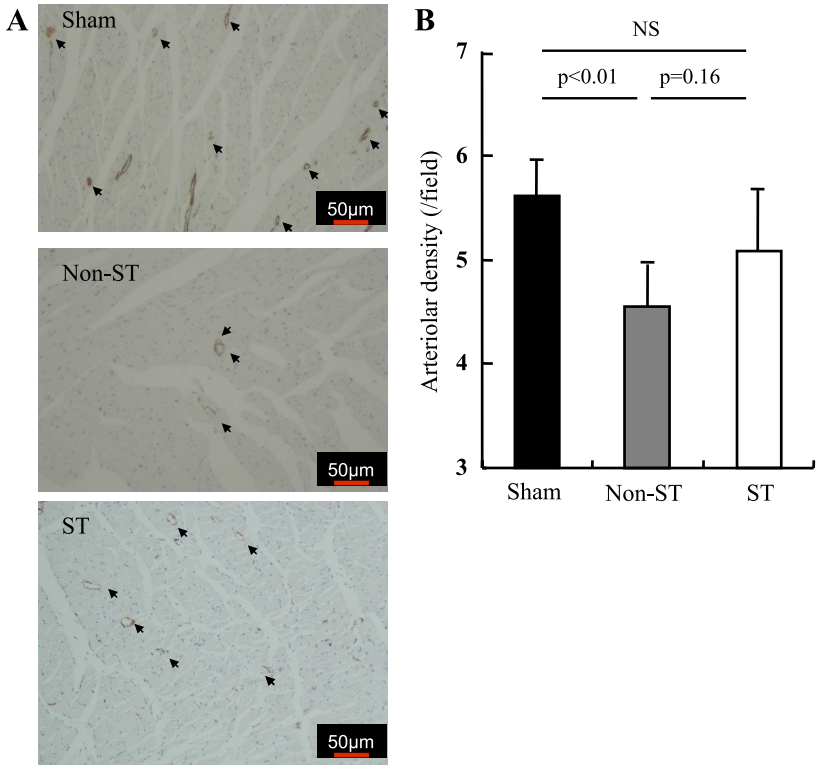

Fig. 3. Effects of ST on arteriole density in noninfarcted myocardium at 28 days after MI induction. *A*: representative photomicrographs of immunohistochemically stained arterioles (×400). Arrows indicate α-smooth muscle actin-positive microvessels. *B*: quantitative morphometric analysis of arteriole density in sham ($n = 4$), non-ST ($n = 6$), and ST ($n = 7$) groups. NS, not significant. Values are means ± SD.

cantly differ between the two groups. ST increases eNOS expression in the aorta of the cardiomyopathic hamster and in ischemic mouse hindlimb muscles (1, 16), and the present study found upregulated eNOS expression in the noninfarcted myocardium after MI. We did not determine the source of eNOS in myocardium, but eNOS derives from various cells, including vascular endothelial cells and cardiac myocytes. Janssens et al. (17) reported that cardiomyocyte-specific overexpression of eNOS improved LV remodeling after MI. Moreover, in an isolated myocyte study, eNOS coupled muscarinic receptor activation to heart rate control and might play a key role for the cholinergic modulation of cardiac myocyte function (7). Taken together, ST-induced increases in eNOS signaling in vascular endothelium as well as in cardiomyocytes

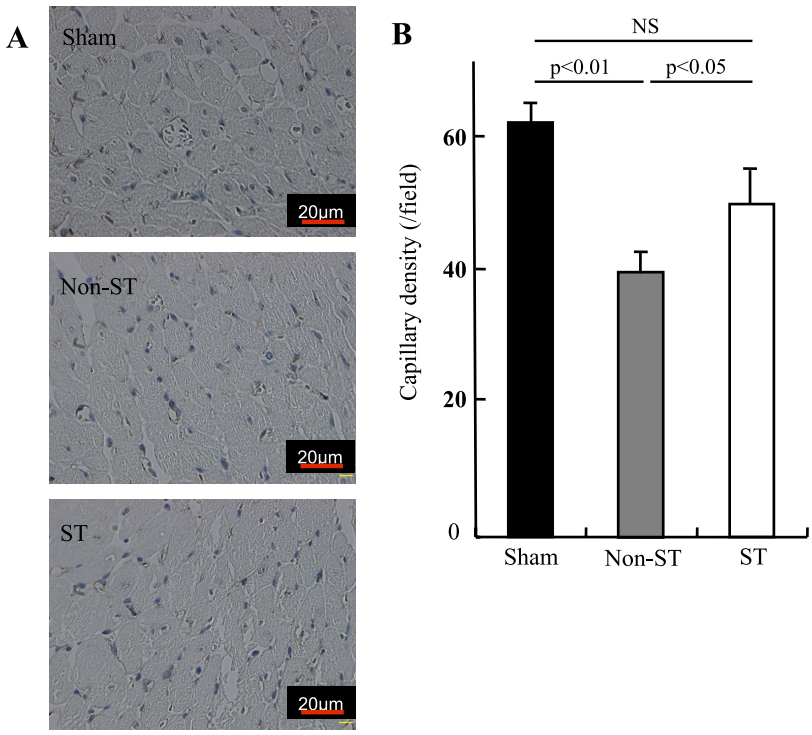

Fig. 4. Effects of ST on capillary density in noninfarcted myocardium at 28 days after MI induction. *A*: representative photomicrographs of capillaries immunohistochemically stained with CD-31 (×800). *B*: quantitative morphometric analysis of capillary density in sham ($n = 4$), non-ST ($n = 6$), and ST ($n = 7$) groups. Values are means ± SD.

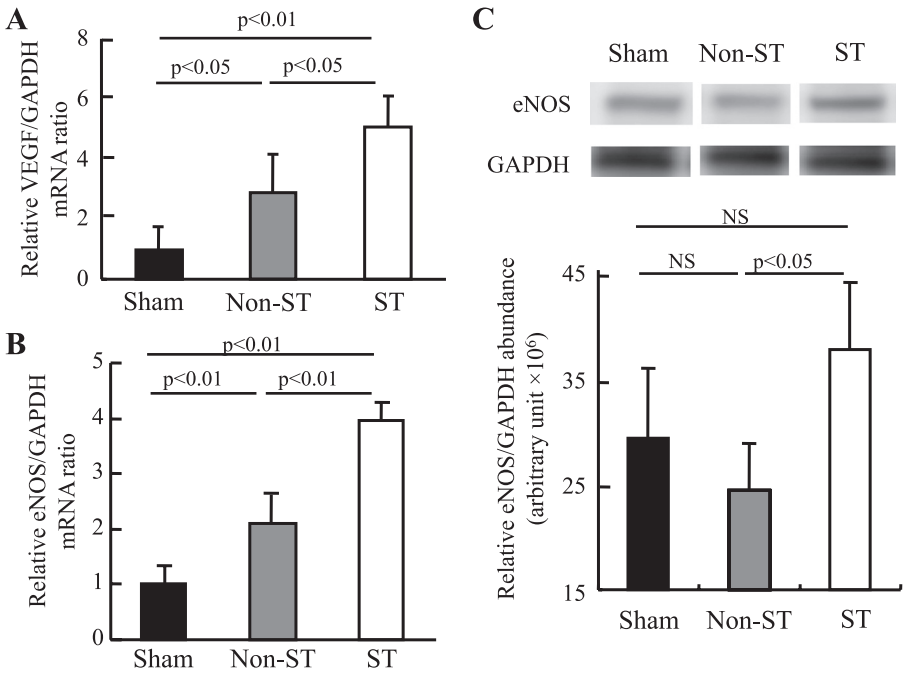

Fig. 5. Effects of ST on vascular endothelial growth factor (VEGF, *A*) and endothelial nitric oxide synthase (eNOS) gene expression (*B*) in noninfarcted myocardium at 28 days after MI induction. Each mRNA level was normalized to amounts of GAPDH mRNA. Levels in sham rats were arbitrarily assigned a value of 1.0. *C*: representative examples of Western blots for eNOS in noninfarcted myocardium and densitometric analysis of eNOS protein expression. The no. of rats in *A*, *B*, and *C* were 4, 6, and 7 in sham, non-ST, and ST groups, respectively. Values are means ± SD.

might lead to improved vascular endothelial function and ventricular remodeling, thus helping to inhibit the progression of heart failure.

Alterations in the coronary circulation of hypertrophied hearts, such as a decrease in myocardial vessel density, have been demonstrated (12, 21). Insufficient microvascular adaptation relative to the extent of cardiomyocyte hypertrophy is a key pathophysiological feature that contributes to progressive cardiac dysfunction in heart failure (31, 39). Therefore, enhanced vascularization therapy might hold promise for the prevention of heart failure (10). The present study found larger myocytes and a lower density of arterioles and capillaries in the noninfarcted areas of MI than in sham rats. Thus the relatively low perfusion in noninfarcted, hypertrophied myocardium might contribute to the deterioration of myocyte function and the progression of LV remodeling.

Cardiac angiogenesis is impaired in pressure overload hypertrophy, and promoting cardiac angiogenesis by introducing angiogenic factors restores cardiac dysfunction under cardiac hypertrophy (31). An increase in eNOS expression enhances angiogenesis in the ischemic region through increases in VEGF (2, 43), and NO production might contribute to the angiogenic properties of VEGF (26). Moreover, VEGF might upregulate eNOS protein and NO production (14). Unfortunately, we did not determine heat shock protein (HSP) 90, which may contribute to angiogenesis induced by ST. VEGF stimulates protein kinase B (Akt) and eNOS activation, which are critical modulators of VEGF-induced angiogenesis, and HSP90 is important for the regulation of Akt (28, 33). However, we found here that ST enhanced eNOS and VEGF expression in the noninfarcted myocardium and might lead to increasing the number of vascular densities. These effects of ST might improve ventricular function after MI and prevent cardiac remodeling.

Another beneficial effect of NO is its antihypertrophic properties (23, 25, 30); it directly inhibits cardiomyocyte hypertrophy by activating a cGMP-dependent mechanism (5). Scherrer-Crosbie et al. (32) showed that eNOS plays an important role in limiting LV dilation, dysfunction, and hypertrophy in murine MI. Fraccarollo et al. (9) also reported that long-term treatment with an eNOS enhancer improves LV remodeling and contractile dysfunction after MI. Therefore, enhanced eNOS expression induced by ST might contribute to the inhibition of myocyte hypertrophy and ventricular remodeling after MI.

The influences of ST on cardiac hemodynamics deserve consideration to interpret the present results. The LVEDP was significantly lower in ST than in non-ST rats. Systolic blood pressure also tended to be lower in ST than in non-ST rats, although the difference did not reach statistical significance. ST improved arterial endothelial function via eNOS upregulation (16) and increased peripheral blood flow. These hemodynamic changes induced by ST might contribute, at least in part, to improving ventricular function and cardiac antiremodeling after MI.

In the present study, capillary and arteriolar densities were lower in non-ST rats than in sham rats, despite greater mRNA expression of eNOS and VEGF in non-ST rats. Angiogenesis factors of VEGF and NO were upregulated by myocardial ischemia (3, 11, 22). In the present study, insufficient myocardial perfusion relative to the extent of cardiomyocyte hypertrophy in noninfarcted myocardium of MI rats might promote the expression of eNOS mRNA. The protein level for eNOS in the noninfarcted myocardium of ST and non-ST rats with MI was not significantly different from that of sham rats, whereas the mRNA level for eNOS was significantly upregulated in both MI rats. The precise mechanism of this dissociation remains unclear. However, eNOS activity is subject to control at the posttranscriptional (mRNA stability) level as well as at the posttranslational level (41). Hypoxia might increase Rho kinase expression and activity, leading to a decrease in bio-

availability of NO through decreases in eNOS mRNA stability and eNOS phosphorylation (29, 35).

Limitations. The present study has several limitations. First, the beneficial effects of repeated ST on vascular densities and cardiac antiremodeling after MI would result primarily from upregulated eNOS signaling, but the influence of ST was not evaluated in eNOS knockout animals or under eNOS inhibition by L-NAME. Second, the long-term effects of ST on cardiac remodeling and mortality after MI remain unclear, although the one-month mortality rates from the onset of MI did not differ significantly between ST and non-ST rats in the present study. Further studies are required to determine how long the beneficial effects of ST on cardiac antiremodeling and angiogenesis will be maintained after the cessation of ST. Furthermore, the optimal time to start ST after the onset of MI and its optimal duration remain unclear.

Although limited for these reasons, the present study nevertheless showed that repeated ST increased vascular density in the noninfarcted myocardium and prevented cardiac remodeling after MI in association with upregulated eNOS and VEGF expression. These results suggest that ST is a novel potential nonpharmacological therapy for patients with acute MI or with ischemic cardiomyopathy.

DISCLOSURES

None.

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