Research Article

Desipramine Pretreatment Improves Sympathetic Remodeling and Ventricular Fibrillation Threshold after Myocardial Ischemia

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Abnormal increase in sympathetic nerve sprouting was responsible for the ventricular arrhythmogenesis after myocardial infarction. This study investigated whether the norepinephrine transporter inhibitor, desipramine, can modulate sympathetic remodeling and ventricular fibrillation threshold (VFT) after myocardial ischemia-reperfusion. Rats were administered desipramine (0.8 mg/kg, IV) before or after myocardial ischemia. VFT, infarct size, tyrosine hydroxylase (TH) and growth-associated protein 43 (GAP43)-positive nerve fibers were measured after one week. The VFT of preischemic treatment group was 11.0 ± 2.65 V and significantly higher than that of control ischemic group (7.2 ± 1.30 V, $P < 0.05$). Infarct size in the preischemic treatment group ($23.3 \pm 2.4\%$) was significantly lower than that in the control ischemic group ($30.8 \pm 1.3\%$, $P < 0.05$) and the delayed application group ($27.1 \pm 2.6\%$, $P < 0.05$). The density of TH and GAP43-positive nerve fibers in the control ischemic group was significantly higher than that in the other three groups ($P < 0.05$). The density of nerve fibers improved after desipramine treatment. Moreover, there was a negative correlation between the VFT and both TH and GAP43-positive nerve fiber density in the infarct border zone ($P < 0.05$). Desipramine treatment before acute myocardial ischemia can decrease infarct size, improve sympathetic remodeling, and increase VFT and electrical stability of ischemic hearts. Desipramine appears to cause myocardial ischemic preconditioning.

1. Introduction

A large amount of norepinephrine (NE) is released from sympathetic nerves during acute myocardial ischemia. NE participates in ischemic preconditioning (IPC) during short-term myocardial ischemia, which can reduce myocardial injury induced by long-term myocardial ischemia and the incidence of arrhythmias. However, a large amount of NE released during long-term myocardial ischemia has cardiac and neurological toxicity, resulting in a significant increase in the incidence of arrhythmias [1–3]. The effect of excessive release of NE on sympathetic nerves and the electrophysiology of myocardial cells after ischemia is not clear. It is known that GAP43, a protein expressed in the growth cones of sprouting axons, is a marker of nerve sprouting. Furthermore, TH is the rate-limiting enzyme of NE synthesis, which serves as not only a marker of sympathetic nerve terminals but also an indirect indicator of sympathetic activity. The

combination of GAP43 and TH can precisely reflect the sprouting of sympathetic nerves. In addition, sympathetic sprouting and sympathetic remodeling reach a peak at one week after myocardial infarction [4, 5]. The findings of sympathetic remodeling and its electrophysiological implications suggest that drugs that affect the release or storage of NE may have important effects on infarct size and arrhythmias. Therefore, the purpose of this study was to use desipramine, a norepinephrine transporter (NET) inhibitor, to increase the concentration of NE before myocardial ischemia and reduce the excessive release of NE by an NET-mediated mechanism during acute myocardial ischemia. One week after myocardial ischemia-reperfusion, the VFT and infarct size were measured and the distribution of nerve fibers in ventricular muscle was determined. In addition, the relationship between nerve hyperplasia and VFT was evaluated. The results of these studies suggest that desipramine pretreatment may cause IPC.

2. Materials and Methods

2.1. Animal Preparation. The experiment protocol conformed to the Guideline for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the Institutional Animal Care and Use Committee. Eighty SD male rats, weighing 200–250 g, were supplied by the animal experiment center of Wuhan University (no. 2003-2004), China. All rats were randomly divided into one of four groups with 20 rats in each group: a control ischemic group, a preischemic treatment group, a delayed application group, and a sham-operated group. After intraperitoneal anesthesia, the pericardium was incised and the anterior wall of the left ventricle was exposed. The left anterior descending (LAD) coronary artery was isolated under the left atrial appendage and carefully clamped for 30 minutes followed by reperfusion [6]. Myocardial ischemia was confirmed by elevation of the ST segment by more than 0.2 mV in leads I, II, and aVL. In the preischemic treatment group, desipramine (0.8 mg/kg IV) was administered [6] five minutes before ischemia, and then the LAD was clamped for 30 minutes followed by reperfusion. In the delayed application group, desipramine (0.8 mg/kg IV) was given by the tail vein after clamping the LAD for ten minutes. Reperfusion was performed after thirty minutes of LAD clamping. In the sham-operated group, rats were operated without clamping the LAD. Postoperatively, each rat received 200,000 IU penicillin intramuscularly twice daily for 3 days and was fed a standard diet for one week.

2.2. Determination of VFT. One week after the operation, 10 rats were randomly selected from each group. These rats underwent a second thoracotomy to expose the hearts. A bipolar needle pacing electrode was inserted in the infracted border zone about 1 mm deep. The pacing electrode in the sham-operated group was placed on the corresponding part of the heart. A train of rectangular 10 ms pacing pulses was administered for 30 seconds at a frequency of 60 Hz with a DF-5A type electrophysiological stimulation apparatus (Suzhou Dongfang Electronic Instrument Factory, China). Successive pulse trains were separated by a period of 1 min. The pacing voltage of the first pulse train started at 4 V and was increased in 1 V increments. The VFT was the minimum voltage that induced ventricular fibrillation.

2.3. Determination of Infarct Size. Myocardial infarct area was determined by triphenyltetrazolium chloride staining [7]. The hearts of the remaining rats in the four groups were removed and sectioned into 2 mm thick slices and incubated in phosphate buffer (pH 7.4) containing 1% triphenyltetrazolium chloride for 20 min to visualize infarcted tissue. Normal tissue was stained red-brown, and infracted tissue was not stained. The infarct size in each heart was expressed as the weight of infarcted area divided by the total weight of the heart.

2.4. Immunohistochemical Staining. After measuring the VFT, the hearts of the four groups were removed. These hearts were cut into two parts along the long axis and infarcted area, one part was placed in a refrigerator at −70°C, and another part was immediately placed in 4% paraformaldehyde for 18–24 hours. These parts were then embedded in paraffin, sliced perpendicular to the epicardium, and then subjected to H&E or immunohistochemical staining. Antibodies including anti-GAP43 antibody and anti-TH antibody (rabbit anti-GAP43 and anti-TH, AbD serotec, 1 : 450, resp.), were used for immunohistochemical staining. Details of the staining techniques have been published elsewhere [8]. Nerve densities were determined by a computer-assisted image analysis system (Image-Pro Plus 3.0, Media Cybernetics, USA). Each slide was examined under a microscope to select three fields randomly. The computer then automatically calculated the area occupied by the nerves in the field. The nerve density was the area occupied by the nerves divided by the actual tissue area examined (um^2/mm^2). The mean density of nerves in these three selected fields was used to represent the nerve density of that slide.

2.5. RT-PCR. TH and GAP43 primers were synthesized according to the template. The primers are shown in Table 1. The total RNA from the cardiac muscle samples was extracted and purified using a TRIzol reagent kit (Invitrogen, USA). Total RNA was reverse transcribed into complementary DNA (cDNA) strands using a cDNA synthesis kit, according to the manufacturer's protocol. The Opticon-2 real-time PCR reactor (MJ Research, USA) and real-time PCR kit were employed based on the manufacturer's instruction. The RT-PCR conditions were 70°C/5 min, 42°C/90 min for reverse transcription; the polymerase chain reaction condition included predenaturing at 94°C for 5 min, then 35 cycles of 94°C for 30 s, and 60°C for 30 s, 72°C for 45 s, and 72°C for 10 min. In this experiment, GAPDH was used as the housekeeping gene. Gene products (4 uL of TH, GAP43, and GAPDH) were mixed with 1 uL of sample buffer. The sample was placed on a 1.5% agarose gel, and 120 V electrophoresis was performed for 25 min. The TH, GAP43, and GAPDH band areas and absorbance (*A*) were calculated by the computer image analysis system. Semiquantitative analysis was performed using with the band area and *A*.

2.6. Statistical Analysis. All values were expressed as the mean ± the standard deviation. Groups were compared using analysis of variance. Linear correlation analysis was used to determine the correlation of the indices between sympathetic nerve density and VFT. Data were analyzed by SPSS13.0 software. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Animal Model. In the animal model preparation, two rats died after surgery, one in the control ischemic group and one in the delayed application group. One rat in the preischemic treatment group died because of excessive bleeding. All rats survived in the sham-operated group. The heart in the infarct area after myocardial ischemia was pale.

TABLE 1: Primer sequence and amplicon size of genes validated by RT-PCR.

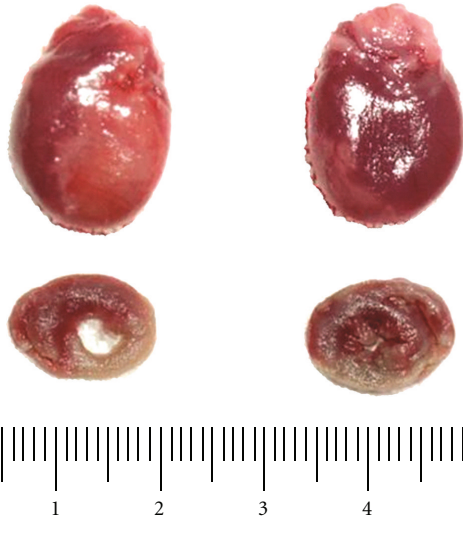| Gene name	Accession no., NM	Primer sequence	Amplicon size, bp
TH	012740	F: 5′-TCCTCACCTATGCATTCACCTGAG-3′	390
		R: 5′-ATAGTTCCTGAGCTTGTCCTTGGC-3′	
GAP43	017195	F: 5′-TGCCTGCTGCTGTCACTGA-3′	254
		R: 5′-GGCAGGAGAGACAGGGTTCA-3′	
GAPDH	017008	F: 5′-GAAACCTGCCAAGTATGATG-3′	191
		R: 5′-ACCAGGAAATGAGCTTGACA-3′	

FIGURE 1: TTC staining of a rat heart one week after myocardial ischemia-reperfusion. The heart in the infarct area was pale.

There was a clear distinction between the infarcted and non-infarcted areas (Figure 1). H&E staining also showed a clear demarcation between necrotic and healthy tissue (Figure 2).

3.2. VFT and Infarct Size. The VFT of the preischemic treatment group was 11.0 ± 2.65 V and was higher than that of the control ischemic group (7.2 ± 1.30 V, $P < 0.05$). Infarct size in the preischemic treatment group was $23.3 \pm 2.4\%$ and significantly lower than that in the control ischemic group ($30.8 \pm 1.3\%$, $P < 0.05$) and the delayed application group ($27.1 \pm 2.6\%$, $P < 0.05$) (Table 2).

3.3. Distribution and Density of Nerve Fibers. TH- and GAP43-positive nerve fibers was particularly evident in myocardial tissue sections after staining (Figures 3 and 4). Compared with sham-operated group, TH- and GAP43-positive nerve fibers in ventricular muscle in control ischemia group increased significantly, thickened, or gathered into a network structure, distributed disorderly. Compared with control ischemia group, nerve fibers after treatment with desipramine significantly improved; the distribution of nerve fibers normalized. The change of preischemic treatment group was more obvious. TH- and GAP43-positive nerve fiber density in control ischemia group was significantly higher than

TABLE 2: Infarct size and VFT in the four groups (mean ± SD).

Group	Infarct size (%)	VFT (V)
Sham-operated group		13.0 ± 2.12
Control ischemic group	30.8 ± 1.3	$7.2 \pm 1.30^{*}$
Preischemic treatment group	$23.3 \pm 2.4^{\star}$	$11.0 \pm 2.65^{\star}$
Delayed application group	$27.1 \pm 2.6^{\star\#}$	$10.4 \pm 3.05^{\star}$

$^{*} P < 0.05$ when compared with the sham-operated group.
$^{\star} P < 0.05$ when compared with the control ischemic group.
$^{\#} P < 0.05$ when compared with the preischemic treatment group.

that of sham-operated group ($P < 0.05$). The ration of TH/GAP43 in control ischemia group was 82.8% and was higher than sham-operated group 68.8% ($P < 0.05$, Table 3). These results indicate that there was hyperplasia of sympathetic nerves.

3.4. Correlation between Nerve Fiber Density and VFT. There was a significant negative correlation between the VFT and both the TH- and GAP43-positive nerve fiber density in the infarct border zone ($P < 0.01$). The correlation coefficient (R) was -0.91 and -0.87, respectively, and the R^2 of linear correlation was 0.83 and 0.76, respectively, (Figure 5).

3.5. RT-PCR. The RT-PCR products of TH, GAP43, and GAPDH were consistent with the expected amplified fragments. The electrophoretic bands were clear and nonhybrid (Figures 6 and 7). TH and GAP43 mRNA expression among the four groups was highest in the control ischemic group. In this group, the ratios of band area × A to GAPDH for TH and GAP42 were 2.05 ± 0.60 and 1.96 ± 0.83, respectively. These values were significantly higher compared with the other three groups ($P < 0.05$). TH and GAP43 mRNA expression in the preischemic and delayed application groups were slightly higher than that in the sham-operated group, but the differences were not significant ($P > 0.05$).

4. Discussion

Myocardial ischemia is associated with a marked accumulation of NE in ischemic tissue. NET is a micromembrane protein that exists in sympathetic presynaptic membranes. Its main functions are to reuptake NE from the synaptic cleft back into the presynaptic membrane, regulate the NE level in the synaptic cleft, terminate nerve impulse signals, and maintain the sensitivity of receptors to neurotransmitter [9].
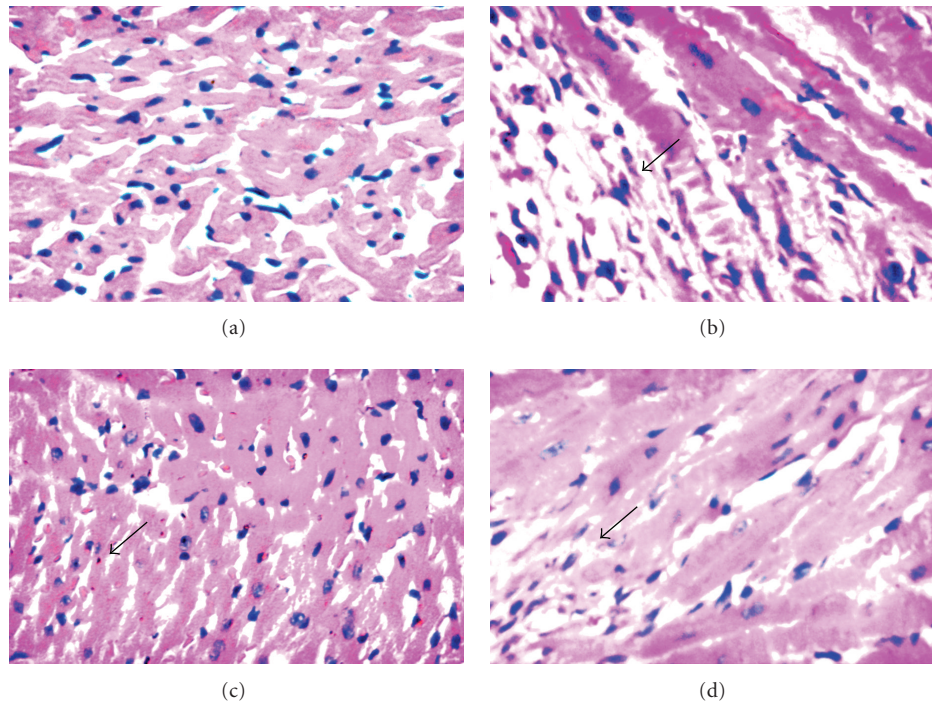
FIGURE 2: H&E staining of rat myocardial cells; arrows show the infarcted tissue (×400). (a) Sham-operated group. Structure of myocardial cells is normal. (b) Control ischemic group. Myocardial necrosis is obvious. (c) Preischemic treatment group. (d) Delayed application group. Myocardial necrosis reduced in (c) and (d).
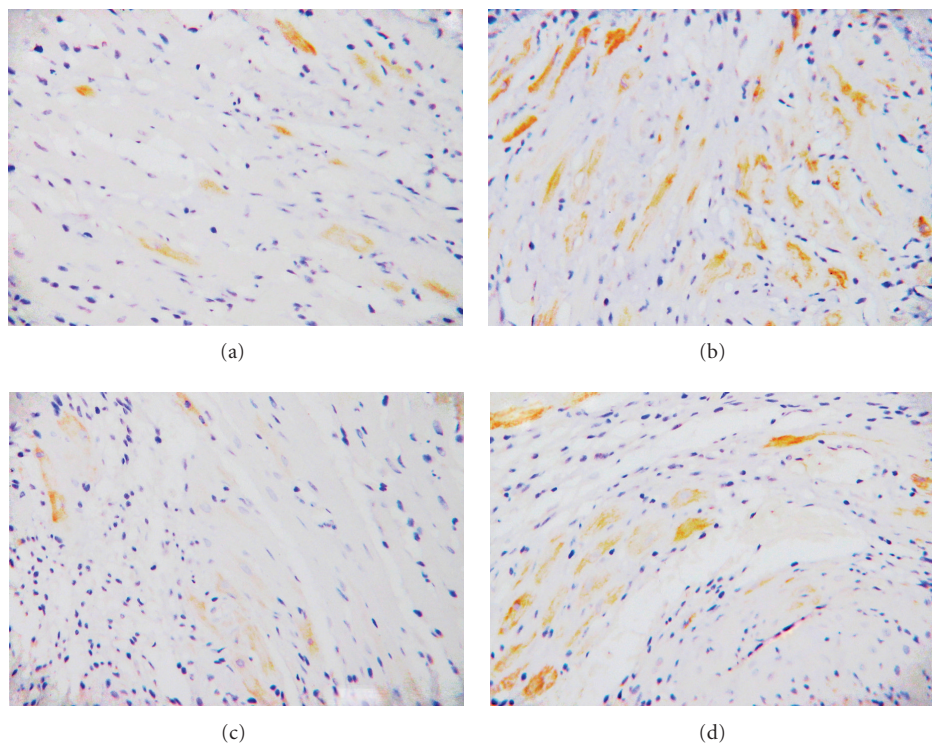

FIGURE 3: TH immunohistochemical staining of the infarct border zone (DAB color, ×200). (a) Sham-operated group. The distribution of TH-positive nerve fibers is orderly. (b) Control ischemic group. TH-positive nerve fibers in ventricular muscle increased significantly it is thickened and disorderly. (c) Preischemic treatment group. (d) Delayed application group. The distribution of nerve fibers after treatment with desipramine significantly improved. The change of preischemic treatment group was more obvious.

TABLE 3: TH- and GAP43-positive nerve fiber density in the infarct border zone (um^2/mm^2, mean ± SD).

Group	TH	GAP43	TH/GAP43 (%)
Sham-operated group	12717.5 ± 4703.0	18538.7 ± 9415.1	68.6 ± 9.8
Control ischemic group	126717.5 ± 19900.3*	153042.5 ± 20386.8*	82.8 ± 11.3*
Preischemic treatment Group	46702.5 ± 8878.6*☆	61121.2 ± 4223.5*☆	76.4 ± 12.1*☆
Delayed application group	82472.5 ± 17902.6*☆#	102042.5 ± 18943.6*☆#	80.8 ± 10.5*

*$P < 0.05$ when compared with the sham-operated group.
☆$P < 0.05$ when compared with the control ischemic group.
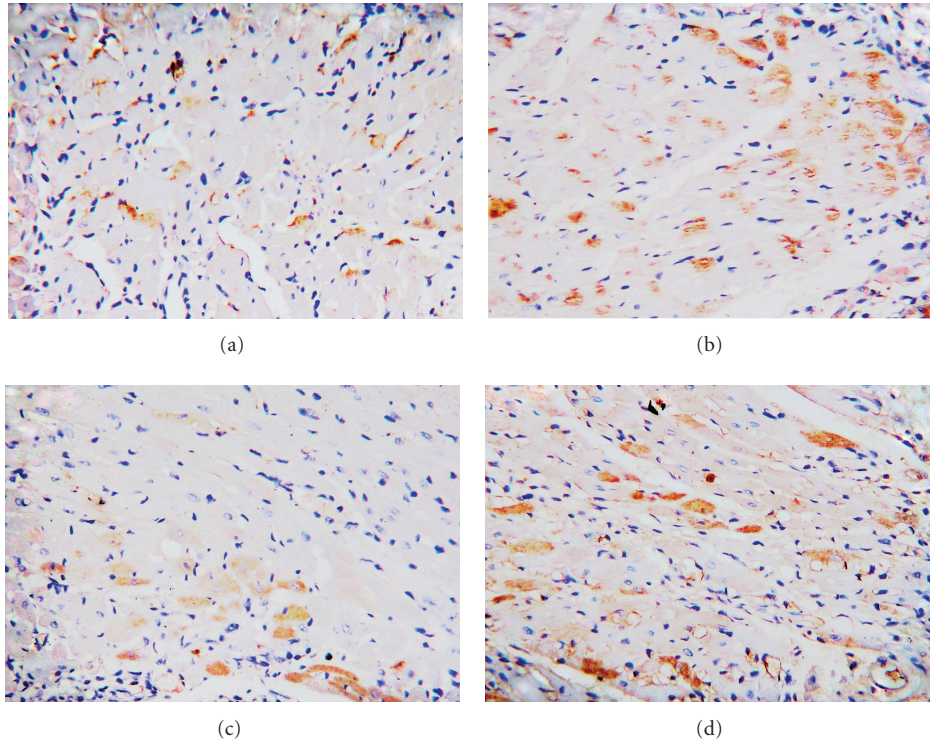#$P < 0.05$ when compared with the preischemic treatment group.

(a)

(b)

(c)

(d)

FIGURE 4: GAP43 immunohistochemical staining of the infarct border zone (DAB color, ×200). (a) Sham-operated group. The distribution of GAP43-positive nerve fibers is orderly. (b) Control ischemic group. GAP43-positive nerve fibers in ventricular muscle increased significantly, it is also thickened and disordered. (c) Preischemic treatment group. (d) Delayed application group. The distribution of GAP43-positive nerve fibers after treatment with desipramine significantly improved.

However, when myocardial ischemia is longer than 10 min, the release of NE becomes nonexocytotic and is thought to involve NET-mediated efflux of NE in reverse of its normal transport direction [10].

A brief episode of myocardial ischemia confers myocardial protection against a following prolonged myocardial ischemia. This effect is known as IPC. Many studies concluded that IPC can reduce myocardial infarct size, reduce apoptosis, attenuate myocardial stunning, and reduce arrhythmias. Several possible triggers for IPC have been postulated, including adenosine, NE, and bradykinin [11, 12]. Previous studies on the effect of IPC have focused primarily on infarct size limitation [13], but its effect on cardiac sympathetic nerve injury is not known. During myocardial ischemia, massive myocardial NE release into the interstitial space was observed, caused by a nonexocytotic mechanism

reflecting sympathetic nerve injury. A dramatic increase in NE in the synaptic cleft binds to β-adrenergic receptors, producing positive inotropic and chronotropic effects. This increases myocardial oxygen demand and may cause coronary artery constriction that can further aggravate myocardial ischemia [14]. Recently, the myocardial release of NE after prolonged ischemia was attenuated by a preceding period of transient ischemia in rat and rabbit hearts, suggesting a beneficial effect of IPC on sympathetic nerve injury. NET played an important role in the beneficial effect. In the present study, we showed that the administration of the NET inhibitor, desipramine, before myocardial ischemia improved myocardial ischemia-reperfusion injury tolerance and reduced myocardial infarct size. This is consistent with Richardt et al.'s research [6]. The reason is that desipramine pretreatment can increase the concentration of NE in the
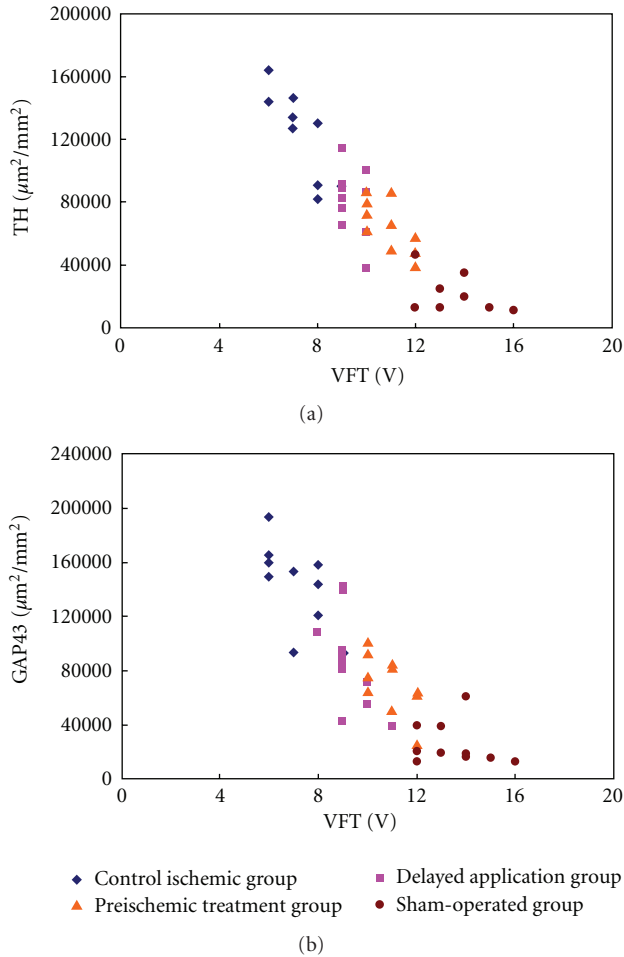
(a)

(b)

Figure 5: Graph of linear correlation analysis between VFT and TH- (a) or GAP43- (b) positive nerve fiber density.

(a)

(b)

Figure 6: (a) RT-PCR products by electrophoresis of TH and GAPDH in the infarct border zone. (b) Ratio of TH band area × A to GAPDH in the infarct border zone. M: marker; 1: sham-operated group; 2: control ischemic group; 3: preischemic treatment group; 4: delayed application group.

(a)

(b)

Figure 7: (a) RT-PCR products by electrophoresis of GAP43 and GAPDH in the infarct border zone. (b) Ratio of GAP43 band area × A to GAPDH in the infarct border zone. M: marker; 1: sham-operated group; 2: control ischemic group; 3: preischemic treatment group; 4: delayed application group.

synaptic cleft and blood, and this may cause preconditioning. At the same time, desipramine reduces NET-mediated NE release and toxic products and free radicals produced from NE when myocardial ischemia is sustained. This can ultimately reduce the damage of myocardial cells and sympathetic nerves.

In this study, we observed that TH- and GAP43-positive nerve fibers in infarct border zone in the control ischemic group increased significantly compared with the sham-operated group. Furthermore, the ratio of TH/GAP43 was significantly higher in the control ischemic group. These results indicate that there was hyperplasia of sympathetic nerves, and this is consistent with Vracko et al.'s and Nori et al.'s research [15, 16]. Desipramine can improve the distribution of TH- and GAP43-positive nerve fibers in infarct border zone and reduces the density of nerve fibers. The reason may be that desipramine decreases sympathetic nerve injury during acute myocardial ischemia and reduces nerve regeneration after myocardial ischemia, thus normalizing the distribution of nerve fibers in ventricular muscle. This study also found that TH and GAP43 mRNA expression levels in the preischemic treatment group were significantly
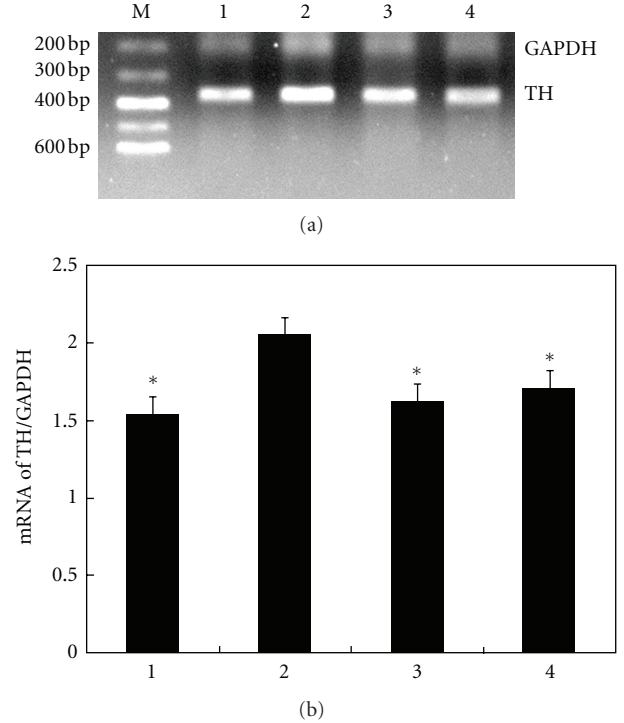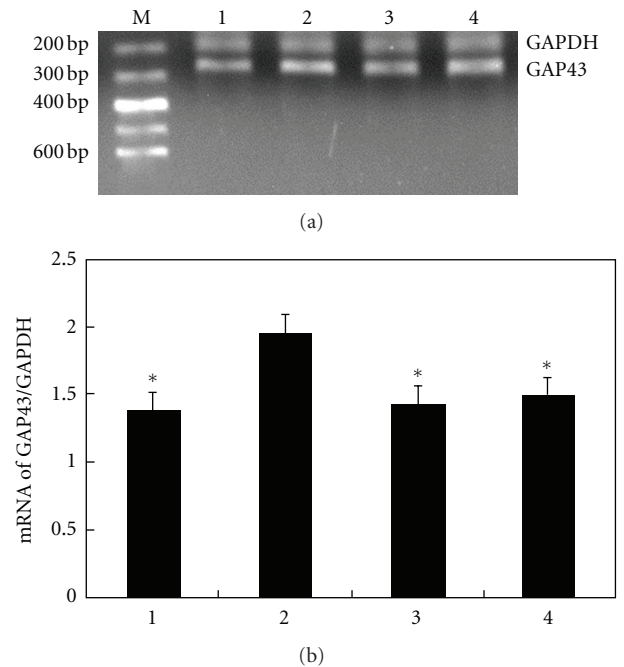
lower than that in the control ischemic group, which is consistent with the changes of TH and GAP43 protein levels. We suggest that desipramine may also influence nerve growth after myocardial ischemia at the gene level, although the specific mechanism remains to be elucidated.

The VFT is an important indicator to evaluate the occurrence of malignant arrhythmias. We observed that the VFT in the control ischemic group with sympathetic hyperplasia was significantly reduced. Since desipramine normalized the distribution of sympathetic nerves, the VFT should also increase. Statistical analysis showed that there was a negative correlation between sympathetic nerve density and the VFT. It is known that sympathetic remodeling after myocardial ischemia changes the electrophysiological properties of the ischemic heart, which lowers the VFT and increases the occurrence of arrhythmias [17, 18]. In the present study, sympathetic remodeling increased nerve hyperplasia in the infarct border zone and presumably the concentration of NE. The concentration gradient of NE in the normally innervated region and in the region with nerve hyperplasia may cause a dispersion of myocardial repolarization and excitability leading to arrhythmias. Exposure of myocardial cells in the infarct border zone to chronically elevated concentrations of catecholamines changes the signal transduction pathway of the receptor, reduces the delayed rectifier potassium current (I_{ks}) and transient outward potassium current (I_{to}), and extends the calcium current (I_{Ca}) and myocardial action potential duration (APD) [17, 19]. I_{ks} and I_{to} increase and APD shortens in the normal myocardial cells when sympathetic nerve are activated, and this increases myocardial repolarization heterogeneity and electrical instability [17, 19, 20]. Desipramine reduces sympathetic nerve injury, improves the distribution of nerves, and reduces myocardial infarct size. This increases the heart's electrical stability and VFT, which should reduce the likelihood of arrhythmias.

The incidence of ventricular arrhythmia and sudden cardiac death increases in the first week after myocardial infarction. The current study found that the decrease in VFT was closely related to sympathetic remodeling after myocardial infarction. IPC is an important self-protection mechanism in the heart and provides a novel approach to prevent myocardial ischemia in the clinical setting. Several different drugs or endogenous substances may cause IPC. In this study, IPC appeared to be induced by desipramine pretreatment. It reduced the acute release of NE and improved sympathetic remodeling and VFT one week after myocardial ischemia-reperfusion. Ultimately, it may have reduced the possibility of arrhythmias. However, further studies are necessary since there are no trials of desipramine in patients with acute myocardial infarction.

4.1. Study Limitations. The number of rats in this study was relatively small. In addition, we were not able to measure the concentration of NE in the synaptic cleft. Large prospective trials will be needed to confirm these results.

5. Conclusions

Desipramine treatment before acute myocardial ischemia can decrease infarct size, improve sympathetic remodeling, and increase VFT and electrical stability of ischemic hearts. These changes should result in an antiarrhythmic effect. Desipramine appears to cause IPC.

Conflict of Interests

The authors declare no conflict of interest.

Acknowledgment

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