Differential expressions of nNOS and iNOS in the rostral ventrolateral medulla induced by electroacupuncture in acute myocardial ischemia rats

XIA Chun-Mei, CHEN Jun, WANG Jin, FAN Ming-Xin, XIAO Fen, CAO Yin-Xiang, LI Li, SHEN Lin-Lin, ZHU Da-Nian*

Department of Physiology and Pathophysiology, Shanghai Medical College of Fudan University, Shanghai 200032, China

Abstract: Increasing lines of evidence has been accumulated that nitric oxide (NO) and nitric oxide synthase (NOS) distribute plentifully in the rostral ventrolateral medulla (RVLM) and contribute to cardiovascular regulation. In the present study, the expressions of neuronal and inducible isoform of NOS (nNOS and iNOS) were observed in the RVLM of acute myocardial ischemia (AMI) Wistar rats experienced electroacupuncture (EA) treatment, thereby the cardiovascular effects of NO in the RVLM were investigated and the mechanism of acupuncture effect on AMI was inferred. The results indicated that in the AMI rats, cardiac functions were markedly attenuated with high serum level of brain natriuretic peptide (BNP) and norepinephrine (NE), the number of nNOS-immunoreactive cells and nNOS mRNA expression in the RVLM area were increased, while those of iNOS were lowered. EA at "Neiguan" acupoints (Pe 6) 30 min daily for successive 5 d resulted in an improvement of the cardiac functions, decreases in NE and BNP levels; it also increased the expression of iNOS and decreased the expression of nNOS in the RVLM. These results suggest that the curative effect of acupuncture on AMI is possibly attributable to the differential regulation of NOS/NO in the RVLM, leading to decreased sympathetic outflow and improvement of cardiac functions.

Key words: rostral ventrolateral medulla; myocardial ischemia; electroacupuncture; neuronal nitric oxide synthase; inducible nitric oxide synthase

电针诱导心肌缺血大鼠延髓头端腹外侧区 nNOS 和 iNOS 差异表达

夏春梅, 陈军, 王锦, 樊明欣, 肖芬, 曹银祥, 李莉, 沈霖霖, 朱大年*

复旦大学上海医学院生理与病理生理学系, 上海 200032

摘 要: 许多研究表明, 延髓头端腹外侧区 (rostral ventrolateral medulla, RVLM) 的 NO/NOS 系统参与心血管活动的中枢调节。本实验以结扎 Wistar 大鼠左冠状动脉前降支法建立急性心肌缺血 (acute myocardial ischemia, AMI) 动物模型, 观察针刺 “内关” 穴改善 AMI 大鼠的心功能作用, 同时检测大鼠 RVLM 区神经元型一氧化氮合酶 (neuronal nitric oxide synthase, nNOS) 和诱导型一氧化氮合酶 (inducible nitric oxide synthase, iNOS) 表达的变化。进而探讨针刺治疗 AMI 的中枢机制。实验观察显示, AMI 大鼠心功能各项指标减低, 伴随意周血去甲肾上腺素 (norepinephrine, NE) 和脑钠肽 (brain natriuretic peptide, BNP) 水平显著升高, 同时 RVLM 区 NOS 阴性神经元数和 NOS mRNA 表达升高, 而 iNOS 水平则降低。针刺“内关”穴 (Pe 6) (每天 30 min, 连续 5 天) 改善心功能, 降低 AMI 大鼠血清中 NE 和 BNP 的水平, 同时升高 iNOS 并降低 NOS 在 RVLM 的表达。以上结果提示, 针刺治疗心肌缺血的同时可以调节 iNOS/NO 和 nNOS/NO 在 RVLM 的变化, 这可能与针刺通过调节 RVLM 区的 NO 含量进而降低交感传出, 从而改善 AMI 大鼠的心功能有关。

关键词: 延髓头端腹外侧区; 心肌缺血; 电针; 神经元型一氧化氮合酶; 诱导型一氧化氮合酶

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*Corresponding author. Tel: +86-21-54237405; E-mail: dznzhu@shmu.edu.cn
Autonomic dysfunction in the form of hyperactive sympathetic and impairment of vagal control of the heart has been associated with an adverse outcome after acute myocardial ischemia (AMI)\(^1\). Investigation has shown that uncomplicated AMI is associated with a protracted sympathetic neural hyperactivity\(^2\). Longhurst proved that acupuncture was effective in improving myocardial ischemia\(^3\). The effect of acupuncture on cardiovascular diseases is related to the modulation on abnormal function of the autonomic nervous system. The rostrum ventrolateral medulla (RVLM) is considered as a key area for controlling the peripheral sympathetic tone and maintaining normal arterial pressure\(^4\). Nitric oxide (NO) in the RVLM plays a very important role in the control of sympathetic outflows and the improvement of myocardial infarction and heart failure\(^5\). Chan et al.\(^6\) demonstrated that the prevalence of neuronal nitric oxide synthase (nNOS) over inducible nitric oxide synthase (iNOS) activity in the RVLM, and the associated dominance of sympathoexcitation over sympathoinhibition might underlie the maintenance of sympathetic vasomotor outflow by the endogenous NO.

Previous studies indicated that the curative effect of acupuncture on hypertension or AMI could be blocked when L-NNA, a non-specific NOS inhibitor, was administered into the ventral periaqueductal gray (vPAG) or the RVLM, respectively\(^7\)\(^8\). Researches have also proved that the curative effect of acupuncture on cardiovascular diseases is related to the overexpression or low expression of NOS isoenzymes, i.e. endothelial NOS (eNOS), nNOS and iNOS in the central nervous system\(^9\)\(^10\). Kim et al. reported that acupuncture resulted in change in the expression of NOS in the brainstem of spontaneous hypertensive rats\(^11\). We also observed that the curative mechanism of acupuncture was related to the changes of nNOS and iNOS expressions in the RVLM of stress-induced hypertensive rats\(^12\). Our study has shown that the effect of electroacupuncture (EA) on myocardial ischemia is mediated by NO in the RVLM\(^13\). Therefore, it is important to observe the expressions of both iNOS and nNOS and their alteration in the RVLM of rats with myocardial ischemia.

1 MATERIALS AND METHODS

1.1 Animal preparations

All procedures conformed to the standards established in the Guide for Care and Use of Laboratory Animals. Experiments were performed on male Wistar rats (250-300 g) and were randomly divided into sham (control; \(n=6-7\)), AMI (AMI for 5 d; \(n=6-7\)) and AMI + EA (AMI for 5 d followed by EA treatment; \(n=6-7\)) groups. The rats were anesthetized with 10% chloral hydrate (300 mg/kg body weight, intraperitoneally). An arterial catheter was inserted into the femoral artery for measurement of blood pressure (BP), and another catheter was inserted into the left ventricle of the heart via the right carotid artery for recording intraventricular pressure, via 2 separate pressure transducers, then the two catheters were connected to a bioelectric signals processing system (Model SMUP-A, Department of Physiology, Shanghai Medical College of Fudan University) from which the mean arterial pressure (MAP) and cardiac function data were obtained and analyzed. During the experiment, the rectal temperature of the animal was monitored and kept at (37.5±0.5) °C.

1.2 Experimental induction of myocardial ischemia

The myocardial ischemia model group underwent permanent ligation of the left anterior descending (LAD) coronary artery as described by Pfeffer et al.\(^16\). Animals were narcotized with 10% chloral hydrate (300 mg/kg body weight, intraperitoneally), intubated, and ventilated with a ventilator (DHX-150, Chengdu Instrument Company, China). Following thoracotomy of the operated group animals, the left descending coronary artery was ligated with a 6/0 silk suture by piercing the pericardial membrane. Successful ligation was tested by visual inspection for pallor of the involved myocardium and ST segment elevation =0.1 mV on electrocardiogram (ECG), and was further confirmed by micro-structural observation. Sham-operated rats underwent the same operation without ligation of the coronary artery and served as controls. After induction of myocardial ischemia, animals’ mortality rate was approximately 30%-40% during the first hour after surgery, because of ventricular arrhythmias or dyspnoea. Twenty-four hours later, the survival rate was 90%.

1.3 Application of EA

Two stainless steel needles were inserted into both “Neiguan” acupoints (Pe 6), corresponding to that of human beings, which are located in the interosseal muscles between the radius and the ulna of the distal medial thoracic limb at the level of 3 mm superior to the wrist...
joint in rats. The electric impulses were derived from a medical stimulator (G6805-2, Shanghai Medical Apparatus) at a frequency of 4-20 Hz alternatively, 0.5 ms duration and at the intensity (4 mA) just strong enough to elicit slight twitches of the foot. Application of EA was continued for 30 min daily for consecutive 5 d.

1.4 Tetrazolium chloride (TTC) and hemoyxin-eosin (HE) staining
At the end of 5 days of LAD ligation, the survival animals were narcotized with 10% chloral hydrate (300 mg/kg body weight, intraperitoneally). 0.1% TTC (2,3,5-triphenyltetrazolium, purchased from Sigma Chemical Company) was used to identify the infarcted left ventricular myocardium\textsuperscript{17}. The hearts were excised, transversely sliced, 2.0-2.5 mm thick, crossing a plane parallel to the atrioventricular groove, cut from apex to base into equal thick 5 slices, and incubated in 0.1% TTC. Myocardial infarct size was measured by planimetry of photographs from each gross slice and histologic section using classical criteria of necrosis. In viable myocardium, TTC is converted by dehydrogenase enzymes to a red formazan pigment that stains tissue dark red\textsuperscript{17}. The infarcted myocardium that did not take TTC stain, where the dehydrogenase enzymes were drained off, remained pale in color. The sections of the heart were fixed in 4% phosphate buffered formalin for 48 h then dehydrated in alcohol and embedded in the paraffin tissue dark red\textsuperscript{17}. The infarcted myocardium that did not take TTC stain, where the dehydrogenase enzymes were drained off, remained pale in color. The sections of the heart were fixed in 4% phosphate buffered formalin for 48 h then dehydrated in alcohol and embedded in the paraffin.

1.5 Immunohistochemical processing
Rats experienced 5 days of LAD ligation were anesthetized with pentobarbital (50 mg/kg body weight, i.p.) and perfused through the ascending aorta with 300 mL heparinized saline followed by 400 mL freshly prepared 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.2). The brain was removed and post-fixed at 4 ºC overnight. The fixed brain was placed in 20% sucrose until the brain sunk to the bottom, and then placed in 30% sucrose in 0.1 mol/L phosphate buffer (pH 7.2) at 4 ºC overnight. The brain was placed in the coronal plane and slices were made at a thickness of 30 µm on cryostat. Immunohistochemical visualization was performed by using a conventional avidin-biotin-peroxidase complex (ABC) procedure. All experiments were conducted at room temperature, unless stated otherwise. Free-floating brain slices were pretreated for 30 min in methanol containing 3% H2O2 to inactivate endogenous peroxidase and for 1 h in 0.01 mol/L phosphate buffered saline (PBS) (pH 7.4) with 5% normal goat serum, subsequently, with a rabbit polyclonal anti-nNOS (diluted 1/1 000; Sigma), mouse monoclonal anti-iNOS (diluted 1/1 000; Sigma) diluted in 0.01mol/L PBS with 0.1% bovine serum albumin. Preparations were incubated at 37 ºC for an hour then incubated at 4 ºC overnight with the diluted primary antibody. After being rinsed with PBS, sections were subjected to either anti-rabbit or biotinylated anti-mouse IgG and subsequently to ABC (Vector laboratories, Burlingame, CA). Then, 3,3-diaminobenzidine (DAB, KPL Corporation) was applied to visualize immunostaining. The evaluation of stained cells was quantitatively assessed through Image Measurement Version 1.00 (Department of Physiology and Pathophysiology, Shanghai Medical College of Fudan University, China). In the control experiment, nNOS or iNOS immunoreactive cells were not apparent when the respective nNOS or iNOS antibody had been omitted. The NOS immunoreactivity reactivity in the brainstem nuclei were expressed as the number of positive cells in a microscopic area (200 µm × 200 µm) as described\textsuperscript{18}. The micrographs were quantified using a microscope with reticule grid to measure the number of positive cells containing color staining in 8-10 non-overlapping tissue sections in RVLM. An averaged number of positive cells in each slide for each animal were obtained. The quantification for all subjects was determined in a blinded fashion before comparison of different groups. The orders of measurements between control and intervention animals were randomized. Six rats were used for each defined group.

1.6 Immunofluorescent staining
The brain sections were prepared and tackled as above (Immunohistochemical processing) described, except that the sections were incubated with the secondary antibody with fluorescein isothiocyanate (FITC-labeled goat-anti-rabbit IgG) (Beyotime institute of biotechnology Co. Ltd., Jiangsu, China) or carboxymethylindocyanine (Cy3-labeled goat-anti-mouse IgG)) diluted at 1:1 000 and 1:500 in provided solution, respectively, washed in PBS and mounted on gelatinized glass slides, air dried and coverslipped with anti-fading reagent. Immunofluorescence was observed under a Leica FW 4000 (Leica QWin system) fluorescence microscope.

1.7 RNA extraction and real-time PCR
Brain was dissected under RNase-free conditions and samples of the RVLM area were rapidly frozen in liquid N2. Samples were homogenated in 1 mL of Trizol Reagent (Invitrogen Life Technologies) per 50-100 mg of tissue. Total RNA concentration and purity were quantified spectrophotometrically at λ=260 nm and 260 nm versus 280
nm, respectively. Reverse transcription (RT) was performed in a reaction volume of 20 µL as follows: First prepared the annealing mixture containing RNA 5 µg and 0.5 µg/µL random primer 1 µL, added RNase-free H2O to a final volume of 10 µL, then prepared the RT cocktail containing 5 × RT buffer (250 mmol/L Tris-HCl, pH 8.3, 200 mmol/L KCl, 40 mmol/L MgCl2, 5 mmol/L DTT; Promega) 4 µL, 2.5 mmol/L dNTP mix (2.5 mmol/L each dATP, dGTP, dCTP and dTTP) 4 µL, RNase inhibitor 1 µL (Promega), and MMLV reverse transcriptase (Promega) 1 µL. For each RT reaction, 10 µL of the pre-warmed RT cocktail was transferred to the 10 µL annealing mixture containing RNA 5 µg and 0.5 µg/µL in a reaction volume of 20 µL as follows: First prepared × RT buffer (250 mmol/L Tris-HCl, pH 8.3, 200 mmol/L dNTP mix 2.5 mmol/L each dATP, dGTP, dCTP and dTTP) 4 µL, RNase inhibitor 1 µL (Promega), and MMLV reverse transcriptase (Promega) 1 µL. For each RT reaction, 10 µL of the pre-warmed RT cocktail was transferred to the 10 µL annealing mixture. They were incubated at 37 ºC for 60 min, and then heated to 95 ºC for 5 min and chilled on ice. The cDNA templates were stored at -20 ºC. Real-time PCR for nNOS and iNOS was performed on Rotor-Gene 3000 Realtime PCR (Corbett Research) and used a reaction mix [Taq polymerase (Promega) 3 U, 10 × PCR buffer, MgCl2 2.5 µL, 1.5 µL dNTP mix 2.5 µL, SyberGreen (Molecular Probes) end concentration 0.25×, 1 µL of each forward and reverse PCR primers (10 µmol/L), 1 µL of cDNA template, and then added water to a final volume of 25 µL] as real-time PCR reactive system. The primers were designed by Primer 5.0 Rotor-gene 6.0 (Corbett Research). The following primers were used: nNOS: 5’-CACAGGAGGAGCGTCG-TGTA-3’ (sense), 5’-AAGGCGGTGGATCATTACATATA-3’ (antisense), iNOS: 5’-CCCTCCTCCTAACCACCAA-3’ (sense), 5’-CCGCCAAGTGCTCAGTGGC-3’ (antisense), β-actin: 5’-CTCTAATGGCAAACAGTGC-3’ (sense), 5’-GTACTCTGCTTGTGCTGATCC-3’ (antisense). Each sample was subjected to 35 cycles of denaturation (94 ºC for 20 s), annealing (58 ºC for 20 s), elongation (72 ºC for 10 s), and single acquisition (86 ºC for 10 s), collected fluorescence after single acquisition. To confirm the amplification specificity, PCR products were subjected to a melting curve analysis and 2% agarose gel electrophoresis followed by ethidium bromide staining. The concentration of each gene is generated directly by Rotor-Gene Real-Time Analysis Software 6.0 (Build 14). The levels of mRNA were quantified by using the standard curve method, constructed with serial dilutions of control mRNA. The relative quantification of the target gene was determined by calculating the ratio of the target gene to housekeeping gene β-actin.

1.8 Radioimmunoassay of brain natriuretic peptide (BNP) and norepinephrine (NE) in the serum
To measure BNP and NE levels in the serum, the blood was collected by internal jugular venous puncture, centrifuged immediately and then the serum was stored at -20 ºC. BNP and NE levels were measured using radioimmunoassay kits, which were obtained from Phoenix Pharmaceuticals, Inc., Belmont; CA. Assays were performed following the procedure recommended by the manufacturer.

1.9 Statistical analysis
The results were expressed as mean±SEM. Differences were determined by one-way analysis of variance using SPSS 11.0 for windows. Individual groups were compared using Dunnett’s test. Differences with P<0.05 were considered statistically significant and the number of animals (n) in each group was given.

2 RESULTS

2.1 Infarct size of the left ventricle and histology of infarcted myocardium
The infarcted area was not stained by TTC. Five days post-infarction, myocardial infarct size was determined in 54 slices by TTC technique. In the AMI + EA group, the infarcted area in the left ventricle was decreased by (21.7±2.8)% compared with that in the AMI group, and the difference was statistically significant (P<0.05, n=7). Histological HE staining of myocardial infarction tissue was shown in Fig. 1: the cardiac muscle fibers showed obvious acidophilic alteration; the coagulation necrosis was observed in the middle layer of the myocardium during the first week of post-infarction; inflammatory cell infiltration was found; both inflammatory lymphocytes and macrophages could be seen, as in human beings[19].

2.2 Cardiac functional changes in the AMI model
On day 5 post-infarction in the AMI group, except heart rate (HR) and MAP, almost all the indices of the cardiac functions decreased significantly as compared with those in the sham control group, including left ventricular systolic pressure (LVSP) (P<0.01, n=7), -dP/dt (P<0.01, n=7) and +dP/dt (P<0.05, n=7), while left ventricular end diastolic pressure (LVEDP) increased significantly (P<0.01, n=7). After EA treatment on 5 days post-ischemia, MAP almost decreased to the control level (P>0.05, n=7) and cardiac functions improved in the AMI rats, including LVSP (P<0.01, n=7), -dP/dt (P<0.05, n=7) and +dP/dt (P<0.05, n=7), while LVEDP decreased from (26.1±3.8) mmHg to (10.7±5.7) mmHg (P<0.05, n=7) (Table. 1).

2.3 Changes in serum BNP and NE concentrations
Serum BNP concentration in AMI group [(153.77±10.07)
pg/mL] increased compared with that in the sham-control group [[67.5±8.02] pg/mL, \( P<0.05, n=6 \)], while EA treatment decreased the serum BNP concentration [[100.71±8.56] pg/mL, \( P<0.05, n=6 \)] (Fig. 2A). Plasma level of NE was significantly higher in the AMI group [[451±47] pg/mL] compared with that in the sham-control group [[247±30] pg/mL, \( P<0.05, n=6 \)]. After EA treatment at “Neiguan” on 5 days post-ischemia, NE level was reduced to [317±35] pg/mL (\( P<0.05, n=6 \)) (Fig. 2B).

### 2.4 The number of nNOS- and iNOS-immunoreactive cells in the RVLM

In the RVLM, both the number and visualization of nNOS-immunoreactive cells in AMI rats had significant increases compared with that in the sham group (\( P<0.05, n=6 \)), while

![Image](image_url)

**Fig. 1.** The infarcted manifestation of the left ventricle (TTC staining) and histology (HE staining) of infarcted myocardium. *A:* Infarced manifestation was pale. *B:* Sham-control myocardium. *C:* Cardiac muscle fibers showed obviously acidophilic alteration on day 5 post-infarction. The coagulation necrosis was observed in the myocardium during the first week post-infarction, both lymphocytes and macrophages could be seen, and numerous cells with features of fibroblasts were present. Scale bar, 20 µm.

### Table 1. HR, MAP and other indices of cardiac functions in different groups

<table>
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<th>Sham (n=7)</th>
<th>AMI (n=7)</th>
<th>AMI + EA (n=7)</th>
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<tr>
<td>HR (beats/min)</td>
<td>405±8.07</td>
<td>415±8.90</td>
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<tr>
<td>MAP (mmHg)</td>
<td>89±3.58</td>
<td>123±3.33 *</td>
<td>101±2.65 *</td>
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<tr>
<td>LVEDP (mmHg)</td>
<td>4.5±3.6</td>
<td>26.1±3.8 **</td>
<td>10.7±5.7 *</td>
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<tr>
<td>LVSP (mmHg)</td>
<td>180.2±15.2</td>
<td>120.6±8.6 **</td>
<td>170.7±9.0 **</td>
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<tr>
<td>-dP/dt (mmHg/s)</td>
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<td>3237.6±250.1 **</td>
<td>4217.6±337.2 **</td>
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<tr>
<td>+dP/dt (mmHg/s)</td>
<td>6794.2±820.6</td>
<td>4160.3±655.9 *</td>
<td>6203.2±671.8</td>
</tr>
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Data were expressed as mean±SEM. \( *P<0.05, \) \( **P<0.01 \) vs sham group; \( \*P<0.05, \) \( \**P<0.01 \) vs AMI group.

![Image](image_url)

**Fig. 2.** The serum concentrations of norepinephrine (NE) and brain natriuretic peptide (BNP) in rats. NE (*A*) and BNP (*B*) concentrations in the serum from sham-operated control (n=6), AMI, AMI + EA groups (n=6) were shown. Bars represented mean±SEM values for each group. \( *P<0.05 \) vs sham group; \( \*P<0.05 \) vs AMI group.
the number of nNOS-immunopositive cells were decreased after 5 days of EA treatment ($P<0.05, n=6$) (Fig. 3). iNOS-immunopositive cells decreased after AMI treatment ($P<0.01, n=6$), but there was an obvious up-regulation of iNOS-immunopositive cells after 5 days of EA treatment ($P<0.05, n=6$) (Fig. 4). In the RVLM, the nNOS-immu-
noreactivities were restricted to the rostral and medial parts with clear positive cells and fibers, while nNOS-immunoreactivities cells were small and sparsely distributed (Fig. 3, 4).

2.5 mRNA expressions of nNOS and iNOS in the RVLM

The electrophoresis of RT-PCR products of NOS from the RVLM was shown in Fig. 5. Relative quantification of the mRNA levels of nNOS in the RVLM was increased after AMI-treatment ($P<0.01$, $n=6$), while EA treatment for 5 days decreased it ($P<0.05$, $n=6$) (Fig. 6A). The ratio of iNOS/β-actin mRNA was significantly decreased in the AMI group ($P<0.01$), while EA treatment for 5 days up-regulated the iNOS mRNA expression ($P<0.05$, $n=6$) (Fig. 6B).

3 DISCUSSION

In our present study, myocardial ischemia was induced in rats by ligating the left descending coronary artery. This AMI model exhibited high level of plasma NE and suffered abnormal cardiac functions[20]. It was observed in the present study that EA treatment decreased plasma NE concentration in the AMI rat model, indicating that sympathetic outflow was reduced by acupuncture “Neiguan” acupoint processing. Many studies have confirmed that EA at “Neiguan” acupoint could reduce oxygen consumption and enhance the myocardial contractility in the ischemic area; so that it contributes to the recovery of the cardiac functions[21-23]. Our present research confirmed this opinion and indicated that this was accompanied by decreased sympathetic outflow. Furthermore, acupuncture decreased the augmented serum BNP concentration in AMI rats. BNP measurement can indirectly reflect post-ischemia area and degree[24,25]. High level of BNP is accompanied by high level of NE and extended infarcted area[26,27]. The decreased plasma BNP level and TTC staining result in AMI rats induced by acupuncture showed acupuncture could protect the non-infarcted myocardium from infarcting as reported[28].

Our data showed that EA treatment for 5 days led to up-regulation of nNOS but a marked decrease of iNOS in the mRNA and protein expressions in the RVLM of AMI rats. Following the wide use of different selective NOS isoenzyme inhibitor, it was recognized that NO produced by different NOS and/or specific brain area could cause different actions on central cardiovascular regulation[29]. Chan et al. reported that the reduction or enhancement of sympathetic vasomotor outflow was elicited when selective inhibitor of nNOS or iNOS was respectively applied in the RVLM[30]. In 2007, a study suggested that nNOS participated
in the regulation of autonomic function by decreasing sympathetic output to the periphery, which suggested that gene expression of nNOS was increased during the states of heightened sympathetic activity[30].

Our present study showed that a significant increase of nNOS in RVLM of rats with heightened sympathetic activity was coincident with this view at the cellular and molecular level. Reversely, acupuncture-induced decrease of nNOS expression in RVLM of rats might attenuate sympathetic activity, which might be the central mechanism of curative effect of acupuncture on AMI. The further explanation was that the NO derived from nNOS in the RVLM induced sympathoexcitation via activation of both NMDA and non-NMDA receptors, while sympathoinhibition elicited by the NO generated by iNOS was mediated by GABA	extsubscript{A} receptors[31]. In AMI, the normal sympathoexcitation over sympathoinhibition[32] balance was broken, which will attenuate the sympathetic inhibition and increase the sympathetic excitation so that the sympathetic outflow is in disorder, which would lead to increase of oxygen consumption in post-infarcted heart and further exacerbation of post-ischemia contractile forces and further progression towards heart failure[33]. Graham et al.[3] showed that this hyperactivity arose because of an impairment of reflexes from cardiac receptors.

EA at “Zusanli” or “Neiguan” acupoints using low current and low frequency activates pathways, which release GABA, opioid etc. to inhibit RVLM neurons and thereby decrease sympathetic outflow[34-37]. These responses had potential therapeutic effects on hypertension, arrhythmias and cardiac ischemia[4]. Our present study indicated that EA could improve the cardiac functions in AMI rats, which was combined with a decrease of nNOS expression and an increase of iNOS expression in the RVLM. These results could be easily understood, for, acupuncture could exert its curative effect on AMI through activation of central sympathetic inhibitory pathway and attenuation of central sympathetic excitatory pathway, which was consistent with the report by Chan et al.’s, as well as our previous work[14,15].

Altogether, these observations suggested that EA regulated different isoforms of NOS in the RVLM or other central areas which might become possible to decrease the progress of increased central sympathetic outflow, thereby slowed down heart failure thereafter. This effect might be related to the changes in the release of inhibitory and excitatory amino acid neurotransmitters in the RVLM[38]. The detailed mechanisms and roles of NO/NOS systems in the RVLM induced by acupuncture in AMI states are complicated and remain to be fully explored and examined.

REFERENCES

Xia Chun-Mei et al.: Expression of NOS in RVLM Induced by Acupuncture in Acute Myocardial Ischemia Rats


