Reduced nitric oxide in the rostral ventrolateral medulla enhances cardiac sympathetic afferent reflex in rats with chronic heart failure

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Abstract: The purpose of this study was to determine the effect of nitric oxide (NO) in the rostral ventrolateral medulla (RVLM) on the central integration of the cardiac sympathetic afferent reflex (CSAR) in normal rats and in rats with coronary ligation-induced chronic heart failure (CHF). Under α-chloralose and urethane anesthesia, mean arterial pressure, heart rate and renal sympathetic nerve activity (RSNA) were recorded at baseline and during elicitation of the CSAR evoked by electrical stimulation of the cardiac afferent sympathetic nerves in sino-aortic denervated and cervical vagotomized rats. A cannula was inserted into the left RVLM for microinjection of NO synthase inhibitor, S-methyl-L-thiocitruline (MeTC) or NO donor, S-nitroso-N-acetyl-penicillamine (SNAP). The CSAR was tested by electrical stimulation (5, 10, 20 and 30 Hz at 10 V for 1 ms) of the afferent cardiac sympathetic nerves. It was observed that (1) the responses of RSNA to stimulation were enhanced in rats with CHF; (2) MeTC (80 nmol) potentiated the responses of RSNA to stimulation in sham rats but not in rats with CHF; (3) SNAP (50 nmol) depressed the enhanced RSNA response to stimulation in CHF rats but had no effect in sham rats; and (4) MeTC increased the baseline RSNA and MAP only in sham rats, but SNAP inhibited the baseline RSNA and MAP in both sham and CHF rats. These results indicate that reductance of NO in the RVLM is involved in the augmentation of CSAR in CHF rats.

Key words: cardiovascular physiology; nitric oxide; heart failure; cardiac sympathetic afferent reflex; rostral ventrolateral medulla

延髓头端腹外侧区一氧化氮与慢性心力衰竭大鼠心交感传入反射的关系

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摘要: 为观察延髓头端腹外侧区 (rostral ventrolateral medulla, RVLM) 一氧化氮 (NO) 在慢性心力衰竭 (chronic heart failure, CHF) 大鼠心交感传入反射 (cardiac sympathetic afferent reflex, CSAR) 中的作用, 实验在左心房受体神经支配的结扎冠状动脉诱发的 CHF 大鼠和假手术 SD 大鼠进行。记录电刺激心交感传入神经中枢前后的血压和肾交感神经活动 (renal sympathetic nerve activity, RSNA) 变化以评价 CSAR。结果显示: (1) CHF 大鼠的 CSAR 显著增强; (2) RVLM 微量注射 NO 合酶 (NOS) 抑制剂 MeTC 增强对照组大鼠的 CSAR 但对 CHF 大鼠的 CSAR 无显著影响; (3) RVLM 微量注射 NO 供体 S-nitroso-N-acetyl-penicillamine (SNAP) 抑制 CHF 大鼠增强的 CSAR; (4) S-methyl-L-thiocitruline (MeTC) 仅增强对照组大鼠基础水平的 RSNA, 而 SNAP 抑制对照组和 CHF 大鼠基础水平的 RSNA。结果表明 RVLM 中内原性 NO 的减少是导致 CHF 大鼠 CSAR 增强的重要机制之一。

关键词: 心血管生理学; 一氧化氮; 心力衰竭; 心交感传入反射; 延髓头端腹外侧区

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Neurohumoral excitation is an important characteristic of chronic heart failure (CHF) and chronic sympathetic excitatory state may contribute to further hemodynamic deterioration. It has been shown that plasma norepinephrine concentration is positively correlated with 5-year mortality rates in patients with CHF, but the origin of the sympathoexcitation has not been clarified. It has been reported that the sympathoexcitation of CHF is related to abnormal arterial baroreceptor reflex and cardiopulmonary reflex control of sympathetic outflow. However, similar increase in plasma norepinephrine was still found in chronically sino-aortic baroreceptor denervated dogs with pacing-induced CHF. Therefore, abnormal cardiovascular inhibitory reflexes are not the sole mechanism for the enhanced sympathetic activity in the CHF state. It has been shown that the cardiac sympathetic afferent reflex (CSAR) is also involved in the enhancement of sympathetic outflow. The CSAR is a sympathetic excitatory reflex and appears to be independent of baroreflex mechanisms. The augmentation of the reflex, which can be initiated by myocardial ischemia and by CHF, may contribute to the increase in sympathetic efferent nerve activity in CHF. However, relatively little knowledge is available related to the mechanisms involved in this augment.

Recent studies suggest that nitric oxide (NO) may play an important role in regulating cardiovascular activities. In addition to being a mediator of the endothelial control of vascular smooth muscle, NO is involved in intracellular signaling in numerous tissues, including the nervous system. Increasing line of evidence shows that blockage of the central NO synthesis results in sympathoexcitatory effects and suggests that endogenous NO inhibits sympathetic outflow. These effects are involved in several nuclei including the nucleus tractus solitarii (NTS), the rostral ventrolateral medulla (RVLM) and the paraventricular nucleus (PVN). However, the effect of NO in the RVLM on the CSAR in the CHF state is still unknown. The purpose of the present study was to test the hypothesis that an abnormal NO in RVLM is involved in the enhancement of the CSAR in rats with coronary ligation-induced CHF.

1 MATERIALS AND METHODS

Thirty-six male Sprague-Dawley rats weighing 360–420 g (Nanjing Medical University, Nanjing, China) were used for all experiments. 

1.1 Model of chronic heart failure. CHF was produced by coronary artery ligation as previous described. All rats were anesthetized by pentobarbital sodium (50 mg/kg, i.p.). Surgery was carried out using sterile techniques. The trachea was incubated to facilitate mechanical ventilation. A left thoracotomy was performed through the fifth intercostal space. After retracting the lung, the pericardium was opened to expose the heart. The left coronary artery was ligated using a suture (6.0) near its branch point from the aorta, between the pulmonary artery outflow tract and left atrium. Following these maneuvers the heart was placed in its original position and the thorax was closed. The air within the thorax was evacuated, allowing the rats to resume spontaneous respiration and recover from anesthesia. Analgesics (Nubain-stadol, 1 ml/kg, s.c.) were administered after surgery. The rats were caged in an environment with ambient temperature maintained at 22°C and humidity at 30–40%. Laboratory chow and tap water was available ad libitum. The sham rats were treated the same as the heart failure rats except that their coronary arteries were not ligated. The final experiment was carried out 6–8 weeks after coronary ligation or sham surgery.

1.2 Surgery. Acute experiments were carried out 6–8 weeks following coronary ligation. Each rat was anesthetized with urethane (800 mg/kg i.p.) and α-chloralose (40 mg/kg i.p.). Supplemental doses of anesthetic were administered at 1/10 of the initial dose per hour. A midline incision in the neck was made, and the trachea was cannulated to facilitate mechanical ventilation. The carotid sinus area was exposed bilaterally. Each carotid sinus nerve was identified and cut. All other nerve fibers that were visible in the area of the carotid sinus were also cut. The carotid bifurcation and the common carotid arteries were stripped of adventitial tissues from 4 mm below the bifurcation to 4 mm above. The vessels were painted with 10% phenol solution to destroy any remaining nerve fibers in this area. Each vagus was then identified in the neck, tied, and sectioned. A carotid artery was catheterized for measurement of mean artery pressure (MAP) and heart rate (HR). The effectiveness of baroreceptor denervation was determined by recording the change in HR to venous injection of phenylephrine (20 µg/kg). This dose evoked an increase in BP between 25–40 mmHg. Baroreceptor denervation was assumed to be complete if HR did not change more than 5 beats/min in response to the intervention.

1.3 Stimulation of cardiac sympathetic afferent nerve. The chest was opened through the left second intercostal
space in rats. The left cardiac sympathetic afferent nerve was identified, tied, and ligated. A pair of stainless steel stimulating electrodes was placed on the central end of this nerve. The nerve-electrode junction was insulated electrically from the surrounding tissue with a silicone gel (Wacker Sil-Gel, 604 A and B). The stimulus was delivered with a stimulator (Grass S88, Astro-Med, West Warwick, RI) and a stimulus isolation unit. The frequencies of stimulation varied at 5, 10, 20 and 30 Hz with a constant voltage of 10 V. The pulse width was kept at 1 ms and each stimulus lasted 30 s. Stimuli were delivered in random sequences in each experimental protocol. The interval between each stimulus was at least 1 min. The RSNA responses to electrical stimulation were used to evaluate the central gain of the CSAR.

1.4 Placement of microinjection cannulae into the RVLM. The rats were placed in a stereotaxic instrument (Stoelting, Chicago, USA) and the skull was exposed through an incision on the midline of the scalp. After the bregma was identified, cannulae were positioned in the RVLM. The coordinates for the RVLM were determined from the Paxinos and Watson’s rat atlas \[21\], which were 12.7 mm posterior, 2.1 mm lateral to the bregma, and 10.1 mm ventral to the zero level. A cannula (o.d. 0.5 mm and i.d. 0.1 mm) connected to a microsyringe (0.5 µl; Model 7000.5, Hamilton) was advanced into the RVLM with a manipulator (Model 310, Stoelting, USA). The volume of microinjection was 100 nl (100 nl in 1 min), and the controls for each group were injected with saline (100 nl).

At the end of each acute experiment, a catheter was advanced through the carotid artery into the left ventricle (LV) to determine LV pressures. The LV end-diastolic pressure (LVEDP) and maximum of the first derivative (LV)dP/dtmax) were determined to provide a functional index of cardiac contractile state. Finally, fast green dye was injected into the RVLM for histological verification of injection site. The rat was euthanized with an overdose of anesthetic (pentobarbital sodium 100 mg/kg, i.v.). The brain was removed from the skull, placed in 10 % formalin. The brains were sectioned and the microinjection site was verified.

1.5 The renal sympathetic nerve activity (RSNA) recording. The left kidney, renal artery and nerves were exposed through a left retroperitoneal flank incision. The renal sympathetic nerves were identified and dissected free of the surrounding connective tissue. The nerve was immersed in a warm mineral oil bath and was placed on a pair of platinum-iridium recording electrodes. The signal was amplified with a Grass direct current preamplifier (Model P18D, Astro-Med. West Warwick, RI) with low frequency cutoff set at 30 or 100 Hz and high frequency cutoff at 1 or 3 kHz. The amplified discharge was monitored on a storage oscilloscope (Model 121 N, Tektronix, Beaverton, OR), and then imported to a computer system with other parameters. A voltage integrator (Buxco Electronics, Inc, Model 1801) was used for quantifying the raw renal sympathetic nerve activity. Background noise was determined after section of the central end of the renal nerve at the end of the experiment. This value was subtracted from all the integrated values of RSNA. The raw nerve activity, integrated nerve activity, arterial pressure and heart rate were recorded on a PowerLab data acquisition system (Model 16S, ADInstruments Inc, Mountain View, CA) and stored on disk until analyzed.

1.6 Experimental protocol

1.6.1 The CSAR in sham and CHF rats. The responses of the RSNA to electrical stimulation of cardiac sympathetic afferent nerves were compared in sham rats (n=6) and CHF rats (n=6).

1.6.2 Microinjection of S-methyl-L-thiocitruline (MeTC) into the RVLM. The effects of microinjection of NO synthase inhibitor, MeTC and normal saline (NS) into the RVLM on the responses of the RSNA to electrical stimulation of cardiac sympathetic afferent nerves were determined in sham rats (n=6) and CHF rats (n=6).

1.6.3 Microinjection of S-nitroso-N-acetyl-penicillamine (SNAP) into the RVLM. The effects of microinjection of NO donor, SNAP and NS into the RVLM on the responses of the RSNA to electrical stimulation of cardiac sympathetic afferent nerves were determined in sham rats (n=6) and CHF rats (n=6).

1.7 Drugs. MeTC and SNAP were obtained from Sigma. All drugs were dissolved in NS separately.

1.8 Statistical analysis. The RSNA was expressed as the percent change from control (before stimulation). The percent changes in the RSNA induced by cardiac sympathetic afferent nerves stimulation were plotted for each group and were used as an index of the central sensitivity of the CSAR. Ten seconds of the integrated RSNA and MAP immediately before cardiac sympathetic afferent stimulation and the last 10 s of the responses to stimulation were averaged. A two-way repeated-measure analysis of variance, followed by the Newman-Keuls test for post hoc analysis, was used when multiple comparisons were made. All statistical analyses were done using com-
puter software (SigmaStat, SPSS, Chicago, IL). All data are expressed as mean ± SE, \( P<0.05 \) was considered statistically significant.

2 RESULTS

2.1 Effects of coronary ligation on baseline hemodynamics, heart weight and infarction area

Table 1 summarizes some characteristics of sham and CHF rats. The rats with coronary ligation had myocardial infarctions of 32.2 ± 11.0% of the left ventricular (LV) surface. Sham rats had no observable damage to the myocardium. Although there was a scar in the infarcted area, which was very thin, the heart weight and the ratio of heart weight to body weight were significantly greater in CHF rats than in the sham rats, suggesting compensatory hypertrophy of the non-infarcted region of the myocardium.

There were no statistically significant differences in baseline MAP, diastolic arterial pressure (DAP) and heart rate (HR) between the sham and CHF rats. However, the systolic arterial pressure (SAP), pulse pressure (PP), left ventricle peak systolic pressure (LVSP) and \( \frac{dP}{dt_{max}} \) were decreased and the left ventricular end-diastolic pressure (LVEDP) was significantly increased in CHF rats compared with the sham rats. These histological and functional data show the presence of myocardial damage and suggest a decreased contractile function in CHF rats.

2.2 CSAR augmented in rats with CHF

The RSNA responses to varying frequencies of stimulation of the cardiac sympathetic afferent nerves were used to evaluate the CSAR in 6 sham rats and 6 CHF rats. The RSNA was increased during stimulation. In most rats, RSNA increased immediately after stimulation were delivered, and reached its maximal level within 15 s. The RSNA responses to 20 and 30 Hz of stimulation in the CHF rats were significantly increased compared with the sham rats, but the RSNA response to stimulation did not increase significantly in the sham rats (Fig. 1).

2.3 Effects of microinjection of MeTC into the RVLM

The effects of microinjection of NO synthase inhibitor, MeTC (80 nmol) into the RVLM were determined in 6 sham rats and 6 CHF rats (Fig. 2). MeTC significantly augmented the RSNA responses to stimulation compared with NS in sham rats. However, MeTC had no significant effect on the RSNA responses compared with NS in CHF rats. Similarly, microinjection of MeTC into the RVLM significantly increased the baseline RSNA and MAP compared with NS in sham rats, but did not affect the baseline RSNA and MAP in the CHF rats (Table 2).

2.4 Effects of microinjection of SNAP into the RVLM

The effects of microinjection of NO donor, SNAP (50

| Table 1. Heart weight, infarct size and baseline hemodynamics after 6–8 weeks of coronary ligation or sham surgery in rats |
|------------------|------|------|
| BW (g)           | Sham| CHF |
| HW (g)           | 408.6±5.8 | 401.7±5.4 |
| HW/BW (g/kg)     | 1.33±0.03 | 1.64±0.03*** |
| IS (% LV area)   | 0   | 32.2±2.6*** |
| SAP (mmHg)       | 117.6±3.7 | 95.3±2.6*** |
| DAP (mmHg)       | 72.8±3.8 | 69.8±4.3 |
| PP (mmHg)        | 44.8±3.4 | 24.0±3.5** |
| MAP (mmHg)       | 90.7±3.2 | 83.7±3.0 |
| HR (beats/min)   | 320.8±16.1 | 369.2±11.6 |
| LVSP (mmHg)      | 139.4±4.9 | 110.4±3.8*** |
| LVEDP (mmHg)     | 0.16±0.85 | 11.4±1.1*** |
| \( \frac{dP}{dt_{max}} \) (mmHg/s) | 3575.5±83.3 | 2236.2±115.6*** |

BW, body weight; HW, heart weight; IS, infarct size; LV, left ventricle; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; PP, pulse pressure; MAP, mean arterial pressure; LVSP, left ventricular peak systolic pressure; LVEDP, left ventricular end-diastolic pressure; \( \frac{dP}{dt_{max}} \), maximum of the first differentiation of left ventricular pressure. Data are given as mean±SE (n=18 for each group). **P<0.01, ***P<0.001 compared with the sham rats.

Fig. 1. RSNA response to varying frequencies of electrical stimulation of cardiac sympathetic afferent nerves in the sham and CHF rats. The RSNA response to the stimulation (20 and 30 Hz) was significantly enhanced in CHF rats. Values are expressed as mean ± SE; *P<0.05 compared with the sham rats.

Fig. 2. Effects of microinjection of MeTC into the RVLM

The effects of microinjection of NO synthase inhibitor, MeTC (80 nmol) into the RVLM were determined in 6 sham rats and 6 CHF rats (Fig. 2). MeTC significantly augmented the RSNA responses to stimulation compared with NS in sham rats. However, MeTC had no significant effect on the RSNA responses compared with NS in CHF rats. Similarly, microinjection of MeTC into the RVLM significantly increased the baseline RSNA and MAP compared with NS in sham rats, but did not affect the baseline RSNA and MAP in the CHF rats (Table 2).
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SNAP significantly inhibited the RSNA responses to stimulation compared with NS in CHF rats, but only induced a tendency of RSNA response decrease compared with NS in sham rats. As shown in Table 2, microinjection of SNAP into the RVLM significantly attenuated the baseline RSNA and MAP compared with NS in both sham rats and the CHF rats.

3 DISCUSSION

The CSAR is a sympatho-excitatory reflex and is activated by an increase in cardiac pressure and dimensions as well as by various substances that may be augmented in the myocardium at the CHF state[12]. In the present study we found that the CSAR was significantly augmented in coronary ligation-induced CHF rats. Microinjection of NO synthase inhibitor MeTC into the RVLM significantly enhanced the CSAR induced by stimulation of cardiac sympathetic afferent nerve and concomitantly increased baseline MAP and RSNA in sham rats, but had no significant effects on CSAR, baseline MAP and RSNA in CHF rats. Microinjection of NO donor SNAP into the RVLM significantly attenuated the CSAR in CHF rats and decreased baseline RSNA and MAP in both sham rats and CHF rats.

It is well known that NO plays an important role in regulating sympathetic outflow, and increasing evidence shows that blockage of the central NO synthesis results in sympatho-excitatory effects and suggests that endogenous NO inhibits sympathetic outflow[14,15,22]. These effects are involved in several nuclei including the nucleus tractus solitarii (NTS)[15], the rostral ventrolateral medulla (RVLM)[15] and the paraventricular nucleus (PVN)[15]. The RVLM is the final area for integration of sympathetic outflow and arterial blood pressure within the brain stem. The neurons in the RVLM are a site of convergence of

Table 2. Baseline change after microinjection of MeTC and SNAP into the RVLM

<table>
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<tr>
<th></th>
<th>Sham</th>
<th>CHF</th>
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<tr>
<td></td>
<td>RSNA (%)</td>
<td>MAP (mmHg)</td>
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<tr>
<td>Normal saline</td>
<td>-0.9±3.5</td>
<td>-0.1±0.7</td>
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<tr>
<td>MeTC 80 nmol</td>
<td>12.6±2.8*</td>
<td>6.3±1.4**</td>
</tr>
<tr>
<td>Normal saline</td>
<td>0.2±2.5</td>
<td>-0.2±1.4</td>
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<tr>
<td>SNAP 50 nmol</td>
<td>-7.1±2.2*</td>
<td>-5.5±1.7*</td>
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Data are given as mean ± SE. *P<0.05, **P<0.01 compared with normal saline.

Fig. 2. Effects of microinjection of MeTC into the RVLM on the RSNA response to varying frequencies of electrical stimulation of cardiac sympathetic afferent nerves in sham rats and CHF rats. Values are expressed as mean±SE; *P<0.05, **P<0.01, ***P<0.001 compared with NS; #P<0.05 compared with sham rats.

Fig. 3. Effects of microinjection of SNAP into the RVLM on the RSNA response to varying frequencies of electrical stimulation of cardiac sympathetic afferent nerves in sham rats and CHF rats. Values are expressed as mean±SE; *P<0.05 compared with NS; #P<0.05 compared with sham rats.
central pathways subserving many cardiovascular reflexes as well as responses evoked from higher brain regions. Immunohistochemical study has confirmed that the NOS is distributed in the RVLM. It was reported that microinjection of L-NMMA, an inhibitor of NO synthase, caused distinct increases in sympathetic nerve activity and arterial blood pressure, and the effects of NO synthase inhibition were counteracted by the NO-donor [15]. These results showed that NO in the RVLM was inhibitory to sympathetic outflow. As yet, the mechanism of NO inhibiting sympathetic outflow is unclear. On the other hand, it is still unknown whether the abnormal NO in the RVLM is involved in the enhancement of CSAR in CHF state. We found that microinjection of MeTC into the RVLM significantly increased the RSNA responses to electrical stimulation in sham rats. The data showed that inhibition of production of endogenous NO in RVLM augmented the CSAR, which indicated that NO in the RVLM inhibited the central gain of the CSAR. However, microinjection of MeTC into the RVLM of CHF rats did not significantly augment the increase of CSAR, which suggests that NOS has already been inhibited and the endogenous level of NO in RVLM is lower in CHF state. Therefore, the level of NO in the RVLM can be further decreased little by microinjection of MeTC into RVLM. So application of MeTC in CHF rats has no significant effect on CSAR, indicating that the inhibition of NOS in RVLM leading to the decrease of endogenous level of NO may be one of the mechanisms responsible for the augment of CSAR and the over-activity of sympathetic-adrenal system in the CHF state. Microinjection of NO donor SNAP into the RVLM significantly attenuated CSAR in both sham rats and CHF rats, which indicated that the increase of NO in RVLM inhibited the enhanced CSAR in CHF rats. Although NOS in the RVLM was inhibited in CHF, the CHF rats were still sensitive to NO. Therefore, the decrease of endogenous NO in the RVLM may play an important role in the enhancement of CSAR in CHF state, which may result in sympatho-excitatory effect.

In conclusion, the present study showed that the CSAR was enhanced in CHF rats and the decrease of NO in the RVLM was involved in the augmentation of CSAR in CHF rats.

REFERENCES

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