Effects of nNOS antisense in the paraventricular nucleus on blood pressure and heart rate in rats with heart failure

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Wang, Yu, Xue-Fei Liu, Kurtis G. Cornish, Irving H. Zucker, and Kaushik P. Patel. Effects of nNOS antisense in the paraventricular nucleus on blood pressure and heart rate in rats with heart failure. Am J Physiol Heart Circ Physiol 288: H205–H213, 2005. First published August 26, 2004; doi:10.1152/ajpheart.00497.2004.— Using neuronal NO synthase (nNOS)-specific antisense oligonucleotides, we examined the role of nitric oxide (NO) in the paraventricular nucleus (PVN) on control of blood pressure and heart rate (HR) in conscious sham rats and rats with chronic heart failure (CHF). After 6–8 wk, rats with chronic coronary ligation showed hemodynamic and echocardiographic signs of CHF. In sham rats, we found that microinjection of sodium nitroprusside (SNP, 20 nmol, 100 nl) into the PVN induced a significant decrease in mean arterial pressure (MAP). SNP also induced a significant decrease in HR over the next 10 min. In contrast, the NOS inhibitor Nω-monomethyl-L-arginine (L-NMMA, 200 pmol, 100 nl) significantly increased MAP and HR over the next 18–20 min. After injection of nNOS antisense, MAP was significantly increased in sham rats over the next 7 h. The peak response was 27.6 ± 4.1% above baseline pressure. However, in the CHF rats, only MAP was significantly increased. The peak magnitude was 12.9 ± 5.4% of baseline, which was significantly attenuated compared with sham rats (P < 0.01). In sham rats, the pressor response was completely abolished by α-receptor blockade. HR was significantly increased from hour 1 to hour 7 in sham and CHF rats. There was no difference in magnitude of HR responses. The tachycardia could not be abolished by the β1-blocker metoprolol. However, the muscarinic receptor antagonist atropine did not further augment the tachycardia. We conclude that NO induces a significant depressor and bradycardiac response in normal rats. The pressor response is mediated by an elevated sympathetic tone, whereas the tachycardia is mediated by withdrawal of parasympathetic tone in sham rats. These data are consistent with a downregulation of nNOS within the PVN in CHF.

oligonucleotides; gene expression; sympathetic nerve activity

A PATHOPHYSIOLOGICAL HALLMARK of chronic heart failure (CHF) is sympathoexcitation (9). Several studies have demonstrated that the magnitude of this excitation is adversely related to the prognosis of CHF (5). Although the precise mechanisms responsible for these relations are unclear, impaired neurohumoral regulation of sympathetic outflow may contribute to this sympathoexcitation in CHF. The neurohumoral substances thought to play a major role include angiotensin II (21), endothelin-1 (23), arginine vasopressin (11), and atrial natriuretic factor (39). Nitric oxide (NO) is a gaseous neuromodulatory substance that plays an important role in several central pathways, including the regulation of sympathetic tone (10). Our previous data showed that the neuronal isoform of NO synthase (nNOS) mRNA is substantially decreased in animals with experimental CHF at various central sites, including the paraventricular nucleus (PVN) (19, 25). Furthermore, NADPH-diaphorase staining demonstrated a decrease in nNOS-positive neurons in the PVN of rats with CHF (45). The PVN, which is located in the hypothalamus, is a central site that is known to be involved in autonomic and neuroendocrine regulation (36, 37). Neurons from the PVN project to several regions in the central nervous system (CNS), including the nucleus tractus solitarius (33, 34), the rostral ventrolateral medulla (RVLM) (6, 28, 42), and the intermediolateral cell column of the spinal cord (4, 15). These structures are known to be important in the regulation of sympathetic outflow. Several laboratories have investigated the role of NO within the PVN. The NO donor sodium nitroprusside (SNP) has been shown to decrease blood pressure (BP) (14), renal sympathetic nerve activity (RSNA), and heart rate (HR) (44), whereas blockade of NOS produced an increase in BP, RSNA, and HR (44). A recent study that evaluated the firing activity of identified PVN neurons showed that application of an NO donor inhibited the firing activity of RVLM-projecting PVN neurons (17). These studies suggest that NO plays a sympathoinhibitory role in the PVN. Zhang et al. (43) showed that the NO-mediated inhibition of RSNA was blunted in rats with CHF, which is consistent with the decrease in nNOS expression in CHF.

Because most studies that have investigated the role of NO within the PVN on the regulation of BP and sympathetic nerve activity have been carried out in the anesthetized state and it is well known that anesthesia can alter autonomic function, we designed the present study to be performed in the conscious, chronically instrumented state.

Antisense oligodeoxynucleotide (ODN) technology was used to prevent translation of nNOS messenger RNA to nNOS protein within the PVN. The purpose of this study was to determine whether there are alterations in the BP and HR responses to the sustained blockade of nNOS production within the PVN and whether there were important differences between CHF and sham rats. We hypothesized that the response to ODN administration would be attenuated in CHF rats.

METHODS

Model of Heart Failure

These experiments conformed with the “Guiding Principles in the Care and Use of Animals” of the American Physiological Society. All experiments were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (180–200 g body wt) underwent coronary artery ligation

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as described elsewhere (27). The rats were randomly assigned to one of two groups: a heart failure (CHF) group and a sham-operated (sham) group. Each rat was anesthetized with an anesthetic cocktail (55 mg/kg ketamine and 10 mg/kg xylazine im). The trachea was intubated, and the rat was placed on a small-animal ventilator. A left thoracotomy was performed, and the heart was exposed. In the CHF group, the left coronary artery was ligated with a 6-0 suture between the pulmonary artery outflow tract and the left atrium as it exited the aorta. The sham rats underwent thoracotomy and manipulation of the heart, but the coronary artery was not ligated. After these procedures, the thorax was closed and the chest was evacuated with a small chest tube. The trachea was extubated after the rat began to recover from the anesthesia, and the chest tube was removed. The rats were then maintained on standard chow with water ad libitum for 6–8 wk.

Transthoracic echocardiography was performed with an Acuson Sequoia 512C ultrasound system (Siemens) using an Acuson 15L8 probe at week 6 under anesthesia (65 mg/kg ketamine and 1.5 mg/kg acepromazine im). Left ventricular (LV) end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), LV end-diastolic volume (LVEDV), and LV end-systolic volume (LVESV) were measured. Fractional shortening (FS) was calculated as follows: \( \text{FS} = \left( \frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD}} \right) \times 100 \). Ejection fraction (EF) was calculated as follows: \( \text{EF} = \left( \frac{\text{LVEDV} - \text{LVESV}}{\text{LVEDV}} \right) \times 100 \). Rats recovered from the anesthesia 30 min later.

**General Surgery**

At 6–8 wk after thoracotomy, a radiotelemetry device (model TAI1PA-C40, Physiotel, Data Sciences International, St. Paul, MN) was implanted for the measurement of arterial pressure in the conscious state. With the rat under pentobarbital sodium (70 mg/kg ip) anesthesia, a central abdominal incision was made and a radiotelemetry device was secured to the intraperitoneal space. The sensing catheter of this device was inserted into the left femoral artery against blood flow. The signals received by the device were processed and digitized as radiofrequency data, which were recorded and stored in a computer with the Dataquest IV system (Data Sciences International). The measured parameters were arterial BP, mean arterial pressure (MAP), and HR. These parameters were displayed and stored in a PowerLab system (AD Instruments, Milford, MA).

After implantation of the radiotelemetry device, the rat’s head was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and the skull was exposed. A small burr hole was made on the right side. A cannula (CMA/11) was inserted through this hole. The tip of the cannula was placed according to the coordinates described by Paxinos and Watson (26): 1.8 mm posterior, 0.4 mm lateral to the bregma, and 7.8 mm ventral to the dura. The cannula was secured in place by jewelers’ screws and dental cement. After implantation of the cannula, rats were housed singly and allowed to recover from the surgical intervention for 1 wk.

**Experimental Protocols**

**Responses to SNP and N\(^2\)monomethyl-L-arginine.** In a group of sham rats, we examined the effect of SNP and N\(^2\)monomethyl-L-arginine (L-NMMA) on MAP and HR. On the day of the experiment, BP and HR were recorded continuously for 60 min starting at ~9 AM. A 60-min average was calculated as baseline data. The NO donor SNP (20 nmol, 100 nl) was gradually injected through the implanted cannula within the PVN in the conscious state over a period of 1 min. MAP and HR were recorded every 10 min for 3 consecutive hours starting at ~8 AM. The average of 30 min represents each reduced data point.

To determine whether the response to exogenous administration of ODN into the PVN was mediated by α- or β-adrenergic receptors or muscarinic receptors, 16 sham rats were divided into 3 groups that were subjected to α-receptor blockade with phentolamine (5 mg/kg ip), β\(_1\)-receptor blockade with metoprolol (3 mg/kg ip), or muscarinic receptor blockade with atropine (1 mg/kg ip). Intraperitoneally injected saline was used as control. These agents were administered intraperitoneally every hour for 7 consecutive hours starting 30 min before ODN microinjection. The rats were given nNOS antisense ODN with receptor blockade, mismatch ODN with receptor blockade, or antisense ODN with saline treatment in random order.

On the day of the terminal experiment, the rat was anesthetized with urethane (0.75 g/kg ip) and α-chloralose (70 mg/kg ip). The temperature was kept between 36 and 37°C. The right carotid artery was dissected, and a 3.5-F catheter transducer (Millar Instruments, Houston, TX) was advanced into the LV. This was also connected to a PowerLab system for recording LV pressure and maximum rate of change in pressure (dP/dt\(_{max}\)).

At the end of the experiment, the rats were euthanized and the brain was removed and fixed in 10% formalin for ≥24 h. The brain was frozen, and serial transverse sections (30 μm) were cut using a cryostat (IHC, model CT, International-Harris Cryostat, Minneapolis, MN) at ~20°C. The sections were mounted on microscope slides and stained with 1% neutral red. The presence of the needle tract within the PVN was verified microscopically. The heart was dissected free of adjacent tissues and lungs. The ventricles were separated from the atria, and the right ventricular free wall was dissected from the septum. The atria and both ventricles were rinsed, blotted, and weighed. The LV was opened with an incision along the septum from base to apex. Incisions were made in the LV so that the tissue could be pressed flat. The circumferences of the LV and the region of infarcted tissue were outlined on a clear photograph taken by a digital camera. Infant size was calculated and expressed as a percentage of LV surface area on the basis of the surface areas measured by the SigmaScan program (SPSS Science, Chicago, IL).

**Western Blot Analysis of nNOS in the PVN**

At 4 or 6 h after ODN injection, the rats were deeply anesthetized with pentobarbital sodium (70 mg/kg ip), and the brains were immediately removed and frozen on dry ice. The brains were blocked in the coronal plane and cut into 300-μm-thick sections in a cryostat. A 15-gauge needle was used to punch the PVN from the ODN-injected side and from the contralateral noninjected side. The punches were homogenized in ice-cold TriReagent (Molecular Research Center, Cincinnati, OH) using a sonicator (GraLab 545).

The protein was extracted according to the protocol described by the Molecular Research Center. Protein content in the SDS supernatant was determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Protein (6 μg) was mixed with SDS-PAGE buffer containing β-mercaptoethanol and heated at 100°C for 5 min. Then protein was fractionated in a 7.5% polyacrylamide gel along with molecular weight standards, transferred to an Immobilon
membrane, and subjected to a Western immunoblotting protocol (20). The membrane was probed with monoclonal anti-nNOS antibody (Transduction Laboratories, Lexington, KY) and peroxidase-conjugated goat anti-mouse IgG, and the signal was detected using the enhanced chemiluminescence immunoblotting detection system (Pierce). The film was digitized using a Kodak digital camera, and the net intensity was determined using Kodak ID Image Analysis software.

**Immunohistochemical Staining of nNOS in the PVN**

One section (20 μm thick) of every five serial sections was prepared for immunohistochemical staining. The sections were rinsed in PBS for 15 min and then in acetone-methanol (1:1) for 20 min and PBS for 5 min (1% BSA and 0.2% Triton X-100). Nonspecific staining was blocked by 2% normal goat serum (Jackson Immuno Research Laboratories, West Grove, PA), 0.2% Triton X-100, and 0.1% sodium azide for 4 h at room temperature. Sections were incubated with primary antibody of mouse anti-rat nNOS IgG (1:100; Transduction Laboratories) and 0.2% Triton X-100 overnight at 4°C and then washed and incubated with goat anti-mouse IgG (1:100; Molecular Probes, Eugene, OR), Hoechst 33258 (antinuclei; Molecular Probes), and 0.2% Triton X-100 for 3 h at room temperature. Then they were rinsed three times in PBS and 0.2% Triton X-100 and mounted. The sections were evaluated under epifluorescence in a DMR research microscope (Leica Microsystems, Wetzlar, Germany) equipped with a digital camera (Magnafire, Optronics, Goleta, CA). Photomicrographs were displayed using Adobe Photoshop (San Jose, CA) image-editing software without further adjustment to maintain the true nature of the findings.

**Data Analysis**

The responses of MAP and HR to SNP, L-NMMA, and ODN are expressed as percent change above baseline (i.e., without treatment). The baseline changes of MAP and HR in response to SNP or L-NMMA treatment were compared by paired t-test. The changes in MAP and HR in response to ODN and the Western blot data were subjected to a two-way ANOVA followed by Bonferroni’s procedure for post hoc analysis to determine the difference between groups. The changes in MAP and HR in response to ODN were subjected to a repeated-measures two-way ANOVA followed by Bonferroni’s procedure for post hoc analysis to determine the difference between groups and between time periods. P < 0.05 was considered statistically significant. Values are means ± SE.

**RESULTS**

**Baseline Hemodynamics in Sham and CHF Rats**

Table 1 shows the values for MAP, HR, dP/d\(\text{max}\), and LV end-diastolic pressure and echocardiography data from the four groups. Both CHF groups exhibited a significantly lower MAP than the corresponding sham groups. There was no significant difference in the body weight and HR among the four groups. All CHF rats exhibited significantly higher LV end-diastolic pressure, LVEDD, LVESD, LVESV, and LVEDV and lower dP/d\(\text{max}\), FS, and EF than the corresponding sham groups. The average infarct size in CHF rats was 42 ± 3.6%.

**Effect of SNP and L-NMMA Within the PVN on MAP and HR in Normal Conscious Rats**

Figure 1 illustrates the sites of microinjection in the PVN. Microinjection of SNP into the PVN induced a significant decrease in MAP from minute 4 to minute 10 in conscious, freely moving rats (average \(-5.1 \pm 1.4%\) from a baseline of 105 ± 5.6 mmHg; Fig. 2A). SNP also induced a significant decrease in HR over the next 10 min (average \(-8 \pm 3.2%\) from a baseline of 365 ± 13.6 beats/min; Fig. 2B). In contrast, the NOS inhibitor L-NMMA significantly increased the MAP (average 17.3 ± 5.0% from a baseline of 102 ± 7.6 mmHg) and HR (average 13 ± 4.5% from a baseline of 375 ± 15.9 beats/min) over the next 18–20 min (Fig. 2).
section clearly shows a suppression of the nNOS signal (green fluorescence) in the antisense-treated side. This change occurred throughout the rostrocaudal extension of the PVN without specificity of cellular compartments (e.g., magnocellular or parvocellular).

Effect of Microinjection of nNOS Antisense Into PVN on MAP and HR in Conscious, Freely Moving Sham and CHF Rats

The administration of nNOS antisense into the PVN induced a significant increase in MAP in sham rats over the next 7 h (Fig. 4A). The peak response occurred at hour 4, and the magnitude was 27.6 ± 4.1% above baseline pressure. The baseline values are shown in Table 1. However, in the CHF rats, MAP was significantly increased only at hour 4. The peak magnitude was 12.9 ± 5.4% of baseline, which was significantly lower than in sham rats ($P < 0.01$). In addition, at hours 3, 5, and 6, the magnitude of the MAP response was significantly lower for CHF than for sham rats. There was no significant difference in the MAP in mismatch ODN-treated sham or CHF rats. The HR was significantly increased from hour 1 to hour 7 in sham and CHF rats after antisense microinjection (Fig. 4B). The peak magnitudes were 19.7 ± 5.8% and 18.3 ± 2.8%, respectively. There was no significant difference in the magnitude of the HR responses between these two groups. In addition, there was no significant difference in the HR responses in mismatch ODN-treated sham and CHF rats. In experiments in which the cannula site was outside the PVN, we did not find changes in MAP and HR in response to nNOS antisense ODN.

Effect of α- or β-Receptor Blockade on Antisense-Induced Change in MAP and HR in Sham Rats

Figure 5A illustrates that prior intraperitoneal administration of the α-receptor blocker phentolamine completely abolished the pressor response induced by PVN administration of antisense in sham rats. The baseline values are shown in Table 2. There was no significant difference in the MAP responses in the antisense or mismatch ODN-injected sham rats during treatment with phentolamine. In fact, phentolamine induced a slight depressor response in antisense-microinjected rats. These results suggest that...
the pressor effect induced by the nNOS antisense is mediated by an increase in α-adrenergic sympathetic outflow.

In antisense-treated rats, the HR response was significantly increased by phentolamine over the first 6 h compared with non-phentolamine-treated rats. This may be the result of baroreflex activation induced by the hypotensive effect of phentolamine. After phentolamine treatment, antisense induced a further significant increase in HR at hours 2 and 3 compared with the mismatch ODN treatment (Fig. 5B).

In contrast, metoprolol treatment significantly reduced the MAP response in antisense-treated sham rats at hours 1 and 2 compared with non-metoprolol-treated rats (Fig. 6A). This may be due to a depression in myocardial contractility by metoprolol. However, antisense treatment still increased MAP over the next 6 h compared with mismatch ODN treatment in metoprolol-treated rats.

Metoprolol did not abolish the HR response to antisense administration. Compared with the mismatch ODN, antisense
induced a significant increase in HR over the first 6 h during β1-receptor blockade (Fig. 6B).

Effect of Atropine on Antisense-Induced Changes in MAP and HR in Sham Rats

The effects of atropine on MAP and HR in antisense- or mismatch ODN-treated sham animals are illustrated in Fig. 7. In atropine-treated rats, antisense resulted in a significant increase in MAP, especially at hours 4 and 5 (Fig. 7A). In antisense-treated rats, we observed a reduction in MAP 3–6 h after treatment with atropine. The mechanism for this reduction is not clear; however, it may be due to compromised cardiac function induced by the resultant tachycardia.

There were no differences in HR between rats given mismatch and those given antisense ODN after atropine administration. Furthermore, there was no additional increase in HR induced by antisense in rats pretreated with atropine. In antisense-treated rats, atropine significantly increased the HR at hours 5 and 6 (Fig. 7B). The baseline values are shown in Table 2.

DISCUSSION

The results of the present study indicate that nNOS antisense ODN evokes a sympathoexcitatory response when administered into the PVN. The excitatory effect of unilateral administration of nNOS antisense ODN within the PVN was blunted in rats with CHF. This is consistent with a decrease in nNOS protein in the PVN in CHF. It is also consistent with a decrease in responsiveness to NO in CHF (43).

The dose and time course of nNOS antisense ODN are based on NADPH-diaphorase staining and Western blot analysis using the same antisense ODN. We found that 1 mM nNOS antisense ODN for 6 h yields the maximal inhibition on gene expression without apparent neuronal or vascular injury after microinjection. On the other hand, the mismatch ODN did not alter nNOS protein levels or hemodynamics.

The role of NO within the PVN on cardiovascular regulation has been studied by several groups. It has been well demonstrated that endogenous nNOS is localized in the PVN (22, 31, 38) and that central NO, which acts as a neuronal modulator, is involved in a variety of physiological responses, such as neuronal firing, ion channel modulation, and modification of neurotransmitter release (10). Because the PVN is known to be an integrative center for the sympathetic nervous system, NO is expected to be an important regulator of central sympathetic outflow (20, 24, 44). Horn et al. (14) showed that perfusion with NO-containing aCSF or microinjection of SNP into the PVN induced a significant decrease in BP. Li et al. (20) showed that delivery of an nNOS adenovirus into the PVN is more effective than delivery of a β-galactosidase adenovirus in suppressing RSNA in normal rats. Direct electrophysiological evidence suggested that the NO donor NONOate inhibited the firing activity of RVLM-projecting PVN neurons and that the nNOS inhibitor 7-nitroindazole increased basal firing activity (17). All these studies support the notion that endogenous NO within the PVN exerts an inhibitory influence on sympathetic outflow. Interestingly, in the present study, we observed that the physiological changes induced by antisense precede the changes in nNOS protein levels. The maximal changes in MAP and HR occurred at hour 4, and a modest but significant increase in MAP was seen at hour 1. However, nNOS protein was significantly reduced at hour 6. These data may indicate that only slight changes in nNOS protein in the PVN can induce significant changes in MAP and HR. Although this observation is of some concern, it is unlikely that antisense ODN exerted nonspecific effects, because there were no effects of mismatched ODN. Therefore, we cannot explain this protein-function disconnect in the time-course response, but...

Table 2. Baseline MAP and HR of sham rats before autonomic blockade and ODN treatment

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<td>α-blocker</td>
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<td>β-blocker</td>
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<td>MAP, mmHg</td>
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<td>104 ± 6.4</td>
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<td>HR, beats/min</td>
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<tr>
<td>Atropine</td>
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Values are means ± SE; n, no. of rats. PVN, paraventricular nucleus; ODN, oligodeoxynucleotide; MM, mismatch; AS, antisense.
this may be related to our inability to detect significant early changes in protein suppression. In this study, we also observed that the magnitude of the changes in MAP and HR in response to a nonspecific NOS antagonist, L-NMMA, and a specific nNOS antagonist, nNOS antisense, was similar. This observation indicates that nNOS may be the major NOS isoform that contributes to the endogenous NO production in the PVN. However, we cannot rule out a contribution from endothelial NOS.

The CHF state is characterized by sympathetic nervous activation. The degree of activation is prognostic for survival rate. Changes in several neurohumoral factors in the CNS are believed to contribute to this sympathoexcitation (8, 16, 29, 46). Our data indicate that blockade of the synthesis of the neuromodulator NO induced a smaller increase in BP in rats with CHF. This is consistent with the idea that NO synthesis of NO is reduced in CHF. Several studies have shown that nNOS is decreased in the hypothalamus (25, 45) in animals with CHF. Our Western blot data also confirm that nNOS protein is decreased in the PVN of CHF rats compared with sham rats. Because the pressor responses to L-NMMA and nNOS antisense were similar in these experiments, we cannot rule out a contribution from endothelial NOS.

The precise mechanism for this reduction is unclear. However, several studies suggest that nNOS expression is modulated by numerous physiological and pathological stimuli, such as neuronal injury and synaptic plasticity (3, 7). Many of these processes are 

Ca^2+ dependent. Sasaki et al. (32) showed that nNOS transcription was regulated by 

Ca^2+ influx through a cAMP response element-binding protein family transcription factor-dependent mechanism. Because NOS catalytic activity is also 

Ca^2+ dependent, many substances, such as endothelin (30), angiotensin II (13), and glutamate (41), may modulate NOS activity and expression in response to increases in intracellular 

Ca^2+. A change in the concentration of these substances in CHF may contribute to a reduction of nNOS synthesis and/or activity (17, 43).

In this study, we found that the pressor response to PVN administration of nNOS antisense was completely abolished by \( \beta \)-adrenergic receptor blockade, clearly a sympathetic response. The tachycardia response to nNOS antisense was not blocked by \( \beta \)-adrenergic receptor blockade but was abrogated after atropine. This suggested that the HR response was mediated by a parasympathetic component. It has been shown that anatomically and functionally segregated PVN neurons project to sympathetic- and parasympathetic-related autonomic targets in the CNS, which includes the dorsal vagal complex (DVC) (1, 17). Using electrodes to stimulate the PVN, Stauss et al.
(35) reported that the sinus node was more responsive to parasympathetic than to sympathetic stimulation at higher stimulation frequencies. Their data also showed a gradual decrease in HR in response to increasing stimulation frequencies during β-adrenergic receptor blockade (35). Li et al. (17) observed that NO donors inhibited the firing activity of DVC-projecting PVN neurons. On the basis of these findings, it may be expected that nNOS antisense would decrease, rather than increase, HR. In contrast, we observed an increase in the HR after nNOS antisense treatment. This is consistent with previous reports in anesthetized rats where NO inhibitors were microinjected into the PVN (18, 20). The difference between our findings and other studies may be due to different experimental preparations. Stauss et al. recorded HR changes immediately after PVN stimulation, whereas we recorded HR for several hours after treatment. In addition, Li et al. recorded from inhibitory PVN neurons, which project to the DVC. It is possible that NO modulates different PVN neurons in different ways: Using whole cell patch-clamp recordings from PVN neurons in a hypothalamic slice preparation, Bains and Ferguson (2) reported that NO depolarized type II PVN neurons (parvocellular neurons), not type I neurons (magnocellular neurons).

CHF is characterized by significant autonomic dysfunction consisting of sympathetic activation, parasympathetic withdrawal, and peripheral organ unresponsiveness (12). There is an imbalance of the sympathetic and parasympathetic nervous systems in CHF. However, we did not find a significant difference in the HR response to intra-PVN administration of nNOS antisense in CHF and sham rats. These data suggest that the extent of withdrawal of parasympathetic nerve activity by antisense is similar in both groups. This may indicate that basal nNOS levels are similar in PVN parasympathetic-driving neurons in sham and CHF rats.

In conclusion, the data presented here suggest that nNOS antisense acting within the PVN increases BP via activation of the sympathetic nervous system, whereas increases in HR are mediated by depression of vagal outflow. In CHF, the BP response to nNOS antisense was blunted. We believe that this finding represents a loss of nNOS production by PVN neurons in CHF. Our data provide further evidence for the importance of central NO mechanisms within the PVN in the sympathetic-excitation in CHF.

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