Blunted nitric oxide-mediated inhibition of renal nerve discharge within PVN of rats with heart failure

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Zhang, Kun, Yi-Fan Li, and Kaushik P. Patel. Blunted nitric oxide-mediated inhibition of renal nerve discharge within PVN of rats with heart failure. Am J Physiol Heart Circ Physiol 281: H995–H1004, 2001.—We have demonstrated a decreased neuronal nitric oxide (NO) synthase (nNOS) message in the hypothalamus of rats with heart failure (HF). Subsequently, we have demonstrated that NADPH diaphorase (a commonly used marker for nNOS activity) positive neurons are decreased in paraventricular nuclei (PVN) of rats with coronary artery ligation model of HF. The goal of the present study was to examine the influence of endogenous NO within the PVN on renal sympathetic nerve discharge (RSND) during HF. In α-chloralose- and urethane-anesthetized rats, an inhibitor of NO synthase, N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) microinjected into the PVN (50, 100, and 200 pmol in 50–200 nl) produced a dose-dependent increase in RSND, blood pressure, and heart rate in control and HF rats. These responses were attenuated in rats with HF compared with control rats. On the other hand, the NO agonist, sodium nitroprusside, microinjected in PVN produced a dose-dependent decrease in RSND and blood pressure in control and HF rats. These responses were less in rats with HF compared with control rats. These data suggest that the endogenous NO-mediated effect within the PVN of HF rats is less potent in suppressing RSND compared with control rats. These data support the conclusion that the NO system within the PVN involved in controlling autonomic outflow is altered during HF and may contribute to the elevated levels of renal sympathoexcitation commonly observed in HF.

The paraventricular nucleus (PVN) is known to be a site of integration for autonomic and endocrine cardiovascular responses (34, 35). Neuroanatomic and electrophysiological data (13, 33, 35) have indicated that the PVN is reciprocally connected to other areas of the central nervous system that are involved in cardiovascular function. Neuroanatomic studies (27, 31, 32) using retrograde tracing techniques have shown that the PVN is a major source of forebrain input to the sympathetic nervous system. Functional studies have also implicated the PVN in cardiovascular regulation. Electrical or chemical stimulation of the PVN appears to increase blood pressure primarily via activation of the sympathetic nervous system (12, 25, 26). Therefore, it is reasonable to speculate that the alterations in the neuronal activity of PVN are possibly responsible for alterations in overall sympathetic nerve activity (34, 35). The PVN is a nucleus on which a number of neurotransmitters converge to influence its neuronal activity (34, 35). Some of these neurotransmitters are excitatory and some are inhibitory. It can be viewed that the coordinated actions of excitatory and inhibitory neurotransmitters in the PVN determine the neuronal activity of the PVN. Either the increased excitatory stimuli or decreased inhibitory stimuli into the PVN would lead to the increased neuronal activity of PVN, and consequent increased sympathetic outflow.

Administration of a nitric oxide (NO) donor, sodium nitroprusside (SNP), into the PVN has been shown to elicit a decrease in renal sympathetic nerve activity, arterial blood pressure, and heart rate (10, 39). Because the PVN is a forebrain integrative site for the sympathetic nervous system, the depressor effect of NO applied to the PVN led to the suggestion that NO in this nucleus is inhibitory to sympathetic outflow (10, 39). Consistent with this contention, administration of N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) or nitro-L-arginine methyl ester (L-NAME) [inhibitors of NO synthase (NOS)], within the PVN increases renal sympathetic nerve discharge (RSND), which indicates that endogenous NO within the PVN is inhibitory to sympathetic outflow (39).

In recent years, we have begun to examine the involvement of central cardiovascular processing mechanisms in the elevated neurohumoral drive during heart failure (HF) (17–20, 22, 39, 41). One key area within the hypothalamus that is important in dictating sympathetic outflow is the PVN (34, 35), which demonstrates an increased neuronal activity during HF (22, 36). The HF condition is known to produce attenuated vasodilation in response to agonists known to operate via an NO mechanism (5). Concomitantly, levels of endogenous endothelial NOS (eNOS) protein and mRNA for eNOS in peripheral tissue are reduced in the HF state (30). However, there are no studies examining the NO system within the central nervous system.
in HF, except for a few recent studies from our laboratory (16, 20, 41). We (20) recently showed that the genetic message for neuronal NOS (nNOS) is decreased in the hypothalamus of rats with HF. This decrease was observed in a section of the hypothalamus that contained several nuclei. Although the majority of the NOS within this section of the hypothalamus were within the PVN and NOS, we cannot be certain that the downregulation is primarily within the PVN. We subsequently stained the PVN for nNOS-positive neurons by using the NADPH diaphorase staining method (41), known to correlate with the immunocytochemistry technique (3). The results demonstrate that there were decreased nNOS-positive neurons in PVN of rats with HF compared with sham-operated control rats (41). This set of observations lead us to hypothesize that there are altered NO mechanisms within the PVN in the regulation of sympathetic outflow during HF, and that this altered mechanism may contribute to the increased sympathetic nerve activity during HF.

To test this hypothesis, this study was designed to examine 1) if the endogenous NO within the PVN contributes to the regulation of sympathetic outflow in rats with heart failure, and 2) if the response of renal sympathetic nerve activity to the microinjection of SNP, an NO donor into the PVN is altered in rats with HF.

METHODS

Induction of Heart Failure

Male Sprague-Dawley rats weighing 210–280 g were obtained from Sasco Breeding Laboratories (Omaha, NE) and were assigned randomly to one of two groups (sham-operated control and HF group). HF was produced by coronary artery ligation, as previously described (6, 11, 24). Briefly, each rat was anesthetized with pentobarbital sodium (50 mg/kg ip) and the trachea was cannulated to facilitate mechanical ventilation. A left thoracotomy was performed and the heart was lifted from the thorax. The left coronary artery was ligated near its branch point from the aorta, between the pulmonary artery outflow tract and left atrium. Sham-operated rats underwent thoracotomy and manipulation of the heart but the coronary artery was not ligated. After these maneuvers were performed, the heart was returned to its original position and the thorax was closed. The air within the thorax was removed, allowing the rats to resume spontaneous respiration. The trachea was closed, the neck incision was sutured, and the rats were allowed to recover from anesthesia. Analgesics (Nubain-Stadol, 1 ml/kg sc) were administered after surgery, followed by codeine phosphate and acetaminophen (Tylenol with codeine elixir) in drinking water for 2 days postsurgery. Each rat was caged individually in an environment with ambient temperature maintained at 22°C and humidity at 30–40%. Laboratory chow (Purina) and tap water were available ad libitum. The acute experiments were conducted 6–8 wk after ligation of the left coronary artery (HF group) or sham surgery in control rats. At the end of each acute experiment, a 2-Fr micromanometer-tipped catheter (Millar Instruments) was advanced through the right carotid artery into the left ventricle (LV) to determine LV pressures. LV end-diastolic pressure (LVEDP) was determined from the LV pressure recording. LVEDP measurement was determined to provide a functional index of cardiac contractile function.

All of the procedures used in this study were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and the experiments were conducted according to the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society.

General Surgery

On the day of the experiment, the rat was anesthetized with urethane (0.75 g/kg ip) and α-chloralose (70 mg/kg ip). The left femoral vein was cannulated with polyethylene tubing (PE-50 filled with saline) for drug administration if necessary, and the left femoral artery was cannulated and connected to a computer-based data-acquisition system (MacLab) via a pressure transducer (model P231D, Gould) for recording of arterial blood pressure and heart rate. The rat was then placed in a stereotaxic apparatus (Davis Kopf Instruments; Tujunga, CA), and a small burr hole was placed in the skull. The coordinates for the right PVN were determined from the Paxinos and Watson (23) rat atlas, which is 1.8 mm posterior, 0.4 mm lateral to the bregma, and 7.8 mm ventral to the dura. A cannula (outer diameter 0.5 mm and inner diameter 0.1 mm) connected to a microsyringe (0.5 μl; model 7000.5, Hamilton) was advanced into the right PVN with a manipulator (model Z-1, Narishige, Japan). The left kidney was exposed through a left retroperitoneal flank incision. A branch of the renal nerve was isolated from the adipose and connective tissues. The distal end of the nerve was ligated, and the nerve was placed on a thin, bipolar steel electrode. The nerve-electrode junction was insulated electrically from the surrounding tissues with mineral oil. The electrical signal from the electrode was linked via a high-impedance probe (H1P5) to a Grass P511 band-pass amplifier (gain, 10–50 × 1,000) with high- and low-frequency cutoffs of 1,000 Hz and 100 Hz. The output from the Grass amplifier was directed to a Grass integrator, which rectifies the signal and integrates the raw nerve discharge. The output of the Grass integrator was displayed as an integrated voltage that is proportional to the renal nerve discharge. The average rectified signal [resistor-capacitor circuit (RC) filtered with a time constant of 0.5 s] was then recorded and stored for later analysis in a computer-based data-acquisition system (MacLab). Efferent RSND at the beginning of the experiment was defined as basal nerve discharge. All renal nerve activity recordings were corrected by subtraction of background noise, defined as the signal remaining after administration of hexamethonium (20 mg/kg iv) or postmortem. The response of RSND to the administration of drugs into the PVN during the experiment was subsequently expressed as a percentage change from the basal value.

At the end of the experiments, monastral blue dye was injected into the brain for histological verification of injection. After the rat was killed with intravenous injection of KCl, the brain was removed and fixed for 4% formaldehyde for at least 24 h. The brain was then frozen, and serial transverse sections (30 μm) were cut with the use of a cryostat (model CT, IEC, International-Harris) at −20°C. The sections were thaw mounted on microscope slides and stained with 1% aqueous neutral red staining procedures. Presence of the blue dye within the PVN was verified under a microscope with ×40 magnification. The sites of termination of the injector tracts are illustrated in Fig. 1. Injector placements were found throughout the rostrocaudal extent of the PVN. Among the 39 injections into the PVN, 31 injections were in...
the PVN area, whereas 8 injections were outside the PVN. Among the 31 injections that were within the PVN, 15 PVN injections were in sham-operated and 16 injections were in rats with HF. The placements within the PVN would be expected to result in a distribution of drug that would result in ~90% of the infusate being restricted to the PVN region (29).

Experimental Protocols

Experiment 1. Experiments were performed to examine if the endogenous NO in the PVN is inhibitory to renal sympathetic outflow, and if this inhibitory effect is reduced in rats with HF. In eight sham-operated control rats and eight rats with left descending coronary artery ligated, an inhibitor of NO synthase, \(N^\text{G}\)-monomethyl-L-arginine (L-NMMA) was consecutively injected (50, 100, and 200 pmol in 50, 100, and 200 nl, respectively, using 1 mM solution of L-NMMA over 2 min) into the PVN in intervals of 20–30 min. The vehicle solution was artificial cerebrospinal fluid composed of (in mM) 132 NaCl, 3.0 KCl, 0.65 MgCl\(_2\), 1.5 CaCl\(_2\), and 24.6 NaHCO\(_3\), with 3.3 glucose adjusted to pH 7.4, for all compounds. The responses in mean arterial blood pressure, heart rate, and RSND over the 20–30 min were recorded after each dose of L-NMMA. To substantiate the conception that any responses of blood pressure, heart rate, and RSND to L-NMMA are mediated by the blockade of NOS within the PVN and not a peripheral action, in five normal rats, intravenous injection of 500 pmol of L-NMMA (500 nl of 1 mM solution of L-NMMA in saline over 2 min) were examined. The responses of changes in arterial blood pressure, heart rate, and RSND were recorded after intravenous injection of 500 pmol of L-NMMA.

Experiment 2. To determine whether the response to exogenous administration of NO into the PVN is different between sham-operated control rats (\(n = 7\)) and rats with HF (\(n = 8\)), SNP (50, 100, and 200 nmol in 50, 100, and 200 nl, respectively, using 1 M solution of SNP over 2 min) was injected consecutively into the PVN in intervals of 20–30 min. The responses in mean arterial blood pressure, heart rate, and RSND over 20–30 min were recorded after each dose of SNP.

Experiment 3. The responses of blood pressure, heart rate, and RSND to L-NMMA were collected in a group of rats (\(n = 8\)) in which the injection site was not in the PVN area; this group consisted of both sham-operated rats (\(n = 4\)) and HF rats (\(n = 4\)). This group is termed the anatomical control group. This group is also used as a time control because these experiments were carried out over the same time frame as all the experiments mentioned above.

Experiment 4. To determine if the reduced RSND response to L-NMMA in HF rats was due to a ceiling effect (elevated basal RSND in HF rats that cannot be increased any further), we determined if RSND in HF rats can be increased further above the response to L-NMMA with another stimulus known to maximally activate RSND (4). The RSND response to blocking the airway of rats with HF and sham-operated rats for 40 s was elicited. Such a stimulus is known to increase RSND by 150–200% of baseline activity. These responses were compared with responses to the maximal dose of L-NMMA (200 pmol) in rats with HF.

Cardiac histology. After the experiment was completed, the heart was removed, weighed, and fixed in 4% formaldehyde. The heart was then divided into four major segments from the atrium to the apex: A, B, C, and D. As described previously (21, 22), sections A and D were discarded, and sections B and C were processed routinely for histological study. Briefly, the segments were dehydrated with increasing levels of alcohol, followed by embedding the tissues in paraffin. Segments were sliced (10-μm thick) and then stained with phosphotungstic acid hematoxylin. The images of these sections were projected onto a screen, where the lengths of the endocardial circumference and endocardial infarct segment of the LV were measured from the projected silhouettes. The infarct size was expressed as a fraction of the total cross-sectional endocardial and epicardial circumferences of the left ventricle.

Data Analysis

Responses of RSND to the various doses of drugs were expressed as the percentage of change over the basal value. Responses of arterial blood pressure and heart rate to drugs were expressed as the difference between the basal value and the value after each dose of drug. The data were subject to repeated two-way analysis of variance, followed by Newman-Keuls test for multiple comparisons (38). \(P < 0.05\) was considered statistically significant.

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Fig. 1. Schematic representations of serial sections from the rostral (−1.4) to the caudal (−2.12) extent of the region of the paraventricular nucleus in experiments 1 and 5. The distance (in mm), posterior to bregma, is shown for each section (see Ref. 23). Closed circles are the sites of termination of the injections of \(N^\text{G}\)-monomethyl-L-arginine (L-NMMA) or sodium nitroprusside (SNP), which are considered to be within the paraventricular nucleus (PVN) region from sham-operated rats, whereas * represents the site of termination of an injection of L-NMMA or SNP, which is considered to be outside the PVN region from a heart failure rat. Open squares are the sites of termination of the injections of L-NMMA or SNP, which are considered to be outside of the PVN region. AH, anterior hypothalamic nucleus; f, fornix; 3V, third ventricle. Horizontal bar = 0.5 mm.
RESULTS

General Data

Table 1 summarizes the salient characteristics of sham-operated and HF rats utilized in the present study. For all of the experiments, any rat subjected to coronary artery ligation that failed to display myocardial infarcts <30% of the LV wall was excluded from the study. Accordingly, the rats retained in the HF group exhibited ~49% of the endocardial surface. Sham rats had no observable damage to the myocardium. The minimum ventricular thickness was significantly less in HF rats than in the sham group, indicating transmural damage to the myocardium. Heart weight was significantly greater in HF rats than in shams, suggesting compensatory hypertrophy of non-infarcted regions of the myocardium. LVEDP was elevated significantly in HF rats compared with sham rats. Furthermore, lung weight was significantly greater in HF rats than in sham rats. In ~15% of rats with HF, we observed pleural and abdominal effusion, indicating that retention of water occurs in these rats. Thus both histological and functional data disclose the presence of myocardial damage and suggest a decreased contractile function in the rats with HF.

The coronary ligation model of HF results in a wide range of LV dysfunction. LVEDP was reported (7, 24) as being related to LV infarct. LV infarcts of <30% show no significant elevation of LVEDP (6 mmHg); those with 30–39% LV infarction show a significant increase in LVEDP (13 mmHg), and those with LV infarction of >39% show a greater increase in LVEDP (28 mmHg) (7). LV infarcts of >46% show overt signs of congestive heart failure (24). Pfeffer et al. (24) found that 86% of rats with infarcts >46% of the left ventricle had LVEDP >20 mmHg. Hostetter et al. (11) observed similar findings. The observation of increased LVEDP, cardiac hypertrophy, and histological damage to the myocardium of 40–49% in the present study suggest that the rats in HF group had decreased cardiac contractile function and experienced HF.

The basal arterial pressure, heart rate, and RSND in the various groups are presented in Table 2. There were no statistically significant differences in basal arterial pressure, heart rate, or RSND between the sham and HF groups. RSND was termed 100% at the start of the experiment. We have observed that RSND, arterial blood pressure, and heart rate do not change significantly over the time frame of this experiment. This was evident in experiment 3 (Table 2), where we missed the PVN in both sham-operated rats and HF rats.

Table 2. Baseline values for blood pressure, heart rate, and RSND in each treatment group of rats

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>Mean Blood Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>RSND, mV/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-NMMA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>8</td>
<td>90 ± 4.6</td>
<td>360 ± 37.5</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Heart failure</td>
<td>8</td>
<td>95 ± 3.2</td>
<td>382 ± 22.5</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>SNP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>7</td>
<td>107 ± 3.3</td>
<td>409 ± 26.6</td>
<td>2.9 ± 0.8</td>
</tr>
<tr>
<td>Heart failure</td>
<td>8</td>
<td>100 ± 2.6</td>
<td>388 ± 19.4</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Miss PVN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-NMMA-miss</td>
<td>4</td>
<td>96 ± 5</td>
<td>374 ± 22</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>SNP-miss</td>
<td>4</td>
<td>93 ± 4</td>
<td>393 ± 40</td>
<td>2.8 ± 0.4</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n, no. of rats. l-NMMA, NG-monomethyl-l-arginine; RSND, renal sympathetic nerve discharge; SNP, sodium nitroprusside; PVN, paraventricular nucleus; miss PVN, injections 0.5 mm away from the boundaries of the PVN. There were no statistically significant differences among the groups.

Table 1. Characteristics of chronic heart failure rats (6–8 wk) and sham-operated rats

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13–15</td>
<td>14–16</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>377 ± 23</td>
<td>368 ± 7</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>98 ± 4</td>
<td>97 ± 3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>380 ± 32</td>
<td>387 ± 21</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>1,038 ± 90</td>
<td>1,489 ± 76*</td>
</tr>
<tr>
<td>Heart wt/body wt, mg/g</td>
<td>3.2 ± 0.1</td>
<td>4.1 ± 0.1*</td>
</tr>
<tr>
<td>Lung wt/body wt, mg/g</td>
<td>4.1 ± 0.3</td>
<td>10.7 ± 1.9*</td>
</tr>
<tr>
<td>Infarct size, % LV endocardial</td>
<td>0</td>
<td>48.9 ± 1.7*</td>
</tr>
<tr>
<td>Infarct size, % LV epicardial</td>
<td>0</td>
<td>39.8 ± 2.0*</td>
</tr>
<tr>
<td>Minimum thickness of LV, mm</td>
<td>1.5 ± 0.20</td>
<td>0.4 ± 0.03*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>2.9 ± 0.5</td>
<td>22.5 ± 3.0*</td>
</tr>
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</table>

Values represent means ± SE; n, no. of rats. LV, left ventricular; LVEDP, LV end-diastolic pressure. *P < 0.05 vs. sham-operated rats.

In normal rats, we also examined the possibility that the observed effects of NOS inhibitors were mediated by their peripheral effect. Intravenous administration of 500 pmol of l-NMMA (n = 5) elicited no significant change in RSND (0.6 ± 4.4%), blood pressure (5.0 ± 0.3 mmHg), or heart rate (1 ± 2).

Experiment 2. Microinjection of the NO donor SNP (50, 100, and 200 pmol) produced significant decreases in efferent RSND, arterial blood pressure, and heart rate relative to l-NMMA in HF rats, the responses were significantly blunted compared with sham-operated control rats. These data indicate that blockade of endogenous NO synthesis in rats with HF is less effective in raising the RSND, blood pressure, and heart rate in rats with HF.
**Experiment 3.** In rats without the PVN, we did not see any response to administration of L-NMMA (Fig. 5). Similarly, microinjection of SNP outside the PVN did not change RSND, arterial pressure, and heart rate (Fig. 6). The data are presented separately for the L-NMMA and SNP to avoid missing the opposing effects of sympathoexcitation and inhibition, respectively, of these substances. We termed these groups the “anatomic control” groups. These groups can also be used as “time controls” because these experiments were carried out over the same time frame as all of the experiments mentioned above. There were no significant changes in RSND, arterial pressure, and heart rate over the time frame of these experiments to microinjection of L-NMMA or SNP in adjacent sites within the hypothalamus in either the sham-operated or HF rats.

**Experiment 4.** In an attempt to determine if the reduced RSND response to L-NMMA in rats with HF was due to an elevated basal RSND in HF rats that cannot be increased any further, we determined whether RSND in heart failure rats can be increased further above the response to L-NMMA response with another maximal stimulus. The RSND response to blocking the airway of HF rats for 40 s was fivefold higher than that elicited by L-NMMA (Fig. 7). These data demonstrate that RSND can increase above the maximal dose of L-NMMA given within the PVN.

**DISCUSSION**

The results of the present study indicate that blockade of the endogenous NO mechanism by the microinjection of L-NMMA into the PVN increases the efferent RSND, mean arterial pressure, and heart rate in both sham-operated control rats and rats with HF. However, these responses to L-NMMA were significantly reduced in rats with HF compared with the sham-operated control group. Conversely, microinjection of NO donor, SNP, into the PVN resulted in the significant decreases in efferent RSND and mean arterial pressure (but not heart rate) in the sham-operated control group but not in the rats with HF. These data suggest that the reduced renal sympathoinhibition mediated by endogenous NO within the PVN may contribute to the elevated sympathetic nerve activity during HF.

Several observations suggest that the responses reported here are primarily from actions on neurons within the PVN. First, the criteria for successful placement of the cannula were based on previous work (29) indicating that, with the microinfusion method, ~90% of the distribution of radiolabeled injectate (100 nl) was within a radius of 0.6 ± 0.1 mm. Therefore, any placement that was within the rostrocaudal and mediolateral boundaries of the PVN and within 0.5 mm in the dorsoventral plane was considered acceptable because a majority of the injected dose would encompass the PVN. Second, it is unlikely that L-NMMA acted at sites in adjacent areas because the histochemistry demonstrates a strong and concentrated density of NOS-positive neurons in the PVN exclusively (2, 15, 37). Third, injections of either L-NMMA or SNP >0.5 mm away from the rostrocaudal and mediolateral bound-

![Fig. 2. Segments of original recordings from individual rats demonstrating the starting baseline parameters and the peak changes in arterial blood pressure (BP), heart rate (HR), and renal sympathetic nerve discharge (RSND) by administration of L-NMMA into the PVN in a sham-operated (A) and a heart failure rat (B) into the PVN. Shown in each panel are baseline parameters 0, 50, 100, and 200 pmol of L-NMMA into the PVN. Bottom: time scale = 20 s (horizontal bar).](image-url)
aries of PVN failed to produce response in RSND. Thus it seems probable that the effects we observed are due primarily to actions of the injectates within the PVN region.

Since Garthwaite et al. (9) first demonstrated that NO was acting as a neuronal messenger in cerebellar granule cells, NO has been reported (8) to be involved in various physiological activities as a nonconventional neurotransmitter. The presence of NOS-positive neurons in the PVN of the hypothalamus suggests NO may serve as a physiological regulator of the sympathetic nervous system. Horn et al. (10) observed that both perfusion of the PVN with NO-containing cerebrospinal fluid and microinjection of SNP into the PVN elicited a significant reduction of arterial blood pressure. These effects of NO within the PVN were attributed to inhibition of neurons mediating sympathetic outflow via the intermediolateral call column of the spinal cord (10). This initial functional evidence is supported more recently by direct measurement of RSND (39, 40), showing that endogenous NO within the PVN is involved in the regulation of sympathetic outflow.

If NO is a physiological regulator of sympathetic outflow within the PVN, it is reasonable to speculate that any alterations in NO mechanisms within the PVN will influence the sympathetic nerve activity and cardiovascular system. Patel et al. (22) reported that the neuronal activity of PVN was elevated during HF.

Fig. 3. Change in RSND (A), change in arterial BP (B), and change in HR (C) to the microinjection of L-NMMA into the PVN in sham-operated control (n = 8) and heart failure rats (n = 8). Values are means ± SE. *P < 0.05 vs. control.

Fig. 4. Change in RSND (A), change in arterial BP (B), and change in HR (C) to the microinjection of SNP into the PVN in sham-operated control (n = 8) and heart failure rats (n = 8). Values are means ± SE. *P < 0.05 vs. control.
We hypothesize that NO is inhibitory to renal sympathetic outflow via its actions in the PVN, and this NO input into the PVN decreases during HF and hence contributes to increased neuronal activity of the PVN and the elevated sympathetic nerve activity (Fig. 8). In the present study, the function of endogenous NO to regulate sympathetic outflow during HF in the PVN was investigated. The microinjection of L-NMMA into the PVN elicited significant increases in efferent RSND, mean arterial blood pressure, and heart rate in sham-operated control rats. These effects of L-NMMA were significantly reduced in rats with HF compared with those observed in the control group. It might be argued that HF rats have an increased basal sympathetic nerve activity and the given dose of L-NMMA maximizes the renal sympathetic nerve activity, which cannot be increased further. The latter possibility is unlikely because RSND could be further elevated via a noncardiac reflex (blocking the airway) (Fig. 7). This blunted response of endogenous NO indicates that NO, an inhibitory physiological regulator of RSND within the PVN is not as effective during HF as it is in control rats. This can probably be attributed to either the decreased activity of nNOS (41) and/or production of nNOS (20) and, hence, the reduced synthesis of NO within PVN during HF (Fig. 8). It might also be possible that the reduced renal sympathoinhibition by NO in the PVN is accounted for by the reduced response of neurons in the PVN to NO during heart failure. In this

![Graphs of changes in RSND, arterial BP, and HR to the microinjection of L-NMMA (A), SNP (B), and sodium nitroprusside (C).](http://ajpheart.physiology.org/)

Fig. 5. Changes in RSND (A), arterial BP (B), and HR (C) to the microinjection of L-NMMA (50, 100, and 200 pmol) to the area of brain, which were outside the PVN of sham-operated (n = 3) and heart failure rats (n = 1). Values are means ± SE (n = 4).

Fig. 6. Changes in RSND (A), arterial BP (B), and HR (C) to the microinjection of SNP (50, 100, and 200 nmol) to the area of brain, which were outside the PVN of sham-operated (n = 2) and heart failure rats (n = 2). Values are means ± SE (n = 4).
study, we also examined the response to administration of NO donor, SNP, into the PVN during HF. SNP, when injected into PVN, elicited significant decreases in RSND and arterial blood pressure in the control group. The reduction of sympathetic outflow and arterial blood pressure to SNP injection into the PVN was not observed in rats with HF, which indicated that the response of PVN neuron to NO is abnormal during the HF condition. These observations suggest that some mechanism downstream after the production of NO is also altered in rats with HF. These would include the action of NO directly or indirectly (via cGMP) on excitability of neurons within the PVN to mediate renal sympathetic nerve activity.

At the present, the precise cellular mechanism through which NO acts within PVN to inhibit sympathetic outflow is unknown. This action of NO could be direct or indirect. Horn et al. (10) observed that perfusion of PVN with NO-cerebrospinal fluid led to an increase in the concentrations of some amino acids in the perfusate, including γ-aminobutyric acid (GABA). The central GABA system exerts a tonic inhibitory influence over the sympathetic nervous system. Intracerebroventricular injections of a GABA agonist produced a decrease in arterial blood pressure, heart rate, and peripheral sympathetic nerve activity (1). Conversely, the administration of GABA antagonists, such as bicuculline methiodide, suggests that the endogenous GABA system may exert a tonic inhibitory effect on the sympathetic nervous system (14, 28). The effects of sympathoexcitation with L-NMMA within the PVN could also be blocked by GABA mechanisms (40). Thus it was proposed that the effect of NO within PVN may be mediated by the release of GABA. If this is true, the production of GABA or the actions of GABA within the PVN may be blunted due to decreased NO synthesis within the PVN and blunted response of the PVN neuron to NO during HF. This altered GABA mechanism may account for the blunted inhibitory responses in RSND to administration of SNP within the PVN. The possibility that these NO responses may be related to an altered GABA mechanism remains to be examined.

In conclusion, the present study demonstrates that NO within the PVN regulates the sympathetic outflow via an inhibitory mechanism. Decreased NO input and abnormalities in post-NO mechanisms within the PVN may contribute to the increased sympathetic nerve activity observed during HF.

HF is characterized by an increased sympathetic nerve activity. The mechanisms that underlie increased sympathetic nerve activity are not clear. We have reported that the neuronal activity of PVN is increased during HF. This change in neuronal activity may be related to the imbalance between excitatory and inhibitory inputs coming into the PVN during heart failure. This study demonstrates that decreased endogenous NO input and abnormalities in post-NO mechanisms within the PVN may contribute to the
increased sympathetic nerve activity observed during HF. The factors involved in altering the NO mechanism in the PVN during HF remains to be examined. Future research will be necessary to establish the origin and nature of the stimuli that activate or inhibit these mechanisms to regulate sympathetic outflow. Further investigations are required to define the precise nature of afferent signaling, interactions with sympathoexcitatory mechanisms such as glutamate and angiotensin II within the PVN, and the mechanisms of NO-mediated sympathoinhibition.

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REFERENCES


