Effect of muscimol and L-NAME in the PVN on the RSNA response to volume expansion in conscious rabbits

Chi Wai Ng, Robert De Matteo, and Emilio Badoer

School of Medical Sciences, Royal Melbourne Institute of Technology University, Bundoora 3083, Melbourne, Victoria, Australia

Submitted 8 December 2003; accepted in final form 1 June 2004

Ng, Chi Wai, Robert De Matteo, and Emilio Badoer. Effect of muscimol and L-NAME in the PVN on the RSNA response to volume expansion in conscious rabbits. Am J Physiol Renal Physiol 287: F739–F746, 2004; 10.1152/ajprenal.00431.2003.—In the present study, we have investigated whether the hypothalamic paraventricular nucleus (PVN) contributed to the reflex reduction in renal sympathetic nerve activity (RSNA) normally elicited by volume expansion in the conscious rabbit. RSNA was monitored after volume expansion (Dextran 70, 2 ml/min for 30 min) in animals microinjected into, and outside, the PVN with muscimol (10 nmol), to acutely inhibit neuronal function. Because nitric oxide within the PVN inhibits RSNA, we also examined the effect of L-N^3-nitro-L-arginine methyl ester (L-NAME; 20 nmol) to block nitric oxide synthase. Compared with vehicle, the reduction in RSNA elicited by volume expansion was abolished by injection of muscimol into the PVN. The effect was specific to the PVN because microinjections of muscimol outside the PVN had no effect on the response. L-NAME microinjected into or outside the PVN had no effect on the RSNA response. The findings suggest that the PVN is essential in the central pathways mediating the renal sympathetic nerve response elicited by elevations in plasma volume but that nitric oxide does not play a major role.

The hypothalamic PVN has direct projections to the sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord (27), and it also directly projects to important autonomic brain regions such as the rostral ventrolateral medulla (RVLM; see Refs. 4 and 8), which is believed to be the site of the tonic generation of sympathetic nerve activity. Additionally, there are neurons in the PVN that send collaterals to both the spinal cord and RVLM (8, 28). Thus these direct and indirect pathways connecting the PVN to sympathetic preganglionic motoneurons are likely to contribute to the effects on sympathetic nerve activity that can be elicited from the PVN (4, 8, 28).

A role of the PVN in the neural responses elicited by volume changes was first suggested by electrophysiological work that showed changes in the firing rates of PVN spinoally projecting neurons could be elicited by volume expansion (21). In agreement with that work, we have utilized Fos as a marker of neuronal activation to show that reductions in blood volume activate spinoally projecting neurons (2). Further evidence that indicates the PVN is an important contributor to the reflex renal responses after elevations in blood volume arises from the studies using kainic or ibotenic acid to destroy neurons in the PVN. These studies, performed in the anesthetized rat, have shown that the increase in renal blood flow and the reduction in RSNA in response to a volume load could be attenuated by lesions of the PVN (12, 22). Thus these studies have suggested that the PVN plays an important role in the cardiac-vasomotor reflex.

Most studies to date have been performed in anesthetized animals. However, the presence of anesthesia can dampen reflex responses and markedly alter the cardiovascular responses that can be elicited by stimulating or inhibiting the PVN. For example, the blood pressure and sympathetic nerve activity responses induced by stimulation of the PVN in anesthetized and conscious animals are diametrically opposite (6, 16). Thus the first aim of the present study was to examine, for the first time in the conscious animal, the role of the PVN in the renal sympathoinhibition induced by volume expansion. This was performed by using muscimol, which acts on GABA receptors, to inhibit neuronal function.

The PVN contains many neurochemicals, but the nature of the neurotransmitters within the PVN that may be mediating the reduction in RSNA in response to volume expansion is unknown. A potentially important inhibitory neurotransmitter is nitric oxide. Nitric oxide is an atypical neurotransmitter in that it is not stored but rather produced on demand after activation of nitric oxide synthase. The enzyme has been found to be rate limiting for the production of nitric oxide (15). However, nitric oxide does not play a major role in the reflex reduction in RSNA in response to volume expansion (2004). The PVN contains many neurochemicals, but the nature of the neurotransmitters within the PVN that may be mediating the reduction in RSNA in response to volume expansion is unknown. A potentially important inhibitory neurotransmitter is nitric oxide. Nitric oxide is an atypical neurotransmitter in that it is not stored but rather produced on demand after activation of nitric oxide synthase. The enzyme has been found to be rate limiting for the production of nitric oxide (15). However, nitric oxide does not play a major role in the reflex reduction in RSNA in response to volume expansion (2004).

The increase in blood volume is sensed by receptors located in the heart, and inhibition of these prevents the reflex reduction in RSNA elicited by an increase in blood volume (5). This has been referred to as the cardiac-vasomotor reflex. In chronic volume overload conditions like heart failure, there is an abnormality in the reflex response so that the reduction in RSNA is attenuated. This is believed to contribute to the autonomic disturbances observed in heart failure, such as an abnormal elevation in sympathetic nerve activity to the kidneys.

The afferents relaying information about blood volume travel from the heart to the brain via the vagus nerve. In the brain, these afferents terminate in the nucleus of the solitary tract (NTS) in the dorsomedial medulla oblongata. The pathways from the NTS that are subsequently involved are not known. However, the hypothalamic paraventricular nucleus (PVN) is known to receive direct projections from the NTS, and it has been suggested that the autonomic reflex changes elicited by a volume load involve the PVN (see below and Refs. 12 and 17).

Address for reprint requests and other correspondence: E. Badoer, School of Medical Sciences, RMIT Univ., PO Box 71, Bundoora 3083 Melbourne, Victoria, Australia (E-mail: emilio.badoer@rmit.edu.au).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
in neurons and terminals within the PVN (7), suggesting that it can influence neuronal function within this brain region. Indeed, the PVN contains the highest concentration of nitric oxide synthase within the hypothalamus. Nitric oxide is inhibitory on neurons, and microinjections of nitric oxide donors in the PVN elicit reductions in RSNA. Conversely, inhibition of nitric oxide synthase within the PVN produces increases in RSNA. Thus nitric oxide appears to mimic the effects elicited after volume expansion. Whether this neurotransmitter, within the PVN, mediates the renal sympathoinhibition induced by volume expansion in the conscious animal is not known.

Thus the aims of the present study were 1) to investigate the effect of inhibiting neuronal function, within the PVN, on the reflex renal nerve reduction elicited by volume expansion and 2) to investigate the role of nitric oxide in this reflex response by blocking nitric oxide synthase within the PVN. To determine whether the PVN was specifically involved in the responses, we microinjected these same drugs in areas outside, but adjacent to, the PVN. We performed our experiments in conscious rabbits to alleviate any confounding effects of anesthesia.

MATERIALS AND METHODS

Both male and female New Zealand White rabbits (2.5–3.0 kg) were used in this study and were obtained from Monash Animal Services (Monash University, Clayton, Victoria) or Nanawie Stud Farm (Geelong, Victoria). All experimental protocols were approved by the Monash University and RMIT University Animal Ethics Committees and conform to the Guiding Principles for Research Involving Animals and Human Beings (1) and guidelines set out by the National Health and Medical Research Council of Australia.

Surgical Procedures

Before the experimental days, the animals underwent surgical procedures performed under general anesthesia using ketamine hydrochloride (40 mg/kg sc) and xylazine hydrochloride (5.0 mg/kg sc), with additional ketamine (20 mg/kg sc) administered every 30 min. Diazepam (5 mg) was administered subcutaneously as premedication. Analgesia (buprenorphine hydrochloride, 60 μg im) was administered after each surgical procedure to alleviate any postoperative pain, and an antibiotic (chloramphenicol, 100 mg sc) was administered routinely after each operation. At least 2 wk separated each surgical procedure.

Implantation of PVN guide cannulas. Under general anesthesia, the head of the rabbit was placed in a David Kopf stereotaxic apparatus. The head clamps were modified so that a small pin protruded from each clamp to allow the clamps to fix on the zygomatic arch. A longitudinal incision was made on the head to expose both the bregmoids and lambroid sutures. The head was positioned so that bregma and lambda were on the same horizontal plane. The head was considered level when the coordinate was determined. The head was considered level when the dorsoventral (DV) coordinate was determined. The head was considered level when the anterior-posterior (AP) coordinate was determined.

Blood pressure was monitored using an indwelling arterial catheter connected to a pressure transducer. The signal was recorded using a MacLab data acquisition system (ADInstruments). MAP and HR were determined electronically using the blood pressure signal.

Experimental Protocols

On the experimental day, after a quiet rest period of at least 1 h, muscimol (a GABA receptor agonist), NO(3)-nitro-l-arginine methyl ester (l-NAME; a nitric oxide synthase inhibitor), or vehicle was microinjected (200 nl) intracerebrally in the conscious rabbit. Later (3–15 min), Dextran (Dextran 70; a plasma volume expander; Baxter Healthcare, New South Wales, Australia) was infused intravenously (2 ml/min for 30 min). Mean arterial pressure (MAP), heart rate (HR), and RSNA were monitored before the intracerebral injections and for 60 min after the start of the infusion. Muscimol (10 nmol/200 nl per side, n = 4) was injected bilaterally in the PVN, 15 min before the start of the Dextran infusion, on one experimental day. On another day, vehicle (Ringer solution) replaced muscimol. The experimental days were separated by 48 h, and the administration of vehicle or muscimol was randomized. In a separate series, performed in four rabbits, the same protocol was followed, except the injections of vehicle or muscimol were centered outside the PVN (defined as 0.5 mm or more from the PVN boundary).

Similar experimental procedures were performed with l-NAME (20 nmol/200 nl per side, n = 8), microinjected in the PVN of the conscious rabbit. In these experiments, l-NAME was microinjected 3 min before the start of the Dextran infusion. In a separate group of animals, vehicle (Ringer solution) replaced l-NAME (n = 8). In separate rabbits, microinjections of l-NAME (n = 8) and vehicle (n = 8) were made in areas adjacent to the PVN.

The intracerebral microinjections were performed using a stainless steel injection needle, which extended 5 mm past the end of the fixed guide cannula. The microinjection needle was connected by thin tubing to a 100-μl Hamilton syringe. The tubing was calibrated, and the injections were made using a micromanipulator with a micrometer attached to the plunger of the syringe, which enabled the accurate microinjection of the volume required.

Monitoring Cardiovascular Variables
Raw RSNA was amplified using a low-noise differential amplifier (ENG models 187B and 133; Baker Institute, Melbourne, Australia), filtered (band pass 100–5,000 Hz), rectified, and integrated at 0.5-s intervals. The threshold was set visually to cut out background nerve activity during quiet periods between bursts. We have found that this method provides a similar estimate of noise level as after a maximum pressor response. The average integrated RSNA over 1- to 2-min periods was calculated and expressed as a percentage of the resting period before each stimulus (5).

Brain Histology

After the completion of all experiments, 200 nl Pontamine sky blue or fluorescent beads were injected intracerebrally to assist in determining the site of injection. Rabbits were deeply anesthetized with pentobarbitone sodium (60 mg/kg iv), injected with 1,000 U heparin intravenously, and then perfused transcardially with 1 liter of 0.1 M PBS (pH = 7.2) followed by 1 liter of 4% paraformaldehyde in phosphate buffer. The brain was removed and stored in fixative solution containing 20% sucrose. The hypothalamus was cut into sections (40 μm thick), and every fourth serial section was taken for histological examination. The sections were mounted on subbed slides and allowed to dry before being counterstained with cresyl violet and coverslipped with DePex mounting medium (BDH Laboratory Supplies).

The sections were examined using light microscopy, and the site of the dye injection was recorded in relation to the PVN. An injection site was categorized as "in the PVN" if it was <0.5 mm from the boundary of the PVN. The positions of the injection sites relative to the PVN were drawn on maps.

Statistical Analysis

The basal resting MAP and HR levels were compared using a paired or an unpaired Student’s t-test as appropriate. Data were expressed as the changes from the resting levels, and comparisons between treatments were made using two-way ANOVA, followed by comparisons between time points using Student’s t-test and applying Bonferroni’s modification to compensate for multiple comparisons.

In the case of RSNA, it is well known that a comparison of absolute levels of sympathetic nerve activity between animals and between days is inappropriate for technical reasons. Thus data were expressed as a percentage of the resting level before volume expansion, and the changes were compared between treatments as described above.

RESULTS

Muscimol Microinjections

Effect of muscimol (10 nmol/side) on the responses elicited by volume expansion. INSIDE THE PVN. In animals pretreated with vehicle, volume expansion elicited an increase in MAP of ~8 mmHg and HR of ~40 beats/min (Fig. 1). RSNA was reduced markedly by ~50% in response to volume expansion in the vehicle-treated group. RSNA fell to the maximal plateau level within 15 min of the start of volume expansion, and it remained markedly reduced for the duration of the observation period (Fig. 1).

When muscimol was microinjected in the PVN, volume expansion did not increase MAP, in contrast to the vehicle pretreatment day (P < 0.05, between-groups 2-way ANOVA; Fig. 1). The increase in HR elicited by volume expansion in the muscimol pretreated group, however, was not significantly different between the two treatment days (Fig. 1). Muscimol microinjected in the PVN completely blocked the decrease in RSNA in response to volume expansion (Fig. 1), which contrasted dramatically with the response observed on the vehicle pretreatment day. Thus there was a significant difference between the treatments (P < 0.05, overall ANOVA) and at every time point after the plateau level had been reached (Fig. 1).

OUTSIDE THE PVN. When vehicle was microinjected outside of the PVN, volume expansion elicited a small increase in MAP and only small changes in HR (Fig. 2). The reflex RSNA was reduced by ~50%, which was similar to that seen when vehicle was microinjected in the PVN (Fig. 2).

When muscimol was microinjected outside of the PVN, there was no increase in MAP, but HR was increased in response to volume expansion, and both were significantly different compared with the vehicle pretreatment (P < 0.005, between groups, 2-way ANOVA; Fig. 2). When muscimol was microinjected outside of the PVN, the reflex reduction in RSNA elicited by the volume expansion was not affected (Fig. 2). This contrasted with that observed when muscimol was microinjected in the PVN (compare Figs. 1 and 2).

Effect of L-NAME (20 nmol/side) on the responses elicited by volume expansion. INSIDE THE PVN. In this series of experiments, volume expansion resulted in a small increase in MAP and HR when vehicle was microinjected in the PVN (Fig. 3). RSNA was reduced by ~45% in response to the volume expansion (Fig. 3). These effects were similar to the control responses we observed in the muscimol series.

After L-NAME pretreatment, there was no effect on the MAP response, elicited by volume expansion, compared with the vehicle group (Fig. 3). By contrast, the HR re-
The response was significantly greater in the l-NAME-treated group compared with the vehicle-treated group (P < 0.01, between-groups, 2-way ANOVA; Fig. 3). The decrease in RSNA in response to volume expansion was not statistically different between the l-NAME- and vehicle-pretreated groups (Fig. 3).

**OUTSIDE THE PVN.** The MAP and HR responses elicited by volume expansion were not significantly different after l-NAME or vehicle microinjected outside the PVN (Fig. 4). l-NAME microinjected outside of the PVN did not produce a marked change in the RSNA response, elicited by volume expansion, compared with vehicle (Fig. 4). RSNA was reduced by ~45% by volume expansion in both groups (Fig. 4).

**Effect of muscimol on basal MAP and HR.** Before volume expansion, MAP was slightly higher in the animals treated with muscimol compared with animals treated with vehicle. This difference was statistically significant (P < 0.05, Table 1). Basal HR levels were not significantly different between treatments (Table 1).

OUTSIDE THE PVN. In this series, basal MAP was not significantly different between the vehicle and muscimol treatment days (Table 1). However, HR was significantly lower before volume expansion on the muscimol treatment day compared with the vehicle treatment day (P < 0.05, Table 1).

**Effect of l-NAME on basal MAP and HR.** Before volume expansion, basal MAP levels were not significantly different in the l-NAME or vehicle groups (Table 1). However, the basal HR was significantly lower in the l-NAME group (P < 0.05, Table 1).

**OUTSIDE THE PVN.** The basal levels of MAP and HR were not significantly different between vehicle-treated and l-NAME-treated days before volume expansion (Table 1).

**Effect on basal RSNA.** Although it is not possible to compare basal RSNA between days or between animals, we did observe that muscimol in the PVN increased resting RSNA (52 ± 41%, n = 4) within animals, but this effect was not statistically significant. A similar effect was observed when muscimol was injected outside the PVN (mean change = 105 ± 72%, n = 4). l-NAME had no effect on resting RSNA within animals (~8 ± 7% inside n = 8; ~4 ± 11% outside, n = 8).

**Intracerebral microinjection sites.** Microinjections in the PVN were located throughout the rostral-caudal extent of the PVN. The sites are schematically shown in Figs. 5 and 6. Microinjections that were found to be outside of the PVN were located predominantly dorsal or caudal to the PVN (Figs. 5 and 6).

**DISCUSSION**

In the present study, we found that inhibition of the PVN with muscimol, in the unanesthetized rabbit, prevented the normal reflex reduction in RSNA elicited by volume expansion. This effect was specific to the PVN. This study shows, for the first time in the conscious animal, that the PVN is essential in the renal nerve response elicited by an elevation in circulating blood volume. We also examined whether nitric oxide...
was a potential neurotransmitter mediating the reflex reduction in RSNA. The findings did not provide evidence for a major role of nitric oxide within the PVN in this response.

Muscimol is a GABA receptor agonist that inhibits neurons. In the present study, the microinjection of muscimol in the PVN abolished the renal sympathoinhibition normally elicited by volume expansion. Microinjections of muscimol in areas adjacent to, but outside, the PVN had no effect on the normal reflex RSNA response. Thus the results indicate that neurons in the PVN are crucial in the renal nerve response elicited by volume expansion. Changes in resting RSNA after intracerebral muscimol have not complicated this conclusion.

An increase in circulating blood volume elicits the reflex reduction in RSNA by activating receptors located on the heart and often referred to as cardiac mechanoreceptors (5, 10, 30). The present results are the first in a conscious animal and agree with very recent findings in the anesthetized rat in which the GABA receptor antagonist, bicuculline, microinjected in the PVN blocked the renal sympathoinhibition elicited by the activation of the cardiac mechanoreceptors (31). Furthermore, the present results are also in agreement with studies in which the PVN was lesioned. In those studies, the reduction in renal nerve activity, or the increase in renal blood flow, elicited by volume expansion in the anesthetized rat was attenuated by the destruction of the PVN (12, 22). Thus the present study, together with these earlier studies, provides direct evidence.

Table 1. MAP and HR before volume expansion in rabbits pretreated with muscimol (10 nmol/side), L-NAME (20 nmol/side), or respective vehicle microinjected inside or outside the hypothalamic PVN

<table>
<thead>
<tr>
<th></th>
<th>Inside PVN</th>
<th></th>
<th>Outside PVN</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP, mmHg</td>
<td>HR, beats/min</td>
<td>MAP, mmHg</td>
<td>HR, beats/min</td>
</tr>
<tr>
<td>Vehicle</td>
<td>68.7±0.9</td>
<td>261±27</td>
<td>75.7±1.8</td>
<td>295±21</td>
</tr>
<tr>
<td>Muscimol</td>
<td>72.5±1.3*</td>
<td>225±17</td>
<td>69.2±3.9</td>
<td>221±24*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>70.0±3.5</td>
<td>267±17</td>
<td>73.7±2.9</td>
<td>255±8</td>
</tr>
<tr>
<td>L-NAME</td>
<td>72.1±2.9</td>
<td>222±9*</td>
<td>67.9±2.4</td>
<td>231±10</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rabbits. MAP, mean arterial pressure; HR, heart rate; PVN, paraventricular nucleus; L-NAME, N\textsuperscript{\textsubscript{G}}-nitro-L-arginine methyl ester. *P < 0.05 compared with vehicle.
that the PVN plays a critical role in the renal sympathetic nerve response induced by volume expansion.

The PVN contains many different subgroups based on morphology and neuroanatomic projections. Of particular interest for the regulation of sympathetic nerve activity are the neurons in the PVN that (1) project to the intermediolateral nucleus of the spinal cord, (2) project to the RVLM, and (3) send collaterals to both regions (8, 28). PVN neurons that project to the spinal cord can directly influence sympathetic nerve activity. On the other hand, the PVN neurons that project to the RVLM influence sympathetic nerve activity indirectly via the RVLM, which is an essential region involved in the generation of sympathetic nerve activity and also heavily innervates the intermediolateral nucleus of the spinal cord (8, 15). Those PVN neurons that send collaterals to both important autonomic sites could directly and indirectly influence sympathetic nerve activity. Electrophysiological evidence indicates that the activity of spinally projecting PVN neurons is influenced by volume expansion (21), suggesting that this subgroup of PVN neurons contributes to the sympathetic nerve response induced by perturbations in blood volume. Consistent with this view are the findings, using the protein Fos as a marker of neuronal activation, that showed neurons in the PVN with projections to the spinal cord were activated by hypotensive and nonhypotensive hemorrhage (3). Similar evidence suggests that a proportion of PVN neurons projecting to the RVLM may also contribute to the central pathways mediating the reflex sympathetic nerve responses elicited by alterations in blood volume (4).

In the present study, we found there was a small increase in arterial pressure accompanying the volume expansion. This effect was attenuated by muscimol microinjected in the PVN. Thus it could be argued that the abolition of the reflex reduction in RSNA in response to volume expansion by muscimol was a secondary effect resulting from the lack of a pressor response in that group. There are several reasons that argue against this explanation. First, the underlying assumption of that argument is that the reduction in RSNA in response to volume expansion is solely mediated by arterial baroreceptors. This is not the case. Indeed, the predominant peripheral sensors mediating this response in the conscious rabbit are found in the heart (5). Second, in the present study, microinjection of muscimol in areas outside the PVN also attenuated the increase in blood pressure but had no effect on the reduction in RSNA.

Nitric oxide is an unconventional neurotransmitter produced by the enzyme nitric oxide synthase. The PVN has one of the highest concentrations of this enzyme within the hypothalamus. Microinjections in the PVN of nitric oxide donors or inhibitors of nitric oxide synthase indicate that nitric oxide elicits reductions in RSNA, a response that is similar to that elicited by volume expansion (33). In the present study, we microinjected L-NAME, the nitric oxide synthase inhibitor, in the PVN but could not significantly attenuate the normal renal sympathoinhibition induced by volume expansion. This work is in agreement with the conclusions reached by Yang and Coote (31) but contrasts with the report by Li et al. (19), who found that local inhibition of nitric oxide synthase in the PVN attenuated the reduction in RSNA elicited by volume expansion.

In the present study, L-NAME in the PVN enhanced the HR response elicited by volume expansion. Thus reducing the production of nitric oxide in the PVN may raise the activity of specific PVN neurons, resulting in the enhanced response observed in the conscious rabbit. This has not been seen in rats (19).

The role of nitric oxide within the PVN in volume regulation, therefore, still appears controversial. The reasons for the differences in the findings are not clear but may be related to the methodology, species, and the presence or absence of anesthesia. In the present study, we used 20 nmol/side L-NAME, a dose that we have found attenuated the reflex renal sympathoinhibition induced by intravenous hypertonic saline (unpublished observations). Similar doses have been used previously to inhibit nitric oxide synthase after intracerebral administration in the rabbit and rat (13, 34); indeed, in the rat, even lower doses appear effective (31). Anesthesia is known to interfere with cardiovascular reflexes, and perhaps this may contribute to the difference between our findings and those of Li et al. (19), since the present study was performed in conscious animals. We performed our experiments in the rabbit, whereas other studies have been performed in the rat;
thus, it is also possible that a species difference could contribute to the different conclusions reached by different studies.

In the present study, muscimol in the PVN influenced the RSNA response but not the HR response, whereas L-NAME influenced the HR but not the RSNA responses. This suggests that the PVN has a critical role in the RSNA response elicited by volume expansion but a more subtle influence on the HR response. Additionally, the data suggest that there is a separation of the mechanisms that mediate these effects at the level of the PVN.

Interestingly, several reports suggest there is an interaction between nitric oxide and GABA within the PVN. The effects of nitric oxide may be mediated by GABA because inhibition of GABA receptors prevents the sympathoinhibitory action of nitric oxide (34). Furthermore, nitric oxide may be acting presynaptically or postsynaptically to enhance GABA function or release (14, 18, 29). It is not possible to administer the GABA receptor antagonist bicuculline in the PVN of the conscious rabbit because of behavioral disturbances that are elicited (6); thus, we could not explore this aspect.

**Functional Significance**

The kidneys are innervated by sympathetic nerves, the terminals of which impinge on the afferent and efferent arterioles, the tubules, and the juxtaglomerular cells. Changes in RSNA can influence renal blood flow, glomerular filtration rate, renal tubular reabsorption of sodium and water, and renin release (9, 11). Thus a brain region capable of having a key role in the reduction in RSNA that is predominantly mediated by mechanoreceptors located on the heart (23) and decreases renal blood flow while increasing urinary flow rate and sodium excretion. The present findings using muscimol indicate that the PVN is essential for the reflex reduction in RSNA, in agreement with previous lesion work (12, 22). This suggests that the PVN could play a critical role in the changes in renal function elicited by volume expansion. Indeed, the PVN is critical in the renal blood flow changes elicited by volume expansion and appears to have important roles in urinary flow rate and sodium excretion (19, 22).

The reflex changes elicited by volume expansion are impaired in conditions such as heart failure, hypertension, and diabetes (24–26, 32), and this impairment may contribute and aggravate the conditions (35). Thus the PVN may be a critical central nucleus involved in autonomic dysfunction observed in these conditions. There is now growing evidence in heart failure to support an important role of the PVN in the abnormal sympathetic nerve regulation characteristic of that condition (20).

In the present study, we found that inhibition of neuronal function within the PVN with muscimol abolished the renal sympathoinhibition elicited by volume expansion in the conscious animal. The effect of muscimol was specific for the PVN, since microinjection of the drug in areas outside, but adjacent to, the PVN did not affect the RSNA response. This work highlights the critical importance of the PVN in the central pathways mediating the reduction in RSNA elicited by volume expansion. The major advantage of this work is that it was performed in conscious animals, alleviating the complications that often occur in the presence of anesthesia.

We also found that inhibition of nitric oxide synthesis within the PVN, as well as outside the PVN, did not significantly affect the normal renal sympathoinhibition elicited by volume expansion. Thus the present findings do not provide evidence for a major role of nitric oxide within the PVN in this response. However, it should be noted that there is evidence in the literature that both agrees and disagrees with this view. Thus the role of nitric oxide within the PVN in the renal sympathetic nerve response induced by increases in circulating blood volume remains controversial.

**GRANTS**

This work was supported by the National Health and Medical Research Council of Australia, the National Heart Foundation of Australia, and RMIT University.

**REFERENCES**


