Inhibition of the Hypothalamic Paraventricular Nucleus in Spontaneously Hypertensive Rats Dramatically Reduces Sympathetic Vasomotor Tone

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Abstract—Experimental evidence indicates that the hypothalamic paraventricular nucleus modulates sympathetic vasomotor tone and blood pressure and that this modulation is altered in some cardiovascular diseases. This study tested the hypothesis that this nucleus exerts a more significant tonic excitatory modulation of basal sympathetic vasomotor activity in spontaneously hypertensive rats. In anesthetized, artificially-ventilated rats, bilateral microinjections of the GABA<sub>A</sub> receptor agonist, muscimol (1 to 1.5 nmoles per side), into the paraventricular nucleus produced a depressor and sympathoinhibitory response that did not recover. When compared with normotensive rats, this response was more marked in spontaneously hypertensive rats, where lumbar sympathetic nerve discharge was reduced by 75±3% and mean arterial pressure fell from 119±7 mm Hg to 58±3 mm Hg. Blockade of excitatory and inhibitory amino acid receptors in the rostral ventrolateral medulla significantly attenuated this response. Microinjections of small volumes (<20 nL) of GABA were used to localize precisely the responsive region of the paraventricular nucleus. Unilateral injections of GABA into the dorsomedial cap of the paraventricular nucleus induced a brisk depressor (decrease of 42±4 mm Hg), sympathoinhibitory (decrease by 72±2%), and bradycardic (decrease of 77±16 bpm) response. The mechanisms underlying the sympathoinhibition after inactivation of the paraventricular nucleus are not elucidated, but evidence discussed suggests the involvement of a supracollicular sympathoinhibitory pathway. The results presented demonstrate that the paraventricular nucleus exerts a powerful, tonic effect on the control of sympathetic vasomotor tone under basal conditions in anesthetized rats and that this is enhanced in spontaneously hypertensive rats. (Hypertension. 2002;39:275-280.)

Key Words: hypothalamus ■ blood pressure ■ sympathetic nervous system

The hypothalamic paraventricular nucleus (PVN) is one of the five major sympathetic premotor neuron cell groups. In addition to this connection to sympathetic preganglionic neurons, the PVN is also in a position to influence vasomotor sympathetic nerve discharge (SND) via direct connections with the rostral ventrolateral medulla (RVLM). The RVLM is another of the sympathetic premotor neuron groups whose activity is essential for the maintenance of SND, reflex regulation of blood pressure, and coordination of blood flow to different organs on demand. Through these and other connections the PVN is ideally placed to exert effects on the central regulation of SND and blood pressure.

Excitation of the PVN by a variety of means, including microinjection of excitatory amino acid receptor agonists or disinhibition through microinjection of the GABA<sub>A</sub> receptor antagonist, bicuculline, induces large increases in SND in anesthetized, normotensive animals. These studies indicate that the PVN tonically influences blood pressure through activation of SND and that this is modulated by a tonic GABA-ergic inhibition.

Ablation of the PVN in hypertensive rats results in decreased blood pressure, suggesting that aberrant control of the activity of the PVN may play a role in the generation or maintenance of hypertension. Consequently, the aim of this study was to determine whether the PVN plays a role in the tonic maintenance of SND and blood pressure in the spontaneously hypertensive (SH) rat, where increased SND plays a major role.

Methods

All experiments were performed in accordance with the Australian National Health and Medical Research “Code of Practice for the Care and Use of Animals for Scientific Purposes” and were approved by the Howard Florey Institute Animal Experimentation Ethics Committee. Experiments were performed on age-matched (14 to 17 weeks) male SH or Wistar-Kyoto (WKY) rats obtained from the Animal Resource Center (Canning Vale, W.A., Australia). They
were housed in pairs at the Howard Florey Institute for at least 1 week before experimentation and given ad libitum access to standard rat chow and water.

After induction of anesthesia (sodium pentobarbitone [60 mg/kg IP]) rats were tracheotomized, artificially-ventilated (with oxygen-enriched room air containing 1.2 to 1.8% isoflurane), and prepared for recording of blood pressure (femoral artery), heart rate, and lumbar SND (5-k gain, 100-Hz–3-kHz filter) as described in detail previously.3,14

Microinjections (15 to 150 nL) were made through glass micropettes (tip diameter of 20 to 40 μm), using a brief pulse of nitrogen. The PVN was localized using stereotaxic coordinates to a site where an injection of the GABA_A receptor antagonist bicuculline (Research Biochemicals International) gave a large increase in blood pressure and lumbar SND as described previously.5,15 Generally this was located 0.5 mm lateral to the midline, 1.5 mm caudal to bregma, and 7.5 to 8.0 mm ventral to the dorsal surface of the brain. The RVLM was localized by mapping the extent of the facial nucleus.3 All injectants contained a 1 to 5% concentration of rhodamine-labeled microspheres (Molecular Probes) for histologic verification of the injection site. At completion of the experiment the animals were given a bolus IV injection of the α_2 receptor agonist, clonidine (Sigma Chemical Co; 250 μL of 2.5 mmol/L solution) to determine the baseline noise in the sympathetic nerve recording. The rats were then killed by an overdose of pentobarbital anesthetic, and the brains were removed, placed in 10% formalin, and processed to demonstrate the injection site as previously described.13

Protocol 1. Aim: To Determine the Effect of Inactivation of the PVN on MAP, HR and Lumbar SND

After localization of the PVN and recovery from the effect of the bicuculline injection, the GABA_A agonist muscimol (100 to 150 nL of a 10 mmol/L solution) was injected bilaterally into the PVN of the SH (n=5) and WKY (n=5) rats.

Protocol 2. Aim: To Localize Precisely the Subregion of the PVN From Which Inhibition Produced Large Decreases in MAP and Lumbar SND

In 4 SH rats, small (15 to 20 nL) microinjections of GABA (50 mmol/L) were made in a grid through the region of the PVN and surrounding nuclei (1.0 to 2.5 mm caudal to bregma, 0.5 and 1.0 mm lateral to midline, and 7.0 to 9.0 mm ventral to the dorsal surface of the brain). The brief responses were readily repeatable and enabled multiple injections to be made throughout the region in each animal. At least 5 to 10 minutes lapsed between each injection to enable MAP and lumbar SND to stabilize at control levels.

Protocol 3. Aim: To Examine Whether the Response to Inactivation of the PVN Involved the RVLM

In 4 SH rats, after localization of the responsive part of the PVN, bilateral microinjections of a mixture of the GABA_A receptor antagonist bicuculline (4 mmol/L) and the nonselective excitatory amino acid receptor antagonist kynurenic acid (50 mmol/L) were made into the RVLM (150 to 200 nL per side). Microinjections of the mixture of bicuculline and kynurenic acid were made because these block excitatory and inhibitory amino acid receptors without markedly altering basal SND or MAP. Five to 10 minutes after completion of these injections, muscimol was then injected bilaterally into the PVN in a manner identical to that described in protocol 1. Data were analyzed using Spike2 software (Cambridge Electronic Design). Mean arterial pressure was derived from the recording of arterial pressure and calculated as diastolic pressure plus one-third of the pulse pressure. Sympathetic nerve recordings were rectified; noise was subtracted, using the level obtained after intravenous administration of clonidine, and the remaining signal integrated with a 1- or 10-second time constant. Control values were obtained from an average of the 60-second period immediately before each injection. Experimental values were obtained from a mean across 30 seconds, approximately 5 to 8 minutes after the injection or when the decrease was maximal. Data are expressed as mean±SEM. Responses were analyzed statistically using Student’s t test (paired or unpaired as appropriate). Significance was taken at P<0.05.

Results

After completion of all surgical procedures and the establishment of a stable anesthetic plane, the SH rats displayed a significantly higher resting MAP than the WKY rats (119±7 versus 99±3 mm Hg, P<0.05). Heart rate did not differ significantly among animals and in all experiments, except those in which GABA was injected into the PVN (protocol 2), showed no consistent changes in response to any intervention. Consequently, HR responses are not discussed further. In initial experiments bicuculline (50 nL of a 2 mmol/L solution) was injected into the PVN. After correctly positioned injections, a prompt increase in both MAP and lumbar SND was observed, as has been described previously.5,15

Protocol 1. Aim: To Determine the Effect of Inactivation of the PVN on MAP, HR and Lumbar SND

Microinjections of 100 to 150 nL of the GABA_A receptor agonist, muscimol (10 mmol/L), induced a rapid and prolonged decrease in both lumbar SND and MAP in both the SH and WKY rats (Figures 1 and 2). In the SH rat, lumbar SND decreased by 75±3% and MAP fell from 119±7 mm Hg to 58±3 mm Hg. In the WKY rat, lumbar SND decreased by 47±7% and MAP fell from 99±3 mm Hg to 65±7 mm Hg.

Figure 1. Representative traces from an SH and a WKY rat showing, from top to bottom, arterial blood pressure, MAP, HR, lumbar SND, and integrated lumbar SND (10-second time constant). The arrows denote the time of a bilateral microinjection of 150 nL of a 10 mmol/L solution of muscimol into the PVN of an SH (left) and a WKY (right) rat. The inset shows lumbar SND on an expanded time scale (horizontal bar is 1 second and vertical bar is 50 μV) from the control (A, SH rat; A’, WKY rat), post-muscimol (B, SH rat; B’, WKY rat) and post-clonidine (C, SH rat; C’, WKY rat) periods. The sympathoinhibition of remnant lumbar SND in the WKY rat after an increase in systemic pressure with phenylephrine is shown. The remnant SND in the SHR was similarly barosensitive. The asterisk denotes an electrical artifact in the record.
In the WKY rat increasing systemic arterial pressure inhibited the remnant lumbar SND (Figure 1). In several experiments lumbar SND and MAP were monitored for several hours after the injection of muscimol into the PVN. No sign of a return of these variables toward control levels was observed in this time.

Microinjections of muscimol were confined to the region of the PVN medial to the magnocellular cell group. Because of the relatively large injection volume and the long biologic half-life of muscimol, the site of action could not be determined accurately.

Protocol 2. Aim: To Localize Precisely the Subregion of the PVN From Which Inhibition Produced Large Decreases in MAP and SND

Small microinjections (15 to 20 nL) of GABA (50 mmol/L) were made sequentially in a grid pattern through the region of the PVN in 4 SH rats. Most microinjections produced no change in any of the variables measured. However, microinjections made in the region 0.5 mm lateral to the midline, 1.3 to 2.0 mm caudal to bregma, and 7.7 to 8.4 mm ventral to the dorsal surface of the brain produced rapid and large decreases in MAP, lumbar SND, and HR (Figure 3). In the most responsive area a unilateral microinjection of GABA produced a 42±4 mm Hg decrease in MAP, a 72±2% decrease in lumbar SND, and a 77±16 bpm decrease in HR. All responses reached their maximum within 10 to 20 seconds of the injection, with the maximal HR decrease occurring within 5 seconds. All variables returned to their basal levels within 2 to 3 minutes of injection. Combination of the injection site maps from each animal indicated that the most robust and rapid response was obtained from the region of the dorsal cap of the PVN. As demonstrated in Figure 3, the response to GABA remained even when injections were made into more ventral regions of the PVN. These responses were slower in onset and smaller in magnitude, suggesting that they were caused by leakage of the injected GABA up the injection track. However, an involvement of other, more ventral, parts of the PVN cannot be conclusively ruled out. Microinjections into sites 0.5 mm lateral to the responsive region produced no or very small effects.

Protocol 3. Aim: To Examine Whether the Response to Inactivation of the PVN Involved the RVLM

Bilateral microinjections of 200 nL of a mixture of bicuculline and kynurenic acid into the RVLM of the SH rat resulted in a gradual increase in MAP and lumbar SND in most animals (Figure 4), although these changes did not reach statistical significance. After the injection of bicuculline and kynurenic acid into the RVLM, pulse modulation of lumbar SND was dramatically attenuated, as was the inhibition of

Figure 2. Grouped data showing the change in MAP and lumbar SND in response to microinjection of muscimol (1 to 1.5 nmoles/side) into the PVN of the SH and WKY rat. Open bars indicate the control preinjection values and hatched bars the values 5 to 8 minutes after completion of the bilateral microinjection. Values were compared by paired t test, and the asterisks denote levels of probability value (* P<0.05; ** P<0.01; *** P<0.001).

Figure 3. A representative example of the MAP, HR, and lumbar SND (integrated with a 10-second time constant) responses to unilateral microinjection of 15 to 20 nL of GABA into five sites in one track through the region of the PVN (1.7 mm caudal to bregma and 0.5 mm lateral to the midline). The center of each of the injections is shown on the schematic map of the region (modified from Swanson29). AHC indicates anterior hypothalamic complex; f, fornix; PaDC, dorsomedial cap of the paraventricular nucleus; PaLM, lateral magnocellular part of the paraventricular nucleus; Re, nucleus reuniens of the thalamus; VMH, ventromedial hypothalamic nucleus; and 3V, third ventricle.
lumbar SND in response to an increase in systemic blood pressure (Figure 4). The change in HR in response to a pressor challenge was also altered from a long-lasting bradycardia under control conditions to a very short-lasting bradycardia followed by a long-lasting tachycardia (Figure 4). The mechanisms underlying this response are not clear. All microinjections into the RVLM were made within 500 μm of the caudal pole of the facial nucleus, and ventral to the compact subgroup of the nucleus ambiguus. After blockade of excitatory and inhibitory amino acid receptors in the RVLM of the SHR, the magnitude of both the depressor (−61±6 mm Hg before and −25±5 mm Hg after RVLM blockade, P<0.01) and sympathoinhibitory (−75±3% before and −29±4% after RVLM blockade, P<0.001) responses to microinjection of muscimol in the PVN were significantly reduced (Figures 4 and 5).

**Discussion**

These results demonstrate that under basal conditions in anesthetized rats, tonically active neurons located in the dorsomedial cap of the PVN powerfully excite lumbar SND and influence MAP. The sympathoinhibition and depressor response produced by inhibition of the PVN is marked in normotensive WKY rats but is more pronounced in the SH rats, where lumbar SND is almost abolished. This response to inhibition of the PVN was attenuated after blockade of excitatory and inhibitory amino acid receptors in the RVLM, indicating that a relay through this medullary nucleus is involved.

In addition to the effects on MAP and lumbar SND, small unilateral microinjections of GABA into the PVN produced a rapid bradycardia. The rapid time-course and magnitude of this response is suggestive of an effect on cardiac parasympathetic efferent activity, although this has not been directly determined in these experiments.

Several studies indicate that the dorsal cap of the PVN contains neurons that project to the RVLM or to the sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord.2,3,16 A small proportion of these neurons project to both sites.2,3,16 Activation of the PVN results in sympathoexcitatory effects in pressor and hypotensive situations.3,6,15—although there is some evidence to indicate that the...
PVN may inhibit renal SND. Thus, anatomic and functional evidence supports the view that the PVN can provide an excitatory drive to sympathetic vasomotor nerves.

Could removal of a tonic excitatory drive from the PVN be sufficient to explain these observations? After midcollicular decerebration SND and MAP are maintained at, or near, control levels, although a brief decrease in inferior cardiac nerve activity and blood pressure is observed after decerebration in anesthetized cats. Thus, the prevailing view is that under basal conditions in anesthetized animals, supracollicular nuclei do not exert marked effects on resting SND and MAP and that the circuitry responsible for the generation of sympathetic vasomotor tone resides caudal to the midbrain. Given that the medullary neuronal circuits can maintain “normal” levels of MAP and SND, it is unlikely that removal of an excitatory drive from the PVN, no matter how powerful, can explain the observations in this study. Two possible explanations, both involving an active inhibition of the medullary sympathetic tone generating circuits by a supracollicular group of neurons, seem most likely. First, removal of the excitatory input from the PVN could unmask a tonic inhibitory input to RVLM and/or intermediolateral cell column. If the source of this inhibitory input were supracollicular, it, too, would be removed in a decerebration preparation, allowing medullary circuits that generate sympathetic tone to continue unabated. An alternative possibility might be that inactivation of the PVN disinhibits a descending inhibitory input to the RVLM and/or intermediolateral cell column. Either way, these results are best explained by the existence of a supracollicular group of neurons that inhibit the medullary circuits generating sympathetic vasomotor tone. The location of this inhibitory influence and whether it is a direct inhibitory input or an excitatory input to medullary inhibitory interneurons remains to be determined.

This conclusion indicates that whereas medullary circuits might generate sympathetic tone, their activity is powerfully modulated by a combination of descending excitatory and inhibitory inputs. Although such a proposal is supported by considerable data derived from experiments in conscious animals, it is novel in regard to the generation of SND under basal conditions in anesthetized animals. Aberrant control of SND and, hence blood pressure, might then arise from an imbalance between these inputs. This study demonstrates the profound effect of one of these excitatory inputs, under basal conditions, in anesthetized rats. Considerable experimental evidence implicates altered drive to the sympathetic nervous system from the rostral forebrain in the maintenance of several forms of hypertension, including SH, DOCA salt, and renovascular hypertensive rats. In this study we demonstrate that the response to removal of the PVN excitatory input is more pronounced in the SHR than in the WKY. This observation in the SHR may be because of the loss of an increased excitatory input from the PVN or an increased efficacy of the proposed remnant inhibitory input. These experiments do not distinguish between these possibilities but do suggest that the balance and magnitude of the descending influences on neural circuits generating SND is abnormal in this model of essential hypertension.

Previous studies in conscious hypertensive rats and their controls have not observed such dramatic changes in MAP after inhibition of the PVN. Apart from the obvious presence of an anesthetic in the current experiments, the reason for this difference is not clear. The dose of muscimol used in the present experiments is about 10-fold higher than that used by Martin and Haywood. This might suggest that a large region of the PVN needs to be inhibited to observe the changes in MAP and SND. However, brisk depressor and sympathoinhibitory responses were obtained with small, localized injections of GABA, suggesting that the effective area is reasonably localized. Making such injections of GABA in conscious rats might prove fruitful, but technically very difficult. Another possible explanation is that different hypothalamic regions might play different roles depending on the animal’s state. For example, inhibition of the dorsomedial hypothalamus has little effect in anesthetized rats, but causes large decreases in MAP in conscious rats. Whatever the reason for the observed differences, the current experiments clearly show that under some circumstances the PVN can play a major role in maintenance of tonic SND.

After inhibition of the PVN with muscimol, MAP and lumbar SND remain depressed for several hours—in fact no recovery was ever seen. Although the long half-life for muscimol is clearly responsible, it is surprising that no compensatory mechanisms exist to counteract the decreases in MAP and return perfusion to normal levels. Previous studies indicated that even after extensive electrolytic lesions of the RVLM, increases in plasma angiotensin and vasopressin compensate to maintain normal blood pressure. Similarly, while a decrease in inferior cardiac nerve activity and blood pressure was observed immediately after midbrain transection in cats these variables had returned to control levels within 30 minutes. In the current experiments it is quite likely that the muscimol would have inhibited the activity of PVN magnocellular neurons, thus removing vasopressin as a compensatory pressor agent. However, the systemic renin-angiotensin system should have been activated by such a dramatic decrease in blood pressure. This lack of effective compensation for the effect of removal of the activity of the PVN requires further study.

Conclusions
These studies demonstrate that a small subregion of the hypothalamic paraventricular nucleus exerts a powerful tonic excitatory effect on sympathetic vasomotor tone generation and blood pressure control under basal conditions in anesthetized rats. This effect is more pronounced in the SH rat, suggesting that an imbalance in descending supracollicular influences on SND may be at least partly responsible for the aberrant control of SND in this model of hypertension and hence contribute to the increased blood pressure.

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References