Glutamatergic Inputs in the Hypothalamic Paraventricular Nucleus Maintain Sympathetic Vasomotor Tone in Hypertension

De-Pei Li, Hui-Lin Pan

Abstract—The paraventricular nucleus (PVN) of the hypothalamus is critical to the regulation of sympathetic output. The PVN hyperactivity is known to cause increased sympathetic nerve activity in spontaneously hypertensive rats (SHRs). The purpose of this study was to determine whether glutamatergic input to the PVN contributes to heightened sympathetic outflow in hypertension. Lumbar sympathetic nerve activity, mean arterial blood pressure, and heart rate were recorded from anesthetized SHRs and Wistar–Kyoto (WKY) rats. Bilateral microinjection of an N-methyl-D-aspartate receptor antagonist, 2-amino-5-phosphonopentanoic acid, or a non-N-methyl-D-aspartate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione, into the PVN dose-dependently decreased lumbar sympathetic nerve activity, mean arterial blood pressure, and heart rate in SHRs but not in WKY rats. Bilateral microinjection of kynurenic acid into the PVN also significantly decreased lumbar sympathetic nerve activity, mean arterial blood pressure, and heart rate in SHRs but not in WKY rats. Furthermore, microinjection of gabazine, a specific GABA_A receptor antagonist, into the PVN increased lumbar sympathetic nerve activity, mean arterial blood pressure, and heart rate in both SHRs and WKY rats. Notably, this response was significantly attenuated in SHRs compared with that in WKY rats. In addition, kynurenic acid abolished the sympathoexcitatory and pressor responses to microinjection of gabazine into the PVN in both SHRs and WKY rats. Thus, this study provides new functional evidence that resting sympathetic vasomotor tone is maintained by tonic glutamatergic input in the PVN in hypertension. (Hypertension. 2007;49:1-10.)

Key Words: excitatory amino acids  ▪  hypothalamus  ▪  sympathetic nervous system  ▪  synaptic transmission  ▪  autonomic nervous system  ▪  NMDA receptors

Hypertension is a significant risk factor for cardiovascular, renal, and cerebrovascular disorders. Increased sympathetic activity is often associated with hypertension and may contribute to the pathogenesis and maintenance of hypertension.1,2 Previous studies suggested that altered central mechanisms are responsible for the elevated sympathetic outflow and arterial blood pressure in hypertension.3,4 Lesions of the PVN in hypertensive rats, such as SHRs, Dahl salt-sensitive rats, and rats with renal hypertension, decrease blood pressure.5–11 Furthermore, inhibition of the PVN with muscimol reduces the blood pressure and sympathetic nerve activity in SHRs.12 Thus, hyperactivity of PVN neurons may directly and indirectly influence the sympathetic outflow and arterial blood pressure, especially in hypertension. We have shown recently that the excitability of PVN presympathetic neurons is increased in SHRs13; however, the underlying mechanisms causing hyperactivity of PVN neurons and heightened sympathetic vasomotor tone in hypertension remain elusive.

As a major excitatory neurotransmitter, glutamate regulates sympathetic outflow in the central nervous system, including the PVN.14–16 An abundance of glutamate-immunoreactive synapses17–19 and the N-methyl-D-aspartate (NMDA) and -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor mRNA and proteins exist in the PVN.20,21 The glutamatergic synaptic inputs to the PVN neurons are from glutamatergic interneurons within the PVN, subnuclei of the hypothalamus, and telencephalic regions.14,22,23 Microinjection of glutamate or NMDA into the PVN increases arterial blood pressure, sympathetic nerve activity, and plasma norepinephrine concentration in anesthetized and conscious rats.24,25 Furthermore, electrophysiological
studies using the hypothalamic slice preparation have shown that glutamatergic stimulation can excite PVN neurons and that blockade of glutamatergic transmission reduces spontaneous and evoked postsynaptic activity.14,16,26

Previous studies have shown that microinjection of the glutamate receptor antagonist alone into the PVN has little effect on the sympathetic output and arterial blood pressure in normotensive rats.15,27 These data suggest that the glutamatergic input in the PVN is not critically involved in tonic control of sympathetic vasomotor tone in normotensive conditions. However, little is known about whether glutamatergic input in the PVN plays a role in the regulation of resting sympathetic outflow and blood pressure in hypertension. In this study, we determined the contribution of glutamatergic input to the PVN in the maintenance of sympathetic vasomotor tone in SHRs.

Methods

Animals

Experiments were carried out on age-matched (12 to 13 weeks old, 280 to 350 g) male WKY rats and SHRs (Taconic, Germantown,
The experiments were approved by the Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center and conformed to the National Research Council Guide for the Care and Use of Laboratory Animals.

We measured blood pressure in all of the WKY rats and SHRs using a noninvasive tail-cuff system (Model 29-SSP, IITC Life Science). Blood pressure was measured every day for 1 week before the final experiment. In the SHRs, blood pressure started to increase at the age of 8 weeks and reached a stable hypertensive level at 13 weeks of age.

### Lumbar Sympathetic Nerve Activity and Blood Pressure Recording

Rats were initially anesthetized using 2% halothane in O2, and halothane was discontinued after a mixture of α-chloralose (60 to 75 mg/kg) and urethane (800 mg/kg) was given intraperitoneally. The depth of anesthesia was assessed for adequacy before surgery by the absence of paw withdrawal response to a noxious pinch. The trachea was cannulated for mechanical ventilation using a rodent ventilator (CWE) with 100% O2. Expired CO2 concentration was monitored with a CO2 analyzer (Capstar 100, CWE) and maintained at 4% to 5% by adjusting the ventilation rate (50 to 70 breaths per minute) or tidal volume (2 to 3 mL) throughout the experiment. Arterial blood pressure was measured with a pressure transducer through a catheter placed into the left carotid artery. Heart rate (HR) was monitored by triggering from the pulsatile arterial blood pressure. The right femoral vein was cannulated for intravenous administration of drugs. Supplemental doses of α-chloralose and urethane were administered as necessary to maintain an adequate depth of anesthesia.

A small branch of the left lumbar postganglionic sympathetic nerve was isolated through a retroperitoneal incision made while using an operating microscope. The lumbar sympathetic nerve was then cut distally to ensure that afferent activity was not recorded. The nerve was next immersed in mineral oil and placed on a stainless steel recording electrode. The nerve signal was amplified (20 000 to 30 000 Hz) and band-pass filtered (100 to 3000 Hz) by an alternating current amplifier (model P511, Grass Instruments), and the LSNA was monitored through an audio amplifier. The LSNA and mean arterial blood pressure (MAP) were recorded using a 1401-PLUS analog-to-digital converter and Spike2 system (Cambridge Electronic Design) and stored on the hard disk of a computer. Respective noise levels were subtracted from the nerve activity, and the percentage change in LSNA from the baseline value was calculated.

### PVN Microinjections

For PVN microinjections, rats were placed in a stereotactic frame (Kopf Instruments). The dorsal surface of the skull was exposed, and a small hole was drilled to expose the brain. A glass microinjection pipette (tip diameter: 20 to 30 μm) was advanced into the PVN. The stereotactic coordinates used were as follows: 1.6 to 2.0 mm caudal.
to the bregma, 0.5 mm lateral to the midline, and 7.0 to 7.5 mm ventral to the dura.15,27,28 The injection sites of the PVN were first verified by the depressor responses to microinjection of 5 nmol \( \text{H}^{9253} \)-aminobutyric acid (GABA; 20 nL, 250 mmol/L). Drugs were pressure ejected using a calibrated microinjection system (Nanoject II, Drumond Scientific Co) and monitored using an operating microscope. After microinjection of the drugs, the glass pipette was left in place for 1 to 2 minutes to ensure adequate delivery of the drug to the injection site. The pipette was then withdrawn and immediately placed at the respective stereotactic coordinates for injection into the contralateral PVN. GABA microinjections were separated by a 10- to 15-minute interval to allow recovery of the depressor response. The PVN vasomotor site was considered to have been located when GABA injection decreased the MAP by \( \text{H}^{11350} \)10 mm Hg.28

The stereotactic coordinates at which the previous GABA microinjection elicited the greatest depressor responses were used in the same rat for the subsequent microinjection of glutamate and GABA receptor antagonists. This approach was chosen to identify the pressor region of the PVN as described previously.27 In total, \( \text{H}^{11349} \)6 microinjections of GABA in the PVN were performed in each rat.

Kynurenic acid (Kyn), 2-amino-5-phosphonopentanoic acid (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and gabazine were obtained from Sigma-Aldrich. All of the drugs were dissolved in saline (pH adjusted to 7.25). We used variable doses of antagonists with the same volume (50 nL) to determine the dose–response effect starting from the lowest concentration in an ascending manner. After injection of 1 dose of the drug, the pipette was withdrawn and loaded with a different concentration of the drug solution. The pipette was then repositioned in the PVN using the identical stereotactic coordinates. Microinjection of the next dose of AP5, CNQX, gabazine, or Kyn was performed 40 to 45 minutes after the previous injection and when the LSNA, MAP, and HR returned to the baseline level. The following doses of the drugs were used: Kyn, 0.15 to 5 nmol/50 nL; AP5, 0.16 to 1.4 nmol/50 nL; CNQX, 0.22 to 1.8 nmol/50 nL; and gabazine, 0.3 to 10 nmol/50 nL. The microinjection doses for Kyn,15 AP5,15 CNQX,29 and gabazine30,31 were selected from previous studies and tested in the pilot experiments.

Histological Analysis
The location of the pipette tip and diffusion of the injectant in the PVN were examined and confirmed histologically in all of the rats. The drug solutions (glutamate and GABA antagonists) contained 5% rhodamine-labeled fluorescent microspheres (0.04 \( \text{H}^{9262} \)m, Molecular Probes) to allow us to estimate drug dispersion throughout the PVN and its surrounding area. At the completion of the experiment, the rat brain was rapidly removed and fixed in 10% buffered formalin solution overnight. Frozen coronal sections (40 \( \text{H}^{9262} \)m thick) were cut on a freezing microtome and mounted on slides. Rhodamine-labeled fluorescent regions were identified using an epifluorescence microscope and plotted on standardized sections from the Paxinos and Watson atlas.32 Rats in which the pipette tip was misplaced outside the PVN were excluded from data analysis.

Data Analysis
Values are presented as mean \( \pm \) SEM. Data were analyzed using Spike2 software. The MAP was derived from the pulsatile arterial blood pressure and calculated as the diastolic pressure plus one third of the pulse pressure. Sympathetic nerve signals were rectified and integrated offline with 1-s time constant. The background noise was subtracted using the level obtained after the rats were euthanized with an overdose of sodium phenobarbital. Control values were obtained by averaging the signal over a 60-s period immediately before each injection. Response values after each intervention were averaged over 30 s when the maximal responses occurred. To compare the responses of the MAP, LSNA, and HR to the antagonists microinjected within experimental groups of WKY rats and SHRs, a repeated-measures ANOVA with Dunnett’s posthoc test was performed. A 2-way ANOVA with Bonferroni’s posthoc test was used to compare the responses of the MAP, LSNA, and MAP to the microinjections.
was used to compare responses between experimental groups. 

Results

This study was carried out using a total of 51 rats, including 24 WKY rats and 27 SHRs. The MAP in conscious rats measured by the noninvasive tail-cuff technique was 148.9 ± 13.5 mm Hg for SHRs, which was significantly higher than that in WKY rats (98.1 ± 8.5 mm Hg; P < 0.05).

After completion of all of the surgical procedures and establishment of stable anesthesia, the SHRs displayed a significantly higher MAP (143.6 ± 6.8 mm Hg) than the WKY rats did (87.5 ± 5.4 mm Hg). Also, the HR was significantly higher in SHRs (358.4 ± 8.9 bpm) than that in WKY rats (320.4 ± 9.2 bpm). The distribution of microinjection sites within the PVN was not different between WKY rats and SHRs (Figure 1). In all of the rats included in the data analysis, the area of the fluorescent microsphere spread was 0.20 to 0.40 mm around the injection site. The spread of the dye did not penetrate to the third ventricular ependymal lining. Spread of the dye, however, was not consistently observed in a nucleus outside of the PVN. Two SHRs and 3 WKY rats were excluded from the study because of micropipette misplacement and the injectant’s spreading outside of the PVN.

Effect of Microinjection of AP5 Into the PVN

To examine the role of glutamatergic input in the PVN in controlling sympathetic vasomotor tone, we first examined the function of NMDA receptors in the PVN in WKY rats and SHRs. Bilateral microinjection of the specific NMDA receptor antagonist AP5 (0.16 to 1.4 nmol/50 nL) into the PVN had no significant effect on the LSNA, MAP, and HR in WKY rats (n = 6). However, bilateral microinjection of AP5 decreased the LSNA, MAP, and HR in 8 SHRs in a dose-dependent manner (Figures 2 and 3). The maximal reductions in the LSNA, MAP, and HR in response to AP5 microinjection were 24.1 ± 8%, 27.6 ± 2.8 mm Hg, and 14.8 ± 5.6 bpm, respectively. The latency of the response to AP5 was 0.38 ± 0.1 minutes. The peak responses of the LSNA, MAP, and HR occurred within 3 minutes of AP5 microinjection in SHRs. The recovery times for the LSNA, MAP, and HR were not significantly different (28.3 ± 3.2 minutes for the LSNA, 27.6 ± 3.6 minutes for the MAP, and 29.2 ± 1.1 minutes for the HR).

Effect of Microinjection of CNQX Into the PVN

We next determined the function of non-NMDA receptors in the PVN in controlling the LSNA, MAP, and HR in WKY rats and SHRs. Bilateral microinjection of a selective non-NMDA receptor antagonist CNQX into the PVN had no significant effect on the LSNA, MAP, and HR in WKY rats (n = 6). However, bilateral microinjection of CNQX (0.22 to 1.8 nmol/50 nL) dose-dependently reduced the LSNA, MAP, and HR in 8 SHRs (Figure 4). The maximal reductions in the LSNA, MAP, and HR in response to CNQX (1.8 nmol/50 nL) were 23.6 ± 1.9%, 20.6 ± 2.8 mm Hg, and 16.5 ± 8.2 bpm.
respectively. The latency of the response of the LSNA, MAP, and HR to CNQX injection was 0.36 ± 0.06 minutes. The peak responses of the LSNA, MAP, and HR occurred ≈3 minutes after CNQX injection in 6 SHRs. The recovery times for the LSNA and MAP were not significantly different (25.6 ± 2.9 minutes for the LSNA, 26.2 ± 2.1 minute for the MAP, and 27.5 ± 3.1 minutes for the HR).

Effect of Microinjection of Gabazine and Kyn Into the PVN

Ionotropic glutamate receptors are involved in sympathoexcitatory and pressor responses to the blockade of GABA<sub>A</sub> receptors with bicuculline in the PVN in normotensive rats. Furthermore, microinjection of bicuculline produces an attenuated increased in the LSNA and MAP in SHRs. Therefore, we determined the role of ionotropic glutamate receptors in the sympathoexcitatory responses to blocking GABA<sub>A</sub> receptors in the PVN in SHRs. Because bicuculline methiodide can excite neurons through its effect on small-conductance Ca<sup>2+ </sup>-activated K<sup>+</sup> channels (SK channels) in addition to blocking GABA<sub>A</sub> receptors, another GABA<sub>A</sub> receptor antagonist, gabazine, was selected for this study. Bilateral microinjection of gabazine (0.3 to 10.0 nmol/50 nL) dose-dependently increased the LSNA, MAP, and HR in 6 normotensive WKY rats (Figures 6 and 7). The onset latency of the response was 0.8 ± 0.2 minutes, and the peak response appeared 2.8 ± 0.5 minutes after gabazine microinjection. The maximal increases in the LSNA, MAP, and HR in response to the highest dose of gabazine injected into the PVN were 79.8 ± 4.7%, 42.6 ± 2.4 mm Hg, and 35.5 ± 3.3 bpm, respectively, in WKY rats. The increases in the LSNA and MAP elicited by gabazine microinjections were significantly attenuated in 7 SHRs (Figure 7). The maximal increases in the LSNA, MAP, and HR were 47.5 ± 2.8%, 33.4 ± 2.1 mm Hg, and 21.8 ± 2.0 bpm, respectively, in SHRs. The LSNA and ABP returned to baseline levels 25 to 35 minutes after each injection.
dose of gabazine injected. The mean recovery time for the LSNA, MAP, and HR was not significantly different between WKY rats (28.3 ± 2.5 minutes) and SHRs (29.6 ± 3.1 minute).

In the same animals, we also determined the role of blockade of ionotropic glutamate receptors in the sympathoexcitatory response to microinjection of gabazine into the PVN in WKY rats and SHRs. Thirty minutes after the injection of the last dose of gabazine, bilateral microinjection of Kyn (5.0 nmol/50 nL) into the PVN was performed. Kyn alone had no effect on the LSNA, MAP, and HR in 6 WKY rats but significantly decreased the LSNA, MAP, and HR in 7 SHRs (Figures 6 and 7). Three minutes after microinjection of Kyn, gabazine (10 nmol/50 nL) was injected into the PVN using the same coordinates. Subsequent gabazine injection in the presence of Kyn failed to increase the LSNA, MAP, and HR in both WKY rats and SHRs (Figures 6 and 7).

**Discussion**

To our knowledge, this is the first study to determine the role of glutamatergic input in the PVN in controlling resting sympathetic vasomotor tone in SHRs. We found that blockade of NMDA receptors with AP5 or non-NMDA receptors with CNQX in the PVN produced a similar dose-dependent sympathoinhibitory effect in SHRs but not in WKY rats. We also observed that the sympathoexcitatory responses to disinhibition of the PVN with gabazine were almost abolished in the presence of Kyn in WKY rats and SHRs. Therefore, this study provides new information that increased glutamatergic input in the PVN contributes importantly to the maintenance of resting sympathetic vasomotor tone in SHRs. Removal of GABAergic inhibition in the PVN can augment sympathetic outflow in SHRs, which depends on increased excitatory glutamatergic input.

The most salient finding of this study is that blockade of ionotropic glutamate receptors in the PVN in SHRs caused a profound decrease in the LSNA, MAP, and HR. The excitability of PVN presympathetic neurons is finely regulated by both excitatory glutamatergic and inhibitory GABAergic inputs. Conflicting results have been reported on the effect of microinjection of glutamate into the PVN on sympathetic vasomotor tone. For example, microinjection of glutamate into the PVN decreases or has no effect on arterial
blood pressure and renal sympathetic activity in rats.25,38 Others have shown that glutamate injection into the PVN increases arterial blood pressure and plasma norepinephrine concentrations.6 This discrepancy is likely because, in addition to stimulation of glutamate receptors, microinjection of glutamate into the PVN concurrently activates inhibitory GABAergic interneurons.39 Therefore, to study the role of glutamatergic input in the PVN in SHRs, we used ionotropic glutamate receptor antagonists. Consistent with previous studies showing that Kyn injected into the PVN has little effect on the sympathetic outflow and blood pressure in normotensive rats,15,27 we observed that microinjection of AP5, CNQX, or Kyn into the PVN had no significant effect on LSNA, MAP, and HR in WKY rats. These data strongly suggest that, under normal conditions, the glutamatergic input in the PVN does not contribute significantly to the resting sympathetic vasomotor tone. Importantly, we found that microinjection of AP5 or CNQX into the PVN had similar inhibitory effects on the LSNA, MAP, and HR in SHRs. These data strongly suggest that, under normal conditions, the glutamatergic input in the PVN does not contribute significantly to the resting sympathetic vasomotor tone. Importantly, we found that microinjection of AP5 or CNQX into the PVN had similar inhibitory effects on the LSNA, MAP, and HR in SHRs. Furthermore, blocking of NMDA and non-NMDA receptors with Kyn produced an effect that was almost identical to that of AP5 and CNQX, suggesting that both NMDA and non-NMDA receptors are essential to mediate the increased glutamatergic input to the PVN to maintain elevated sympathetic vasomotor tone in SHRs. Thus, glutamatergic input in the PVN is critically important to maintain the sympathetic vasomotor tone in SHRs.

It is not clear whether the increased glutamatergic input in the PVN is maintained by glutamate release within the PVN in SHRs, because we did not perform microinjections in the nearby structures surrounding the PVN. Also, because of the "inherited" problems (eg, drug diffusion) with the microinjection techniques, injection of the drug into the PVN is not always precisely controlled. The glutamatergic synaptic input to the PVN presympathetic neurons originates mostly from interneurons within the PVN, the medial and lateral preoptic area, the suprachiasmatic nucleus, the anterior and lateral hypothalamus, the thalamic PVN, and some telencephalic regions (including the lateral septum, the bed nucleus of the stria terminalis, and the amygdala).14,22,26 It should be noted that stimulation of the dorsomedial hypothalamus with bicuculline or NMDA can increase sympathetic vasomotor tone in normotensive rats.40,41 Although our study is focused on the PVN, we do not exclude the possibility that increased glutamatergic input may occur in the dorsomedial hypothalamus or other brain nuclei in SHRs. Previous studies have shown that the tonic glutamatergic input to the brain stem also has an important role in the maintenance of basal blood pressure in both SHRs and Dahl salt-sensitive hypertensive rats.42,43 The rostroventrolateral medulla vasomotor neurons receive excitatory glutamatergic input from the PVN.44,45 In this regard, Kyn can block the excitation of rostroventrolateral medulla vasomotor neurons elicited by stimulation of the PVN.46 Therefore, it is possible that PVN neuronal hyperac-
tivity is an important source of excitatory drive to the rostroventrolateral medulla vasomotor neurons in SHRs.

The excitability of the PVN neurons depends on the balance between the excitatory and inhibitory input. Removal of tonic GABAergic inhibition in the PVN can result in excessive excitatory input to the PVN presympathetic neurons. We, thus, determined whether glutamatergic input contributes to increased vasomotor tone induced by blockade of GABA$_B$ receptors in the PVN in SHRs. We observed that microinjection of gabazine into the PVN caused a large increase in the sympathetic vasomotor tone in both WKY rats and SHRs, but the sympathoexcitatory effect of gabazine was attenuated in SHRs. We also found that Kyn nearly abolished the sympathoexcitatory effect of gabazine in the PVN in both groups. These findings suggest that increased glutamatergic input is responsible for the sympathoexcitatory response to disinhibition of the PVN in both WKY rats and SHRs. We noted a smaller difference in increased MAP compared with a larger difference in the LSNA response to gabazine between the WKY rats and SHRs. These data suggest that the increase in the MAP may be limited by a higher afterload in SHRs. It remains unclear if and to what extent reduced GABAergic tone in the PVN contributes to increased glutamatergic input to PVN presympathetic neurons in SHRs.

Perspectives
This study provides important functional evidence that the glutamatergic input in the PVN plays an important role in controlling the basal sympathetic vasomotor tone in SHRs. Increased glutamatergic input may contribute to the hyperactivity of PVN presympathetic neurons in SHRs. The augmented glutamatergic input in the PVN also could be an important source of excitatory drive to the brain stem vasomotor neurons to increase the sympathetic outflow in SHRs. Furthermore, enhanced glutamatergic input and NMDA receptor function may play a role in the plasticity of GABA$_A$ and GABA$_B$ receptors in the PVN in SHRs. Further studies are warranted to test this hypothesis and to identify the sources for increased glutamatergic input to PVN presympathetic neurons in SHRs. Based on the findings from the present study, it is tempting to speculate that NMDA receptor antagonists may have a therapeutic effect in the treatment of neurogenic hypertension.

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Disclosures
None.

References

28. Li DP, Pan HL. Role of GABA(A) and GABA(B) receptors in paraventricular nucleus in control of sympathetic vasomotor tone in hypertension. *J Pharmacol Exp Ther*. 2007;320:615–626.


45. Pyner S, Coote JH. Identification of branching paraventricular neurons of the hypothalamus that project to the rostroventrolateral medulla and spinal cord. *Neuroscience*. 2000;100:549–556.


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