Sympathoexcitation by PVN-Injected Bicuculline Requires Activation of Excitatory Amino Acid Receptors

Qing Hui Chen, Joseph R. Haywood, Glenn M. Toney

Abstract—Acute blockade of γ-aminobutyric acid (GABA)-A receptors in the hypothalamic paraventricular nucleus (PVN) increases mean arterial pressure (MAP), heart rate (HR), and sympathetic nerve activity (SNA). However, the underlying neural mechanisms have not been fully determined. We tested the hypothesis that responses to GABA-A receptor blockade in the PVN require activation of local ionotropic excitatory amino acid (EAA) receptors. MAP, HR, and renal SNA responses to unilateral PVN microinjection of bicuculline methobromide (BIC, 0.1 nmol) were recorded before and after ipsilateral PVN injection of either vehicle (saline), the nonselective ionotropic EAA receptor antagonist kynurenic acid (KYN), the NMDA receptor antagonist D(-)-2-amino-5-phosphonopentanoic acid (AP5), or the non-NMDA receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoline-7-sulfonamide disodium (NBQX). Responses to PVN-injected BIC were unaltered by vehicle injection. In contrast, injection of KYN (7.2 nmol; n = 4) nearly abolished ABP and renal SNA responses to BIC (P < 0.01) and significantly attenuated (P < 0.05) HR responses as well. Similarly, graded doses of AP5 (0.6, 3, and 6 nmol) and NBQX (0.26, 1.3, and 2.6 nmol) reduced responses to PVN-injected BIC in a dose-related manner, with the 3 nmol (n = 7) and 1.3 nmol (n = 6) doses producing maximal effects (P < 0.05). KYN, AP5, and NBQX did not affect baseline parameters. Effects of a cocktail containing AP5 (3 nmol) and NBQX (1.3 nmol) were greater (P < 0.01) than either antagonist alone and were not statistically different from KYN. These data indicate that cardiovascular and renal sympathetic responses to acute GABA-A receptor blockade in the PVN require local actions of EAAs at both NMDA and non-NMDA receptors. (Hypertension. 2003;42[part 2]:725-731.)

Key Words: heart failure ■ hypertension, arterial ■ sympathetic nervous system ■ arterial pressure

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) plays a pivotal role in the central regulation of cardiovascular function. One site where GABA has a tonic influence on arterial blood pressure (ABP), heart rate (HR), and sympathetic nerve activity (SNA) is the hypothalamic paraventricular nucleus (PVN). Indeed, studies conducted over the past 12 years have documented that acute blockade of GABA-A receptors in the PVN leads to large-amplitude increases in ABP, HR, and SNA.1–4 However, the neural mechanisms that underlie these responses have yet to be fully elucidated. The ability of GABA-A receptor blockade to produce such dramatic cardiovascular effects indicates that the PVN has not only a functionally high level of inhibitory GABAergic tone but a local source of membrane excitation as well. This is the case because inhibition by GABA-A receptors is due to hyperpolarization of membrane potential. Consequently, blockade of GABA-A receptors only removes hyperpolarization; alone, it does not cause membrane potential to depolarize to the threshold for action potential formation. The latter requires either an intrinsic or extrinsic source of excitation. Intrinsic mechanisms of excitation such as pacemaker potentials have not been described among sympathetic and cardiovascular regulatory neurons of the PVN and thus the likely source would seem to arise from extrinsic synaptic input. The PVN is innervated by a number of excitatory transmitters,5–9 but among the most prominent is L-glutamate. Not only does the PVN contain an abundance of glutamatergic nerve terminals, recent evidence also indicates that neurons in autonomic regions of the nucleus express ionotropic excitatory amino acid (EAA) receptors in high density.10 Given the wealth of inhibitory GABAergic and EAA inputs to the PVN, it is reasonable to postulate that interactions between these two opposing influences may provide an important mechanism to regulate neuronal excitability. In this study, we tested the hypothesis that full manifestation of cardiovascular and sympathetic responses to blockade of GABA-A receptors in the PVN requires actions of EAs mediated through ionotropic receptors. These results were presented previously in abstract form.11
Methods

Experiments were performed in 49 male Sprague-Dawley rats (weight, 350 to 450 g) anesthetized with a mixture of α-chloralose (80 mg/kg) and urethane (800 mg/kg) given intraperitoneally. An adequate depth of anesthesia was assessed before surgery by the absence of pedal and corneal reflexes and by failure to withdraw the hind limb in response to pinching the paw. Animals were instrumented with an arterial catheter inserted into the aorta through a femoral artery. The catheter was connected to a strain-gauge transducer to measure ABP. HR was obtained from the R-wave of the ECG (lead I). A catheter was also placed in the left femoral vein to administer drugs. After tracheal cannulation, rats were paralyzed with gallamine triethiodide (25 mg/kg per hour IV) and artificially ventilated with oxygen-enriched room air. After paralysis, anesthesia was monitored by the stability of HR and ABP, and supplements equal to 10% of the initial dose were given when needed. End-tidal CO₂ was measured by a noninvasive infrared CO₂ monitor (Novametrix, Wallingford, CT) and maintained between 35 to 40 mm Hg by adjusting ventilation rate (80 to 100 breaths per minute) and/or tidal volume (2.0 to 3.0 mL). Body temperature was held at 37°C with a water-circulating pad. All experimental and surgical procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Recording Renal Sympathetic Nerve Activity

A flank incision was made to expose the left kidney. A renal nerve bundle was isolated from surrounding tissue, mounted on a stainless steel wire electrode (A-M Systems, Inc, 0.127-mm OD), and covered with a silicon-based impression material (Coltene, Light Body) to insulate the recording from body fluids. The recorded signal was directed to an AC amplifier equipped with half-amplitude filters (band pass, 100 to 1000 Hz) and a 60-Hz notch filter. The processed signal was rectified, integrated (30-ms time constant), and digitized at a frequency of 1000 Hz and stored on computer disk.

Microinjection of Drugs

Animals were placed in a stereotaxic head frame, and the skull was leveled between bregma and lambda. A section of skull was removed so that a single barreled glass microinjection pipette could be lowered vertically into the left PVN, using a piezoelectric microdrive (Burleigh Instruments, Inc). The following stereotaxic coordinates were used (in mm): caudal to bregma, 1.6 to 2.0; lateral to midline, 0.5 to 0.7; ventral to dura, 7.0 to 7.5. The final position of the microinjector was typically located on the initial placement or with only one adjustment to a deeper (50 to 100 μm) site. As an anatomical control, BIC was delivered just outside the PVN, 1.7 to 2.0 mm lateral to midline. Injected compounds were purchased from Sigma and included the GABA-A receptor antagonist BIC (0.1 nmol), the endogenous EAA receptor ligand L-glutamate (10 nmol), the non-competitive ionotropic EAA receptor antagonist kynurenate (KYN, 0.7 nmol), the selective ionotropic NMDA receptor antagonist D(-)-2-amino-5-phosphonovaleric acid (AP5, 0.6, 3.0, and 6.0 nmol), and the non-NMDA receptor selective antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamido disodium (NBQX, 0.26, 1.3, and 2.6 nmol). Vehicle and EAA receptor antagonist compounds were injected at two sites located ~0.1 mm rostral and caudal to each BIC microinjection site to ensure complete and uniform coverage. For all EAA receptor antagonist injections, half the total dose (listed above) was injected at each site. AP5 and NBQX were dissolved in saline (pH adjusted to 7.4). KYN was prepared in saline made basic by titrating with 1N NaOH until dissolved. The solution pH was then adjusted to 7.3 with 1N HCl. All compounds were microinjected at a volume of 100 nL per site with a pneumatic pump (WPI, Inc.). The volume of each injection was determined by measuring the movement of the fluid meniscus within the microinjector pipette with a dissecting microscope equipped with an eyepiece reticle.

Experimental Protocol

Animals were allowed to stabilize for at least 1 hour after surgery, at which time the response to PVN-microinjected BIC was tested. ABP, HR, and renal SNA were allowed to rise to a maximum (~5 to 10 minutes) before removing the microinjector. Recorded variables typically returned to baseline within 30 minutes. Next, depending on the experiment, KYN, AP5, NBQX, or a cocktail of AP5+NBQX was injected into the PVN. A BIC microinjector was then reintroduced into the PVN and responses were tested 20 and 120 minutes later. To control for possible nonspecific effects of the injected volume, responses to PVN injected BIC were determined before and after vehicle injections of saline. Finally, responses to PVN-microinjected L-glutamate (10 nmol) were recorded before and after injection of KYN to insure efficacy of drug action.

Histology

At the end of each experiment, 100 nL of an Evans blue dye solution (2% in saline) was injected into the PVN to mark the BIC injection site. Brains were removed and postfixed for 24 hours at 4°C in 0.1 mol/L PBS containing 4% paraformaldehyde. Tissue containing the hypothalamic PVN was cut into 40-μm-thick coronal sections, and microinjection sites were identified using bright-field microscopy.

Data Analysis

Systolic and diastolic pressures were determined from the raw ABP signal. Mean arterial pressure (MAP) was determined by adding one third of the pulse pressure to the diastolic pressure. Renal SNA was determined as an average of the rectified, integrated signal. Baseline values of all recorded variables were obtained by averaging a 3-minute segment of data recorded immediately before each treatment. MAP, HR, and renal SNA responses to BIC in the PVN were measured by averaging a 60-second period centered on the maximal response. MAP, HR, and renal SNA responses to PVN injected BIC were measured before, 20 minutes after, and 120 minutes after PVN microinjection of vehicle, KYN, AP5, NBQX, or an AP5+NBQX cocktail.

Responses to BIC in the PVN were compared before and after each microinjected compound by means of a 1-way repeated-measures ANOVA. For analyses that yielded a significant interaction, pairwise comparisons were made by using the Bonferroni multiple comparison test. To ensure that recorded variables returned to baseline between treatments, baseline values were similarly compared. To compare responses across treatment groups, a 2-way ANOVA was performed with a Tukey post hoc test for pairwise comparisons. All values in the text and figures are expressed as mean±SEM. Differences were considered statistically significant at a critical value of P<0.05.

Results

Histology

Histological examination of brain sections showed that microinjection tips were consistently lowered to within 50 to 100 μm of the dorsal cap region of the PVN. Microinjection sites marked by injection of dye suggest that exposure to BIC was mainly confined to superior portions of the nucleus near the dorsal cap region and extended to include a portion of the ventrolateral subnucleus without rupturing the ependyma of the third cerebral ventricle (Figure 1).

Effect of PVN Injection of Bicuculline on MAP, HR, and Renal SNA

PVN microinjection of BIC (0.1 nmol) significantly increased MAP, HR, and renal SNA. Responses began within 20 to 30 seconds, reached a peak within 5 to 10 minutes, and gradually returned toward the premicroinjection baseline within ~30 minutes. Compared with the control response to PVN-
injected BIC, responses recorded 20 and 120 minutes after PVN injection of vehicle (saline) were unchanged (n=4) (Figure 2). Among this group, BIC produced consistent changes in MAP (30±6, 35±4, and 31±6 mm Hg, respectively), HR (32±9, 33±6, and 23±8 bpm, respectively) and renal SNA (63±14%, 73±6%, and 64±18% increase). Resting MAP, HR, and renal SNA remained unchanged throughout the ~3-hour experimental period (Table). BIC effects appeared to be site-specific because microinjections deliberately placed lateral to the PVN (n=3) failed to significantly change MAP (3±1 mm Hg), HR (1±2 bpm), or renal SNA (11±9% increase).

**Effects of Kynurenate on Responses to PVN-Injected Bicuculline**

To test whether increases in MAP, HR, and renal SNA evoked by GABA-A receptor blockade involved local actions of EAAs, responses to PVN-injected BIC were examined before and after ipsilateral PVN microinjection of 7.2 nmol of the nonselective ionotropic EAA receptor antagonist KYN (n=4). KYN had no significant effect on resting MAP, HR, or renal SNA (see Table) but nearly abolished both the MAP (25±3 to 4±4 mm Hg; P<0.01) and the renal SNA (61±10% to 7±12% increase; P<0.01) (Figure 3 and Figure 5) response and significantly attenuated the HR (44±9 to 22±13 bpm; P<0.05) response. Responses to PVN microinjection of BIC after KYN recovered within 120 minutes.

The efficacy of EAA receptor blockade was tested by recording MAP, HR, and renal SNA responses to PVN microinjection of l-glutamate (10 nmol, n=5) before and after PVN injection of KYN. PVN injection of KYN significantly reduced (P<0.05) both the pressor (7±1 to 2±1 mm Hg) and renal sympathoexcitatory (28±4% to 7±1% increase) response to l-glutamate. Also, the HR response tended to be smaller (9±2 to 3±1 bpm) after KYN treatment, but the effect did not reach statistical significance (P<0.08). Responses to PVN microinjection of l-glutamate after KYN recovered within 120 minutes.

**Effects of AP5 and NBQX on Responses to PVN Injection of Bicuculline**

The role of local NMDA receptors in the response to PVN injection of BIC was examined by testing responses before and after delivering the NMDA receptor–selective antagonist AP5 into the ipsilateral PVN. Microinjection of AP5 had no significant effect on resting MAP, HR, or renal SNA (see Table). A 0.6 nmol dose of AP5 tended to reduce MAP (28±3 to 20±5 mm Hg) and renal SNA (80±12% to 66±10% increase) responses to PVN-microinjected BIC without reaching statistical significance (P<0.08). HR responses to BIC were unaltered by this dose of AP5 (22±7 to 21±6 bpm). Microinjection of either a 5- or 10-fold-higher dose (3.0 nmol, n=7, or 6.0 nmol, n=5) significantly attenuated the MAP (3.0 nmol, 32±4 to 16±3 mm Hg, P<0.05; 6.0 nmol, 32±4 to 13±3 mm Hg, P<0.01) and renal SNA (3.0 nmol, 81±9% to 35±4% increase, P<0.05; 6.0 nmol, 71±5% to 33±6% increase, P<0.01) responses to BIC without significantly altering HR responses (3.0 nmol, 29±5 to 24±4 bpm; 6.0 nmol, 30±7 to 31±7 bpm). Effects of the 3.0- and 6.0-nmol doses of AP5 were not significantly different, indicating that the 3.0-nmol dose is maximally effective. Figure 4A shows an example of the response to PVN-injected BIC after microinjection of the 3.0 nmol dose of AP5; Figure 5 summarizes the group data for this response.

The role of local non-NMDA receptors in the response to PVN-injected BIC was examined by testing responses before
Effect of PVN Microinjected Compounds on Resting MAP, HR, and Renal SNA

<table>
<thead>
<tr>
<th>Injected Compound</th>
<th>n</th>
<th>MAP, mm Hg</th>
<th>Post-20 Minutes</th>
<th>Post-120 Minutes</th>
<th>HR, beats/min</th>
<th>Post-20 Minutes</th>
<th>Post-120 Minutes</th>
<th>Renal SNA, V</th>
<th>Post-20 Minutes</th>
<th>Post-120 Minutes</th>
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<tr>
<td>KYN, 7.2 nmol</td>
<td>4</td>
<td>100±6</td>
<td>95±8</td>
<td>98±10</td>
<td>425±11</td>
<td>420±15</td>
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<tr>
<td>APS, 3.0 nmol</td>
<td>7</td>
<td>95±5</td>
<td>89±2</td>
<td>95±2</td>
<td>414±10</td>
<td>414±11</td>
<td>409±11</td>
<td>0.4±0.1</td>
<td>0.4±0.1</td>
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</tr>
<tr>
<td>NBQX, 1.3 nmol</td>
<td>6</td>
<td>110±6</td>
<td>112±6</td>
<td>104±5</td>
<td>424±13</td>
<td>423±20</td>
<td>412±7</td>
<td>0.4±0.1</td>
<td>0.4±0.1</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>AP5 + NBQX</td>
<td>5</td>
<td>94±7</td>
<td>88±8</td>
<td>95±9</td>
<td>433±9</td>
<td>413±9</td>
<td>417±3</td>
<td>0.4±0.1</td>
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<td>0.5±0.1</td>
</tr>
<tr>
<td>Saline</td>
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<td>102±5</td>
<td>101±5</td>
<td>96±6</td>
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<td>426±13</td>
<td>425±14</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
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</tr>
</tbody>
</table>

Values are mean±SE. MAP indicates mean arterial pressure; HR, heart rate; SNA, sympathetic nerve activity.

and after delivering the non-NMDA receptor selective antagonist NBQX into the PVN. A 0.26 nmol dose (n=5) did not significantly alter MAP (24±2 to 22±2 mm Hg), HR (27±5 to 33±7 bpm), or renal SNA (75±16% to 81±17% increase) responses evoked by PVN-injected BIC. Use of 5-fold (1.3 nmol; n=6) and 10-fold (2.6 nmol; n=8) higher doses of NBQX significantly attenuated the pressor (1.3 nmol, 25±3 to 11±4 mm Hg, P<0.05; 2.6 nmol, 34±4 to 16±5 mm Hg, P<0.01) and renal sympathoexcitatory (1.3 nmol, 79±9% to 36±11% increase, P<0.05; 2.6 nmol, 66±4% to 37±6% increase, P<0.05) responses to BIC and tended to reduce HR responses, but the latter effect did not reach statistical significance (1.3 nmol, 17±4 to 12±3 bpm; 2.6 nmol, 27±6 to 20±6 bpm). Effects of the 1.3- and 2.6 nmol doses of NBQX are not significantly different, suggesting that the 1.3 nmol dose is maximally effective. Figure 4B shows an example of the response to PVN-injected BIC after microinjection of 1.3 nmol of NBQX; Figure 5 shows the summary data from this group.

To further demonstrate contributions by both NMDA and non-NMDA receptors in mediating responses to PVN microinjection of BIC, a cocktail solution containing a mixture of AP5 (3.0 nmol) and NBQX (1.3 nmol) was used. The cocktail (n=5) significantly attenuated both the pressor (40±6 to 12±5 mm Hg, P<0.01) and renal sympathoexcitatory (82±10% to 21±9% increase, P<0.01) responses to BIC without significantly altering the HR response (25±4 to 15±7 bpm). These data are summarized in Figure 5. Inhibitory effects of cocktail on responses to PVN-injected BIC (MAP, 70%; HR, 40%; renal SNA, 74%) were quantitatively larger than those produced by injection of either AP5 alone (MAP, 50%; HR, 17%; renal SNA, 57%) or NBQX alone (MAP, 56%; HR, 29%; renal SNA, 54%), suggesting that effects of NMDA and non-NMDA receptor blockade were at least partially additive. This is supported by the fact that effects of cocktail and KYN, the nonselective EAA receptor antagonist, were not statistically different (Figure 5). Responses to PVN microinjection of BIC after AP5, NBQX, or cocktail recovered within 120 minutes.

Figure 3. ABP (bottom), renal SNA (middle), and HR (top) responses to microinjection of GABA-A receptor antagonist BIC into the hypothalamic PVN before (control), 20 minutes after, and 120 minutes after ipsilateral PVN microinjection of the nonselective ionotropic EAA receptor antagonist KYN (7.2 nmol). Each 100-nL injection of BIC (arrowhead) was completed over a period of ~2 minutes. Note that ABP, renal SNA, and HR were markedly increased after control administration of BIC into the PVN (left). Responses to BIC were nearly abolished 20 minutes after PVN microinjection of KYN (middle) and began to recover within ~120 minutes (right). All tracings were recorded in the same animal.

Figure 4. ABP (bottom), renal SNA (middle), and HR (top) responses to microinjection of the GABA-A receptor antagonist BIC into hypothalamic PVN before (control), 20 minutes after, and 120 minutes after ipsilateral PVN microinjection of the non-NMDA receptor antagonist AP5 (3.0 nmol, A) or the non-NMDA receptor antagonist NBQX (2.6 nmol, B). BIC was injected in a volume of 100 nL over a period of ~2 minutes (arrowhead). Note that ABP, renal SNA, and HR increased markedly in response to PVN injection of BIC. Compared with control responses to BIC (A and B, left), those recorded 20 minutes after PVN microinjection of AP5 (A, middle) or NBQX (B, middle) were significantly reduced and recovered within ~120 minutes (A and B, right). Tracings in A and B were recorded in two separate animals.
The present study supports this conclusion by showing that responses to GABA-A receptor blockade in the PVN were nearly prevented by prior microinjection of kynureinate, a nonselective ionotropic EAA receptor antagonist.

Since autonomic regions of the PVN have been shown recently to express both NMDA and non-NMDA receptors,\(^{10}\) we also examined effects of the selective receptor antagonists AP5 and NBQX and found that injection of either antagonist reduced BIC-evoked ABP and renal SNA responses by \(\approx 50\%\), without significantly altering BIC-induced tachycardia. These findings suggest that both NMDA and non-NMDA receptors contribute to sympathetic responses evoked by PVN injection of BIC.

An unresolved question is why prior blockade of EAA receptors in the PVN attenuated MAP and renal SNA responses to PVN injected BIC more than it attenuated the tachycardic response. One possibility is that anesthesia might have differentially affected sympathetic and parasympathetic neural circuits that both control HR. In addition, HR can be influenced by humoral and hemodynamic factors\(^{14}\) that may have been relatively unchanged by EAA receptor blockade in the PVN. Alternatively, it could be that BIC accessed cardiac regulatory neurons in the PVN, where EAA receptor blockade was incomplete. Finally, at least some PVN neurons that influence HR responses to BIC may not be regulated by EAA inputs and thus may not be affected by local EAA receptor blockade. That PVN-mediated control of HR and SNA may differ is underscored by recent data reporting that in hypertensive animals, HR responses to BIC are maintained, whereas BIC-induced increases in plasma catecholamines and ABP are blunted.\(^{2,15}\) Whether this reflects different chemical mediators within the PVN or divergent afferent influences remains to be determined. Clearly, additional work is needed to elucidate the neural mechanisms controlling HR response to PVN-injected BIC.

An important question that arises from the present findings is whether actions of EAAs underlie cardiovascular and sympathetic responses more generally when GABA-mediated inhibition in the PVN is reduced. Although considerably more work is needed to fully address this question, tentative support for this possibility comes from Patel and coworkers,\(^{16}\) who showed that pressor and sympathoexcitatory responses to PVN microinjection of the nitric oxide synthase (NOS) inhibitor l-NAME were significantly reduced by local NMDA receptor blockade. On the basis of available evidence, inhibition of NOS activity appears to reduce GABA-mediated inhibition by reducing tonic augmentation by nitric oxide, although the mechanism for this effect has not been established.\(^{17,18}\) Nevertheless, these findings are consistent with a role for EAAs in producing responses when GABA-mediated restraint is reduced, in this case by inhibiting nitric oxide-induced facilitation of GABAergic activity.

Another question is whether blockade of EAA receptors reduces responses to PVN-injected BIC because it interrupts tonic excitation that is revealed on removal of GABAergic inhibition or whether EAA receptor blockade prevents the actions of glutamate released from neurons recruited in response to GABA-A receptor blockade. That muscimol-induced inhibition of PVN neuronal activity as well as PVN...
lesions have been reported to acutely reduce resting ABP and SNA in anesthetized rats^{19–21} suggests that autonomic neurons of the PVN might be tonically activated by excitatory inputs and their activity may contribute to ongoing SNA.

Although in vivo studies have not established whether EAA inputs contribute significantly to ongoing activity of PVN autonomic neurons, in vitro electrophysiologic studies have shown that parvocellular neurons do exhibit spontaneous EAA-mediated postsynaptic currents.\textsuperscript{22} This is not unexpected, given that CNS neurons typically do receive tonic EAA input. Nevertheless, it should be emphasized that data from the present study do not provide direct support for tonic EAA actions in controlling sympathetic activity and blood pressure. This is the case because PVN injections of EAA receptor antagonists were without significant affect on resting ABP or renal SNA. One explanation for these findings is that effects of reduced PVN neuronal activity during EAA receptor blockade could have been masked by processing at synapses in the brain stem and/or spinal cord. What is evident is that a great deal more must be learned before conclusions can be reached concerning the role of EAA transmission in regulating the tonic activity of cardiovascular and sympathetic regulatory neurons of the PVN.

The lack of effect of EAA receptor blockade in the PVN is reminiscent of similar responses reported for the rostral ventrolateral medulla (RVLM). Ito and Sved\textsuperscript{23} showed that KYN microinjection into the RVLM failed to reduce ongoing arterial pressure. However, chemical inhibition of the caudal ventrolateral medulla to remove putative GABA-mediated inhibition of RVLM resulted in a robust increase in ABP, and this was reversed by blockade of ionotropic EAA receptors in the RVLM. Thus, both the PVN and RVLM could be organized similarly such that removal of tonic inhibition is required to observe effects of excitatory amino acids on neuronal discharge and cardiovascular function. This could be the result of a dynamic balance between excitation and inhibition such that blockade of excitatory effects is balanced by a decrease in local inhibition. This could be accomplished if EAA inputs simultaneously targeted both sympathetic regulatory output neurons as well as local GABAergic neurons/terminals. It is interesting that Paquet and Smith\textsuperscript{24} have recently reported that EAA receptors are indeed expressed on GABA-containing terminals of the PVN.

Another question that remains unanswered is the source(s) of EAA input that provides the necessary excitation to mediate cardiovascular and sympathetic responses to PVN injection of BIC. Available evidence indicates that cell groups in a variety of nearby hypothalamic structures could subserve this role, including cells in the suprachiasmatic nucleus as well as the anterior, ventromedial, and dorsomedial hypothalamicus.\textsuperscript{25} Evidence also suggests that glutamatergic neurons reside within the PVN,\textsuperscript{6} and these, too, could provide tonic or stimulated excitatory input during GABA-A receptor blockade. Finally, little is known concerning the specific physiological stimuli that drive glutamatergic activity to autonomic neurons of the PVN. In a study by Kubo et al,\textsuperscript{26} blockade of either NMDA or non-NMDA receptors in the PVN was shown to significantly attenuate the pressor response to carotid body chemoreceptor stimulation. Additional studies are needed to determine the extent to which other sensory modalities modulate cardiovascular function by activating EAA inputs to PVN.

In summary, the present study demonstrates that full manifestation of pressor, tachycardic, and renal sympathoexcitatory responses to acute GABA-A receptor blockade in the PVN requires activation of local NMDA and non-NMDA EAA receptors. These data highlight a potentially important mechanism whereby interactions between excitatory and inhibitory amino acid neurotransmitters govern the activity of sympathetic and cardiovascular regulatory neurons in the PVN. Plasticity in either or both of these systems may provide a substrate for altering the activity patterns in sympathetic nerves under physiological and pathophysiologic conditions.

**Perspectives**

The hypothalamic PVN is a key site where GABA tonically regulates cardiovascular function. Of key importance are studies establishing that GABAergic function in the PVN is reduced in diseases such as hypertension\textsuperscript{4} and heart failure,\textsuperscript{4} thereby contributing to elevated sympathetic activity. However, recent data by Haywood et al\textsuperscript{15} raise an additional possibility. These authors showed that in rats with chronic hypertension, the response to PVN GABAergic inhibition is augmented, not reduced, and yet the affinity (Kd) and number (Bmax) of GABA-A receptors in the PVN are unchanged. These data suggest that the increase in SNA could reflect an increase in excitatory drive to the PVN. The present study indicates that excitatory actions of glutamate may be key to increasing SNA acutely when PVN GABA-A receptors are blocked. Whether glutamatergic actions increase through mechanisms that are independent or dependent on a reduction in GABAergic control remains to be established. Whichever the case may be, results from the present study raise the possibility that changes in glutamatergic/GABAergic interactions within the PVN could represent an important mechanism driving elevated SNA in cardiovascular disease.

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**References**


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