Cardiovascular Responses to Bicuculline in the Paraventricular Nucleus of the Rat

Douglas S. Martin, Teodoro Segura, and Joseph R. Haywood

The present study was undertaken to determine whether $\gamma$-aminobutyric acid in the paraventricular nucleus contributes to the regulation of cardiovascular function. Blood pressure and heart rate were recorded and plasma catecholamines were measured in conscious rats receiving microinfusions of either artificial cerebrospinal fluid or a $\gamma$-aminobutyric acid antagonist, bicuculline methiodide, bilaterally into the paraventricular nucleus. Artificial cerebrospinal fluid had no effect on any of the recorded variables. In contrast, infusion of bicuculline into the region of the paraventricular nucleus produced increases in blood pressure (20±2 mm Hg), heart rate (110±11 beats/min), and plasma concentrations of norepinephrine (640±107 pg/ml) and epinephrine (1,266±267 pg/ml). Pretreatment with a ganglionic blocking agent abolished both the blood pressure (−1±2 mm Hg) and heart rate (5±18 beats/min) effects. Bilateral adrenal medullectomy reduced the changes in plasma norepinephrine concentrations (81±14 pg/ml) significantly and abolished the changes in plasma epinephrine concentrations (5±4 pg/ml). Conversely, adrenal medullectomy reduced the pressor effects (18±2 mm Hg) only slightly while the heart rate responses were attenuated (42±9 beats/min) by approximately 50%. These results suggest that an endogenous $\gamma$-aminobutyric acid system exerts a tonic inhibitory effect on the sympathetic nervous system at the level of the paraventricular nucleus of the hypothalamus. (Hypertension 1991;18:48–55)

A growing body of evidence suggests that $\gamma$-aminobutyric acid (GABA) plays an important role in central cardiovascular control. Intracerebroventricular injections of GABA agonists produced decreases in arterial pressure, heart rate, and peripheral sympathetic nerve activity. Conversely, intracerebroventricular administration of GABA antagonists, such as bicuculline methiodide (BMI) or picrotoxin, resulted in marked increases in blood pressure and heart rate due to an increase in sympathetic nervous system activity. In addition, alterations in GABA function have been implicated in the pathogenesis of hypertension.

It has been proposed that a forebrain periventricular GABA system exerts a tonic inhibitory influence on the sympathetic nervous system. At present, the available evidence suggests that this GABAergic site is located within the hypothalamus. A number of regions within the hypothalamus contain relatively high concentrations of GABA, including the anterior hypothalamic area, the posterior hypothalamic area, and the paraventricular nucleus (PVN) of the hypothalamus. Recent studies suggest that GABA exerts a tonic depressor effect within the posterior hypothalamic region. On the other hand, the PVN has been suggested as a site of integration for autonomic and endocrine cardiovascular responses. Neuroanatomic and electrophysiological data have indicated that the PVN is reciprocally connected to other areas of the central nervous system that are involved in cardiovascular function. Recent studies using pseudorabies virus retrograde tracing techniques have shown that the PVN is a major source of forebrain input to the sympathetic nervous system.

Functional studies have also implicated the PVN in cardiovascular regulation. A number of studies have shown that electrical or chemical stimulation of this region can influence arterial blood pressure. In conscious rats, PVN stimulation appears to increase blood pressure primarily via activation of the sympathetic nervous system. In addition, the PVN appears to be involved in the pathogenesis of hypertension. Electrolytic or chemical ablation of the PVN attenuated the development of high blood pressure in spontaneous hypertensive rats, deoxycorticosterone-salt, and Dahl salt-sensitive hypertension. Lesions of the PVN also reversed the hypertension associated with aortic baroreceptor deafferentation and figure-8
renal wrap (unpublished observations from our laboratory) in the rat. Finally, it has been demonstrated that the PVN can modulate both the sympathetic and parasympathetic components of the baroreceptor reflex.\textsuperscript{59,60} Thus, there is evidence that the PVN is part of the neuronal circuitry involved in central nervous system control of cardiovascular function. Consequently, the neurochemical signals that modulate the activity of the PVN are of considerable interest.

Accordingly, the present study was undertaken to determine whether GABA might be involved in modulating the activity of neurons in the PVN associated with the control of sympathetic neuronal outflow. To assess the role of endogenous GABA, a GABA-A receptor antagonist, BMI, was administered into the PVN of conscious rats while blood pressure and heart rate were monitored. The role of the sympathetic nervous system in the responses to BMI was assessed both pharmacologically and by measurement of plasma catecholamine levels. In addition, the importance of adrenal catecholamines was determined in animals subjected to bilateral adrenal medullectomy.

Methods

Surgical Procedures

Male Sprague-Dawley rats (275–300 g) were maintained in rooms with constant temperature (24°C) and a 14-hour light/10-hour dark cycle. The animals were allowed free access to standard laboratory chow and tap water. On the day of surgery, the rats were anesthetized with an intraperitoneal injection of chloral hydrate (300 mg/kg) and placed in a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, Calif.). With the skull level between bregma and lambda, 23-gauge stainless steel guide cannulae were directed bilaterally at the PVN at a 10° angle from vertical using the following coordinates: 2.0 mm posterior, 1.7 mm lateral to bregma, and −6.2 mm ventral from dura. The animals were allowed at least 7 days to recover from the stereotaxic surgery.

To assess the role of adrenal catecholamines, some animals were subjected to bilateral medullectomy. The adrenal gland was exposed via a retroperitoneal incision, and after clearing the connective tissue, a small cut was made in the adrenal cortex. Pressure and heart rate were allowed to return to control levels. A separate group of animals was used to assess the importance of adrenal catecholamines. BMI was infused as described previously, into the PVN of rats subjected to bilateral medullectomy (n=6) or sham medullectomy (n=8). Blood samples were taken as described above in some of the animals. At the end of the experiments, the animals subjected to medullectomy or sham operation were anesthetized and the adrenal glands were quickly removed, weighed, frozen at −20°C, and stored at −70°C.

Experimental Protocols

On the day of the experiment, the animals were brought to the recording area and allowed to acclimate for approximately 30 minutes. The arterial catheter was then connected to a pressure transducer (Cobe Laboratories Inc., Lakewood, Colo.) for the recording of arterial blood pressure. Heart rate was derived from the pulsatile blood pressure signal (Coulbourn Instruments, Lehigh Valley, Pa.). These variables were recorded for a baseline period of at least 60 minutes before any interventions.

One group of animals (n=7) received artificial cerebrospinal fluid (aCSF) while a second group (n=15) received BMI (Research Biochemicals Inc., Natick, Mass.). Stainless steel injectors (30 gauge), which extended 0.7–1.0 mm beyond the end of the guide cannulae, were attached via PE-20 polyethylene tubing to 1.0-μl microsyringes (Hamilton Co., Reno, Nev.) and backfilled with either aCSF or a 2×10⁻³ M solution of BMI. The injectors were inserted into the PVN guides, and blood pressure and heart rate were allowed to return to control levels. aCSF or BMI was then infused bilaterally (Razel Scientific Instruments, Stamford, Conn.) at a rate of 5 nl/min for a period of 20 minutes (total volume 100 nl/site) to achieve a steady-state response. One-milliliter arterial blood samples were collected during the baseline period and during the plateau phase of the responses to aCSF or BMI for the determination of plasma norepinephrine and epinephrine concentrations by radioenzymatic assay.\textsuperscript{44} The blood samples were replaced with an equal volume of 0.9% saline. After recovery, some of the animals receiving BMI were then treated with a ganglionic blocking agent, chlorisondamine hydrochloride (11 mg/kg s.c.) (n=5). The BMI infusions were repeated after ganglionic blockade after blood pressure and heart rate had stabilized.

A separate group of animals was used to assess the importance of adrenal catecholamines. BMI was infused, as described previously, into the PVN of rats subjected to bilateral medullectomy (n=6) or sham medullectomy (n=8). Blood samples were taken as described above in some of the animals. At the end of the experiments, the animals subjected to medullectomy or sham operation were anesthetized and the adrenal glands were quickly removed, weighed, frozen at −20°C, and stored at −70°C. The adrenal catecholamine content was measured by radioenzymatic assay. The concentrations of norepinephrine and epinephrine present in the sham-operated and demedullated adrenal glands are shown in Table 1. The medullectomy procedure reduced adrenal norepinephrine and epinephrine concentrations by over 99%.

Histology

At the end of the experiments, the animals were anesthetized with sodium pentobarbital (50 mg/kg) (Abbott Laboratories, Chicago, Ill.). The animals
TABLE 1. Adrenal Norepinephrine and Epinephrine Content in Rats Subjected to Sham Operation or Bilateral Adrenal Medullectomy

<table>
<thead>
<tr>
<th>Group</th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>SH</td>
<td>554±144</td>
<td>781±146</td>
</tr>
<tr>
<td>MEDX</td>
<td>0.3±0.1*</td>
<td>0.1±0.1*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of adrenal content of norepinephrine and epinephrine expressed as picograms per milligram of adrenal gland for sham-operated (SH) (right side n=5, left side n=6) and bilaterally medullectomized (MEDX) (n=5 for both right and left sides) rats.

*p<0.05 compared with SH rats.

were then perfused transcardially with 0.9% saline followed by 10% buffered formalin solution. The brains were removed and 60-μm frozen coronal sections were cut through the region of the PVN. The sections were stained with cresyl violet for histological verification of the injection sites. The sites of termination of the injector tracts are illustrated in Figure 1.

Data Analysis

Data are expressed as mean±SEM. Student's paired t test was used to compare the control values with the respective values during infusion of aCSF or BMI. Responses between aCSF- and BMI-treated animals and between sham and medullectomized rats were compared using an unpaired t test. Absolute values for blood pressure and heart rate were compared using analysis of variance for repeated measures. Post hoc comparisons were made using Scheffe's method. Student's paired t test was used to compare the changes in blood pressure, heart rate, and plasma catecholamines before and after the autonomic blocking agents. Differences were considered to be significant at p<0.05.

Results

Effects of Bicuculline Methiodide on Blood Pressure and Heart Rate

The changes in blood pressure and heart rate in response to microinfusion of aCSF or BMI are illustrated in Figure 2. Microinfusion of vehicle into the PVN had little effect on any of the recorded variables. During the infusion of aCSF, blood pressure averaged 118±4 mm Hg, which was not significantly different from the preinfusion control value of 119±4 mm Hg. Similarly, heart rate remained unchanged during the administration of aCSF (control 390±11 beats/min versus aCSF 385±9 beats/min). In contrast, infusion of BMI into the PVN had marked effects on both blood pressure and heart rate. The time to onset of the effects of the BMI microinfusions was variable and averaged approximately 10±1 minutes. The time to the peak response averaged 11±1 minutes. This latency for the onset of the cardiovascular responses to BMI presumably reflects a time lag associated with drug delivery into the PVN or the time required to achieve a minimally effective concentration of BMI. BMI increased mean arterial pressure by 20±2 mm Hg from the control level of 119±3 mm Hg. Heart rate also increased markedly by 110±11 beats/min from a control level of 377±10 beats/min. The BMI-induced changes in blood pressure and heart rate were significantly greater than...
those observed during the infusion of aCSF. Thus, blockade of GABA-A receptors in the region of the PVN resulted in increases in both arterial blood pressure and heart rate.

**Effect of Bicuculline Methiodide on Plasma Catecholamines**

The changes in plasma norepinephrine and epinephrine concentrations are depicted in Figure 3. In the animals selected for aCSF administration, the basal plasma concentrations of norepinephrine and epinephrine were 286±30 pg/ml and 331±58 pg/ml, respectively. These plasma concentrations were unchanged during the infusion of aCSF. In the BMI-treated rats, the basal plasma concentration of norepinephrine was 283±22 pg/ml, and the basal plasma epinephrine concentration was 257±24 pg/ml. In contrast to the vehicle, administration of BMI into the PVN significantly elevated plasma norepinephrine threefold to 922±114 pg/ml, whereas plasma epinephrine increased sixfold to 1,524±276 pg/ml. These changes in plasma catecholamines were significantly greater than those observed during vehicle administration. Thus, inhibition of tonic GABAergic function in the PVN resulted in a marked activation of the sympathoadrenal system.

**Effects of Ganglionic Blockade on Cardiovascular Responses to Bicuculline Methiodide**

Ganglionic blockade was used to assess the contribution of non-neural effector systems to the cardiovascular responses elicited by BMI. The ganglionic blocking agent chlorisondamine was given after recovery from an initial microinfusion of BMI. Just before the administration of chlorisondamine, blood pressure was 116±3 mm Hg, which was similar to that observed before the initial BMI infusion (112±3 mm Hg). Heart rate also recovered to a level not significantly different from the control level (control 377±22 versus recovery 396±16 beats/min) after the initial BMI infusion. Administration of chlorisondamine resulted in a profound fall in pressure to a plateau level of 60±7 mm Hg. Heart rate also decreased significantly to 318±7 beats/min after injection of chlorisondamine. The BMI-induced changes in blood pressure and heart rate in untreated and ganglion-blocked rats are compared in Figure 4. Infusion of BMI after ganglionic blockade failed to alter mean blood pressure (−1±2 mm Hg) or heart rate (5±18 beats/min) significantly. Therefore, the changes in blood pressure and heart rate in chlorisondamine-treated animals were significantly smaller than those observed in untreated rats. Thus, ganglionic blockade eliminated both the blood pressure and heart rate effects of microinfusing BMI into the PVN, suggesting that these responses were primarily neurally mediated.
Effects of Adrenal Medullectomy on Cardiovascular Responses to Bicuculline Methiodide

Since increases in both plasma norepinephrine and epinephrine implicated the adrenal gland in the BMI-induced responses, bilaterally adrenal-medullectomized rats were challenged with BMI in an effort to determine the importance of adrenal catecholamines in the cardiovascular responses. The effects of BMI on blood pressure and heart rate in these two groups of animals are shown in Figure 5. In the animals subjected to sham medullectomy, infusion of BMI increased blood pressure from 114±2 to 139±3 mm Hg and heart rate from 399±9 to 492±9 beats/min. In the medullectomized animals, BMI caused blood pressure to increase from 116±4 to 134±5 mm Hg. This pressor response was significantly, albeit slightly, smaller than that observed in the sham rats. In the medullectomized rats, the BMI-induced increase in heart rate was significantly smaller (422±10 to 464±12 beats/min) than in the sham-operated rats. Thus, removal of adrenal catecholamines had only a small effect on the blood pressure responses to impairment of GABA tone in the PVN region. On the other hand, the tachycardic responses appear to depend substantially on the release of catecholamines from the adrenal medulla.

Effects of Adrenal Medullectomy on Plasma Catecholamines

The BMI-induced changes in plasma catecholamine concentrations for sham-operated and medullectomized rats are illustrated in Figure 6. In the rats subjected to sham medullectomy BMI increased plasma norepinephrine concentrations approximately twofold from control levels of 232±44 pg/ml, whereas plasma epinephrine concentrations increased fourfold from basal concentrations of 114±34 pg/ml. In contrast, in the medullectomized animals plasma norepinephrine concentrations only increased from 201±25 to 283±29. Basal plasma concentrations of epinephrine were markedly lower in the rats subjected to medullectomy (31±4 pg/ml) in comparison with the sham-operated animals (114±34 pg/ml). Microinfusion of BMI into the PVN of medullectomized rats changed epinephrine concentrations from 31±4 pg/ml to 35±7 pg/ml. The changes in plasma norepinephrine and epinephrine concentrations recorded in the medullectomized rats were significantly smaller than those observed in their sham-operated counterparts. Thus, release of catecholamines from the adrenal medulla appeared to account for the changes in plasma norepinephrine and epinephrine concentrations observed after BMI administration into the PVN.
A study aimed at localizing the site at which BMI may exert these effects suggested that the drug acted on sites within the rostral and caudal hypothalamus. Recently, it has been reported that injections of GABA antagonists or inhibitors of GABA synthesis directly into the posterior hypothalamic area result in increases in blood pressure, heart rate, and sympathetic nervous system activity. Thus, it has been proposed that the posterior hypothalamic area is a site within the caudal hypothalamus at which GABA exerts tonic control over sympathetic function. The results of the present study indicate that the PVN is a site within the rostral hypothalamus at which GABA exerts a tonic inhibitory effect on sympathetic outflow.

The hypothalamus contains a number of sites involved in cardiovascular control. Among these sites, BMI can elicit increases in blood pressure and heart rate when injected into the posterior hypothalamus. It is conceivable therefore that the responses we observed resulted from diffusion of BMI to responsive sites in the hypothalamus other than the PVN. However, several observations suggest that the effects we observed were not due to activation of areas outside the PVN. First, in a separate study we found that a 100 nl microinfusion of radiolabeled bicuculline was distributed in a sphere of approximately 0.6 mm (unpublished observations from our laboratory). Thus, the highest concentrations of drug were localized in the immediate vicinity of the PVN. Second, electrical stimulation of areas 0.5 mm dorsal, lateral, and anterior to the PVN elicited cardiovascular responses smaller than direct stimulation of the PVN. Similarly, injection of excitatory amino acids into the PVN produced greater pressor responses than injection into adjacent areas of the rostral hypothalamus. Finally, injections of BMI into the posterior hypothalamic area are associated with marked escape responses. In contrast, we observed that administration of BMI into the PVN is associated with grooming behavior similar to that reported to occur after injection of kainic acid into the PVN. Thus, it seems probable that the effects we observed were due to a relatively restricted activation of cells in the PVN region.

Arterial pressure increased by approximately 20 mm Hg after BMI microinfusion. A similar pressor response has been reported to occur after electrical or chemical activation of the PVN. We also observed a consistent, marked increase in heart rate of approximately 100 beats/min. In contrast, electrical or chemical stimulation of the PVN has been reported to cause increases decreases or no change in heart rate. The reasons for these variable responses are unknown but may be related to the presence of anesthesia or the recruitment of different subpopulations of neurons within the PVN. In the present study, only those neurons under tonic GABA influence would be disinhibited by BMI. On the other hand, injections of excitatory amino acids presumably act on all cell bodies within the zone of diffusion of the drug, whereas electrical

Discussion

In the present study, we have demonstrated that administration of BMI, a GABA-A receptor antagonist, into the region of the PVN caused increases in blood pressure and heart rate of approximately 20 mm Hg and 100 beats/min, respectively. These cardiovascular responses were abolished after ganglionic blockade. The BMI microinfusions were also associated with approximately a twofold to threefold increase in plasma norepinephrine and a fivefold to sixfold increase in plasma epinephrine concentrations. Thus, impairment of GABA tone in the PVN elicits increases in blood pressure, heart rate, and plasma catecholamine levels, suggesting that PVN neurons involved in cardiovascular function receive tonic inhibitory GABA input.

A growing body of evidence indicates that GABA is involved in the central control of cardiovascular function. Intracerebroventricular injections of BMI restricted to the forebrain ventricles elicited marked increases in heart rate and blood pressure and sympathetic nervous system outflow. Consequently, it has been proposed that a forebrain periventricular GABA system exerts a tonic inhibitory effect on sympathetic nervous system activity. A study aimed

FIGURE 6. Upper panel: Bar graph shows changes (mean±SEM) in plasma norepinephrine concentrations. Lower panel: Bar graph illustrates changes (mean±SEM) in plasma epinephrine concentrations in response to infusion of bicuculline methiodide into the region of the paraventricular nucleus of sham-operated (open bars) and bilaterally medullectomized (hatched bars) rats. Numbers at base of histograms represent baseline values before infusion. *p<0.05.
stimulation has the potential to activate not only cell bodies but also fibers of passage. In any case, our data clearly indicate that neurons in the PVN region involved in cardiovascular function receive tonic GABA input.

The PVN is a complex nucleus and has been implicated in both endocrine and autonomic cardiovascular responses. Consequently, there are several effector systems that may have participated in the blood pressure and heart rate changes we have observed. The PVN contains magnocellular vasopressin (AVP) neurons and increases in the circulating levels of AVP have been reported following glutamate injections into the PVN of anesthetized rats. However, previous reports indicate that AVP does not play a major role in the pressor response caused by electrical stimulation of the PVN. In addition, electrical stimulation of the PVN reportedly elicits an increase in plasma renin activity. Because we did not directly assess the contribution of the AVP or renin-angiotensin systems in the present study, we cannot definitively rule out a contribution by either of these systems.

On the other hand, we found that pretreatment with a ganglionic blocking agent abolished both the blood pressure and heart rate responses to BMI administration. Thus, our findings suggest that the cardiovascular effects of BMI injected into the PVN were neurally mediated. Indeed, we observed a marked activation of the sympathetic nervous system. During the infusion of BMI, the circulating concentrations of norepinephrine and epinephrine increased approximately twofold to threefold and fivefold to sixfold, respectively. The fact that epinephrine levels were increased implicates the adrenal gland in the response to BMI. Our findings are consistent with previous work reporting an increase in adrenal catecholamine secretion during PVN stimulation. Moreover, recent studies using pseudorabies virus retrograde labeling techniques have demonstrated a direct afferent pathway from the PVN to the preganglionic neurons innervating the adrenal gland.

We assessed the role of adrenal catecholamines by microinfusing BMI into the PVN of rats subjected to bilateral adrenalectomy. In the medullectomized animals, the infusion of BMI caused only small changes in plasma norepinephrine and epinephrine concentrations compared with the sham-operated control animals. Similarly, the heart rate responses were significantly attenuated in the medullectomized animals suggesting that a portion of the tachycardic responses to BMI were mediated via increases in the circulating concentrations of epinephrine. In contrast, there was significant, but small, difference in the blood pressure responses between the sham-operated and medullectomized rats. This result is similar to earlier findings that acute bilateral adrenalectomy does not impair the effects of electrical stimulation of the PVN in anesthetized rats. Thus, our results suggest that the adrenal medulla plays an important role in the plasma catecholamine and heart rate responses to BMI. However, it appears that the blood pressure responses elicited by BMI infusions into the PVN are not dependent on adrenal catecholamines or, alternatively, other divisions of the sympathetic nervous system compensate for the loss of the adrenal medulla.

In conclusion, the present study has demonstrated that infusion of a GABA-A receptor antagonist into the PVN of conscious rats results in marked activation of the sympathetic nervous system and leads to increases in arterial blood pressure and heart rate. Thus, the PVN appears to be a forebrain site at which GABAergic activity exerts a tonic inhibitory effect on the sympathoadrenal axis.

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References


KEY WORDS • GABA • bicuculline • adrenal medulla • catecholamines • heart rate
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