The primary inhibitory neurotransmitter in the central nervous system is \( \gamma \)-aminobutyric acid (GABA). Several anatomically and functionally distinct GABAergic mechanisms that contribute to cardiovascular homeostasis have been described. Postsynaptic GABA antagonists, bicuculline methiodide (BMI) or picrotoxin, elicited tachycardia and a modest increase in blood pressure when microinjected into the posterior hypothalamus of anesthetized rats. These cardiovascular changes were shown to be sympathetically mediated. Thus, the posterior hypothalamus appears to contain a sympathoexcitatory system that is inhibited by GABA. We recently performed similar experiments in conscious rats in which electrodes had been chronically implanted onto their splanchnic nerves. We showed that in unanesthetized rats, changes in GABAergic tone within the posterior hypothalamus modulated sympathetic outflow to the cardiovascular system. We also demonstrated that this sympathoexcitatory system, although completely suppressed by endogenous GABA in anesthetized rats, may be active and contribute to basal sympathetic tone in the conscious state.

Elevated sympathetic activity is thought to contribute to the increase in blood pressure in spontaneously hypertensive rats (SHR). Abnormalities in GABAergic inhibition have previously been identified in SHR. In the mature SHR, hypothalamic concentrations of GABA are lower than those of young SHR or age-matched Wistar-Kyoto (WKY) rats. To further elucidate a potential role of altered GABAergic inhibition in the development of hypertension in SHR, we studied the cardiovascular effects of microinjecting a GABA receptor antagonist and a GABA agonist into the posterior hypothalamus of conscious SHR and WKY rats.
Materials and Methods

All experiments were performed in conscious male SHR and WKY rats of the Okamoto strain (Taconic Farms, Inc., Germantown, New York). The rats ranged in age from 12 to 16 weeks. The rats were housed individually in plastic cages with free access to food and water under controlled temperature, humidity, and light periodicity. The rats were allowed at least 3–4 days for adaptation before the surgical procedures were performed.

The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p., Abbott Labs., Chicago, Illinois) and were mounted in a stereotaxic frame. Heart rate was monitored throughout the cannula-lation procedure by means of a cardiotachometer triggered via lead II of the electrocardiogram and was recorded on a chart recorder (Beckman Instr., Shiller Park, Illinois). Body temperature was maintained between 36° and 38° C by intermittent heating with an infrared lamp.

After a craniotomy was performed, a stainless steel guide cannula (22 gauge, 7.0 mm in length) and injector cannula (28 gauge, 12.0 mm in length) were lowered unilaterally by micromanipulator through the cerebral cortex into the posterior hypothalamus at a 10° angle with respect to the midsagittal plane. Target coordinates used were anterior-posterior -1.2 mm, left-right 0.7 mm, and height-depth -9.0 mm with respect to bregma. Proper cannula placement was confirmed by infusion of 25 ng BMI (in 250 nl over a 30-second period) into the area through the inserted injector cannula. Injection at active sites in this manner increased heart rate by at least 75 beats/min. If heart rate failed to increase by 75 beats/min, the cannulas were raised or lowered by 0.3 mm, and the new placement was retested (after 10 minutes) until an active site was located. After heart rate returned to baseline values (approximately 30–60 minutes), the same procedure was repeated on the opposite side. Once both sets of cannulas were positioned for injection into active sites, the guide cannulas were cemented into position with cranioplastic cement (Plastic Products, Roanoke, Virginia). The injector cannulas were removed, and wire dummy cannulas were inserted to seal the guide cannulas. The rats were then removed from the stereotaxic apparatus and allowed to recover in individual cages.

On completion of the experiments, the injection sites were marked by microinjection of 250 nl of 0.1N hydrochloric acid. After the rats were killed by lethal injection of urethane, the brains were removed and placed in a 10% solution of formaldehyde for at least 48 hours. At a later time, the brains were sectioned into 50 μm sections, mounted on slides, and stained with cresyl violet. The exact position of the injection sites was determined by comparison of sections with the atlas of Pellegrino et al.

At least 10–14 days after cannulation of the posterior hypothalamus, the rats were again anesthetized with sodium pentobarbital, and the femoral artery and vein were catheterized. The arterial and venous catheter tips were positioned in the abdominal aorta and inferior vena cava, respectively, caudal to the renal vasculature. The catheters were routed subcutaneously to the nape of the neck and then through a small leather harness fastened around the forequarters of each rat. Experimental protocols were begun 36–48 hours after catheterization.

Specimens for plasma catecholamine determination were obtained from the femoral artery in such a fashion as not to disturb the animal. The blood samples were withdrawn with a cold syringe that contained sodium metabisulfate (5 mM). The specimens were immediately centrifuged, and the plasma was removed and frozen at −70°C until assayed. An equivalent volume of donor blood from a donor rat was infused after withdrawal of the sample. Donor blood was obtained from decapitated rats of the appropriate strain.

Plasma catecholamines were measured by high-performance liquid chromatography with electrochemical detection as described by Goldstein et al. in 1981. In brief, samples were extracted by using the following procedure: plasma sample (0.5 ml), alumina (50 mg, acid washed), and 10 μl of 0.1 M perchloric acid that contained the internal standard of dihydroxybenzylamine (DBHA) (Aldrich Chemical Co., Milwaukee, Wisconsin). The pH was adjusted to 8.6 with 1.0 M Tris-HCl with 2% EDTA. The alumina was washed with water and filtered with a regenerated cellulose membrane filtering system (Amicon Corp., Danvers, Massachusetts). The catecholamines were then desorbed from the alumina with 0.1 M perchloric acid. This extraction procedure resulted in approximately 70% recovery. Standard solutions of norepinephrine, epinephrine, and DBHA were prepared in 0.1 M perchloric acid with an antioxidant and kept frozen in aliquots. The chromatographic mobile phase was 0.15 M monochloroacetic acid buffer with 0.1 mM sodium EDTA, 1% acetonitrile, and sodium octyl sulfate. The solution was adjusted to a pH of 3.0 with NaOH. This assay has a sensitivity of 10 pg and 15 pg for norepinephrine and epinephrine, respectively. The correlation coefficients for the standard curves are 0.97 for norepinephrine and 0.96 for epinephrine. At a concentration of 75 pg/ml, the interassay coefficient of variation for this assay has been 7% for norepinephrine and 13% for epinephrine.

Drugs used in this study included BMI and muscimol (both purchased from Sigma Chemical Co., St. Louis, Missouri). All drugs were dissolved in saline and microinjected bilaterally in a volume of 250 nl over a 30-second period.

Results are expressed as mean±SEM. The data were analyzed by multiple repeated-measures analysis of variance and paired t tests where indicated. Criterion for statistical significance was p<0.05.
Wible et al Hypothalamic GABA in SHR

Results

Microinjection of BMI into the posterior hypothalamus of conscious SHR or WKY rats increased heart rate (Figure 1) and mean blood pressure (Figure 2) in a dose-related fashion. The doses of BMI (1.0, 3.0, and 10.0 ng/side) and saline were microinjected bilaterally into the posterior hypothalamus in a random order (45-60 minutes apart). Baseline heart rate and mean blood pressure were not significantly different before each injection. The cardiovascular effects of BMI began within 1 minute from the beginning of infusion. Changes in heart rate and mean blood pressure reached their maximum between 3 and 7 minutes and returned to baseline within 20-45 minutes depending on the dose of BMI infused. Saline had no significant effect on heart rate or mean blood pressure. The changes in heart rate elicited by BMI were not significantly different before each injection. The increases in heart rate and mean blood pressure elicited by BMI were not significantly different between the two strains of rats. Comparisons were analyzed by multiple repeated-measures analysis of variance (p<0.05).

Microinjection of muscimol into the posterior hypothalamus caused dose-related decreases in heart rate and mean blood pressure in both the SHR and WKY rats (Figure 3). Muscimol (10.0, 30.0, and 100.0 ng/side) was microinjected bilaterally in a random order 2-3 days apart. Although changes in heart rate and mean blood pressure began almost immediately, the peak cardiovascular changes occurred between 15 and 30 minutes after initiation of infusion. The decreases in heart rate and mean blood pressure induced by muscimol began to return toward baseline values after approximately 90-120 minutes. The magnitude of heart rate changes elicited by muscimol was not significantly different between the two strains of rats. In contrast to the changes in heart rate, muscimol caused significantly greater decreases in mean blood pressure in the SHR strain compared with WKY rats.

Hypothalamic microinjection of BMI or muscimol altered circulating levels of catecholamines (Tables 1 and 2). The concentrations of plasma catecholamines were measured 20 minutes before treatment, 5 minutes after BMI or saline infusion, and 20 minutes after muscimol infusion. Baseline concentrations of the plasma catecholamines were not significantly different before treatment, and there was no difference between the two strains of rats. Infusion of BMI (10.0 ng/side) into the posterior hypothalamus increased plasma concentrations of epinephrine and norepinephrine in both the SHR and WKY rats. In contrast, salin had no such effect. When microinjected into the posterior hypothalamus, muscimol (100.0 ng/side) significantly decreased plasma catecholamine levels in the SHR, but not in the WKY rats. The magnitude of change caused by either BMI or muscimol was not significantly different between SHR and WKY rats.

Microinjection of BMI into the posterior hypothalamus caused intermittent increases in locomotor activity. These changes were dose dependent

Figure 1. Line graph showing peak increase in heart rate after bilateral microinjection of saline (250 nl/side) or bicuculline methiodide (BMI) (1.0, 3.0, and 10.0 ng/side) into the posterior hypothalamus of conscious spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. BMI caused significant increases in heart rate compared with saline control. Changes elicited by BMI were not significantly different in the two strains of rats. Comparisons were analyzed by multiple repeated-measures analysis of variance (p<0.05).

Figure 2. Line graph showing peak change in mean blood pressure after bilateral microinjection of saline (250 nl/side) or bicuculline methiodide (BMI) (1.0, 3.0, and 10.0 ng/side) into the posterior hypothalamus of conscious spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Mean blood pressure increased significantly with microinjection of BMI compared with saline control. Increases in blood pressure caused by BMI were not statistically different between the two strains of rats by multiple repeated-measures analysis of variance (p<0.05).
and followed approximately the same time course as the cardiovascular responses. However, during quiescent periods of the behavioral response, cardiovascular parameters remained elevated. In contrast, microinjection of muscimol into the posterior hypothalamus of conscious SHR and WKY rats suppressed locomotor activity. At the highest dose of muscimol injected (100.0 ng/side), the rats were difficult to arouse and displayed ataxia but did not lose the righting reflex. Microinjection of muscimol at smaller doses produced no ataxia and, although the rats slept most of the time, they were easily aroused. Before treatment or after infusion of saline, the rats slept, consumed food, explored their home cage, and groomed themselves. The behavioral responses caused by microinjection of either the GABA antagonist or agonist did not appear to be different between the two strains of rats.

**Discussion**

The hypothalamus may be an important area in the central nervous system for blood pressure control in hypertensive as well as in normotensive animals. A body of evidence has accumulated that suggests that hypertension in different animal models may be associated with altered GABAergic inhibition in several regions of the brain including the posterior hypothalamus. In SHR, lower endogenous hypothalamic GABA concentrations compared with WKY rats have been described. In addition, infusion of muscimol into the cerebral ventricles decreased blood pressure, adrenal nerve activity, and plasma epinephrine values to a greater degree in SHR than WKY rats. In the deoxycorticosterone acetate–salt rat, daily administration of valproic acid, a compound thought to enhance GABAergic transmission in the brain, blunted the development of hypertension.

We recently identified the posterior hypothalamus as a site where blockade of GABAergic inhibition evokes powerful cardiorespiratory stimulation as well as dramatic behavioral changes in the rat. We found that alteration of GABAergic function in the posterior hypothalamus could influence heart rate, blood pressure, and sympathetic tone. Microinjection of BMI, a GABA<sub>A</sub> receptor antagonist, increased sympathetic tone, whereas infusion of muscimol stimulated local GABA receptors and decreased sympathetic tone.

Because the GABA agonist muscimol, when injected into the lateral cerebral ventricles, had a greater effect in SHR than WKY rats and mature SHR were found to have lower GABA levels in the posterior hypothalamus than WKY rats, we reasoned that decreased GABAergic inhibition of sympathetic tone in the posterior hypothalamus may contribute to hypertension in SHR. Therefore, we

**Table 1. Effects of Saline, Bicuculline Methiodide, and Muscimol on Heart Rate, Mean Blood Pressure, and Plasma Catecholamines in Conscious Spontaneously Hypertensive Rats**

<table>
<thead>
<tr>
<th>Injections</th>
<th>Baseline HR (beats/min)</th>
<th>Baseline MBP (mm Hg)</th>
<th>Baseline EPI (pg/ml)</th>
<th>Baseline NE (pg/ml)</th>
<th>Response HR (beats/min)</th>
<th>Response MBP (mm Hg)</th>
<th>Response EPI (pg/ml)</th>
<th>Response NE (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (250 nl/side) (n=10)</td>
<td>373±10</td>
<td>159±4</td>
<td>244±93</td>
<td>312±63</td>
<td>416±16</td>
<td>164±4</td>
<td>247±49</td>
<td>362±78</td>
</tr>
<tr>
<td>BMI (10 ng/side) (n=12)</td>
<td>348±8</td>
<td>161±5</td>
<td>239±31</td>
<td>300±54</td>
<td>500±15*</td>
<td>184±5*</td>
<td>1411±281*</td>
<td>940±158*</td>
</tr>
<tr>
<td>Muscimol (100 ng/side) (n=10)</td>
<td>366±8</td>
<td>162±4</td>
<td>211±47</td>
<td>272±34</td>
<td>318±8*</td>
<td>134±4*</td>
<td>80±12*</td>
<td>169±29*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. HR, heart rate; MBP, mean blood pressure; EPI, epinephrine; NE, norepinephrine; BMI, bicuculline methiodide.

*Significantly different from corresponding baseline value by paired t test (p<0.05).
expected that BMI would exert less of an effect on blood pressure, heart rate, and sympathetic tone in SHR than in WKY rats. In addition, reduced GABAergic tone in the posterior hypothalamus of SHR would be expected to result in an increased response to muscimol.

The changes in heart rate and plasma catecholamines elicited by BMI and muscimol were not statistically different between the SHR and WKY rats. In contrast, muscimol caused a greater decrease in mean blood pressure in the SHR compared with the WKY strain, and although not statistically different, the increases in blood pressure elicited by BMI tended to be greater. However, the abilities of muscimol and possibly BMI to cause greater changes in blood pressure in the SHR may result not from differences in autonomic sympathetic activity but rather from anatomic differences. The vasculature of the mature SHR displays an increased wall-to-lumen ratio indicating hypertrophy of the vascular smooth muscle. Thus, equivalent changes in sympathetic activity in the two strains of rats would result in larger changes in blood pressure in the SHR.

Microinjection of muscimol into the posterior hypothalamus significantly decreased plasma catecholamines in the SHR but not in WKY rats. These results are consistent with earlier observations regarding the effects of muscimol infused into the cerebral ventricular system. Although the ventricular administration of muscimol caused equivalent decreases in splanchic nerve activity in the stroke-prone SHR and WKY rats, adrenal nerve activity and plasma epinephrine decreased to a significantly greater degree in the stroke-prone SHR. The failure of BMI to cause a greater increase in plasma catecholamines in the SHR may be the result of maximal release of adrenal catecholamines in both strains of rats. Differences may have been observed at low doses of BMI. Thus, the ability of GABA in the posterior hypothalamus to regulate the release of adrenal catecholamines may be different in the SHR compared with normotensive rats.

Epinephrine, which is secreted into the systemic circulation from the adrenal gland, can be taken up by sympathetic nerve terminals. This epinephrine can then be rereleased and facilitate neurogenic vasoconstriction by stimulating presynaptic β-receptors.

In this investigation, we were unable to identify differences in resting catecholamine values between SHR and WKY rats. Such differences are not regularly identified, perhaps in part because of artifactual elevation of plasma catecholamines above resting values related to the stress of sample acquisition in some studies. Consequently, we attempted to minimize stress. Our plasma samples were obtained from the conscious, unrestrained rat via an indwelling arterial catheter implanted 2 days earlier. Volume losses related to sample removal were replaced by blood from donor rats. Higher catecholamine values have been reported in stroke-prone strains of SHR compared with WKY rats. Plasma concentrations reported in these studies are in the range of those values reported here. It is possible that strain differences play a role. As indicated in a recent publication, there is considerable strain variability, particularly in the WKY rats, which may explain some of the inconsistencies reported.

The present investigation supports and confirms our earlier findings in normal rats. The posterior hypothalamus contains neurons, tonically inhibited by GABA, that can influence sympathetic outflow. In SHR and WKY rats, this mechanism functions in a similar manner as in normal rats. If differences exist between SHR and WKY rats with respect to their response to GABA A receptor stimulation and blockade at this site, they appear to be subtle. Our data suggest that GABAergic inhibition in the posterior hypothalamus of the SHR may be unable to suppress properly the release of catecholamines from the adrenal medulla. It is also possible that SHR differ from WKY rats with respect to GABAergic inhibition at other sites along the neuraxis. The content of GABA in the rostral ventrolateral medulla of the SHR is lower than that found in age-matched WKY rats. Thus, small differences in GABAergic mechanisms in several regions of the central ner-

### Table 2. Effects of Saline, Bicuculline Methiodide, and Muscimol on Heart Rate, Mean Blood Pressure, and Plasma Catecholamines in Conscious Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Injections</th>
<th>Baseline</th>
<th></th>
<th>Response</th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>MBP</td>
<td>EPI</td>
<td>NE</td>
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<tr>
<td>Saline (250 nl/side)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=12)</td>
<td>372±10</td>
<td>117±4</td>
<td>302±107</td>
<td>358±47</td>
</tr>
<tr>
<td>BMI (10 ng/side)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=12)</td>
<td>372±12</td>
<td>115±1</td>
<td>187±27</td>
<td>313±51</td>
</tr>
<tr>
<td>Muscimol (100 ng/side)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=12)</td>
<td>361±11</td>
<td>107±4</td>
<td>242±55</td>
<td>252±34</td>
</tr>
</tbody>
</table>
| Values are mean±SEM. HR, heart rate; MBP, mean blood pressure; EPI, epinephrine; NE, norepinephrine; BMI, bicuculline methiodide. *Significantly different from corresponding baseline value by paired t test (p<0.05).
vous system may contribute to the overall expression of hypertension in the SHR.

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