Hypothalamic GABA and Sympathetic Regulation in Spontaneously Hypertensive Rats

James H. Wible Jr., Joseph A. DiMicco, and Friedrich C. Luft

The posterior hypothalamus contains a sympathoexcitatory system that can be modulated by changes in GABAergic tone. We tested the hypothesis that the GABAergic mechanism in the posterior hypothalamus is altered in spontaneously hypertensive rats (SHR) compared with the Wistar-Kyoto (WKY) control rats. Blood pressure and heart rate were continuously measured in the conscious state; blood samples were obtained for determination of plasma catecholamine concentrations. Bilateral microinjections of the GABA\textsubscript{A} receptor antagonist bicuculline methiodide into the posterior hypothalamus increased heart rate and blood pressure in a dose-related fashion and increased plasma catecholamine concentrations in both SHR and WKY rats. The responses were not significantly different between the two strains of rats. Microinjections of the GABA\textsubscript{A} receptor agonist muscimol in this same region caused dose-related decreases in both heart rate and blood pressure in SHR and WKY rats. Although the decreases in heart rate caused by muscimol were not significantly different between the SHR and WKY rats, the decreases in blood pressure were significantly greater in SHR compared with WKY rats. Further, microinjection of muscimol caused a significant decrease in plasma catecholamines in SHR but not in WKY rats. These data indicate that in SHR and WKY rats the posterior hypothalamus contains a sympathoexcitatory mechanism that is tonically inhibited by GABA. The ability of muscimol to decrease plasma catecholamines selectively in SHR and to cause greater decreases in blood pressure, suggests that the GABAergic mechanisms in the posterior hypothalamus of the SHR and WKY rats may differ. (Hypertension 1989; 14:623–628)

The primary inhibitory neurotransmitter in the central nervous system is \textit{G-aminobutyric acid} (GABA). Several anatomically and functionally distinct GABAergic mechanisms that contribute to cardiovascular homeostasis have been described.\textsuperscript{2–4} 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.\textsuperscript{5} These cardiovascular changes were shown to be sympathetically mediated.\textsuperscript{5,6} 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.\textsuperscript{7} 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).\textsuperscript{8} Abnormalities in GABAergic inhibition have previously been identified in SHR.\textsuperscript{9} In the mature SHR, hypothalamic concentrations of GABA are lower than those of young SHR or age-matched Wistar-Kyoto (WKY) rats.\textsuperscript{10} 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 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. 11 Proper cannula placement was confirmed by infusion of 25 ng BMI (in 250 nl saline 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 cannula (28 gauge, 12.0 mm in length) were lowered unilaterally by micromanipulator through the appropriate strain.

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. Criteron for statistical significance was p<0.05.
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, saline 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 3. Line graph showing peak decrease in heart rate (top panel) and mean blood pressure (bottom panel) after bilateral microinjection of saline (250 nl/side) or muscimol (10.0, 30.0, and 100.0 ng/side) into the posterior hypothalamus of conscious spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Muscimol decreases heart rate and mean blood pressure compared with saline control. Although changes in heart rate caused by muscimol were not significantly different between the two strains of rats, decreases in blood pressure evoked by muscimol were statistically greater in SHR compared with WKY rats. Comparisons were analyzed 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 GABAA 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
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.16 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.9 Although the ventricular administration of muscimol caused equivalent decreases in splanchnic 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.18 This epinephrine can then be rereleased and facilitate neurogenic vasoconstriction by stimulating presynaptic β-receptors.19

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.20 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.21 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,22 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.23 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 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>
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<tbody>
<tr>
<td>Saline (250 nl/side)</td>
<td>372±10</td>
<td>117±4</td>
<td>302±10</td>
<td>358±47</td>
<td>367±11</td>
<td>117±4</td>
<td>229±48</td>
<td>398±50</td>
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<td>(n=12)</td>
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<tr>
<td>Bicuculline Methiodide (10 ng/side)</td>
<td>372±12</td>
<td>115±1</td>
<td>187±27</td>
<td>313±51</td>
<td>493±17*</td>
<td>134±3*</td>
<td>896±138*</td>
<td>727±117*</td>
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<tr>
<td>Muscimol (100 ng/side)</td>
<td>361±11</td>
<td>107±4</td>
<td>242±55</td>
<td>252±34</td>
<td>305±7*</td>
<td>94±4*</td>
<td>234±57</td>
<td>190±22</td>
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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.

References

Hypothalamic GABA and sympathetic regulation in spontaneously hypertensive rats.

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