Plasticity of GABAergic control of hypothalamic presympathetic neurons in hypertension

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Hypertension is a well-recognized risk factor for morbidity and mortality due to stroke, coronary artery disease, heart failure, and chronic renal disease. The sympathetic nervous system plays an important role in the pathogenesis of hypertension (1, 2, 21). Almost all the animal models of hypertension, including spontaneously hypertensive rats (SHR), renin transgenic rats, Dahl salt-sensitive rats, and deoxycorticosterone acetate-salt rats, exhibit an increase in sympathetic activity (6, 21, 39). There is also evidence of elevated sympathetic nerve activity in hypertensive patients (2, 16, 17, 28). The brain function altered in hypertensive animals occurs predominantly in the hypothalamus and brainstem (1, 10, 12, 14, 20). The hypothalamic paraventricular nucleus (PVN) plays an important role in the control of sympathetic outflow through projections to the intermediolateral cell column of the spinal cord and the rostral ventrolateral medulla (RVLM) (11, 37, 38). However, the cellular and molecular mechanisms underlying heightened sympathetic vasomotor tone in hypertension remain poorly understood.

GABA is the most important inhibitory neurotransmitter in the brain. The inhibitory actions of GABA in the brain are mediated primarily through ionotropic GABA_A and metabotropic GABA_B receptors. The PVN presympathetic neurons are tonically regulated by GABAergic synaptic inputs (25–27, 29, 31). In this regard, disinhibition of the PVN with the GABA_A receptor antagonist bicuculline increases sympathetic nerve activity, blood pressure, and plasma norepinephrine in anesthetized and conscious rats (29, 31, 41). Previous studies suggest that the GABAergic system is impaired in the hypothalamus in hypertension. For example, microinfusion of the GABA_A antagonist bicuculline into the PVN has no effect on blood pressure in the renal wrap model of hypertension (30). Also, microinjection of the GABA_B agonist baclofen into the presser area of the hypothalamus produces a larger decrease in blood pressure, heart rate, and sympathetic nerve activity in SHR than in Wistar-Kyoto (WKY) rats (40). Furthermore, the number of GABA_A receptor binding sites in the PVN is significantly lower in SHR than in WKY rats (23). However, little information is available about the alteration of GABA receptor function in the PVN in hypertension. Therefore, we used a combination of in vivo retrograde labeling and in vitro whole cell patch-clamp recordings in the hypothalamic slice to examine the tonic GABAergic control of PVN presympathetic neurons in SHR and normotensive rats. This study reveals unexpected functional changes in GABA_A and GABA_B receptors in the control of the firing activity of PVN-RVLM projection neurons in SHR. The plasticity of GABA receptor function in the regulation of these PVN neurons may contribute to the pathogenesis of essential hypertension.

Materials and Methods

Animals

Male Sprague-Dawley (SD) (Harlan, Indianapolis, IN), WKY, and SHR (Taconic, Germantown, NY) at both 6 and 13 wk of age were used for this study. The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the Pennsylvania State University College of Medicine and conformed to the National Institutes of Health guidelines on the ethical use of animals. We measured the blood pressure in each rat using a noninvasive tail-cuff system (model 29-SSP, IITC Life Science, Woodland Hills, CA). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Hills, CA). Blood pressure was measured every day for at least 1 wk before the final experiment. The SHR had an increased blood pressure starting at 8 wk of age and reached a stable state of hypertension ~13 wk after birth.

Retrograde Labeling of RVLM-Projecting PVN Neurons

Rats were anesthetized by intraperitoneal injection of a mixture of ketamine (70 mg/kg) and xylazine (6 mg/kg), and the head of the rat was placed in a stereotaxic apparatus. A burr hole (4 mm in diameter) was made in the occipital bone bilaterally according to the following coordinates (bregma): 11.80–13.0 mm caudal, 1.8–2.2 mm lateral, and 7.8–8.1 mm deep from the surface of the cortex. A rhodamine-labeled fluorescent microsphere suspension (0.04 μm, FluoSpheres, Molecular Probes, Eugene, OR) was injected (Nanojector II, Drummond Scientific, Broomall, PA) bilaterally into the region of the RVLM. The pipette was positioned with a micromanipulator, and the injection of 50 nl FluoSpheres was monitored through a surgical microscope. After injection was completed, the muscle and skin were sutured and the wound was closed. Animals were returned to their cages for 2–5 days, sufficient time to permit the retrograde tracer to be transported to the PVN (27).

Slice Preparations

Coronal hypothalamic slices (300 μm in thickness) containing the PVN were cut with the use of a vibratome (Technical Product International, St. Louis, MO), as described previously (24, 25). Briefly, 2–5 days after tracer injection was completed, the rats were rapidly euthanized under halothane anesthesia. The brain was quickly removed and placed in ice-cold artificial cerebral spinal fluid (aCSF) solution saturated with 95% O2-5% CO2 for 1–2 min. After sectioning was completed, the slices were preincubated in the aCSF at 34°C for at least 1 h before recording. For the recordings, a slice was transferred to a submersible-type recording chamber; continuously perfused (3 ml/min) with aCSF perfusion solution containing (in mM) 124.0 NaCl, 3.0 KCl, 1.3 MgSO4, 2.4 CaCl2, 1.4 NaH2PO4, 10.0 glucose, and 26.0 NaHCO3; and saturated with a gas mixture of 95% O2-5% CO2 at 34°C.

To verify the injection site and diffusion size of FluoSpheres, the brainstem was sectioned to 35 μm in thickness at the injection level immediately after the rat was euthanized. The brainstem slices were viewed, and the injection sites of FluoSpheres were identified under a microscope equipped with epifluorescence illumination, as we described previously (27). The diffusion of the tracer in the RVLM was generally limited to 0.35 mm (bregma) and 7.8–8.1 mm deep from the surface of the cortex. A rhodamine-labeled fluorescent microsphere suspension (0.04 μm, FluoSpheres, Molecular Probes, Eugene, OR) was injected (Nanojector II, Drummond Scientific, Broomall, PA) bilaterally into the region of the RVLM. The pipette was positioned with a micromanipulator, and the injection of 50 nl FluoSpheres was monitored through a surgical microscope. After injection was completed, the muscle and skin were sutured and the wound was closed. Animals were returned to their cages for 2–5 days, sufficient time to permit the retrograde tracer to be transported to the PVN (27).

Electrophysiological Recordings

Whole cell voltage- and current-clamp recordings were performed in a radio frequency-shielded room (24, 25). The recording pipettes were pulled from borosilicate capillaries (1.2 mm OD, 0.68 mm ID; World Precision Instruments, Sarasota, FL) using a micropipette puller (P-97, Sutter Instrument, Novato, CA). The resistance of the pipette was 3–6 MΩ when filled with a solution containing (in mM) 140.0 potassium gluconate, 2.0 MgCl2, 0.1 CaCl2, 10.0 HEPES, 1.1 EGTA, 0.3 Na2-GTP, and 2.0 Na2-ATP, adjusted to pH 7.25 with 1 M KOH (270–290 mosM). The slice was placed in a glass-bottomed recording chamber (Warner Instruments, Hamden, CT) and fixed with a grid of parallel nylon threads supported by a U-shaped stainless steel weight. The slice was perfused at 34°C, maintained by an in-line solution heater and a temperature controller (model TC-324, Warner Instruments). It took ~1.5 min to completely exchange the solution inside the recording chamber at the perfusion speed of 3.0 ml/min. Whole cell recordings from labeled PVN neurons were made under visual control using a combination of epifluorescence illumination and infrared and differential interference contrast optics on an upright microscope (BX51WI, Olympus). Because the labeled RVLM-projection neurons are primarily present in the medial one-third of the PVN between the third ventricle and fornix, labeled PVN neurons in this study were selected for recording. Signals were processed by using a Multiclamp 700A (Axon Instruments, Foster City, CA). Signals were filtered at 1–2 kHz and digitized at 10 kHz using Digidata 1320A (Axon Instruments). The series resistance was compensated by 60–80%. The recording was abandoned if the input resistance, measured with a voltage range of 15–20 mV, changed more than 15% during the recording.

Current-clamp recordings of firing activity. To determine the effect of blockade of GABA_A and GABA_B receptors on the excitability of labeled PVN neurons, the spontaneous firing activity of labeled PVN neurons was recorded by using the whole cell current-clamp technique (26, 27). Recordings of the firing activity of neurons began ~5 min after the whole cell access was established and the firing activity reached a steady state. In all cases, values were obtained and averaged during a 3–5 min recording period before and after drug application. Signals were processed, recorded, and analyzed as described in Electrophysiological Recordings. The junction potential was corrected during off-line analysis.

Voltage-clamp recordings of inhibitory postsynaptic currents and agonist-evoked postsynaptic currents. The spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded at a holding potential of 0 mV, as described previously (26, 27). To study the current elicited by activation of postsynaptic GABA_A receptors, whole cell voltage-clamp recordings were performed. For the recording of GABA_A currents, we used the internal solution containing (in mM) 119.0 CsSO4, 2.4 MgCl2, 0.5 CaCl2, 10.0 HEPES, 5.0 BAPTA, 0.33 GTP-Tris, 5.0 Na2-ATP, 5.0 tetraethylammonium-Cl, and GDPβS, adjusted to pH 7.25 with 1 M CsOH (270–290 mosM). Puff application of GABA was done by using a picospritzer (General Valve, Fairfield, NJ). The puff electrode (~5 to 10 μm) was placed to the recorded cell at a distance of ~100 μm. Positive pressure (6–8 psi and 150-ms duration) was applied to eject aCSF solution containing GABA. The drug concentration was chosen based on the results of preliminary experiments. GABA_A current was elicited by puff application to the GABA_A receptors to the holding potential of 0 mV, based on the reverse potential for GABA_A receptor-mediated Cl− current. A potassium channel blocker cesium and a G protein inhibitor GDPβS were included in the pipette internal solution to block the GABA_A current (3, 34). The specific GABA_A receptor antagonist CGP-55845 was used to eliminate the GABA_A current.

All the drugs were prepared immediately before the experiments and were applied to the recording chamber using syringe pumps unless stated otherwise. Bicuculline methiodide and GDPβS were obtained from Sigma (St. Louis, MO). CGP-55845 was purchased from Tocris (Ballwin, MO).

Data Analysis

Data are presented as means ± SE. The action potentials and sIPSCs were analyzed off-line with a peak detection program (MiniAnalysis, Synaptosoft, Leonia, NJ). The firing rate and membrane potentials were obtained by averaging the frequency and membrane potentials over a period of 3–6 min before, during, and after drug application. For cells displaying intermittent firing activity, the membrane potential was measured when the cell was silent and the membrane potential became stable. For those cells showing tonic activity, the membrane potential was usually estimated 200 ms before initiation of the action potential. We categorized the neurons according to their responses to drug application. The neuron was considered responsive if its firing activity was altered more than >20% after drug application. The junction potential was corrected off-line based on the composition of the internal and external solutions used for recordings.
The sIPSCs were detected by the fast rise time of the signal over an amplitude threshold set above the background noise. The amplitude detection threshold was typically 5–10 pA. We manually excluded the event when the noise was erroneously identified as the sIPSCs by the computer program. The background noise level was typically constant throughout the recording of a single neuron. At least 100 randomly selected sIPSCs were used in each analysis. The amplitude of the GABA\(_{A}\) and GABA\(_{B}\) current was analyzed by using Clampfit 9.0 software. The value of the GABA\(_{A}\) currents was normalized by the cell capacitance and expressed as current density. The effects of drugs on the firing activity, membrane potentials, sIPSCs, and the GABA\(_{A}\) currents were determined by Wilcoxon’s signed-rank test or nonparametric ANOVA (Kruskal-Wallis) with Dunn’s post hoc test. \(P < 0.05\) was considered to be statistically significant.

**RESULTS**

The systolic arterial blood pressure of 13-wk-old SHR was significantly higher than that of SD (6 and 13 wk old), WKY (6 and 13 wk old), and 6-wk-old SHR (Fig. 1). Whole cell current- and voltage-clamp recordings were obtained from a total of 283 FluoroSphere-labeled PVN cells (\(n = 68\) rats). The membrane potentials of labeled PVN neurons displayed no significant difference between groups. The mean membrane potential was \(-59.2 \pm 0.89\) mV and an input resistance of \(498.5 \pm 8.6\) M\(\Omega\). Table 1 shows the membrane potential, input resistance, and cell capacitance of labeled PVN neurons in each group of rats.

**Role of GABA\(_{A}\) Receptors in Controlling Firing Activity of Labeled PVN Neurons in SHR and Normotensive Rats**

Blockade of the GABA\(_{A}\) receptors by bicuculline consistently increases the excitability of the PVN output neurons in SD rats (25, 26). We first determined the effect of blockade of GABA\(_{A}\) receptors on the firing activity of labeled PVN neurons in the normotensive WKY, SD, and 6-wk-old SHR and hypertensive 13-wk-old SHR. The majority of the labeled PVN neurons recorded displayed spontaneous activity in 6-wk-old SHR (10 of 12 cells, 83%), SD (10 of 12 cells, 83%), and WKY (7 of 9 cells, 78%) rats and 13-wk-old SD (9 of 12 cells, 75%) and WKY (6 of 8 cells, 75%) rats. The firing properties of the labeled PVN neurons in 6-wk-old SHR were not significantly different from 6-wk-old SD and WKY rats. A bath application of 20 \(\mu\)M bicuculline significantly increased the firing activity from 1.34 \pm 0.35 to 2.61 \pm 0.46 Hz (\(n = 12\) cells, \(P < 0.05\)) in 6-wk-old SHR (Figs. 2A and 3A). The membrane potentials were depolarized from \(-62.5 \pm 4.1\) to \(-58.1 \pm 3.5\) mV (\(n = 12\) cells, \(P < 0.05\)). Notably, in 13-wk-old SD and WKY rats, the basal firing rate was significantly lower than that in the 6-wk-old SD and WKY groups (0.25 \pm 0.03 vs. 1.87 \pm 0.57 Hz, \(P < 0.05\) in SD and 0.22 \pm 0.07 vs. 1.50 \pm 0.66 Hz, \(P < 0.05\) in WKY rats, respectively) (Fig. 3B). Bath application of 20 \(\mu\)M bicuculline significantly increased the spontaneous firing activity of PVN neurons in both 6-wk-old and 13-wk-old SD and WKY rats in all cells tested (Fig. 3B). Furthermore, in almost all the silent PVN neurons tested in 6 (2 of 2 cells)- and 13-wk-old (3 of 3 cells) SD, 6 (2 of 2 cells)- and 13-wk-old (3 of 4 cells) WKY, and 6-wk-old (2 of 2 cells) SHR, a bath application of 20 \(\mu\)M bicuculline induced depolarization and spontaneous firing activity.

Similar to those in normotensive control rats, the majority of labeled PVN neurons recorded in 13-wk-old SHR displayed spontaneous activity (47 of 59 cells; 79.6%). However, the baseline firing activity of labeled PVN neurons in 13-wk-old SHR (1.85 \pm 0.22 Hz, \(n = 59\) cells) was significantly higher than that in age-matched normotensive SD (0.25 \pm 0.03 Hz, \(P < 0.05\)) and WKY (0.22 \pm 0.07 Hz, \(P < 0.05\)) rats. Among those 47 spontaneously active neurons, a bath application of 20 \(\mu\)M bicuculline increased the firing rate from 2.12 \pm 0.45 to 3.16 \pm 0.51 Hz (\(P < 0.05\)) in only 23 (48.9%) PVN neurons tested. Also, bicuculline depolarized the membrane potential from \(-58.35 \pm 3.25\) to \(-55.23 \pm 2.36\) mV (\(P < 0.05\)) in these 23 neurons (Figs. 2B and 3A). However, bicuculline had no significant effect on the ongoing firing activity in 10 of 47 (21.3%) labeled PVN neurons (2.39 \pm 0.45 vs. 2.46 \pm 0.48 Hz, \(P > 0.05\)). Strikingly, in a group of 14 (29.8%) cells tested, bicuculline decreased the ongoing firing rate from 2.48 \pm 0.76 to 1.40 \pm 0.43 Hz (\(P < 0.05\)) and hyperpolarized the membrane potential from \(-60.24 \pm 1.65\) to \(-62.35 \pm 1.24\) mV in these neurons (Figs. 2C and 3A).

A bath application of 20 \(\mu\)M bicuculline only depolarized and induced firing in 1 of 12 silent PVN neurons tested in

<table>
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<th>Group</th>
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<th>Membrane Potential</th>
<th>Input Resistance</th>
<th>Cell Capacitance</th>
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<td>42.31 \pm 0.46</td>
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<tr>
<td>13-wk-old WKY</td>
<td>44</td>
<td>(-58.9 \pm 0.62)</td>
<td>498.2 \pm 9.1</td>
<td>41.25 \pm 0.23</td>
</tr>
<tr>
<td>6-wk-old SHR</td>
<td>41</td>
<td>(-58.6 \pm 0.53)</td>
<td>502.4 \pm 8.9</td>
<td>40.12 \pm 0.26</td>
</tr>
<tr>
<td>13-wk-old SHR</td>
<td>95</td>
<td>(-57.9 \pm 0.24)</td>
<td>500.9 \pm 6.2</td>
<td>41.39 \pm 0.11</td>
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Values are means \(\pm SE\); \(n\), number of cells. SD, Sprague-Dawley rats; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; PVN, paraventricular nucleus.

Fig. 1. Systolic blood pressure in normotensive and hypertensive rats. Summary data showing systolic blood pressure measured on day before electrophysiological experiment in 2 age groups of Sprague-Dawley (SD), Wistar-Kyoto (WKY), and spontaneously hypertensive rats (SHR). Data are means \(\pm SE\). \(\ast P < 0.05\) compared with control (Kruskal-Wallis ANOVA followed by Dunn’s post hoc test).
13-wk-old SHR but had no significant effect on the membrane potentials (−61.5 ± 1.98 vs. −60.68 ± 1.63 mV, P > 0.05) and firing activity in 11 of 12 silent labeled PVN neurons. Additionally, a bath application of 50 μM picrotoxin, a chloride channel blocker, decreased the firing activity in 4 of these 14 labeled PVN neurons (2.21 ± 0.92 to 1.32 ± 0.85 Hz, P < 0.05).

In addition to blocking \( \text{GABA}_A \) receptors, bicuculline methiodide may increase the firing activity of PVN neurons through its effect on small conductance \( \text{Ca}^{2+} \)-activated \( \text{K}^+ \) channels (SK channels) (13, 22). Therefore, we determined the effect of apamin, a specific blocker of SK channels, on the firing activity of labeled PVN neurons. A bath application of 100 nM apamin had no significant effect on the firing rate of labeled PVN neurons in both 6-wk-old SD and 13-wk-old hypertensive SHR (Fig. 3, C and D). At 500 nM, apamin also failed to alter the firing activity of seven labeled PVN neurons in 6-wk-old SD rats (data not shown). Similar to the effect of bicuculline, a bath application of 50 μM picrotoxin, a \( \text{GABA}_A \) receptor antagonist and a chloride channel blocker, significantly increased the firing activity of six labeled PVN neurons in 6-wk-old SD rats (from 1.80 ± 0.72 to 3.50 ± 0.88 Hz, P < 0.05, Fig. 3E).

**Role of \( \text{GABA}_B \) Receptors in Regulating Firing Rate of PVN Neurons in SHR and Normotensive Rats**

We next determined the role of \( \text{GABA}_B \) receptors in regulation of firing activity of labeled PVN neurons using a \( \text{GABA}_B \) receptor antagonist CGP-55845 (5). A bath application of 1 μM CGP-55845 did not significantly change the firing activity of labeled PVN neurons in all normotensive groups, including SD (6 and 13 wk old), WKY (6 and 13 wk old), and 6-wk-old SHR (Figs. 4A and 5). Furthermore, a higher concentration of CGP-55845 (5 μM) still had no significant effect on the firing activity of 5 (2 in 13-wk-old SD and 3 in 6-wk-old SHR) labeled PVN neurons (data not shown). In all the silent PVN neurons tested in 6- (n = 1 cell) and 13-wk-old (n = 3 cell) SD, 6- (n = 1 cell) and 13-wk-old (n = 3 cells) WKY, and 6-wk-old (n = 2 cells) SHR, a bath application of 1 μM CGP-55845 did not induce any depolarization and spontaneous firing activity.

In contrast, in 12 of 16 (75%) labeled PVN neurons recorded from the 13-wk-old SHR, a bath application of 1 μM CGP-55845 significantly increased the ongoing firing activity from 1.76 ± 0.44 to 3.38 ± 0.59 Hz (P < 0.05). Also, CGP-55845 produced a depolarization of the cell membrane potential from −59.35 ± 3.48 to −57.21 ± 2.31 mV (n = 12 cells, P < 0.05) (Figs. 4B and 5A). CGP-55845 had no significant effect on the firing rate and membrane potential in 4 of 16 (25%) spontaneous active cells tested. A bath application of 1 μM CGP-55845 significantly depolarized and induced spontaneous firing (from 0 to 1.26 ± 0.56 Hz) in 3 of 4 silent PVN neurons tested in 13-wk-old SHR.

We reasoned that the inhibitory effect of bicuculline on some PVN cells in 13-wk-old SHR might be due to the stimulation of \( \text{GABA}_B \)ergic interneurons, which increases \( \text{GABA} \) release onto PVN projection neurons and subsequently causes inhibition of PVN-RVLM neurons through \( \text{GABA}_B \) receptors. In 8 of 14 neurons inhibited by bicuculline, we further tested the effect of bicuculline on the firing activity of labeled PVN neurons in the presence of 1 μM CGP-55845. CGP-55845 alone increased the firing activity of labeled PVN neurons from 2.55 ± 0.35 to 3.81 ± 0.33 Hz (P < 0.05, Fig. 6). A subsequent application of 20 μM bicuculline failed to
decrease the firing activity of these eight labeled PVN neurons in the presence of CGP-55845 in the 13-wk-old SHR (Fig. 6).

**Postsynaptic GABA<sub>A</sub> Current of Labeled PVN Neurons in Normotensive and SHR Rats**

We then examined the function of GABA<sub>A</sub> receptors in labeled PVN neurons in SHR and normotensive controls. The GABA<sub>A</sub> current was elicited by a puff application of 1 mM GABA at a holding potential of 0 mV (15). Because GABA can activate both GABA<sub>A</sub> and GABA<sub>B</sub> receptors, Cs<sup>2+</sup> and GDP<sub>B</sub>S were included in the recording internal solution to abolish the GABA<sub>B</sub> current. In all six neurons tested (3 in SD and 3 in 13-wk-old SHR), a bath application of 100 μM bicuculline completely eliminated the current induced by puff application of GABA (Fig. 7A). A puff application of GABA induced a large outward current in all normotensive control rats. The peak amplitude of the GABA<sub>A</sub> current was similar in normotensive rats, including SD (6 and 13 wk old, 77.0 ± 7.8 and 85.1 ± 14.1 pA/pF), WKY (6 and 13 wk old, 75.6 ± 9.3 and 87.3 ± 12.0 pA/pF), and 6-wk-old SHR (72.4 ± 8.8 pA/pF, Fig. 7). However, the peak amplitude of GABA<sub>A</sub> current (44.3 ± 3.0 pA/pF) recorded in labeled PVN neurons from 13-wk-old SHR was significantly lower than that in normotensive SD, WKY, and 6-wk-old SHR rats (Fig. 7, A and B).

Furthermore, both the frequency (1.45 ± 0.59 Hz, n = 9 cells) and amplitude (31.48 ± 0.79 pA, n = 9 cells) of GABAergic sIPSCs in labeled PVN neurons from 13-wk-old SHR were significantly lower than those in age-matched normotensive SD and WKY rats and in 6-wk-old SHR controls (Fig. 8).

**DISCUSSION**

This is the first study demonstrating the plasticity of the GABA receptor function in regulation of PVN projection neurons in hypertension. We found that, unlike the stimulating effect of bicuculline on labeled PVN-RVLM cells in normotensive controls, bicuculline failed to increase the firing activity or even inhibited the firing activity of 59.3% cells in 13-wk-old SHR. Furthermore, blockade of GABA<sub>B</sub> receptors significantly increased the excitability of most labeled PVN neurons (75%) in hypertensive 13-wk-old SHR but not in normotensive rats. We also found that the GABA<sub>A</sub> current in labeled PVN neurons was significantly smaller in the hypertensive SHR than in normotensive rats. This study provides novel information that there is not only a decrease in GABA<sub>A</sub> but also an increase in GABA<sub>B</sub> receptor function in GABAergic control of the excitability of PVN-RVLM neurons in SHR.

The PVN is an important brain structure, which regulates sympathetic outflow and plays a critical role in the development or maintenance of high blood pressure in hypertensive animals (1, 10, 37, 38). Lesion of the PVN attenuates the development of hypertension and lowers the blood pressure in...
In the present study, we found that the basal activity of labeled PVN neurons was significantly lower in normotensive 13-wk-old SD and WKY groups compared with the 6-wk-old groups, suggesting that tonic GABAergic inhibition of the PVN presympathetic neurons may be enhanced in adult normotensive rats. However, we observed that there was no significant difference in the basal membrane potentials and input resistance of labeled PVN neurons between 6-wk-old and 13-wk-old normotensive rats, and the peak GABAA currents were slightly but not significantly increased in 13-wk-old than in 6-wk-old normotensive rats. Thus it appears that mechanisms other than GABAA receptor changes may be responsible for this age-dependent decline in the basal activity of PVN-RVLM neurons in normotensive rats. For example, possible changes in nitrergic, noradrenergic, and glutamatergic inputs in the PVN (12, 24, 26) may play a role in the low firing activity of PVN-RVLM neurons in 13-wk-old SD and WKY rats. Importantly, the basal firing activity of labeled PVN neurons in hypertensive 13-wk-old SHR was significantly higher than that in 6-wk-old SHR. Therefore, increased excitability of PVN-RVLM neurons due to inadequate GABAergic inhibition (disinhibition) may play a role in the heightened sympathetic vasomotor tone in adult hypertensive rats. The SHR are the most often used genetic model of hypertension and may resemble, to certain extent, essential hypertension in humans. In addition to using WKY rats as a control strain for SHR, SD rats were also utilized because their genetic background is closer to the SHR (42). Furthermore, young (6 wk old) normotensive SHR were also used as controls in this study to rule out the possibility that the observed change in GABAergic inputs and GABA receptor function in adult SHR is due to differences in genetic background.

It is well established that GABAA receptor-mediated inhibitory GABAergic inputs tonically control the excitability of PVN neurons. In this regard, microinjection of bicuculline into the PVN profoundly increases the arterial blood pressure, sympathetic nerve activity, and plasma norepinephrine in both conscious and anesthetized rats (29, 36, 41). Also, bicuculline increases the excitability of the PVN-RVLM neurons in anesthetic-free brain slices (27). In this study, we found that bicuculline significantly increased the excitability of all labeled PVN neurons in normotensive SD, WKY, and 6-wk-old normotensive SHR. However, in hypertensive 13-wk-old SHR, bicuculline increased the excitability in only 39% of labeled

Fig. 4. Effect of CGP-55845 (CGP) on firing activity of labeled PVN neurons in SHR. A, top: effect of 1 μM CGP on firing activity of labeled PVN neuron in 6-wk-old SHR. A, bottom: firing activity of same cell during control, application of CGP, and washout. B, top: effect of 1 μM CGP on firing activity of labeled PVN neuron in 13-wk-old SHR. B, bottom: spontaneous firing activity of same cell during control, application of CGP, and washout.

Fig. 5. Effect of CGP on firing activity of labeled PVN neurons in SD, WKY, and SHR. n, number of cells. A: effect of CGP on firing activity of labeled PVN neurons in 6- and 13-wk-old SHR. Note that CGP increased firing activity in 12 of 16 labeled PVN neurons in 13-wk-old SHR but had no effect on firing activity in 6-wk-old SHR. B: effect of 1 μM CGP on firing activity of labeled PVN neurons in 6- and 13-wk-old SD and WKY rats. Note that 1 μM CGP had no effect on firing rate of PVN neurons in all groups. Data are means ± SE. *P < 0.05 compared with control; #P < 0.05 compared with 6-wk-old SD or WKY rats (Kruskal-Wallis ANOVA followed by Dunn’s post hoc test).
PVN neurons. Strikingly, bicuculline either decreased or had no effect on 59.3% of the labeled PVN neurons in 13-wk-old SHR. It has been shown that bicuculline-induced sympathoexcitatory response in the PVN may require activation of glutamate and angiotensin II type 1 receptors (7, 8). It remains uncertain whether alterations of glutamate and angiotensin receptors play a role in the decreased effect of bicuculline on the excitability of the presympathetic PVN neurons in SHR. Because bicuculline methiodide may increase the firing activity of PVN neurons through its effect on SK channels (13, 22), we determined the effect of apamin, a specific blocker of SK channels, on the firing activity of labeled PVN neurons. We found that apamin had no significant effect on the firing activity of labeled PVN neurons in both normotensive and hypertensive rats. Therefore, it is less likely that SK channels are involved in the effect of bicuculline on the firing activity of labeled PVN neurons in normotensive and hypertensive rats. These data strongly suggest that the GABAA receptor-mediated GABAergic inhibition of PVN-RVLM neurons is attenuated in hypertensive SHR. We directly examined the functional GABAA receptors of PVN-RVLM neurons at the single cell level in normotensive and hypertensive rats. We found that GABAA current, induced by puff application of GABA, was significantly reduced in 13-wk-old hypertensive SHR compared with all normotensive control groups. Hence, the lack of stimulating effect of bicuculline on the firing activity of most PVN-RVLM neurons is likely due to the fact that the function of the postsynaptic GABAA receptors on PVN-RVLM projec-

tion neurons is reduced in hypertension. We observed the heterogenous responses of the firing of labeled PVN neurons to bicuculline in 13-wk-old SHR. Notably, there was a consistent decrease in the amplitude of GABA-evoked GABAA currents in labeled PVN neurons in SHR. These data suggest that the degree of loss of GABAA receptors and the relative important role of GABAA receptors in tonic control of the firing activity of PVN neurons may be different among subpopulations of

![Fig. 7. GABA current induced by puff application of GABA in labeled PVN neurons in SD, WKY, and SHR. A: GABA current induced by puff application of 1 mM GABA recorded at holding potential of 0 mV. Dashed line indicates puff application of GABA. Note that peak amplitude of GABA current in 13-wk-old SHR was significantly lower than that in SD (6 and 13 wk old), WKY (6 and 13 wk old), and 6-wk-old SHR. Also, Bic (100 μM) completely abolished current elicited by GABA application. B: current density of GABA current in labeled PVN neurons in SD, WKY, and SHR. *p < 0.05 compared with other groups (Kruskal-Wallis ANOVA followed by Dunn’s post hoc test).](http://apjheart.physiology.org/)
PVN-RVLM neurons in hypertensive rats. Furthermore, the inhibitory effect of bicuculline on the firing activity of some PVN neurons in 13-wk-old SHR was unexpected. Because such an inhibitory effect was eliminated by a specific GABA_B receptor antagonist, it is possible that bicuculline stimulates GABAergic interneurons to increase GABA release onto PVN-RVLM projection neurons, which subsequently inhibits the firing activity of these neurons through GABA_B receptors in SHR. Additionally, we found that both the frequency and amplitude of sIPSCs of the PVN neurons were significantly reduced in 13-wk-old SHR than those in normotensive control groups. The decrease in the frequency of sIPSCs in PVN neurons in SHR may reflect a reduced presynaptic GABA release. However, because the postsynaptic GABA_A current of PVN neurons was substantially reduced in SHR, the decrease in both the amplitude and frequency of sIPSCs could also be explained by the impaired function of postsynaptic GABA_A receptors in SHR. These electrophysiological data provide further functional evidence that the GABAergic synaptic input to the PVN-RVLM neurons is decreased in SHR. It is not clear whether the synaptic and/or extrasynaptic GABA_A receptors are altered in SHR. Because the frequency and amplitude of GABAergic sIPSCs are decreased in SHR, it suggests that at least the synaptic GABA_A receptor on PVN-RVLM neurons is reduced in SHR.

Nevertheless, conflicting information exists about the changes in GABAergic system in the PVN in hypertension. For instance, although GABA_A receptor binding sites are significantly decreased in the PVN of SHR (23), there appears to be no difference in the PVN GABA_A receptor binding between renal-wraped and sham-operated rats (18). Furthermore, the initial study by Martin and Haywood (30) shows that microinfusion of bicuculline into the PVN has little effect on blood pressure in rats with renal hypertension. More recent work (18) by the same group, however, suggests enhanced GABAergic function in the PVN in a rat model of renal hypertension. It is possible that GABA_A function in the PVN may be reduced during the onset of renal hypertension (4 days after the establishment of hypertension) (30). However, in chronic renal hypertension (3 wk after hypertension), the GABA_A receptor function may be potentiated in the PVN as an adaptation to the high blood pressure (18). Our data suggest that attenuated GABA_A function of PVN-RVLM neurons is present in 13-wk-old SHR, which likely represent an established state of hypertension (5 wks since initial hypertension). There are currently no clear explanations for these inconsistent results. It is important to recognize that different models of hypertension (renal hypertension vs. SHR) were used in these two studies. Also, the GABA_A function in the PVN was evaluated by using very different techniques. In this regard, the present electrophysiological study was conducted at the single cell level in brain slices in vitro, whereas the study by Haywood et al. (18) used receptor binding and microinjection of bicuculline in the whole animal. Further studies are required to determine whether the GABA_A function in the PVN in the control of vasomotor tone is attenuate in 13-wk-old SHR so that the physiological significance of the in vitro finding can be established.

In contrast to the reduction of GABA_A receptor function, we found that the GABA_B receptor function in the control of the firing activity of PVN-RVLM projection neurons was potenti- ated in hypertensive 13-wk-old SHR. Blockade of GABA_B receptors with CGP-55845 had no significant effect on the firing activity of labeled PVN neurons in all normotensive groups. These data suggest that GABA_B receptors normally do

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Fig. 8. Spontaneously inhibitory postsynaptic currents (sIPSCs) of labeled PVN neurons in normotensive SD, WKY, and SHR. A: sIPSCs recorded from labeled PVN neurons in normotensive 13-wk-old SD and WKY and 6-wk-old SHR and hypertensive 13-wk-old SHR. B and C: frequency and amplitude, respectively, of labeled PVN neurons in 13-wk-old SD (n = 10 cells) and WKY (n = 9 cells) and 6 (n = 10 cells)- and 13-wk-old (n = 10 cells) SHR. Note that both frequency and amplitude of sIPSCs were reduced in 13-wk-old SHR. Data are means ± SE. *P < 0.05 compared with control (Kruskal-Wallis ANOVA followed by Dunn’s post hoc test).
not play a significant role in tonic GABAergic control of the excitability of PVN-RVLM neurons in normotensive rats. However, in hypertensive 13-wk-old SHR, CGP-55845 significantly increased the excitability in 75% of the labeled PVN neurons tested. This suggests that tonic GABAergic inhibition of PVN presympathetic neurons is mediated mainly by GABA_A receptors in hypertensive 13-wk-old SHR. Collectively, these electrophysiological data suggest that GABA_B receptors play a significantly greater role in GABAergic inhibition of PVN-RVLM projection neurons in hypertensive SHR. Notably, increased GABA_A and decreased GABA_B receptor function has been observed in the nucleus of the solitary tract in a rat model of renal hypertension (33). The inhibitory effect of bicuculline on some labeled PVN cells in 13-wk-old SHR is completely unexpected. It is possible that bicuculline may excite the inhibitory GABAergic interneurons, which then indirectly inhibit PVN-RVLM output neurons through GABA_A receptors. In support of this hypothesis, we found that the GABA_A receptor antagonist CGP-55845 completely abolished the inhibitory effect of bicuculline on these cells. Therefore, the GABA_B receptors probably mediate the inhibitory effect of bicuculline on these PVN projection neurons in hypertensive rats.

The molecular mechanisms underlying the plasticity of GABA_A and GABA_B receptors in the PVN of hypertensive rats are not clear. The reduction in GABA_A receptor function in hypertension may result from an alteration of the receptor number and/or its affinity, phosphorylation status of the receptor, and receptor subunit composition (35). Also, the neurotransmitter glutamate can modulate the function of GABA_A and GABA_B receptors in the brain (9, 32). N-methyl-D-aspartate suppresses the GABA_A current through dephosphorylation of GABA_A receptors, an effect mediated by Ca^{2+}-dependent phosphatase calcineurin (9). However, little is known how the GABA_A receptor function is upregulated in the PVN presympathetic neurons in hypertension. Nociceptive inputs from the hindlimb increase the expression of the GABA_B mRNA in dorsal root ganglion neurons and the lumbar spinal dorsal horn (32), suggesting that increased glutamatergic synaptic inputs can upregulate GABA_A receptors.

In summary, our study demonstrates that there is a decrease in GABA_A but an increase in GABA_B receptor function in GABAergic control of PVN-RVLM neurons in hypertensive SHR. This functional change in the GABAergic input may be responsible for increased firing activity of PVN presympathetic neurons and elevated sympathetic outflow in hypertension. A salient finding of this study is that the GABA_A receptor antagonist CGP-55845 completely abolished the inhibitory effect of bicuculline on these cells. Therefore, the GABA_B receptors probably mediate the inhibitory effect of bicuculline on these PVN projection neurons in hypertensive rats.

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