Plasticity of pre- and postsynaptic GABAB receptor function in the paraventricular nucleus in spontaneously hypertensive rats

De-Pei Li,1 Qing Yang,2 Hao-Min Pan,1 and Hui-Lin Pan2

1Department of Critical Care and 2Department of Anesthesiology and Pain Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Submitted 12 March 2008; accepted in final form 13 June 2008

HYPERTENSION IS OFTEN ASSOCIATED with elevated sympathetic nervous system excitability (1, 7, 20, 50). The paraventricular nucleus (PVN) of the hypothalamus plays an important role in the control of sympathetic outflow through projections to the intermediolateral cell column of the spinal cord and rostral ventrolateral medulla (15, 19, 43, 46, 49). Previous studies have suggested that the PVN is critically involved in the development and maintenance of hypertension. For example, lesions of the PVN attenuate the development of hypertension and lower the blood pressure in spontaneously hypertensive rats (SHR) (12). However, the cellular mechanisms regulating the augmented sympathetic outflow in hypertension are not fully known.

γ-Aminobutyric acid (GABA) is a ubiquitous inhibitory neurotransmitter in the central nervous system, including the PVN. The inhibitory actions of GABA in the PVN are mediated primarily through ionotropic GABA_A receptors, which are ligand-gated chloride channels, and metabotropic GABA_B receptors, which are G protein-coupled receptors. GABA_B receptors are distributed in both presynaptic and postsynaptic sites in the hypothalamus (33, 37, 38). Previous studies have shown that the GABA_B receptor function is upregulated in the hypothalamus in hypertension. For example, microinjection of the GABA_B receptor agonist baclofen into the ventromedial hypothalamus produces a larger decrease in blood pressure, heart rate, and sympathetic nerve activity in SHR than in Wistar-Kyoto (WKY) rats (51). Also, microinjection of baclofen into the PVN produces a greater inhibitory effect on sympathetic outflow in SHR than in normotensive WKY rats (29). Furthermore, the GABA_B receptor antagonist CGP-55845 significantly increases the firing activity of PVN presynaptic neurons in SHR but not in WKY rats, suggesting that the GABA_B receptor is tonically involved in the regulation of the excitability of PVN output neurons in SHR (28). Although these studies provide evidence that GABA_B receptor function is enhanced in the hypothalamus in SHR, it remains unclear whether upregulation of GABA_B receptors occurs pre- or postsynaptically in the PVN in SHR.

The GABA_B receptor is a heterodimeric complex composed of a single GABA_B1 subunit for membrane ligand binding and a single GABA_B2 subunit for intracellular signaling through G proteins (4, 6, 22, 23). GABA_B receptors are G protein-coupled receptors, which can hyperpolarize the neurons by activating inwardly rectifying K⁺ channels (32, 39, 45). On the other hand, activation of presynaptic GABA_B receptors inhibits both GABAergic and glutamatergic inputs to spinally projecting PVN neurons (11). To study whether upregulation of GABA_B receptors in the PVN of SHR occurs pre- or postsynaptically, we determined the possible functional changes in pre- and postsynaptic GABA_B receptors in spinally projecting PVN neurons in SHR by using in vivo retrograde labeling and in vitro whole cell recordings in brain slices.

MATERIALS AND METHODS

Animals. Male WKY rats and SHR (11–13 wk old; Harlan, Indianapolis, IN) were used in this study. The surgical procedures and 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.
experimental protocols were approved by the Institutional Animal Care and Use Committee of The University of Texas M. D. Anderson Cancer Center and conformed to the National Institutes of Health guidelines on the ethical use of animals. The blood pressure was measured in each rat by using a noninvasive tail-cuff system (model 29-SSP; IITC Life Science, Woodland Hills, CA). We measured blood pressure every day for at least 1 wk before the electrophysiological experiments.

Retrograde labeling of spinally projecting PVN neurons. Rats were anesthetized by intraperitoneal injection of a mixture of ketamine (70 mg/kg) and xylazine (6 mg/kg), and the spinal cord at the T1 to T4 level was exposed through dorsal laminectomy. A glass pipette (Drummond Scientific, Broomall, PA) filled with rhodamine-labeled fluorescent microsphere suspension (FluoSpheres, 0.04 μm; Molecular Probes, Eugene, OR) was positioned at 500 μm from the midline and 500 μm below the surface of the spinal cord with a micromanipulator. FluoSpheres were pressure-ejected (50 nl, Nanojector II; Drummond Scientific) bilaterally into the intermediolateral cell column region of the spinal cord in three or four separate injections. The microinjection was monitored under a surgical microscope. After injection, the rats were returned to their cages to recover for 3–5 days to permit FluoSpheres to be transported to the PVN. After surgery, rats were treated prophylactically with an antibiotic (enrofloxacin; 5 mg/kg subcutaneously daily for 3 days) and an analgesic (buprenorphine; 0.2–0.5 mg/kg subcutaneously every 12 h for 2 days).

Slice preparation. Hypothalamic slices containing the PVN were prepared from the FluoSphere-injected rats. Briefly, under 2% isoflurane anesthesia, the rat was quickly decapitated, and the brain was immediately removed and placed in ice-cold 95% O2-5% CO2-saturated artificial cerebral spinal fluid (aCSF). A tissue block containing the hypothalamus was glued onto the stage of a vibrating microtome (Technical Product International, St. Louis, MO). Coronal hypothalamic slices containing the PVN were cut 300 μm thick, as described previously (11, 28). The slices were then transferred to a storage chamber to incubate in the aCSF continuously gassed with 95% O2-5% CO2 at 34°C for at least 1 h before electrophysiological recordings were performed. The aCSF solution contained (in mM) 124.0 NaCl, 3.0 KCl, 1.3 MgSO4, 2.4 CaCl2, 1.4 NaH2PO4, 10.0 glucose, and 26.0 NaHCO3.

Electrophysiological recordings. Whole cell voltage-clamp recordings were performed in labeled PVN neurons in brain slices. A slice was placed in a recording chamber (Warner Instruments, Hamden, CT) and held to the bottom of the chamber by nylon mesh attached to a U-shaped stainless steel weight. The recording chamber was continuously perfused (3 ml/min) with aCSF 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. The labeled PVN neurons were briefly identified by using an upright microscope (BX51WI; Olympus, Tokyo, Japan) with a combination of epifluorescence illumination and differential interference contrast optics. The recording electrode was pulled from borosilicate capillaries (1.2-mm outer diameter, 0.68-μm inner diameter; World Precision Instruments, Sarasota, FL) by using a micropipette puller (P-97; Sutter Instruments, Novato, CA). The resistance of the pipette was 3–7 MΩ when it was filled with internal solution containing (in mM) 110.0 Cs2SO4, 2.0 MgCl2, 0.1 CaCl2, 1.1 EGTA, 10.0 HEPES, 2.0 MgATP, and 0.3 Na2GTP (pH was adjusted to 7.25 with 1 M KOH; 280–300 mosmol/kgH2O). After a gigaohm seal was formed, brief negative pressure was used to obtain the whole cell configuration. Signals were processed using an Axopatch 200B amplifier (Molecular Devices, Foster City, CA), filtered at 1–2 kHz, digitized at 20 kHz using Digidata 1320A (Molecular Devices), and saved to the hard drive of a computer.

The spontaneous firing activity of labeled PVN neurons was recorded using the whole cell current-clamp technique. Recording of the firing activity of labeled PVN neurons began about 5 min after the whole cell access was established and the firing activity reached a steady state. Spontaneous firing activity was recorded from the labeled neurons with resting membrane potentials of −50 mV or lower and with action potential overshoot >10 mV.

To assess the postsynaptic GABA<sub>B</sub> receptor function, we recorded the GABA<sub>B</sub> currents induced by direct puff application of baclofen to labeled PVN neurons. To record the postsynaptic current mediated by GABA<sub>B</sub> receptors, we used a pipette solution containing (in mM) 130.0 potassium gluconate, 10.0 NaCl, 1.6 MgCl<sub>2</sub>, 0.1 EGTA, 10 HEPES, 2 MgATP, and 0.3 Na2GTP. The pH was adjusted to 7.25 with 1 M KOH (280–300 mosmol/kg H2O). Puff application of baclofen was done using a Pressure System IIe (Toohey, Fairfield, NJ). The puff pipette (~5-μm tip diameter) was placed ~150 μm away from the recorded cell (30). Positive pressure (~4 lb./in.² and 400-ms duration) was applied to eject baclofen solution onto the recording cell to elicit a current at a holding potential of ~60 mV in the presence of 1 μM tetrodotoxin.

To assess the presynaptic action of baclofen, we recorded the spontaneous and miniature excitatory postsynaptic currents (eEPSCs) and miniature inhibitory postsynaptic currents (sIPSCs), respectively, at a holding potential of ~70 mV in the presence of 10 μM bicuculline. The spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded at a holding potential of 0 mV in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM). The mIPSCs were recorded in the presence of 20 μM CNQX and 1 μM tetrodotoxin. To eliminate the possible postsynaptic action mediated by GABA<sub>B</sub> receptors, a general G protein inhibitor, guanosine 5′-O-(2-thiodiphosphate) (GDPβS; 1 mM), was added into the pipette solution in some experiments (36). A sodium channel blocker, lidocaine N-ethylisobromide (QX-314; 10 mM), was included in the pipette solution to suppress action potential generation.

All the drugs were freshly prepared in aCSF before the recording and delivered by syringe pumps at final concentrations. Baclofen (11, 24) and CGP-55845 (5) were used as GABA<sub>B</sub> receptor agonist and antagonist, respectively. MK-801 (54) and 2-amino-5-phosphonopentanoic acid (AP5) (30) were used to block N-methyl-D-aspartic acid (NMDA) receptors. Bicuculline methiodide and CNQX disodium were used to block GABA<sub>B</sub> and non-NMDA receptors, respectively. The drugs were purchased from Sigma (St. Louis, MO) except for tetrodotoxin and QX-314, which were purchased from Alomone Labs (Jerusalem, Israel).

Data analysis. Data are means ± SE. The firing activity and membrane potentials were analyzed over a period of 5–30 min before, during, and after drug application. The junction potential was corrected off-line based on the composition of the internal and external solution used for the recordings. The amplitude of baclofen-induced currents was analyzed using Clampfit 8.2 (Molecular Devices). The GABA<sub>B</sub> current was presented as current density normalized by cell capacitance. The firing rate, amplitude, and frequency of sEPSCs and sIPSCs were analyzed off-line using a peak detection program (MiniAnalysis; Synaptosoft, Leonia, NJ). Events were detected by setting a threshold above the noise level. The effect of drugs on the firing rate, GABA<sub>B</sub> current, amplitude, and the frequency of sEPSCs and sIPSCs was analyzed using ANOVA with Dunn’s post hoc test. Two-way ANOVA was used to compare the difference of the baclofen-induced inhibitory effect on the frequency and amplitude of sEPSCs and sIPSCs between SHR and WKY rats. P < 0.05 was considered to be statistically significant.

**RESULTS**

The mean systolic arterial blood pressure of 11- to 13-wk-old SHR (220 ± 12 mmHg, n = 29 rats) was significantly higher than that of age-matched WKY rats (132 ± 11 mmHg, n = 32 rats). Whole cell voltage-clamp and current-clamp recordings were performed on a total of 150 FluoSphere-labeled PVN cells (83 from SHR and 67 from WKY rats). The resting membrane potentials, input resistance, cell capacitance,
and access resistance of labeled PVN neurons were not significantly different between WKY rats and SHR (Table 1).

**Effect of baclofen on spontaneous firing activity of labeled PVN neurons in WKY rats and SHR.** We first determined the effect of the GABA<sub>B</sub> receptor agonist baclofen on the spontaneous firing activity of labeled PVN neurons. The majority of the labeled PVN neurons displayed spontaneous discharges in WKY rats (12 of 15 cells, 75.0%) and SHR (11 of 14 cells, 78.6%). The baseline firing rate of PVN neurons was significantly higher in SHR (2.3 ± 0.5 Hz, n = 11 cells) than in WKY rats (0.8 ± 0.3 Hz, n = 12, P < 0.05). We tested the effect of baclofen on the firing activity only in labeled neurons with spontaneous activity. In WKY rats, bath application of 10 μM baclofen for 2 min significantly decreased the firing activity of labeled PVN neurons in 8 of 12 cells (Fig. 1). The membrane potential was hyperpolarized from −59.6 ± 1.5 to −64.3 ± 2.5 mV (P < 0.05) in these eight cells. The firing rate of the remaining four cells did not change significantly (1.6 ± 0.7 vs. 1.5 ± 0.8 Hz) in response to 10 μM baclofen. In SHR, bath application of 10 μM baclofen for 2 min significantly reduced the firing activity of labeled PVN neurons in 9 of 11 cells (Fig. 1). The membrane potential was hyperpolarized from −57.6 ± 1.3 to −63.8 ± 2.6 mV (P < 0.05) in these nine cells. Baclofen failed to change the firing activity in the remaining two cells (from 2.6 to 2.5 Hz in 1 cell and 1.6 to 1.5 Hz in another cell). However, the magnitude of the baclofen-induced decrease in the firing activity of PVN neurons was significantly larger in SHR than in WKY rats (Fig. 1D).

**Baclofen-induced postsynaptic currents in labeled PVN neurons in WKY rats and SHR.** We next determined the postsynaptic current induced by puff application of baclofen to labeled PVN neurons at a holding potential of −60 mV in WKY rats and SHR. Since the capacitance of labeled neurons varied from 32 to 48 pF, we normalized the current by cell capacitance. Puff application of 10 μM baclofen to labeled PVN neurons did not evoke detectable outward currents in WKY rats (n = 4 cells) and SHR (n = 4 cells) (data not shown). When the baclofen concentration was increased to 100 μM, it evoked an outward current in 7 of 13 cells (53.9%) in WKY rats and 12 of 14 labeled PVN neurons (85%) in age-matched SHR (Fig. 2). The baclofen-sensitive current density (normalized by cell capacitance) was significantly higher in SHR than in WKY rats (Fig. 2C).

To examine whether baclofen-induced outward current was mediated through G proteins, we included 1 mM GDPβS, a competitive inhibitor of G proteins, in the internal pipette solution. When 1 mM GDPβS was included in the pipette solution, puff application of 100 μM baclofen failed to produce any outward current in all labeled PVN neurons in both WKY rats (n = 8 cells) or SHR (n = 6 cells) (Fig. 2B). We also determined whether the outward current elicited by baclofen in labeled PVN neurons was mediated by GABA<sub>B</sub> receptors. In a separate group of labeled PVN neurons, neurons with baclofen-sensitive currents were initially identified by puff application of 100 μM baclofen. Bath application of 2 μM CGP-55845, a specific GABA<sub>B</sub> receptor antagonist (44), for 4–5 min completely blocked the baclofen-induced outward current in all neurons tested in both WKY rats (n = 5 cells) and SHR (n = 7 cells) (Fig. 2D).

We then determined whether the increased baclofen-sensitive currents in labeled PVN neurons in SHR resulted from augmented glutamatergic inputs (3, 30, 35). Puff application of 100 μM baclofen elicited an outward current of 25.2 ± 4.36 pA in 13 of 15 labeled PVN neurons in SHR. The slice was then treated with NMDA receptor antagonists AP5 (100 μM, n = 6) or MK-801 (20 μM, n = 7) for 5 min. It has been shown that the NMDA receptor is completely blocked by 100 μM AP5 or 20 μM MK-801 (30, 54). Bath application of AP5 (100 μM) or MK-801 (20 μM) did not produce significant transmembrane current in these labeled PVN neurons in WKY rats and SHR. Puff application of 100 μM baclofen produced similar outward currents in these neurons in the presence of AP5 or MK-801 (Fig. 3).

**Effect of baclofen on sEPSCs in labeled PVN neurons in WKY rats and SHR.** To assess the function of presynaptic GABA<sub>B</sub> receptors on glutamatergic afferent terminals in the PVN in WKY rats and SHR, we tested the effect of baclofen on glutamatergic sEPSCs. Inclusion of the G protein inhibitor GDPβS (1 mM) in the internal pipette solution had no significant effect on the baseline frequency (2.6 ± 0.6 vs. 2.7 ± 0.8 Hz in WKY rats and 5.5 ± 0.8 vs. 5.6 ± 0.9 Hz in SHR) and amplitude (16.3 ± 0.8 vs. 16.2 ± 1.2 pA in WKY rats and 16.5 ± 1.0 vs. 17.5 ± 0.9 pA in SHR) of sEPSCs in either WKY rats or SHR. In WKY rats, bath application of 1 to 20 μM baclofen dose-dependently reduced the frequency, but not the amplitude, of sEPSCs in all of the labeled PVN neurons (Fig. 4, A–E, n = 9 cells). The sEPSCs were completely blocked by 20 μM CNQX (Fig. 4A). The decrease in the frequency of sEPSCs occurred ~2.0 ± 0.4 min after perfusion of baclofen into the recording chamber. In age-matched SHR, baclofen also dose-dependently decreased the frequency, but not the amplitude, of sEPSCs in all of the labeled PVN neurons (Fig. 4, n = 8 cells). In both WKY rats and SHR, the cumulative probability analysis of sEPSCs revealed that the distribution pattern of the interevent interval of sEPSCs was shifted toward the right in response to baclofen. However, the baclofen-induced inhibition of the frequency of sEPSCs at different concentrations was significantly greater in SHR than in WKY rats (Fig. 4F). In a separate group of labeled PVN neurons, blocking GABA<sub>B</sub> receptors with 2 μM CGP-55845 had no significant effect on the basal frequency and amplitude of sEPSCs in either WKY rats (n = 7 cells) or SHR (n = 8 cells) (Fig. 4G).

Bath application of 1 μM tetrodotoxin had no significant effect on the frequency (2.5 ± 0.4 vs. 2.2 ± 0.5 Hz in WKY rats and 4.9 ± 0.6 vs. 4.6 ± 0.5 Hz in SHR) and amplitude

| Table 1. Electrophysiological properties of labeled PVN neurons in WKY and SHR |
|-----------------|---------|-----------------|-----------------|-----------------|
|                 | No. of Cells | Membrane Potential, mV | Input Resistance, MΩ | Cell Capacitance, pF | Access Resistance, MΩ |
| WKY             | 42        | −59.1±4.5        | 492.6±25.6        | 29.7±1.5         | 17.3±1.5          |
| SHR             | 60        | −57.9±3.6        | 516.1±18.9        | 31.9±1.2         | 16.2±1.2          |

Values are means ± SE measured in paraventricular neurons (PVN) of Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR).
Effect of baclofen on sIPSCs in labeled PVN neurons in WKY rats and SHR. To further determine the function of presynaptic GABA<sub>B</sub> receptors on GABAergic terminals in the PVN in WKY rats and SHR, we tested the effect of baclofen on GABAergic sIPSCs. Inclusion of GDP<sub>S</sub> (1 mM) in the recording pipette solution did not change the baseline frequency (3.2 ± 0.4 vs. 2.9 ± 0.6 Hz in WKY rats and 1.8 ± 0.4 vs. 1.9 ± 0.6 Hz in SHR) and amplitude (40.6 ± 4.9 vs. 41.3 ± 4.5 pA in WKY rats and 25.5 ± 3.2 vs. 24.4 ± 2.9 pA in SHR) of GABAergic sIPSCs in either WKY rats or SHR. Bath application of 1, 5, 10, and 20 μM baclofen reduced the frequency of sIPSCs in a dose-dependent fashion without an effect on the amplitude of sIPSCs in both WKY rats and SHR (Fig. 5, A–F). The sIPSCs were completely blocked by 10 μM bicuculline in both WKY rats and SHR (Fig. 5A). The cumulative probability analysis of sIPSCs showed that the distribution pattern of the interevent interval of sIPSCs was shifted toward the right in response to baclofen. The magnitude of inhibition of the sIPSC frequency by different concentrations of baclofen in labeled PVN neurons was significantly smaller in SHR than in WKY rats (Fig. 5F). In a separate group of labeled PVN neurons, blocking of GABA<sub>B</sub> receptors with 2 μM CGP-55845 had no significant effect on the basal frequency and amplitude of sIPSCs in either WKY rats or SHR (Fig. 5G).

Bath application of 1 μM tetrodotoxin did not significantly change the frequency (3.1 ± 0.6 vs. 2.7 ± 0.6 Hz in WKY rats (18.5 ± 5.1 vs. 17.4 ± 4.6 pA in WKY rats and 17.9 ± 4.5 vs. 18.1 ± 5.2 pA in SHR) of sEPSCs in these labeled PVN neurons in WKY rats (n = 7 cells) and SHR (n = 8 cells).
DISCUSSION

Our finding that baclofen produced a greater inhibition of the firing activity of PVN neurons in SHR than in WKY rats is consistent with previous studies showing that the hypothalamic GABA<sub>B</sub> receptor function is enhanced in the hypothalamus in SHR (28, 29, 51). To assess whether the postsynaptic GABA<sub>B</sub> receptor function is altered in spinally projecting PVN neurons in SHR, we compared postsynaptic GABA<sub>B</sub> receptor currents evoked by puff application of baclofen directly to labeled PVN neurons in WKY rats and SHR. We found that the amplitude of baclofen-elicited outward currents in labeled PVN neurons was

![Fig. 2. Baclofen-sensitive currents in labeled PVN neurons in WKY rats and SHR. A: original tracings showing currents elicited by puff application of 100 μM baclofen to labeled PVN neurons in WKY rat and SHR. Arrows indicate puff application of baclofen. B: raw tracings showing undetectable current by puff application of 100 μM baclofen when 1 mM GDPβS was included in the recording pipette solution in WKY rats and SHR. C: summary data showing the baclofen-sensitive current elicited by puff application of 100 μM baclofen in labeled PVN neurons in WKY rats and SHR. D: summary data showing baclofen-sensitive currents in the absence and presence of 2 μM CGP-55845 (CGP) in labeled PVN neurons in WKY rats and SHR. Data are means ± SE. *P < 0.05 compared with the corresponding value in WKY rats.](image)

and 1.8 ± 0.4 vs. 1.6 ± 0.4 Hz in SHR) and amplitude (38.8 ± 4.2 vs. 37.6 ± 4.3 pA in WKY rats and 24.5 ± 3.8 vs. 22.7 ± 3.5 pA in SHR) of sIPSCs in these labeled PVN neurons in WKY rats (n = 7 cells) and SHR (n = 6 cells).

![Fig. 3. Lack of effect of N-methyl-D-aspartic acid (NMDA) receptor antagonists on baclofen-elicited currents in labeled PVN neurons in SHR. A: raw tracings showing that puff application of 100 μM baclofen elicited outward currents in the absence and presence of 2-amino-5-phosphonopentanoic acid (AP5) in a labeled PVN neuron in SHR. B and C: summary data showing baclofen-elicited currents in labeled PVN neurons in SHR before and after blockade of NMDA receptors with AP5 (B) or MK-801 (C).](image)
significantly greater in SHR than in WKY rats. Bath application of CGP-55845, a specific GABAB receptor antagonist (37, 44), or inhibition of G proteins with intracellular dialysis of GDP/βS completely blocked baclofen-elicited outward currents in all PVN neurons tested. These data suggest that the postsynaptic GABAB receptor function is upregulated in the PVN presympathetic neurons in SHR. It is likely that this upregulation of postsynaptic GABAB receptor function contributes to the potentiated inhibitory effect of baclofen on the firing activity of spinally projecting PVN neurons and sympathetic vasomotor tone in SHR (28, 29).

However, little is known about how the postsynaptic GABAB receptor function is augmented in the PVN in hypertension. Previous studies have shown that NMDA receptor activation can increase the receptor gene expression and postsynaptic GABAB receptor function in the spinal cord and hippocampus (3, 13, 35). Since the glutamatergic input in the PVN is enhanced in SHR (27, 30), it is possible that the enhanced glutamatergic input in SHR causes more Ca²⁺ influx into postsynaptic PVN neurons, which could upregulate postsynaptic GABAB receptors through Ca²⁺-dependent phosphorylation or dephosphorylation mechanisms. However, we found that acutely blocking NMDA receptors with AP5 or MK-801 had no significant effect on the baclofen-elicited currents in labeled PVN neurons in SHR. Because acute blockade of NMDA receptors may not be sufficient to inhibit the downstream signaling cascade leading to increased expression of GABAB receptors by enhanced glutamatergic inputs in
we cannot rule out the possibility that chronic blockade of NMDA receptor is required to affect GABAB receptors in SHR. Furthermore, increased angiotensin II concentration and angiotensin receptors in the PVN in SHR (18, 42) also could play a role in the upregulation of GABAB receptors. The physiological significance of increased function of postsynaptic GABAB receptors in PVN presympathetic neurons in hypertension is not fully known. The excitability of PVN presympathetic neurons is tonically controlled by the GABAergic input and both GABAA and GABAB receptors. We have shown previously that the GABAA receptor function in the PVN is attenuated, which contributes to increased firing activity of PVN presympathetic neurons in SHR (29). The GABAB receptor can function as a “brake” to reduce the NMDA action in CA1 neurons (40). Hence, upregulation of postsynaptic GABA_B receptors in the PVN may represent a compensatory response to the reduced GABA_A receptor function and enhanced glutamatergic input to these neurons in SHR and serve to dampen the increased sympathetic outflow in hypertension.

The firing activity of PVN neurons is regulated by excitatory and inhibitory synaptic inputs such as glutamatergic and GABAergic inputs from inside the PVN, suprachiasmatic nucleus, and subfornical organ (2, 14, 16, 52). Previous studies have shown that the GABAergic system may be impaired in the hypothalamus in hypertension (26, 28, 34, 51). Consistent with these previous studies, we found that the basal frequency of GABAergic sIPSCs of labeled PVN neurons was significantly lower in SHR than in WKY rats. In contrast, the basal...
frequency of glutamatergic sEPSCs of labeled PVN neurons was much higher in SHR than in WKY rats (30). It is possible that both increased glutamatergic input and decreased GABAergic input contribute to the high basal firing activity of PVN presynaptic neurons and increased sympathetic vasmotor tone in SHR (28–30). We found that tetrodotoxin had no significant effect on the basal frequency of sIPSCs and sEPSCs in both WKY rats and SHR, which is consistent with the findings from previous studies (10, 11, 21). These results suggest that changes in sIPSCs and sEPSCs in our thin slice preparation are probably caused by altered synaptic transmitter release at the terminal rather than the activity of glutamatergic and GABAergic neurons. However, it is uncertain whether altered synaptic release of glutamate and GABA is due to changes in the release probability or the density of presynaptic terminals in the PVN in SHR. Notably, blocking of GABA<sub>B</sub> receptors with CGP-55845 alone did not significantly alter the frequency and amplitude of sIPSCs and sEPSCs in labeled PVN neurons in either WKY rats or SHR. Thus it is unlikely that the observed difference in the baseline sIPSCs and sEPSCs between SHR and WKY rats is the result of differential activation of GABA<sub>B</sub> receptors at the GABAergic and glutamatergic terminals.

Activation of presynaptic GABA<sub>B</sub> receptors inhibits GABAergic and glutamatergic synaptic inputs to spatially projecting PVN neurons (11). We determined the presynaptic action of baclofen by using the following strategies. According to the quantal hypothesis, presynaptic action can affect the probability of neurotransmitter release. Analysis of the frequency and amplitude of IPSCs or EPSCs has been commonly used to determine pre- and postsynaptic loci of the agents (21, 41, 47). Furthermore, to determine the presynaptic effect of baclofen, we blocked the postsynaptic effect of baclofen by including GDPβS in the pipette internal solution (36). In this study, we observed that baclofen produced a greater inhibitory effect on the frequency of glutamatergic sEPSCs in SHR than in WKY rats. In contrast, baclofen caused a smaller inhibitory effect on the frequency of sIPSCs in SHR than in WKY rats. These findings suggest that the presynaptic GABA<sub>B</sub> receptor function in the control of glutamatergic and GABAergic synaptic inputs to the PVN presynaptic neurons is differentially affected in SHR. The increased GABA<sub>B</sub> receptor function on glutamatergic terminals and reduced GABA<sub>B</sub> receptor function on GABAergic terminals could contribute to the enhanced inhibitory effect of baclofen on the firing activity of PVN neurons and sympathetic vasmotor tone in SHR. We have shown that microinjection of the GABA<sub>B</sub> receptor antagonist into the PVN produces a sympathoexcitatory effect in SHR (29). However, we found that GABA<sub>B</sub> receptor antagonist had no significant effect in the in vitro experiments. It is possible that the lack of the GABA<sub>B</sub> receptor antagonist effect on synaptic glutamate and GABA release is because tonic activation of GABA<sub>B</sub> receptors by endogenously released GABA is reduced in the slice preparation.

It is not clear whether different GABA<sub>B</sub> receptor subunits (i.e., GABA<sub>B1</sub> and GABA<sub>B2</sub>) are differentially expressed at glutamatergic and GABAergic terminals in the PVN in SHR. For example, in the hippocampus, both GABA<sub>B1</sub> and GABA<sub>B2</sub> subtypes are mainly present at glutamatergic axon terminals (25). Also, in the dorsal cochlear nucleus, GABA<sub>B1</sub> is primarily located at glutamatergic synapse but not GABAergic terminals (31). Furthermore, GABA<sub>B</sub> receptor subunits are differentially localized in the subcellular structures in glutamatergic and GABAergic synapses in the globus pallidus (9). Alternatively, changes in the downstream signaling also can preferentially alter the GABA<sub>B</sub> receptor function in glutamatergic and GABAergic terminals in the PVN in SHR.

In summary, data from our study strongly suggest an upregulation of postsynaptic GABA<sub>B</sub> receptor function in PVN presynaptic neurons in SHR, an enhancement of presynaptic GABA<sub>B</sub> receptor control of glutamatergic inputs, and an attenuation of presynaptic GABA<sub>B</sub> receptor control of GABAergic inputs in the PVN in SHR. It has been shown that GABA<sub>B</sub> receptor-mediated depressor response and the GABA<sub>B</sub> mRNA level are increased in the nucleus of the solitary tract in SHR and renal-wrap model of hypertension (8, 17, 48, 53). However, the mechanisms underlying the plasticity of pre- and postsynaptic GABA<sub>B</sub> receptor function in hypertension remain to be determined. The enhanced GABA<sub>B</sub> control of the firing activity of the PVN neurons may represent a potential new target for the treatment of neurogenic hypertension.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants HL60026 and HL77400 and Scientist Development Grant 0635402N from the American Heart Association National Center.

REFERENCES


