Chronic Hypertension Enhances the Postsynaptic Effect of Baclofen in the Nucleus Tractus Solitarius

Weirong Zhang, Myrna Herrera-Rosales, Steve Mifflin

Abstract—Microinjection of the inhibitory neurotransmitter γ-aminobutyric acid B-subtype receptor agonist baclofen into the nucleus tractus solitarius increases arterial blood pressure and sympathetic nerve discharge. The baclofen-induced pressor response is enhanced in chronic hypertension. We hypothesized that a postsynaptic mechanism contributes to the enhanced responses to baclofen in hypertension. We investigated the postsynaptic effect of baclofen on second-order baroreceptor neurons, identified by 1,1′-dilinoleyl-3,3′,3′-tetra-methylindocarbocyanine, 4-chlorobenzenesulphonate labeling of the aortic nerve, in nucleus tractus solitarius slices from sham-operated normotensive and unilateral nephrectomized, renal-wrap hypertensive rats. After 4 weeks, arterial blood pressure was 153±7 mm Hg in hypertensive rats (n=9) and 93±3 mm Hg in normotensive rats (n=8; P<0.05). There was no difference in resting membrane potential (54.5±0.7 versus 53.3±0.6 mV) or input resistance (1.07±0.11 versus 1.03±0.11 GΩ) between hypertensive and normotensive neurons (both n=18). Baclofen induced a net outward current in nucleus tractus solitarius neurons in the presence of 1 μmol/L tetrodotoxin. The EC₅₀ of the baclofen effect was greater in normotensive cells (9.1±3.2 μmol/L; n=5) than hypertensive cells (3.0±0.5 μmol/L; n=7; P<0.05), and baclofen (10 μmol/L) induced a greater decrease in input resistance in hypertensive cells (61±2%; n=6) than in normotensive cells (45±4%; n=9; P<0.05). Both potassium and calcium channels were involved in the baclofen-evoked whole-cell current. The results suggest an enhanced postsynaptic response to activation of inhibitory neurotransmitter γ-aminobutyric acid B-subtype receptors in second-order baroreceptor neurons in the nucleus tractus solitarius in renal-wrap hypertensive rats. This enhanced inhibition could alter baroreflex function in chronic hypertension. (Hypertension. 2007;49[part 2]:659-663.)

Key Words: cardiovascular regulation ■ baroreceptor ■ baroreflex ■ hypertension ■ blood pressure

The nucleus tractus solitarius (NTS) is the first site of baroreceptor afferent integration within the central nervous system.¹² The synaptic transmission of baroreceptor afferents within the NTS is constantly modulated by both excitatory and inhibitory inputs mediated by many neurotransmitters, including the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Microinjection of baclofen, a selective GABA B-subtype (GABAB) receptor agonist, into the NTS results in an increase in arterial pressure, heart rate, and renal sympathetic nerve discharge,⁵⁻⁶ which are expected, because baclofen inhibits NTS neurons that integrate baroreceptor afferent inputs.⁵⁻⁶⁻⁸ This baclofen-induced pressor response is enhanced in several animal models of chronic hypertension, including the spontaneously hypertensive rat,⁹¹⁰ deoxycorticosterone salt–hypertensive rats,¹¹ and 1-kidney, renal wrap models of hypertension.⁶⁻⁸,¹² Baclofen can presynaptically inhibit glutamate release from afferent terminals and postsynaptically induce outward current to reduce neuronal excitability in the NTS.¹³ However, it is not known to what extent postsynaptic GABAB receptor-mediated inhibition contributes to the enhanced baclofen-induced pressor response in chronic hypertension.

Previous studies from this laboratory have demonstrated that renal-wrap hypertension is associated with increased GABAB receptor–mediated inhibition of baroreceptor-evoked discharge in NTS neurons⁸ and increased expression of GABAB receptor mRNA in the NTS.⁷ To clarify GABAB receptor–mediated cellular mechanisms in the neuronal adaptations to chronic hypertension, the present study investigated the postsynaptic effect of baclofen on NTS neurons receiving monosynaptic afferent inputs from baroreceptors and the influence of chronic hypertension on the postsynaptic response to baclofen. We addressed these questions using an in vitro patch-clamp method to directly investigate the postsynaptic effect of baclofen on second-order baroreceptor neurons in the NTS. The results demonstrated that, after chronic hypertension, second-order neurons showed enhanced postsynaptic responses to baclofen. This enhanced postsynaptic baclofen effect could contribute to the enhanced baclofen-induced pressor response observed in chronic hypertension.¹⁴

Methods

All of the experimental protocols in this work were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio.
Surgical Preparation for Labeling Aortic Nerve

Male Sprague–Dawley rats (100 to 125 g, Charles River, Wilmington, MA) were anesthetized with a combination of ketamine (75 mg/kg IP; Ft Dodge) and medetomidine (0.5 mg/kg IP; Pfizer). Under aseptic conditions, crystals of anterograde fluorescent dye 1,1′-dilinoleyl-3,3′,3′-tetra-methylindocarbocyanine, 4-chlorobenzenesulphonate ([DiA] D-3883, Molecular Probes) were gently applied unilaterally to the aortic nerve to visualize baroreceptor synaptic terminals and neurons receiving these synaptic contacts.15–17 The area was then embedded with silicone adhesive (Kwik-Sil, WPI). Anesthesia was terminated by atipamezole (1 mg/kg IP, Pfizer) at the conclusion of the surgical procedures. Postoperative analgesics (Nubaine, IM) were available as needed. The rats were allowed to recover for ≥1 week before performing renal wrap/sham surgery.

Chronic Hypertensive Model

Rats were anesthetized using the ketamine/mDETomidine described above. Hypertension was induced using a figure-8 renal wrap of 1 mm thick) were cut with a sapphire knife and placed in the femoral artery while the animal was under ketamine/mDETomidine anesthesia as described above. After a 2-day recovery period, the blood pressure of the conscious animal was measured by connecting the arterial catheter to a pressure transducer (Kobe) and displayed on a television monitor and an access resistance (DiA) D-3883, Molecular Probes) were gently applied unilaterally to the aortic nerve to visualize baroreceptor synaptic terminals and neurons receiving these synaptic contacts.15–17 The area was then embedded with silicone adhesive (Kwik-Sil, WPI). Anesthesia was terminated by atipamezole (1 mg/kg IP, Pfizer) at the conclusion of the surgical procedures. Postoperative analgesics (Nubaine, IM) were available as needed. The rats were allowed to recover for ≥1 week before performing renal wrap/sham surgery.

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rats than in HT rats (9.1±3.2 μmol/L, n=5 versus 3.0±0.5 μmol/L, n=7; P<0.05). During application of 10 μmol/L baclofen (Figure 1D), HT cells responded with a greater outward current than NT cells (32.6±4.2 pA, n=10 versus 16.3±2.3 pA, n=12; P<0.01). This concentration of baclofen elicited a greater reduction in input resistance in HT cells than in NT cells (39±2% of control, n=6 versus 55±4% of control, n=9; P<0.05).

Current–voltage relationships were investigated during application of 10 μmol/L of baclofen (Figure 2A). Application of 10 μmol/L of baclofen increased the current response to each voltage step. The baclofen equilibrium potential (\(E_b\)) was similar between HT and NT cells (Figure 2B; 77±3 mV, n=7 versus 74±2 mV, n=6; P>0.05). There was a significant increase in slope conductance in all of the second-order neurons during the 10 μmol/L of baclofen application, as calculated from the slope of current–voltage curve between -130 mV and 40 mV. However, HT cells had greater increase in conductance than NT cells (254±31%, n=6 versus 184±13%, n=7; P<0.05).

**GABA\(_B\) Receptors Mediate Baclofen Effect**

In 2 NT cells, application of 20 μmol/L of selective GABA\(_B\) receptor antagonist SCH 50911 did not induce discernible change on holding currents, indicating no tonic activation of postsynaptic GABA\(_B\) receptors in the brain slice from NT rats. To confirm the selective effect of baclofen on GABA\(_B\) receptors, coapplication of 10 μmol/L of baclofen with 20 μmol/L of SCH 50911 did not significantly alter holding current currents, suggesting that baclofen-evoked outward current was because of activation of GABA\(_B\) receptors. After washout, baclofen application induced outward currents of 13.9±1.4 pA.

**Ionic Mechanisms of Baclofen Effect**

Under our experimental conditions, the calculated potassium equilibrium potential was -98 mV, which was more negative than \(E_b\) presented above (74±2 mV; n=6). Thus, a separate group of 16 control NT rats was used to identify the ionic mechanisms of the baclofen-induced current. Application of 1 mmol/L of barium, a nonselective potassium channel blocker, increased the holding current by 1.4±0.5 pA. However, application of 10 μmol/L of barium increased the holding current by 3.9±1.4 pA, indicating that the remaining baclofen-induced current was because of activation of potassium channels.

**Figure 1.** Whole-cell patch clamp recording of second-order baroreceptor neurons in medial NTS. A, left photograph shows a DiO-labeled neuron viewed with fluorescence (left) and brightfield (right). B, recording showing the outward current induced by application of 30 μmol/L of baclofen. The horizontal bar represents a 2-min application time. C, dose–response curve of baclofen-induced outward currents in second-order baroreceptor neurons in the NTS. HT (n=7) rats had significantly lower EC\(_{50}\) than NT rats (n=5; P<0.05). D, baclofen at 10 μmol/L caused significantly greater outward currents in HT cells (n=10) than in NT cells (n=12). All of the experiments were performed in the presence of 1 μmol/L of TTX. **P<0.01, compared with NT neurons.

**Figure 2.** Baclofen alteration of current–voltage (I–V) relationship in second-order NTS neurons. A, current responses to a series of voltage steps (from -130 to -40 mV in 10-mV steps) applied before and at the peak of a baclofen-induced response. Notice the elevated holding current after baclofen application. B, baclofen at 10 μmol/L increased the slope of I–V curves. In HT cells (n=6), there was significantly greater increase in slope (254±31% vs 184±13%; P<0.05) than NT cells (n=7); C, the net baclofen-induced current determined by subtraction of these I–V values is displayed in panel B. All of the experiments were performed in the presence of 1 μmol/L of TTX.
blocker, induced a mild, apparently inward current of 6.9±2.1 pA (n=6) coupled with an increase in membrane resistance (136±9% of control; P<0.05). Coapplication of 10 µmol/L of baclofen with barium significantly reduced the evoked outward current in NTS neurons (8.0±1.2 pA versus 16.3±2.3 pA; P<0.05) and attenuated the baclofen-evoked reduction in input resistance (65±3% of control versus 55±4% of control; P<0.05). Furthermore, after application of 1 mmol/L barium, the $E_0$ shifted to more positive potentials (−63±8 mV; n=5; Figure 3A) than the $E_0$ under control condition (74±2 mV; n=6). These data suggest that the baclofen effect on second-order baroreceptor neurons in the NTS was largely mediated by potassium channels.

Baclofen has been reported to alter calcium currents. Baclofen decreases the action potential discharge of NTS neurons receiving baroreceptor afferent inputs and induces a pressor response. Activation of GABAB receptors with baclofen induces outward currents in second-order baroreceptor neurons in the NTS, which will decrease neuronal excitability. This GABAB receptor-mediated inhibitory effect was enhanced after chronic hypertension. Both potassium and calcium channels were involved in the baclofen-evoked currents in the NTS. Enhanced postsynaptic GABAB receptor function could contribute to the enhanced baclofen-induced pressor response observed in vivo in various animal models of chronic hypertension. Our present study did not examine presynaptic inhibition by baclofen. This component of baclofen-mediated inhibition may or may not be altered in chronic hypertension.

An initial key component of the baroreflex arc is the synaptic integration of baroreceptor afferent inputs within the NTS. Baclofen decreases the action potential discharge of NTS neurons receiving baroreceptor afferent inputs and induces a pressor response. Activation of GABAB receptors can presynaptically inhibit excitatory neurotransmitter release and decrease postsynaptic neuronal excitability via activation of a potassium conductance, thus reducing the transmission of baroreceptor afferent inputs to other sites in baroreflex pathways. An enhanced baclofen-induced pressor response is a well-described phenomenon after chronic hypertension, suggesting an enhanced inhibitory effect of baclofen. However, it is difficult to achieve conclusive evidence on the role of presynaptic and/or postsynaptic mechanisms underlying the baclofen-induced pressor response and its alterations in hypertension using an in vivo approach. The current study used in vitro whole-cell recording methods to directly investigate the postsynaptic responses to baclofen after chronic hypertension. We observed that chronic hypertension significantly enhanced baclofen-induced postsynaptic outward currents. There is significantly lower EC50 for baclofen-induced outward current comparing HT with NT cells, suggesting that chronic hypertension enhanced neuronal sensitivity to baclofen. The results of the current study are consistent with previous biochemical studies on NTS GABAB receptors. A previous study from our laboratory has found that in the NTS of renal-wrap HT rats there is increased expression of GABAB.

**Figure 3.** Ionic mechanisms of baclofen-induced current. A, effect of barium on current–voltage relationship in second-order baroreceptor neurons in the NTS (n=5). Barium itself induced an apparent inward current by blocking potassium channels. In the presence of barium, the baclofen equilibrium potential shifted to more positive level (−63±8 mV). B, when 10 µmol/L of baclofen was coapplied with 200 µmol/L of cadmium (n=5), the baclofen equilibrium potential was shifted to a more negative level comparing with baclofen alone in Figure 2. C, current–voltage relationship of normalized barium currents before and during the application of 10 µmol/L of baclofen (n=7). Inset, an example of voltage-activated barium current inhibited by 10 µmol/L of TTX.

**Discussion**

These results demonstrate that activation of postsynaptic GABA$_B$ receptors with baclofen induces outward currents in second-order baroreceptor neurons in the NTS, which will decrease neuronal excitability. This GABA$_B$ receptor–mediated inhibitory effect was enhanced after chronic hypertension. Both potassium and calcium channels were involved in the baclofen-evoked currents in the NTS. Enhanced postsynaptic GABA$_B$ receptor function could contribute to the enhanced baclofen-induced pressor response observed in vivo in various animal models of chronic hypertension. Our present study did not examine presynaptic inhibition by baclofen. This component of baclofen-mediated inhibition may or may not be altered in chronic hypertension.
receptor mRNA. A similar study reported that baclofen binding in the NTS was increased in spontaneously hypertensive rats when compared with control rats. The enhanced baclofen effect could be mediated by an increased number of GABA<sub>B</sub> receptors and/or increased flow through individual channels on second-order neurons after chronic hypertension.

The changes that we report here are considered adaptations in response to a chronically elevated blood pressure. The exact stimulus, or stimuli, that induces an increase of postsynaptic response to activation of GABA<sub>B</sub> receptors is unknown. The stimulus could be increased excitatory baroreceptor afferent inputs to NTS neurons because of the elevated blood pressure. The stimulus could be systemic and/or local tissue-generated neurohormonal alterations in chronic hypertension (eg, angiotensin).

In the present study, the baclofen equilibrium potential was more positive than the potassium reversal potential. Our result is similar to the baclofen equilibrium potential of −73 mV reported in a previous study in medial NTS under a similar experimental condition. Postsynaptic baclofen effects are primarily mediated by activation of potassium channels. In addition, baclofen also inhibits voltage-dependent calcium channels. Our results confirmed these findings and showed that blocking voltage-dependent calcium channels shifted E<sub>Ca</sub> closer to potassium equilibrium potential, whereas blocking potassium channels shifted E<sub>K</sub> further away from potassium equilibrium potential, suggesting that both channels are involved in the baclofen effect on second-order baroreceptor neurons in the NTS. Future studies will be needed to explore the role of different types of calcium and potassium channels and their relative contributions to the enhanced baclofen-induced inhibition observed in chronic hypertension.

Perspectives
The baroreflex serves to minimize arterial blood pressure fluctuation under physiological conditions and remains functional in chronic hypertension. However, baroreflex regulation of sympathetic discharge is reset to higher pressures in chronic hypertension. It has been proposed that this resetting reflects a new balance between increased excitatory afferent inputs because of increased blood pressure and GABA-mediated inhibition. Such balance would maintain regulatory function of baroreflex during chronic hypertension. Enhanced GABA<sub>B</sub> receptor–mediated inhibition could be a crucial factor in baroreflex adaptation in chronic hypertension. The current study provides direct evidence of an enhanced postsynaptic baclofen effect on second-order baroreceptor neurons in the NTS. This enhanced postsynaptic GABA<sub>B</sub> receptor function could be crucial in modulating baroreflex function in chronic hypertension. Future studies will investigate the effect of chronic hypertension on presynaptic GABA<sub>B</sub> receptor function, including both excitatory and inhibitory inputs to second-order NTS neurons. Furthermore, the impact of chronic hypertension on different ion channels mediating postsynaptic baclofen effects will be investigated.

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Disclosures
None.

References
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