Abstract—The increase in mean arterial pressure evoked by injection of the γ-aminobutyric acid (GABA) B agonist baclofen into the nucleus tractus solitarius (NTS) is greater in spontaneously hypertensive rats and renal wrap chronically hypertensive (CHT) rats compared with normotensive (NT) controls. We report here that the baclofen-induced pressor response (BIPR) is enhanced after acute hypertension (AHT) of only 30 minutes. Sprague-Dawley rats were anesthetized with Inactin, paralyzed, and artificially ventilated. As we previously reported, after unilateral electrolytic ablation of the NTS, microinjection of 40 pmol baclofen into the contralateral NTS of NT rats resulted in a BIPR of 22 ± 1 mm Hg (n = 12). During the infusion of phenylephrine for 30 minutes (AHT), the BIPR was 39 ± 5 mm Hg (n = 10), significantly greater than the response in NT rats (P < 0.01) and no different from the response in CHT rats (39 ± 5 mm Hg, n = 7). Baclofen has both presynaptic and postsynaptic effects. To eliminate the presynaptic component of the baclofen response, sinoaortic denervation (SAD) was performed before the microinjections. The magnitude of the BIPR was 12 ± 1 mm Hg in NT-SAD rats (n = 8), 12 ± 1 mm Hg in AHT-SAD rats (n = 12), and 20 ± 3 mm Hg in CHT-SAD rats (n = 7). The BIPR is enhanced in both CHT and AHT rats. It appears that the increase in baroreceptor afferent input to NTS during phenylephrine-induced AHT provides a greater substrate for presynaptic inhibition by baclofen because the postsynaptic component of the baclofen response is the same in NT-SAD and AHT-SAD. The enhanced BIPR in CHT rats appears to be associated with an enhancement of both the presynaptic and postsynaptic components of the response. (Hypertension. 2001;37[part 2]:619-622.)

Key Words: baroreceptors, baroreflex, electrophysiology, hypertension, renal

The inhibitory amino acid γ-aminobutyric acid (GABA) has been demonstrated to be a potent modulator of neurons within the nucleus of the solitary tract (NTS), the initial site of baroreceptor afferent integration within the central nervous system. In vivo and in vitro studies have demonstrated that the inhibition of NTS neurons and afferent evoked discharge in these neurons by the GABA B receptor agonist baclofen is mediated by both presynaptic and postsynaptic mechanisms.1,2 The microinjection into the NTS of baclofen results in an increase in arterial pressure, heart rate, and sympathetic nerve discharge to the kidney,3–6 which are predictable effects of inhibition of NTS neurons that integrate baroreceptor afferent inputs. This baclofen-induced pressor response (BIPR) is enhanced in chronically hypertensive rats, specifically the spontaneously hypertensive rats (SHR) and deoxycorticosterone (DOCA)-salt5 and 1-kidney, renal wrap models of hypertension.6 The duration that arterial pressure must be elevated before an enhanced BIPR is observed is currently unknown. Furthermore, the relative contributions of presynaptic and postsynaptic inhibitory mechanisms to the BIPR are also unknown.

The present study was designed to answer 2 questions. First, is the enhanced BIPR that is observed in chronic hypertension also observed in acute hypertension? Second, what are the relative contributions of presynaptic and postsynaptic inhibition to the BIPR? To answer the first question, we measured the BIPR in acutely hypertensive (30 minutes of phenylephrine infusion) and chronically hypertensive (4 weeks after unilateral nephrectomy, renal wrap surgery) rats. To answer the second question, we performed similar measurements before and after section of baroreceptor buffer nerves. The results demonstrate that after acute hypertension, the BIPR is enhanced by the same amount as previously reported in chronically hypertensive rats. The results suggest that in normotensive rats, the BIPR is an equal mix of presynaptic and postsynaptic inhibition. The results further suggest that during acute hypertension, the enhanced BIPR is mediated primarily by an increase in the presynaptic component of the BIPR, whereas in chronic hypertension, the enhanced BIPR is mediated by an increase in both the presynaptic and postsynaptic components of the BIPR.
Methods

Successful experiments were performed on adult male Sprague-Dawley rats (375 to 500 g; Charles River Laboratories or Harlan Sprague-Dawley Inc). Rats were housed 2 per cage in a fully accredited (Association for the Assessment and Accreditation of Laboratory Animal Care International and US Department of Agriculture) laboratory animal room with free access to food and water. All rats were given ≥1 week to acclimate before being used for any procedures. The Institutional Animal Care and Use Committee approved all experimental protocols.

Chronic Hypertensive Model

Hypertension was induced with a 1-kidney renal wrap procedure. Rats underwent medetomidine (0.5 mg/kg IP; Pfizer) and ketamine (75 mg/kg IP; Fort Dodge Laboratory). A figure-8 Grollman renal wrap and contralateral nephrectomy were performed on these animals.\(^5\) Control animals consisted of sham-operated rats that were similarly anesthetized and received a unilateral nephrectomy but no wrap of the contralateral kidney or of rats with no surgical procedures before the day of the experiment. Because the responses of both groups of normotensive rats were identical, they were grouped together for analysis. Anesthesia was terminated by atipamezole (1 mg/kg IP; Pfizer) at the conclusion of the surgical procedures. Postoperative analgesic agents (Nubaine IM) were available as needed.

Acute Surgical Preparation

Four to 6 weeks after the initial surgery, hypertensive and sham-operated animals were anesthetized with Inactin (100 mg/kg IP) and placed on a thermostatically controlled heating pad. Body temperature was monitored with a rectal probe and maintained at 36° to 38°C throughout the experiment. After placement of a venous catheter in the femoral vein and cannulation of the trachea, the rat was artificially ventilated with room air supplemented with 100% \(O_2\). Additional anesthetic was administered as needed (10 mg IV) to maintain stable arterial pressure and heart rate at rest and during pinch of the hind paw. Gallamine triethiodide (20 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) 30 min\(^{-1}\) IV) was administered for paralysis. A femoral artery was cannulated, and arterial pressure was measured with a strain-gauge transducer. The rat was then placed in a stereotactic head frame, and an occipital craniotomy was performed to expose the dorsal medulla in the region of the obex.

Denervation of Reflexogenic Areas

To determine the contribution of peripheral afferents to the baclofen-induced responses, after the acute surgical preparation described here, some of the rats underwent bilateral section of the carotid sinus and aortic nerves. In some of these rats, a bilateral section of the vagal nerves was also performed. The microinjection aspects of the protocol did not begin until mean arterial pressure (MAP) returned to 36° to 38°C. Additional anesthetic was administered as needed (10 mg IV) to maintain stable arterial pressure and heart rate at rest and during pinch of the hind paw. Gallamine triethiodide (20 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) 30 min\(^{-1}\) IV) was administered for paralysis. A femoral artery was cannulated, and arterial pressure was measured with a strain-gauge transducer. The rat was then placed in a stereotactic head frame, and an occipital craniotomy was performed to expose the dorsal medulla in the region of the obex.

Microinjection Protocol

We repeated the microinjection protocol originally described by Tsukamoto and Sved.\(^2\) A bipolar stimulating electrode was placed in the NTS using coordinates provided by these authors (0.5 mm rostral to calamus, 0.5 mm lateral to the midline, and 0.5 mm below the surface of the brain). With this electrode, an electrolytic lesion was placed in the right NTS to eliminate reflex buffering of responses to drugs injected into the left NTS. After a recovery period of 30 to 60 minutes, a glass micropipette (outer tip diameter <50 \(\mu\)m) filled with baclofen (dissolved in artificial cerebrospinal fluid, pH adjusted to 7.4) was placed into the contralateral NTS with use of the same stereotoxic coordinates. Baclofen was injected (40 pmol in a 100-nL volume) slowly over 1 to 3 minutes with a pressurized source. Cardiovascular parameters were measured with a MacLab A/D system.

Table 1. Baseline Levels of Blood Pressure

<table>
<thead>
<tr>
<th>Group</th>
<th>Buffer Nerves</th>
<th>SAD</th>
<th>SAD and Vagotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>103 ± 3</td>
<td>105 ± 3</td>
<td>113 ± 6</td>
</tr>
<tr>
<td></td>
<td>(n=12)</td>
<td>(n=8)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>Chronic</td>
<td>132 ± 4</td>
<td>133 ± 3</td>
<td>140 ± 4</td>
</tr>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>Acute</td>
<td>136 ± 3</td>
<td>135 ± 5</td>
<td>138 ± 4</td>
</tr>
<tr>
<td></td>
<td>(n=10)</td>
<td>(n=12)</td>
<td>(n=7)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM with the number of animals in each group given in parentheses.

*Significant differences between groups, \(P<0.05\).

Acute Hypertension and Hypotension

To examine the effects of acute changes in pressure on the BIPR, intravenous infusions of phenylephrine (100 \(\mu\)g/mL) were used to raise the pressure of normotensive rats to the same level as in chronic hypertension for 30 minutes. Intravenous infusions of nitroprusside (250 \(\mu\)g/mL) were used to lower the pressure of hypertensive rats to normotensive levels for 30 minutes. To ensure that the enhanced BIPR was not the result of changes at the vascular level, responses to bolus injections of norepinephrine (0.1 \(\mu\)g) and angiotensin II (30 to 40 ng) were examined during the acute hypertension induced by phenylephrine infusion. A previous study found that pressor responses to phenylephrine, angiotensin II, and vasopressin were not altered in chronic renal wrap hypertension.\(^6\)

Data Analysis

Pulsatile pressure and MAP were viewed and saved for off-line analysis with a MacLab A/D system. Statistical significance was determined with ANOVA with Tukey’s or Dunn’s tests used for post hoc comparisons. Student’s \(t\) test was used to analyze the norepinephrine and angiotensin II bolus injection data. All values are expressed as mean ± SEM, and significance was accepted at \(P<0.05\).

Results

BIPR in Buffer Nerve–Intact Rats

The baseline levels of MAP in the groups of animals studied are presented in Table 1. As previously described in the renal wrap model of hypertension, the microinjection of baclofen into the NTS of chronic hypertensive rats induces a pressor response that is greater than the pressor response induced by identical baclofen injections in normotensive rats (Figure, Table 2).

In the course of these studies, we made the chance observation that acute, spontaneous changes in arterial pressure altered the BIPR. We further examined this phenomenon using phenylephrine to acutely increase MAP in normotensive rats and nitroprusside to acutely lower pressure in chronically hypertensive rats. During phenylephrine infusions that increased the MAP of a normotensive rat to the same MAP as a chronically hypertensive rat for 30 minutes, the BIPR was enhanced (Figure, Table 2). The magnitude of the BIPR measured in acute hypertensive rats was the same as the BIPR observed in chronic hypertensive rats (Figure, Table 2). Furthermore, during nitroprusside infusions that lowered the MAP of chronic hypertensive rats to the same MAP as in normotensive rats for 30 minutes, the BIPR was reduced (Figure). The BIPR measured in these acutely normotensive rats was the same as the BIPR observed in sham, normotensive rats.
Increase in MAP evoked by NTS microinjection of baclofen in (from left to right) sham-operated, normotensive rats; chronically hypertensive, renal wrap rats; normotensive rats during phenylephrine-induced acute hypertension; and hypertensive rats during nitroprusside-induced acute hypotension. The values inside the columns indicate the number of animals in each group and the resting level of pressure before the baclofen injection. *Significant difference compared with sham, normotensive rats.

To determine whether the changes in the magnitude of the BIPR observed after acute increases in MAP were due to alterations in the contractile properties of blood vessels, the pressor response induced by a bolus intravenous injection of norepinephrine (n=6) or angiotensin II (n=7) was measured before and during a 30-minute phenylephrine infusion. Norepinephrine increased MAP by 23±2 mm Hg at the resting level of MAP and by 28±3 mm Hg during phenylephrine infusion (P=0.11). Angiotensin II increased MAP by 38±4 mm Hg at the resting level of MAP and by 41±3 mm Hg during phenylephrine infusion (P=0.67).

**BIPR in Baroreceptor-Denervated Rats**

To determine the contribution of presynaptic inhibition of carotid sinus and aortic afferents to the BIPR, microinjection experiments were performed in rats after bilateral section of the carotid sinus and aortic nerves. Sinoaortic denervation (SAD) did not alter baseline MAP in the 3 groups (Table 1). The effects of SAD on the magnitude of the BIPR are presented in Table 2, and in all 3 groups, the difference in the magnitude of the BIPR between the buffer nerve–intact and SAD animals was significant. In normotensive, SAD animals, the BIPR was 55% of that observed in buffer nerve–intact rats. In the acute hypertensive rats, after SAD, the magnitude of the BIPR was 55% of that observed in buffer nerve–intact, chronically hypertensive rats was enhanced, the BIPR in acute hypertensive, SAD rats was only 31% of that measured in acute hypertensive, buffer nerve–intact rats. After SAD in chronically hypertensive rats, the BIPR was 51% of that observed in buffer nerve–intact, chronically hypertensive rats. The BIPR measured after SAD in the chronically hypertensive rats was significantly greater than the BIPR measured in both normotensive, SAD rats and in acute hypertensive, SAD rats (Table 2). Table 2 also provides the difference between the magnitude of the BIPR measured in the buffer nerve–intact and the SAD animals for the 3 groups.

In addition to arterial pressure baroreceptor afferent inputs, it is possible that cardiopulmonary afferent inputs contribute to the BIPR. Therefore, the effects of bilateral vagotomy, in addition to SAD, were examined. Combining vagotomy with SAD did not significantly alter baseline MAP compared with buffer nerve–intact animals or the effects of SAD alone (Table 1). Combining vagotomy with SAD did not significantly alter the magnitude of the BIPR observed in normotensive rats (11±2 mm Hg), acute hypertensive rats (15±1 mm Hg), or chronic hypertensive rats (19±3 mm Hg) compared with the effects of SAD alone (P>0.05 for all 3 comparisons).

**Discussion**

These results confirm our previous observations in this model of hypertension and those of Tsukamoto and Sved in the SHR and DOCA-salt models of hypertension showing that the BIPR is enhanced in chronic hypertensive rats. The present study extends these observations by finding that the BIPR was enhanced after brief (30-minute) increases in MAP and that the enhanced BIPR observed in chronic hypertensive rats could be reduced to normal levels after brief (30 minutes) reductions in MAP. Furthermore, the present study attempted to estimate the relative contribution of presynaptic and postsynaptic mechanisms to the BIPR by examining the BIPR before and after SAD.

SAD is likely to lead to a number of alterations within the NTS, so interpretation of these data as being the result of solely the removal of afferent input is, no doubt, a simplistic assumption. However, the assumption is not without some merit. Our recent report that describes the responses of NTS neurons to changes in blood pressure found that reductions in blood pressure did not significantly reduce the spontaneous discharge of NTS neurons. Although baroreceptor unloading is not strictly analogous to SAD, it does suggest that removal of afferent input does not alter the spontaneous discharge of NTS neurons as dramatically as one might predict. Our present analysis cannot exclude possible presynaptic effects of baclofen on other excitatory inputs to NTS neurons, whether these inputs originate from the periphery or within the central nervous system. Keeping in mind the limitations and assumptions inherent in this analysis, we propose that the BIPR remaining after SAD reflects the postsynaptic component of the BIPR because the SAD removes the presynaptic component. Subtraction of the BIPR after SAD, the postsynaptic component, from the BIPR observed before SAD reveals the presynaptic component of the BIPR (Table 2, far

**TABLE 2. Increase in Pressure Induced by Microinjection of Baclofen into the NTS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Buffer Nerves</th>
<th>SAD</th>
<th>Intact - SAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>22±2 (100%)</td>
<td>12±1 (55%)</td>
<td>10 (45%)</td>
</tr>
<tr>
<td>Chronic</td>
<td>39±5 (100%)</td>
<td>20±3 (51%)</td>
<td>19 (49%)</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>39±5 (100%)</td>
<td>12±1 (31%)</td>
<td>27 (69%)</td>
</tr>
</tbody>
</table>

Actual increase presented as mean±SEM in mm Hg. Parentheses contain the actual percentage increase relative to the intact condition set at 100%.

*Significant differences between groups, P<0.05.
right column, Intact-SAD). It is possible that vagal, cardiopulmonary afferents might also contribute to the BIPR and alter our estimates of the presynaptic versus the postsynaptic components of the response. Therefore, we also performed experiments in rats with combined SAD and vagotomy.

The results suggest that the BIPR observed in normotensive rats is an equal mix of presynaptic and postsynaptic inhibition. They also suggest that in normotensive rats, vagal afferents do not contribute to the BIPR. In acute hypertensive rats, the enhanced BIPR appears to be the result of an increase in the presynaptic component of the BIPR, the calculated postsynaptic component being identical to that observed in normotensive rats (12±1 mm Hg). A likely explanation for this observation is that the pronounced increase in baroreceptor afferent input to the NTS during acute increases in MAP provides a greater substrate for the presynaptic inhibitory effects of baclofen. Vagal afferents do not contribute to the BIPR observed in acute hypertension.

The situation in chronic hypertension is more complex. The results suggest that there is an increase in both the presynaptic and postsynaptic components of the BIPR. Jones and Thoren report that in chronic hypertension, there is an increase in the number of baroreceptor afferents discharging at resting levels of MAP, and our observation of an increased presynaptic component of the BIPR in chronic hypertension is consistent with the interpretation that there is an increased baroreceptor afferent input to the NTS in chronic hypertension. The increase in the calculated presynaptic component is slightly less than that calculated in acute hypertension, and this could reflect some degree of baroreceptor resetting in chronic hypertension.

Of particular interest is the increase in the postsynaptic component of the BIPR in chronic hypertension, that is, the magnitude of the BIPR observed after SAD. We previously reported that the enhanced BIPR observed in chronic hypertension is associated with an increased expression of GABA_A mRNA in the NTS, and on the basis of the present results, we propose that this increased mRNA results in increased expression of the postsynaptic GABA_B receptor. We have found that the sensitivity of NTS neurons to exogenously applied baclofen is enhanced in chronic hypertension (J. Zhang and S.W. Mifflin, unpublished observations), and this is consistent with the interpretation of enhanced expression of the postsynaptic GABA_B receptor. The exact stimulus for the increased postsynaptic expression of GABA_B receptors remains to be defined, but it could be mediated by neurotransmitters released by baroreceptor afferents or other inputs to NTS neurons, or it could be related to alterations in the levels of a circulating hormone.

The normalization of the BIPR observed in chronic hypertensive rats during acute reductions in blood pressure, produced through intravenous infusion of nitroprusside, was surprising. If the enhanced BIPR observed in chronic hypertensive rats is mediated in part by an increase in the postsynaptic response to baclofen, we predicted that after the acute normalization of MAP, we would still observe an enhanced BIPR. However, we observed a BIPR that was not different from that observed in normotensive rats. The reason for this might be related to the degree to which we lowered MAP with nitroprusside. If there is some degree of receptor resetting in the presynaptic component as discussed earlier, then our decision to reduce MAP to the level observed in normotensive rats may account for this observation. Because there is baroreceptor resetting in chronic hypertension, the level of baroreceptor afferent input may be less in the chronic hypertensive rats than in the normotensive rats compared at the same MAP. This would lead to an overestimate of the presynaptic versus postsynaptic components of the BIPR.

To conclude, these results suggest that there is large degree of plasticity in the cardiovascular responses to the microinjection of baclofen in the NTS. Adaptations occur on an acute (30 minutes) and chronic (4 week) time scale, although we did not precisely define the temporal course of these adaptations. The adaptations appear to result from alterations in peripheral afferent input on a short time scale and alterations in both the level of afferent input and the neurons that integrate these afferent inputs on a longer time scale. It is proposed that these alterations reduce the level of increased excitatory input to NTS neurons due to increased afferent input in hypertension, whether the hypertension is acute or chronic. We have previously reported that the discharge of most NTS neurons is not altered in chronic hypertension; therefore, we propose that this plasticity in the initial processing of cardiovascular afferent information represents an attempt to normalize NTS neuronal discharge in response to increased baroreceptor afferent input and to maintain some degree of reflex buffering capability.

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γ-Aminobutyric Acid\textsubscript{B} Receptor – Mediated Responses in the Nucleus Tractus Solitarius Are Altered in Acute and Chronic Hypertension
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