Enhanced \(\gamma\)-Aminobutyric Acid–B Receptor Agonist Responses and mRNA Within the Nucleus of the Solitary Tract in Hypertension

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Abstract—\(\gamma\)-Aminobutyric acid–B (GABA\(_B\)) receptor function and regulation in the nucleus of the solitary tract (NTS) was examined in Sprague-Dawley rats made chronically (4 to 5 weeks) hypertensive with the one-kidney, figure-8 renal wrap model of hypertension. NTS microinjection of the GABA\(_B\) agonist baclofen produced a pressor response that was enhanced in hypertensive rats compared with the response observed in sham-operated normotensive rats (36±4 mm Hg increase in mean arterial pressure in 8 hypertensive rats compared with 21±2 mm Hg increase in 7 sham-operated normotensive rats, \(P=0.03\)). Responses to microinjection of GABA\(_B\) antagonists (CGP-55845A and SCH-90511), the GABA\(_A\) agonist muscimol, the GABA\(_A\) antagonist bicuculline, and the GABA reuptake inhibitor nipecotic acid were not different comparing normotensive sham-operated and hypertensive rats. Renal sympathetic nerve responses to NTS microinjection of these drugs were not different in hypertensive compared with normotensive rats. Micropunches of the NTS were homogenized and reverse transcriptase–polymerase chain reaction was performed to examine mRNA levels for the GABAB receptor. There was a 3-fold increase in GABA\(_B\) receptor mRNA levels in the caudal NTS of 7 chronically hypertensive rats compared with levels measured in 8 sham-operated normotensive rats (\(P=0.01\)). In conclusion, chronic hypertension is associated with an upregulation of GABA\(_B\) receptor function; however, the tonic activity of the system does not appear to be different between normotensive and hypertensive rats. The upregulation of GABAB receptor function might be due to an increased number of receptors, as suggested by the elevated levels of GABA\(_B\) receptor mRNA measured in the NTS of hypertensive rats. All of these alterations suggest that hypertension is associated with dynamic changes in receptor-mediated mechanisms within the NTS, and these alterations could modify baroreflex regulation of cardiovascular function in hypertension. (Hypertension. 1999;33[part II]:530-536.)

Key Words: kidney \(\rightarrow\) tractus solitarius \(\rightarrow\) hypertension, renal \(\rightarrow\) receptors, aminobutyric acid \(\rightarrow\) baclofen \(\rightarrow\) reverse transcriptase

The nucleus of the solitary tract (NTS) is the brain stem nucleus where baroreceptor afferent fibers make their initial synapse within the central nervous system. Therefore, information regarding the physiology and pharmacology of NTS neurons is critical to our understanding of baroreflex regulation of cardiovascular function. The region of the NTS where baroreceptor afferents terminate contains a high density of both \(\gamma\)-aminobutyric acid–A (GABA\(_A\)) and GABA\(_B\) receptors. A number of in vivo microinjection studies as well as in vivo and in vitro single unit electrophysiological experiments have demonstrated that both GABA\(_A\) and GABA\(_B\) receptors play an important role in the integration of baroreceptor afferent inputs and baroreflex function. Since activation of GABA\(_A\) receptors evokes postsynaptic inhibition and activation of GABA\(_B\) receptors evokes both presynaptic and postsynaptic inhibition of NTS neurons, microinjection of either GABA\(_A\) or GABA\(_B\) agonists into the NTS inhibits NTS neurons, which results in an inhibition of the baroreflex and an increase in mean arterial pressure (MAP). Conversely, NTS microinjection of GABA\(_A\) or GABA\(_B\) antagonists removes tonic GABAAergic inhibition of NTS neurons and results in a fall in arterial pressure.

There is little information regarding adaptive changes in the physiology and pharmacology of NTS neurons in pathological states such as chronic hypertension. Of note are a series of studies by Sved and colleagues that demonstrated an enhanced pressor response to activation of GABA\(_B\) receptors within the NTS in spontaneously hypertensive rat (SHR) and deoxycorticosterone acetate (DOCA)–salt models of hypertension. Conversely, blockade of GABA\(_B\) receptors evoked a greater fall in arterial pressure in both models of hypertension compared with normotensive Wistar-Kyoto rats. NTS microinjections of the excitatory amino acid glutamate or drugs that activate or antagonize GABA\(_A\) receptors produce similar changes in arterial pressure in normotensive and hypertensive rats. All of these data suggest...
that within the NTS of SHR and DOCA-salt hypertensive rats, there is a tonically enhanced GABA_B receptor system. Since microinjection of GABA_B agonists into the NTS of normotensive rats shifts the baroreflex to higher pressures, a tonically enhanced GABA_B input to the NTS of hypertensive animals could reset the baroreflex to higher pressures and contribute to the maintenance of hypertension.

The goals of the present study were to determine whether alterations in GABA_B receptor function occur in another model of hypertension—one-kidney, figure-8 renal wrap—and then to examine a possible mechanism for any observed alterations. In the present study we examined GABA_B receptor function in the NTS in this model of hypertension using microinjection techniques. We also examined whether chronic hypertension altered GABA_B receptor mRNA by quantitation of mRNA for the receptor using quantitative, competitive reverse transcriptase–polymerase chain reaction (RT-PCR). Our results indicate enhanced presor pressor responses to NTS microinjection of GABA_B agonists but not antagonists in hypertensive compared with sham-operated animals. In addition, GABA_B mRNA levels in the NTS were elevated in hypertensive compared with sham-operated animals. Both of these findings suggest an alteration in GABA_B receptor function and regulation within the NTS in chronic hypertension.

Methods

Microinjection Experiments

Adult male Sprague-Dawley rats (350 to 500 g, n = 124) were used in the present studies. Experiments were performed using both the Charles River (n = 93) and Harlan (n = 31) strains. Cardiovascular responses did not differ between the 2 strains, so the data were pooled. All procedures were approved by the Institutional Animal Care and Use Committee. Anesthesia was induced by an intraperitoneal injection of medetomidine (0.5 mg/kg, Pfizer) and ketamine (75 mg/kg, Fort Dodge Laboratory). To induce hypertension, the rats were subjected to the figure-8 Grollman renal wrap procedure combined with contralateral nephrectomy.16,17 Sham-operated rats were similarly anesthetized and received unilateral nephrectomy alone. At the conclusion of surgery, anesthesia was terminated by intraperitoneal injection of atipamezole (Antisedan) (1 mg/kg, Pfizer). All studies were performed 4 to 5 weeks after the induction of hypertension.

Experiments were performed on rats anesthetized with intraperitoneal injections of either a-chloralose (80 mg/kg)/urethane (800 mg/kg, n = 107) or thiobutabarbital (100 mg/kg, n = 17). MAP and renal sympathetic nerve activity (RSNA) responses were not different between the 2 anesthetics, so the data were pooled for analysis. After induction of anesthesia, a femoral artery and vein were cannulated for measurement of arterial pressure and injection of drugs, respectively. Supplemental anesthesia was administered intravenously as determined by the stability of arterial pressure and heart rate with rats under resting conditions and during a pinch of the hind paw. The trachea was intubated and the animal placed in a stereotaxic head frame. After removal of overlying muscles, an occipital craniotomy was performed to expose the caudal medulla. Rectal temperature was monitored and maintained at 37 ± 1°C using a heating pad that circulated warm water. The tracheal cannula was connected to a small-animal respirator (Harvard Apparatus), and positive-pressure ventilation was begun using room air supplemented with 100% O_2. To minimize respiratory movements of the brain stem and respiratory fluctuations in arterial pressure, the animal was paralyzed by an intravenous injection of gallamine (10 to 20 mg), supplemented as needed, typically hourly, and given a pneumothorax. Since this paralytic agent has antimuscarinic properties, heart rate responses were minimal or absent and were not analyzed.

The renal sympathetic nerve was approached retroperitoneally via a flank incision. The nerve was isolated contralaterally to the nephrectomy and placed on bipolar, polytetrafluoroethylene-coated platinum wires with the ends bared. RSNA was measured with a Grass high-impedance probe (HIP-511) and amplified with a Grass P5 series AC amplifier. The output was rectified and integrated (Coulbourn S76 to 01) at time constants of 20 to 50 milliseconds. The zero level of RSNA was designated as the activity that remained after an intravenous injection of phenylephrine sufficient to raise MAP by 40 to 50 mm Hg and silence ongoing discharge in the nerve. The zero level of RSNA was subtracted from the resting RSNA, and this level was designated 100%. Changes in RSNA were expressed as a percentage change from the resting level of 100%.

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We repeated the microinjection protocol described by Sved and Tsukamoto. A bipolar stimulating electrode was placed in the NTS using coordinates provided by these authors (0.5 mm caudal to the calamus, 0.5 mm lateral to the midline, and 0.5 mm below the surface of the brain). Using 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 μm) filled with the drugs being injected was placed into the contralateral NTS using the same stereotaxic coordinates. Drugs were injected slowly over 1 to 3 minutes using a pressurized source (WPI, Pneumatic Picopump PV800), and cardiovascular parameters were measured using a MacLab A/D system.

GABA_B agonists and antagonists were dissolved in artificial cerebrospinal fluid, and pH was adjusted to 7.4. The following drugs were microinjected in a 100 nl volume: baclofen (40 pmol), CGP-55845A (1 nmol), SCH-90511 (1 nmol), muscimol (100 pmol), and nipecotic acid (10 nmol). The doses of agonists used were determined in preliminary experiments (n = 4 to 5 for each drug) and were taken as the dose that produced 70% to 80% of the maximal response. The doses of antagonists used were determined in preliminary experiments and were taken as the dose that reduced the response to microinjection of a selective agonist by > 90%.

Arterial pressure, heart rate, and RSNA (both raw and integrated) were viewed and saved for off-line analysis using a MacLab A/D system. Statistical significance was determined using ANOVA with Scheffe’s post hoc test for comparisons. All values are expressed as mean ± SEM.

Molecular Analyses

Experiments were performed on adult, male Sprague-Dawley rats of the Charles River strain (350 to 460 g, n = 15) 4 weeks after induction of hypertension as previously described. Two days before the experiment, an arterial catheter was placed in the femoral artery while the animal was under medetomidine/ketamine anesthesia (0.5 mg/kg IP and 75 g/kg IP, respectively). After a 2-day recovery period, blood pressure of the conscious animal was measured by connecting the arterial catheter to a pressure transducer (Kobe) and displayed using MacLab or Cambridge Electronic Design A/D converters. Blood pressure was measured for 3 hours, and measurements made during the last hour were used as an index of resting arterial pressure and heart rate.

The methods for quantitative, competitive RT-PCR from brain micro punches were recently described in detail. Briefly, after measurement of conscious MAP and heart rate, rats were anesthetized with pentobarbital (50 mg/kg IP) and the brain stem was quickly removed and frozen in isopentane cooled on dry ice. Using a rodent brain slicer (Brain Tree Scientific, BS 200), a 1-mm-thick section of the caudal medulla was obtained from the calamus to 1 mm caudal to the calamus. The section, still mounted on the razor blade, was frozen under liquid nitrogen to avoid RNA degradation. Using a blunt-end, 20-gauge stainless steel piece of hypodermic tubing (Small Parts Inc), bilateral punches of the NTS were removed under RNase-free conditions. RNA was isolated as per the manufacturer’s protocol (Sigma Chemical Co), and RT was carried out in
20-μL volumes using oligo(dT) and MuLV reverse transcriptase according to the manufacturer’s protocol (Gene Amp RNAS ATP kit, Perkin-Elmer). The internal standard was synthesized by PCR using the method of Celi et al. The GABA\(_B\) receptor primers were designed from the cDNA sequence (Gene Bank No. Y10369) that amplifies both GABA\(_B\)-R1a and GABA\(_B\)-R1b subtypes. Three primers were synthesized. Primer A (GTGACCATGATCCTTTCCAG) consisted of 20 bases corresponding to the 5’ sequence (from 2461 to 2480 bp), and primer B (CAACAGTCGGGACCTTCCTTTT) consisted of 20 bases corresponding to the opposite strand of the target at the 3’ sequence (from 2670 to 2651 bp) and at a predetermined distance from primer A. Amplification using these two primers resulted in a 209-bp PCR product. Another primer, primer C (CAACAGTCGGGACCTTCCTTTTATGGTGTCCTGCGTTTCA), was approximately 40 bp in length: 24 nucleotides at the 3’ end corresponding to primer B and 24 nucleotides from the opposite strand of the target sequence upstream from primer B (from 2620 to 2601 bp). Amplification from primers A and C resulted in a 159-bp PCR product, which was used as an internal standard.

PCR was performed in 50-μL volumes using a thermal cycle (PTC 100, MJ Research). The PCR cycle used for the amplification started with an initial denaturation step at 95°C for 3 minutes; followed by 95°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes for 35 cycles; and a final elongation step of 72°C for 7 minutes. Both products arising from amplification of target message and internal standard were then separated on 2% agarose gels, stained with ethidium bromide, and photographed (DS 34, Polaroid). The photograph was scanned (Scanjet 4C, Hewlett-Packard) and mass measured using a scientific imaging system (Kodak 1D analysis software). The mRNA levels were measured as the mass ratio of the target message to that of internal standard using an appropriate mass ladder with known molecular weights (low mass DNA ladder, BRL). Statistical significance was determined using ANOVA with Scheffé’s post hoc test for comparisons. All values are expressed as mean±SEM.

**Results**

**Microinjection Experiments**

Using the protocol established by Sved and Tsukamoto, we lesioned one side of the NTS and microinjected 100 nL of drug into the contralateral NTS. Control injections of artificial cerebrospinal fluid produced inconsistent changes in MAP of <5 mm Hg in both sham-operated normotensive and hypertensive rats. In sham-operated rats, injection of the GABA\(_B\) agonist baclofen increased MAP from 101±3 to 122±3 mm Hg (n=7) (Figure 1 and Table). In hypertensive rats, identical injections increased MAP from 132±3 to 168±5 mm Hg (n=8). The increase in pressure was significantly greater in the hypertensive (36±4 mm Hg) compared with the sham-operated (21±3 mm Hg) rats (P<0.01) (Table). This enhanced responsiveness was selective for the GABA\(_B\) agonist, as results using the GABA\(_A\) agonist muscimol revealed no such enhanced responsiveness in the hypertensive animals (increase in MAP of 21±2 mm Hg in 9 sham-operated rats and increase of 26±3 mm Hg in 7 hypertensive rats) (Table). These results suggest that in hypertensive animals there is a selective increase in the responses to GABA\(_B\) agonists. Control and response values of MAP are given in the Table.

There was also no difference in the responses to NTS microinjections of the GABA\(_B\) antagonists CGP-55845A (decrease in MAP of 21±4 mm Hg in 7 sham-operated rats and decrease of 19±3 mm Hg in 11 hypertensive rats) and SCH-90511 (decrease in MAP of 17±2 mm Hg in 11 sham-operated rats and decrease of 19±3 mm Hg in 6 hypertensive rats) (Table). In addition, there were also no differences in the responses to NTS microinjections of the GABA\(_A\) antagonist bicuculline (decrease in MAP of 22±2 mm Hg in 9 sham-operated rats and decrease of 20±2 mm Hg in 8 hypertensive rats) or the GABA uptake inhibitor nipecotic acid (increase in MAP of 25±3 mm Hg in 9 sham-operated rats and increase of 24±2 mm Hg in 9 hypertensive rats) (Table).

To examine whether enhanced sympathetic nerve responses mediated the enhanced responses to NTS microinjection of GABA\(_B\) agonists in hypertensive rats, we measured RSNA responses during such injections. Microinjection of baclofen increased RSNA by 13±4% in 6 sham-operated rats and by 13±2% in 8 hypertensive rats. Microinjection of CGP-55845A decreased RSNA by 14±8% in 6 sham-operated rats and by 13±6% in 8 hypertensive rats, and microinjection of SCH-90511 decreased RSNA by 12±6% in

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**Figure 1.** A, Pulsatile arterial pressure (AP), MAP, and RSNA responses to microinjection of baclofen into NTS during the period indicated by vertical dashed lines. Raw RSNA is presented before rectification and averaging. B, Cresyl violet–stained section of caudal NTS illustrating extent of electrolytic lesion on right side of brain and local tissue damage resulting from several microinjections into NTS (arrow on left side of brain). Calibration bar=200 μm.
Group Responses to Microinjection of GABA Receptor Agonists and Antagonists Into the NTS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Group</th>
<th>n</th>
<th>Control MAP, mm Hg*</th>
<th>Response MAP, mm Hg*</th>
<th>Change in MAP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baclofen</td>
<td>Sham</td>
<td>7</td>
<td>101±3</td>
<td>122±3</td>
<td>21±2†</td>
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<tr>
<td>Baclofen</td>
<td>Hypertensive</td>
<td>8</td>
<td>132±3</td>
<td>168±5</td>
<td>36±4</td>
</tr>
<tr>
<td>CGP-55845A</td>
<td>Sham</td>
<td>7</td>
<td>105±3</td>
<td>84±4</td>
<td>−21±4</td>
</tr>
<tr>
<td>CGP-55845A</td>
<td>Hypertensive</td>
<td>11</td>
<td>137±4</td>
<td>118±6</td>
<td>−19±3</td>
</tr>
<tr>
<td>SCH-90511</td>
<td>Sham</td>
<td>11</td>
<td>102±4</td>
<td>84±4</td>
<td>−17±2</td>
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<tr>
<td>SCH-90511</td>
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<td>6</td>
<td>128±3</td>
<td>109±3</td>
<td>−19±3</td>
</tr>
<tr>
<td>Muscimol</td>
<td>Sham</td>
<td>10</td>
<td>108±2</td>
<td>129±3</td>
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<tr>
<td>Muscimol</td>
<td>Hypertensive</td>
<td>9</td>
<td>137±5</td>
<td>164±5</td>
<td>26±3</td>
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<tr>
<td>Bicuculline</td>
<td>Sham</td>
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<td>101±3</td>
<td>79±4</td>
<td>−22±2</td>
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<td>136±4</td>
<td>117±4</td>
<td>−20±2</td>
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<tr>
<td>Nipecotic acid</td>
<td>Sham</td>
<td>9</td>
<td>100±4</td>
<td>125±3</td>
<td>25±3</td>
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<tr>
<td>Nipecotic acid</td>
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<td>9</td>
<td>132±5</td>
<td>157±6</td>
<td>24±2</td>
</tr>
</tbody>
</table>

*For all values in column, sham is less than hypertensive (P<0.01); †Sham is less than hypertensive (P=0.03).

Discussion

The results of the present study demonstrate that within the NTS in the one-kidney, figure-8 renal wrap model of hypertension, GABA<sub>B</sub> receptor responses to agonist are enhanced and the enhanced responses are associated with an increased level of GABA<sub>B</sub> mRNA. The results of the present study differ from those of previous studies regarding the role of GABA<sub>B</sub> receptors in hypertension in several respects. In general, the present results are consistent with those of previous studies that demonstrated a selective enhancement of GABA<sub>B</sub> responses in the NTS of SHR and DOCA-salt hypertensive rats, in that a similar phenomenon occurs in

RT-PCR Analysis of GABA<sub>B</sub> mRNA in NTS

MAP and heart rate of sham-operated animals were 100±5 mm Hg and 360±5 beats/min (n=8), respectively, and MAP and heart rate of renal wrapped animals were 134±6 mm Hg and 365±7 beats/min (n=7). Differences in MAP were significant (P=0.001), and differences in heart rate were not (P=0.585). A standard curve was constructed and demonstrates a linear relationship between the log of the mass ratio of target template and internal standard and the log of the amount of target template added (Figure 2), indicating similar amplification efficiencies for the target template and internal standard. The mRNA levels measured in 100 ng RNA isolated from the micropunches of caudal NTS from sham-operated and renal wrap animals are presented in Figure 3. The mass ratios of the target template (209 bp) to internal standard (159 bp) were significantly greater in hypertensive animals (4.13±0.99) compared with those of sham-operated animals (1.30±0.19) (P=0.010). This demonstrates that there is an increased level of GABA<sub>B</sub> mRNA in the NTS of hypertensive animals compared with sham-operated normotensive animals.

Enhanced Response to GABA<sub>B</sub> Agonist in Hypertension

In general, the present results are consistent with those of previous studies that demonstrated a selective enhancement of GABA<sub>B</sub> responses in the NTS of SHR and DOCA-salt hypertensive rats, in that a similar phenomenon occurs in the present study.

5 sham-operated rats and by 16±5% in 5 hypertensive rats. None of the RSNA responses were significantly different when sham-operated animals were compared with the renal wrap hypertensive animals.

Figure 2. A, Standard curve for GABA<sub>B</sub> mRNA. Curve constructed by plotting the log mass ratios of wild template (209 bp) to that of internal standard (159 bp) against the log input of the wild template. B, Typical gel from which standard curve was derived, showing separation of PCR products of wild-type and internal standard on a 2% agarose gel. Pairs of bands illustrate varying amounts, from 6.25 to 50 pg, of the wild template (top bands, 209 bp) with a constant amount, 25 pg, of internal standard (bottom bands, 159 bp). Note the inverse relationship between the brightness of the wild-type bands, reflecting relative abundance of the endogenous message, and the brightness of the internal standard bands.
MAP responses to activation of GABA_A receptors, might be augmented pressor response could be due to enhanced vaso-

administration of a vasopressin antagonist. Therefore, the into the NTS was significantly reduced after the intravenous pressor response that followed the microinjection of baclofen.

reported previously is due to enhanced inhibition of vasopressin release. Since in conscious animals resting plasma vasopressin levels are normally very low, enhanced inhibition would require an increase in the circulating level in hypertension. In the model of hypertension used in the present study, resting vasopressin levels were elevated in hypertensive rats fed a high sodium diet; however, no difference was observed between normotensive and hypertensive rats fed a normal sodium diet (J.R. Haywood, personal communication, 1998). Therefore, the possibility that the enhanced pressor response is due to enhanced vasopressin release does not appear likely.

A second possible interpretation is that enhanced sympathetic responses occur in other beds. We did not test the responses of other sympathetic nerves, so we cannot exclude this possibility. A third possible interpretation is that the current methods for analysis of sympathetic nerve responses are inadequate to make such between-group comparisons, without a simultaneous measure of end-organ flow or resistance. Because the measure of SNA is not absolute but is relative to the resting level and zero level determined for each animal, the occurrence of a shift in the basal level of SNA between normotensive and hypertensive animals cannot be determined. Indirect measures suggest that SNA is elevated in hypertension, as ganglionic blockade produces a greater fall in MAP in one-kidney, figure-8 renal wrap hypertensive rats compared with sham-operated normotensive rats. Therefore, the same percentage increase in SNA could produce an enhanced vasoconstriction if baseline SNA is elevated in hypertension.

Mechanism for Enhanced Responses to GABA_B Agonist in Hypertension

A variety of physiological and pharmacological mechanisms could underlie the enhanced responses to activation of GABA_B receptors within the NTS observed in this and previous studies. The enhanced pressor response could be the result of increased baroreceptor afferent input to the NTS in hypertension. This increased baroreceptor discharge could increase the discharge of NTS neurons, which would result in enhanced sympathoinhibitory output from the NTS. Under these conditions, the pharmacological inhibition of the NTS, by injection of baclofen, would result in a greater increase in MAP compared with the situation when the sympathoinhibitory output of the NTS is not as great, as in normotension. However, if this were the case one might predict that the effects of any agent that inhibits NTS neurons, eg, muscimol, would be enhanced in hypertension. Clearly, this was not the case in the present and previous studies.

Pharmacological mechanisms for the enhanced pressor response include alterations in the number and/or affinity of GABA_B receptors. Singh and Ticku reported enhanced baclofen binding in the NTS of SHR, and the increase in mRNA for the GABA_B receptor that we observed suggests that the number of GABA_B receptors in chronically hypertensive rats increases. Unfortunately, at present an antibody that would permit immunoblotting (Western) or immunopre-

the one-kidney, figure-8 renal wrap model of hypertension. Therefore, alterations within the NTS in the MAP responses to activation of the GABA_B receptor system, but not in the MAP responses to activation of GABA_A receptors, might be a general characteristic of chronic hypertension. However, in the present study, no difference was found between normotensive and hypertensive rats in their responses to NTS microinjection of GABA_B antagonists. This has important functional implications as it suggests that unlike the SHR and DOCA-salt models of hypertension, in one-kidney, figure-8 renal wrap hypertension GABA_B receptor function is not tonically elevated. A tonic increase in GABA_B receptor function, inferred from the enhanced response to injection of a GABA_B agonist in SHR and DOCA-salt hypertensive rats, has been suggested as a possible contributor to baroreflex resetting in hypertension. However, it is difficult to reconcile this suggestion with the observation that DOCA-salt hypertensive rats, while exhibiting the enhanced response to NTS microinjection of GABA_B agonists and antagonists, do not exhibit an attenuated aortic depressor reflex. Therefore, it is difficult to determine the functional significance of tonically enhanced GABA_B receptor responses as far as baroreflex function is concerned.

The observation that the enhanced pressor response to microinjection of baclofen into the NTS was not accompanied by an enhanced percentage increase in RSNA presents several possible interpretations. The first is that baroreceptor inhibition of sympathetic nerve activity (SNA) under resting conditions is similar in normotensive and hypertensive rats, and the enhanced pressor response results from an augmented release of a circulating hormone. An earlier study by Sved and Sved found that the amplitude and duration of the pressor response that followed the microinjection of baclofen into the NTS was significantly reduced after the intravenous administration of a vasopressin antagonist. Therefore, the augmented pressor response could be due to enhanced vaso-

Figure 3. A, Population means for log mass ratios of GABA_B mRNA for sham-operated normotensive (shaded bar) and renal wrap hypertensive (open bar) rats. Numbers in each bar indicate number of animals from which samples were obtained. B, Gel run from samples obtained from 2 normotensive sham-operated animals and 2 hypertensive animals.
cipitation analyses of the GABA<sub>B</sub> receptor protein directly in our micropunches of NTS is not commercially available. Therefore, this question will remain unresolved until such antibodies are available or a radioligand binding study is performed.

To gain some insight into the molecular regulation of the GABA<sub>B</sub> receptor in hypertension, we analyzed mRNA for the receptor. We found a dramatic increase in GABA<sub>B</sub> mRNA level after 4 weeks of hypertension. Assuming that this mRNA is translated into functional receptor protein, such an increase could result in an enhanced response to GABA<sub>B</sub> receptor agonist. Several factors could induce an increase in GABA<sub>B</sub> receptor mRNA. Increases in synaptic inputs to neurons have been shown to alter gene expression, and the elevated baroreceptor input to NTS neurons might initiate transcription of GABA<sub>B</sub> receptor mRNA. Circulating hormones have also been shown to alter gene expression, and altered levels of angiotensin or catecholamines in chronic hypertension could induce an increased expression of GABA<sub>B</sub> receptor mRNA. The precise stimulus, or stimuli, that induces the increased GABA<sub>B</sub> mRNA levels in chronic hypertension will be the focus of future studies.

**Significance**

In chronic one-kidney, figure-8 renal wrap hypertension, there is an enhanced response to the NTS microinjection of the GABA<sub>B</sub> agonist baclofen. This suggests an upregulation of receptor function in this model of chronic hypertension. However, the responses to microinjection of GABA<sub>B</sub> receptor antagonists are not different when comparing normotensive and hypertensive rats; therefore, it does not appear that there is a tonic “overactivation” of GABA<sub>B</sub> receptors in this model of hypertension. The chronic hypertension is associated with an increased level of GABA<sub>B</sub> receptor mRNA in the caudal NTS.

Insights into the functional significance of alterations in GABA<sub>B</sub> receptor function in hypertension will require more information regarding the specific changes that occur in identified cells within this region. For example, from the present data, we cannot discern whether the increase in GABA<sub>B</sub> mRNA occurs in cells that already possess the receptor or whether it represents expression in a population that previously did not express the receptor. We cannot determine whether the increase in GABA<sub>B</sub> mRNA is a generalized occurrence among NTS neurons or whether the changes occur in a restricted subpopulation of NTS neurons. Our recent iontophoretic study examining the responses of NTS neurons to GABA<sub>B</sub> receptor agonists found that cells which presumably received a monosynaptic aortic depressor nerve input were only slightly inhibited by baclofen, whereas cells receiving a polysynaptic input were markedly inhibited by the GABA<sub>B</sub> agonist. The data presented here might reflect a selective increase in the expression of GABA<sub>B</sub> receptor in the monosynaptic population that could enhance inhibitory effects of exogenously applied agonist.

Of course, the important question is what do these changes tell us about the role of endogenous GABA in hypertension? Changes in GABA<sub>B</sub> receptor function might serve a protective role and prevent overactivation of NTS neurons in response to an increase in pressure and resulting increase in baroreceptor afferent discharge. Overactivation of neurons can lead to depolarization inactivation and, in the face of prolonged depolarization, cell death. Numerous electrophysiological studies have found that in many NTS neurons, activation of baroreceptor afferent inputs evokes a negative feedback inhibition of discharge that limits the duration of the initial excitatory input. This feedback inhibition appears to be primarily mediated by activation of both GABA<sub>A</sub> and GABA<sub>B</sub> receptors, although other mechanisms could also be involved. Enhancement of the GABA<sub>B</sub> component of the feedback inhibition could serve a protective function.

Alternatively, changes in GABA<sub>B</sub> receptor function might serve to dampen excitatory inputs and maintain normal baroreflex buffering in the face of increased arterial pressure. In the absence of any adaptive changes in the baroreflex, resting RSNA would be near zero in hypertensive animals. However, a preliminary examination of baroreflex curves in normotensive and hypertensive rats has shown that in hypertensive animals, the curve is shifted to higher MAP values, with no change in gain around the new operating point (M.V. and S.W.M., unpublished observations, 1998). Adaptive changes within the NTS, such as those described here for the GABA<sub>B</sub> receptor system, could serve to maintain the operating point of the baroreflex in a linear region of the right-shifted reflex curve in hypertensive animals. However, as previously discussed, it is difficult to reconcile a role for GABA<sub>B</sub> receptors in baroreflex resetting given the lack of tonically enhanced GABA<sub>B</sub> receptor function. This is indicated by the similar responses of normotensive and hypertensive rats to antagonist, observed in the present study, and the lack of baroreflex resetting in DOCA-salt hypertensive rats, which exhibit enhanced responses to GABA<sub>B</sub> antagonists.

Finally, changes in GABA<sub>B</sub> receptor function could contribute to the attenuated baroreflex inhibition of cardiovascular function observed in hypertension. During sustained stimulation of the aortic nerve, a significant degree of adaptation was observed in the steady-state depressor response evoked in hypertensive rats compared with normotensive rats, and this enhanced adaptation was blocked by NTS microinjection of GABA<sub>B</sub> antagonist. We have observed a similar enhanced adaptation of aortic nerve–evoked depressor responses in one-kidney, figure-8 renal wrap hypertensive rats (J. Zhang and S.W.M., unpublished observations, 1998). Enhanced GABA<sub>B</sub> receptor function could also mediate the enhanced adaptation, which results in attenuation of steady-state baroreflex responses, in this model of hypertension. It is interesting to consider that this would not require tonic activation of an enhanced GABA<sub>B</sub> receptor system. Our observation that GABA<sub>B</sub> antagonist responses are not increased suggests that there is no tonically enhanced activation of the GABA<sub>B</sub> receptor system in this model of hypertension. The enhanced responses would be evident only during increases in pressure and activation of baroreceptor afferent inputs, beyond the new, hypertensive operating point of the reflex.

The functional significance of the changes described here for the GABA<sub>B</sub> receptor system within the NTS of hypertensive animals could be (1) that the changes dampen responses
to increases in afferent input to protect the neurons from overactivation, (2) that the changes permit normal baroreflex buffering of changes in MAP, even in the face of persistently elevated MAP, and/or (3) that the changes contribute to attenuated steady-state baroreflex inhibition in hypertensive animals. The results of the present study demonstrate that changes in physiological state can result in functional alterations in neurotransmitter systems within the NTS, and these alterations can be associated with alterations in the regulation of neurotransmitter systems within the NTS at the level of the gene.

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References

Enhanced γ-Aminobutyric Acid–B Receptor Agonist Responses and mRNA Within the Nucleus of the Solitary Tract in Hypertension
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