Integration of Aortic Nerve Inputs in Hypertensive Rats

Jing Zhang, Steve W. Mifflin

Abstract—The integration of arterial baroreceptor afferent inputs was studied in renal wrap hypertensive (HT) and normotensive (NT) rats. In anesthetized and paralyzed rats, aortic nerve (AN)-evoked depressor responses were reduced in HT compared with NT rats (P<0.05). We tested the hypothesis that the attenuated baroreflex was associated with altered integration of baroreceptor inputs within the nucleus of the solitary tract. Based on onset latency and the ability of monosynaptic neurons (MSNs) to respond to each of 2 AN stimuli separated by 5 ms, cells in HT and NT rats were divided into 3 groups: short-latency MSNs (SLMSNs), long-latency MSNs (LLMSNs), and polysynaptic neurons (PSNs). A higher percentage of PSNs (73% versus 61%) and a lower percentage of SLMSNs (20% versus 27%) or LLMSNs (7% versus 12%) were found in HT rats (P<0.05). In addition, in HT compared with NT rats, the AN onset latency was greater in PSNs (29.9±1.1 versus 26.7±0.8 ms) but not in SLMSNs (5.0±0.5 versus 5.0±0.3 ms) or LLMSNs (22.9±1.2 versus 24.1±0.7 ms) (P<0.05). Finally, in HT compared with NT rats, the number of PSNs responding to a single AN stimulus with multiple action potentials was increased (40% versus 19%) (P<0.05). This was not observed in SLMSNs (26% versus 13%) or LLMSNs (12% versus 18%). The results indicate that renal wrap hypertension is associated with reduced AN-evoked depressor responses. There were also alterations in the integration of AN afferent inputs within the nucleus of the solitary tract, and these alterations were most marked in the PSN population. (Hypertension. 2000;35[part 2]:430-436.)

Key Words: reflex ■ electrophysiology ■ hypertension, renal

In human1,2 and many animal3–8 models, hypertension is associated with alterations in baroreflex regulatory function. Hypertension-induced changes at the level of the baroreceptor result in, or contribute to, altered reflex function.9–11 Numerous studies have also suggested that alterations within various sites within the central nervous system contribute to the changes in reflex function observed in hypertension.6,8,12–17

The nucleus of the solitary tract (NTS) is the primary site of the termination of baroreceptor afferents within the central nervous system.18 Despite its pivotal role in central cardiovascular regulation, its function in hypertension has received little attention.

The goals of the present study were 2-fold. First was to determine whether alterations in baroreflex function occur in a 1-kidney, renal wrap model of hypertension,19 which, in contrast to genetic models such as spontaneously hypertensive rats (SHR) or Dahl rats, is a surgically induced model of hypertension. This was accomplished through electrical stimulation of the aortic nerve (AN), which eliminated altered transduction by the baroreceptor as a contributing factor to any observed alterations. The second goal was to examine the integration of AN-evoked inputs to neurons in the NTS to determine whether there were any discernible alterations in the integration of baroreceptor afferent inputs during their initial processing within the central nervous system.

Methods

Chronic Hypertensive Model

Successful experiments were performed on 179 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 (AAALAC and USDA) laboratory animal room with free access to food and water. All rats were allowed at least 1 week to acclimate before use in any procedures. All experimental protocols were approved by the institutional animal care and use committee. Hypertension was induced in 48 rats through a 1-kidney, renal wrap procedure.19 Rats were anesthetized with 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 the animals. Control animals consisted of sham-operated rats that were similarly anesthetized and received a unilateral nephrectomy but no wrap of the contralateral kidney or rats with no surgical procedures performed before the day of the experiment. Because the responses of the 2 groups of rats were identical, they were grouped together for analysis. Anesthesia was terminated with atipamezole (1 mg/kg IP; Pfizer) at the conclusion of the surgical procedures. Postoperative analgesic agents (nalbuphine HCl [Nubain] IM) were available as needed.

Acute Surgical Preparation

At 4 to 6 weeks after the initial surgery, hypertensive and sham-operated animals were anesthetized with sodium pentobarbital (60 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 the placement of a venous catheter in the tail vein and cannulation of the trachea, the rat
was artificially ventilated with room air supplemented with 100% O2. Subsequent anesthetic agent was administered as an infusion at a rate of 10 to 20 mg·kg⁻¹·h⁻¹ IV. Gallamine triethiodide (20 mg·kg⁻¹·30 min⁻¹ IV) was administered to induce paralysis. A femoral artery was cannulated, and arterial pressure was measured with the use of a strain-gauge transducer. The depth of anesthesia was monitored on the basis of the stability of arterial pressure and heart rate at rest and during a pinch of the hind paw. The ANs were isolated bilaterally and marked with small loops of black suture. The rat was then placed in a stereotaxic head frame, and an occipital craniotomy was performed to expose the dorsal medulla in the region of the obex. An AN, ipsilateral to the site of central recording, was mounted on a bipolar stimulating electrode with care taken to separate the AN as far as possible from the vagus nerve (4 to 10 mm). The electrode and exposed nerve were covered with a mixture of petroleum jelly and mineral oil. The AN was stimulated with constant-current square-wave pulses of 1-ms width and 500 μA at frequencies and durations indicated in the specific protocols.

AN-Evoked Depressor Responses

Approximately 30 minutes after the completion of the acute surgical procedures, AN-evoked depressor responses were tested by altering AN stimulus frequency. A short-duration, 5-second, variable-frequency stimulus was used to construct the relationship between AN stimulus frequency and mean arterial pressure. To examine adaptive properties, a long-duration, 50-second, 5-Hz stimulus was applied.

Data Analysis

Spike2 data acquisition and analysis software (CED) was used to generate peristimulus time (PST) histograms (bin width 1 ms, 40 sweeps of 0.5-Hz AN stimulation) and rate meter histograms (bin width 1 second). Rate meter histograms (bin width 1 second) were used to analyze AN-evoked changes in arterial pressure and to measure the spontaneous discharge of NTS neurons. PST histograms were used to analyze AN-evoked discharge. Data were analyzed with the use of Statistica software (StatSoft) or a χ² test (2×2 or 2×3 tables). The significance of curve shifts was determined with MANOVA. All data are expressed as mean±SEM, and significance was accepted at P<0.05.

Results

AN-Evoked Depressor Responses

Twenty-two 1-kidney, renal wrap hypertensive (HT) and 24 sham-operated, normotensive (NT) rats were used in the present study. The resting arterial pressure of HT rats was significantly higher than that of NT rats (132±3 versus 115±2 mm Hg, P<0.01). Short-duration AN stimulation periods resulted in frequency-dependent depressor responses (Figures 1A and 1B, top traces), and these responses were...
markedly reduced in HT (Figure 1B) compared with NT (Figure 1A) rats.

Throughout the range of AN stimulus frequencies that were tested, the relationship between AN stimulus frequency and the change in blood pressure, expressed as the percent change relative to the baseline level of blood pressure, was shifted upward so that in HT rats, the AN-evoked fall in blood pressure was markedly reduced (Figure 1C, \(P<0.01\)). This upward shift was also evident if the AN-evoked change in blood pressure was expressed as an absolute change in mean arterial pressure (\(P<0.05\)). At AN stimulus frequencies of 5 Hz, blood pressure fell 15±2 mm Hg in NT rats and 10±2 mm Hg in HT rats. At AN stimulus frequencies of 10 Hz, blood pressure fell 20±2 mm Hg in NT rats and 15±2 mm Hg in HT rats. At AN stimulus frequencies of 20 Hz, blood pressure fell 23±2 mm Hg in NT rats and 18±2 mm Hg in HT rats. At AN stimulus frequencies of 40 Hz, blood pressure fell 23±1 mm Hg in NT rats and 17±2 mm Hg in HT rats. At AN stimulus frequencies of 80 Hz, blood pressure fell 26±2 mm Hg in NT rats and 20±3 mm Hg in HT rats. Therefore, the reduced AN depressor reflex observed in HT rats was independent of the manner used to express the fall in blood pressure.

With longer-duration periods of AN stimulation (up to 50 seconds), it was noted in 11 of 13 NT rats that blood pressure continued to fall throughout the period of AN stimulation. In 7 of these 11 rats, the AN-evoked fall in blood pressure consisted of 2 distinct phases (Figure 1A, lower sweep). As illustrated in Figure 1A, the precise time at which the second phase was apparent was not fixed relative to the onset of the stimulus, and in 4 NT rats, there was no indication of a separation into 2 phases. In 6 of 10 HT rats, no such inflection was observed during the falling phase of blood pressure (Figure 1B, lower sweep). In fact, in 2 of 10 HT rats, there was a trend for blood pressure to adapt or to return to control levels during continuous stimulation. Figure 1D illustrates mean responses during 5-Hz AN stimulation as a function of time. Note that in NT rats, blood pressure continues to fall during the 50-second stimulus period, whereas in HT rats, blood pressure has reached steady state within 10 to 20 seconds.

In NT rats, the response to a second period of AN stimulation was reduced compared with the response during the first period, if the 2 periods occurred within 20 minutes of each other. The reduction was specific for the second, or later, phase of the fall in blood pressure; the initial phase was not altered by repeated stimuli. It was also noted in 5 of 10 HT rats that a similar adaptation or desensitization occurred (Figure 1B, lower sweep).

**AN-Evoked Inputs to NTS Neurons**

Responses to electrical stimulation of the AN were observed in 277 NTS neurons in NT rats and 134 NTS neurons in HT rats. The resting blood pressure of the HT rats (143±3 mm Hg, \(n=48\)) was significantly higher (\(P<0.01\)) than that of the NT rats (116±2 mm Hg, \(n=131\)).

**AN Onset Latencies**

The distribution of onset latencies within the MSN population revealed 2 groups: a short-latency group (SLMSNs), whose onset latencies were in the 3- to 12-ms range, and a long-latency group (LLMSNs), whose latencies were in the 18- to 32-ms range (Figure 2A). In a comparison of NT with HT rats, there was no difference in the onset latencies observed in SLMSNs (5.0±0.3 versus 5.0±0.5 ms, \(P=0.93\)) or LLMSNs (24.1±0.7 versus 22.9±1.1 ms, \(P=0.42\)). However, the AN onset latency was greater (\(P<0.05\)) in PSNs recorded in HT rats (29.9±1.1 ms) than in PSNs recorded in NT rats (26.7±0.8 ms).

Table 1 presents the distribution of SLMSNs, LLMSNs, and PSNs within NT and HT rats. PSNs make up a greater percentage of the total population of AN-evoked neurons and MSNs make up a smaller percentage in HT compared with NT rats (\(\chi^2=6.48, P<0.05\)).

**Spontaneous Discharge**

In NT rats, 72% (199 of 277) of AN-evoked neurons were spontaneously active; this did not differ from the percentage observed in HT rats (78%, 104 of 134). As illustrated in Figure 2C, there was no difference between NT and HT rats in the spontaneous discharge (expressed as action potentials/s) of SLMSNs (5.2±1.1 versus 5.5±1.4), LLMSNs (3.2±1.2 versus 2.6±1.2), or PSNs (7.2±0.7 versus 6.1±0.8). (The next section provides an interesting observation regarding the spontaneous discharge of a subset of the PSN population in HT rats.)

**Number of Evoked Action Potentials**

The number of action potentials evoked by 40 AN stimuli delivered at a frequency of 0.5 Hz are presented in Figure 2B. There was no difference between NT and HT rats in this parameter in SLMSNs (41±3 versus 41±5), LLMSNs (45±4 versus 39±1), and PSNs (43±3 versus 51±3) (\(P>0.05\)).

At low stimulus frequencies, NTs neurons typically responded to each AN stimulus with 1 or, on average, \(<1\) action potential. However, there were neurons in all 3 groups in NT and HT rats that responded to a single AN stimulus with \(>1\) action potential. Figure 3 presents a histogram of the number of action potentials evoked by 40 AN stimuli and the number of neurons exhibiting each pattern of discharge response in NT MSNs (A1) and NT PSNs (B1) rats. The grouping around the 40 on the ordinate indicates that most neurons respond to each stimulus with 1 or \(<1\) action potential; however, there are a number of neurons in which AN stimulation evoked \(>1\) action potential. Figure 3A2 indicates that in the HT rats, MSNs responded as in the NT rats, whereas Figure 3B2 indicates that in HT rats, there was an increase in the number of PSNs responding with \(>1\) action potential to each stimulus. This difference was not significant when the absolute number of evoked action potentials were

<table>
<thead>
<tr>
<th>Table 1. Distribution of AN-Evoked Neurons</th>
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<tr>
<td><strong>Cell Type</strong></td>
</tr>
<tr>
<td>SLMSNs</td>
</tr>
<tr>
<td>LLMSNs</td>
</tr>
<tr>
<td>PSNs</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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*\(\chi^2=6.48, P<0.05\) vs control groups.
compared (Figure 2B). However, if the number of action potentials evoked by 40 AN stimuli was divided by the number of stimuli, the percentage of neurons in which this ratio was $>1$ increased in the PSN group in a comparison of NT with HT rats. Table 2 indicates that in HT rats, a higher percentage of PSNs respond to a single AN stimulus with $>$1 action potential ($P_{<0.01}$).

A closer examination of the PSN population revealed an interesting finding. In NT rats, the spontaneous discharge of PSNs responding to a single AN stimulus with multiple action potentials ($11.6 \pm 2.5$ Hz, $n=32$) was greater ($P_{<0.01}$) than that of PSNs responding to a single AN stimulus with 1 action potential ($4.7 \pm 0.5$ Hz, $n=136$). A similar observation was made in HT rats, in which the spontaneous discharge of PSNs responding to a single AN stimulus with multiple action potentials ($7.5 \pm 1.4$ Hz, $n=39$) was greater ($P_{<0.03}$) than that of PSNs responding to a single AN stimulus with 1 action potential ($4.0 \pm 0.7$ Hz, $n=59$).

The percentages of NTS neurons that responded to AN stimulation with, on average, $<1$ action potential for every stimulus are presented for the 3 cell groups in Table 3. As in Table 2, the number of action potentials evoked by 40 AN stimuli was divided by the number of stimuli. The percentage of neurons in which this ratio was $<1$ was not different in any group in which NT were compared with HT rats.

### AN-Evoked Inhibition

In many NTS neurons, AN stimulation evokes not only an initial excitatory response but also a later period of inhibition. With the use of extracellular recordings, excitatory-inhibitory responses can be identified only in neurons with a spontaneous discharge frequency of $>0.5$ action potential/s. As illustrated in Figure 2D, there was no difference between NT and HT rats in the duration of AN-evoked inhibition in the SLMSNs ($224 \pm 22$ versus $273 \pm 40$ ms), LLMSNs ($328 \pm 60$ versus $556 \pm 165$ ms), and PSNs ($224 \pm 14$ versus $275 \pm 26$ ms).

### Discussion

In human$^{1,2}$ and many animal$^{3-8}$ models, hypertension is associated with alterations in baroreflex regulatory function. The present results provide evidence of blunted AN depressor reflexes in renal wrap, HT rats, and these changes are

### Table 2. Percentage of NTS Neurons With Multiple Evoked Action Potentials in Response to AN Stimulation

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Normotensive, n (%)</th>
<th>Hypertensive, n (%)</th>
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<tbody>
<tr>
<td>SLMSNs</td>
<td>10/76 (13)</td>
<td>7/27 (26)</td>
</tr>
<tr>
<td>LLMSNs</td>
<td>6/33 (18)</td>
<td>1/9 (13)</td>
</tr>
<tr>
<td>PSNs</td>
<td>32/168 (19)</td>
<td>39/98* (40)</td>
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* $\chi^2=13.62$, $P<0.01$ vs control group.

### Table 3. Percentage of NTS Neurons With an Average of $<1$ Action Potential per AN Stimulus

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Normotensive, n (%)</th>
<th>Hypertensive, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLMSNs</td>
<td>47/76 (62)</td>
<td>15/27 (56)</td>
</tr>
<tr>
<td>LLMSNs</td>
<td>17/33 (52)</td>
<td>3/9 (33)</td>
</tr>
<tr>
<td>PSNs</td>
<td>107/168 (64)</td>
<td>51/98 (52)</td>
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associated with alterations in the initial integration of baroreceptor afferent inputs within the central nervous system.

AN-Evoked Responses

It is well established that in hypertension, the relationship between arterial pressure and arterial baroreceptor discharge is “reset,” or shifted to higher pressures.9–11 In the present study, electrical stimulation of the AN was used to bypass the aortic baroreceptors and to eliminate this complication. There is evidence to suggest that in this model of hypertension, sympathetic function is enhanced,7 and reduced baroreflex function could contribute to a tonically elevated level of sympathoexcitation. The present data indicate that reflex function is reduced in HT rats, because equivalent stimuli evoke ‘10% less of percentage change in baseline blood pressure in HT compared with NT rats. A previous study in 1 kidney, renal wrap HT rats found blunted heart rate responses to pharmacologically induced increases in pressure,7 so it appears that both the cardiac and vasomotor limbs of the baroreflex are attenuated in this model of hypertension.

Similarly blunted AN-evoked depressor responses were reported in comparison of Wistar-Kyoto rats with SHR but were not observed in deoxycorticosterone acetate-treated HT rats.6 However, a major difference between renal wrap HT rats and SHR is that in SHR, the significance of the blunted AN responses was dependent on the method used to express the change. The AN responses in SHR were significantly different from those in NT Wistar-Kyoto rats only if the changes in blood pressure were expressed as a percentage change in pressure relative to the baseline level of pressure.6,8 Expression of the change in pressure as an absolute change in pressure resulted in no significant difference between the 2 groups.6 In the present study, HT rats exhibited blunted AN depressor responses regardless of the method used to express the change in pressure.

In NT rats, electrical stimulation of the AN for prolonged periods resulted in a reduction in MAP that occurred in 2 phases, and this has been reported for SHR.6,8 In a few animals, the 2 phases overlapped to an extent that made them difficult to differentiate; however, in most instances, the 2 phases were clearly distinct (Figure 1A). During stimuli of short duration, only the first phase was evident. During repeated periods of AN stimulation, the initial phase was not altered; however, the second, later phase was reduced. Therefore, to observe the second phase, it was necessary to separate the periods of AN stimulation by 20 minutes. The mechanisms that result in this biphasic fall in blood pressure during AN stimulation are not known, but due to the fact that the second phase was not observed in the majority of HT rats, as discussed later, curves relating the AN-evoked fall in blood pressure and AN stimulus frequency were constructed with use of the initial phase only. Therefore, the absolute magnitudes of the AN-evoked depressor responses reported here appear smaller than those reported in previous studies.

In HT rats, both phases of the AN-induced fall in blood pressure were altered. First, only the initial phase was observed even during prolonged AN stimulation (Figure 1B) in the majority of HT rats. In addition, AN-evoked depressor

![Figure 3. Distribution of number of action potentials evoked by 40 sweeps of AN stimulation at 0.5 Hz in MSNs in NT (A1) and HT (A2) rats and in PSNs in NT (B1) and HT (B2) rats. Grouping of responses around 40 on abscissa indicates most cells responded to AN stimulation with 1 action potential for each stimulus. In MSNs, this was not altered in HT; however, in PSN population, a higher percentage of total population of neurons respond with >1 action potential in HT rats. Insets in B1 and B2, action potentials evoked by AN stimulation ([star]stimulus artifact) in a neuron from each group.](http://hyper.ahajournals.org/line_graphics/334_Fig3.png)
responses in HT rats were significantly less than those observed in NT rats, regardless of whether the data were expressed as an absolute change in pressure or as a percentage change relative to baseline. Finally, in some HT animals during prolonged AN stimulation, blood pressure began to adapt and return to baseline levels, as previously described for SHR. In SHR, adaptation was observed only during high-frequency AN stimulation (>20 Hz), whereas in the renal wrap model of hypertension used in the present study, adaptation was observed at frequencies as low as 5 Hz. The significance of these alterations in baroreflex function in hypertension is not known, but the results indicate altered reflex function at a site distal to the baroreceptors.

Single-Unit Recordings

These results do not differentiate possible sites of altered responsiveness in the baroreflex pathway downstream to the aortic baroreceptors. There are no apparent alterations in vascular responsiveness to vasoactive agents in this model of hypertension, because after ganglionic blockade, dose-response relationships for phenylephrine, angiotensin II, and vasopressin were not altered in a comparison of NT rats with HT rats. However, altered function within any or all central nuclei or peripheral neurons participating in the baroreflex or at the level of neuroeffector junction could contribute to the reduced depressor reflex that was observed during electrical stimulation of the AN.

We hypothesized that in HT rats, alterations in the integration of AN afferent inputs within the NTS, the initial site of termination of AN afferents, could contribute to the altered depressor reflexes evoked by AN stimulation. Several observations indicate that this is the case. First, there was a significant redistribution within the various groups of neurons, so in HT rats, a higher percentage of the total population of AN-evoked NTS neurons were classified as PSN or, alternatively, there was a relative reduction in the number of PSNs after AN stimulation with multiple-action potentials. In NT rats, however, only 1 action potential. This could be a reflection of enhanced responsiveness to naturally occurring baroreceptor inputs, or it could reflect enhanced neuronal excitability due to convergent inputs or intrinsic membrane mechanisms.

Without information regarding the functional role of these PSNs, we can only speculate on the functional significance of these changes. The alterations result in an increased responsiveness in some PSNs. If these PSNs are GABAergic, the end result could be dampened transmission within the NTS and blunted reflex function. If the alterations occur in another population of PSNs that relay baroreceptor excitation, enhanced responsiveness could be an adaptation to normalize afferent input and maintain reflex function in response to a reduced afferent input due to receptor resetting. A recent report demonstrated that baroreflex function, assessed as a tonic sympathoinhibitory drive, is present in renal wrap HT rats. Adaptations within the NTS such as those described in this report and others may play a role in the maintenance of some degree of baroreflex function in the setting of altered baroreceptor afferent input during long-term elevations in blood pressure.

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References

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