Reflex Influences on Renal Nerve Activity Characteristics in Nephrosis and Heart Failure

GERALD F. DIBONA, SUSAN Y. JONES, and LINDA L. SAWIN
Department of Internal Medicine, University of Iowa College of Medicine and Department of Veterans Affairs Medical Center, Iowa City, Iowa.

Abstract. Cardiac baroreflex regulation of efferent renal sympathetic nerve activity (ERSNA) is abnormal in nephrotic syndrome (NS). The purpose of the present study was to examine the responses of amplitude and frequency of synchronized ERSNA discharge in anesthetized NS rats subjected to reflex maneuvers that alter the activity of and the interaction between aortic and cardiac baroreceptors. Steady-state ERSNA was analyzed in three groups of anesthetized rats: control, NS, and congestive heart failure (CHF) (in the latter, the defect in cardiac baroreflex regulation of ERSNA is peripheral rather than central). In protocol A, analysis was performed during control, after bilateral aortic depressor nerve section, after bilateral cervical vagus nerve section, and during central vagus nerve stimulation (VAGSTIM). In protocol B, analysis was performed during control, after bilateral cervical vagus nerve section, after bilateral aortic depressor nerve section, and during central aortic depressor nerve stimulation in anesthetized NS rats subjected to reflex maneuvers that alter the activity of and the interaction between aortic and cardiac baroreceptors. Steady-state ERSNA was analyzed in three groups of anesthetized rats: control, NS, and congestive heart failure (CHF) (in the latter, the defect in cardiac baroreflex regulation of ERSNA is peripheral rather than central). Whether afferent vagal nerve activity was increased by cardiac baroreceptor stimulation with intravenous volume expansion in anesthetized (3) or conscious (4) rats or by direct electrical stimulation of the afferent vagus nerve in anesthetized rats (4), there was lesser inhibition of ERSNA in NS rats compared with control rats. This defect was specific in that reductions in heart rate and mean arterial pressure were similar in NS and healthy rats. Basal ERSNA has been examined in conscious, chronically instrumented NS rats with arterial and cardiac baroreflexes intact (5). Sympathetic peak detection analysis of ERSNA was used to measure the synchronized bursts (peaks) that are characteristic of postganglionic ERSNA. This enabled a description of the number of active fibers, which is related to peak amplitude, the firing frequency of the active fibers, which is related to the peak-to-peak interval, and the extent of firing synchrony of the active fibers, which is related to the peak duration (6). NS rats had increased heart rate compared with control rats, so that the frequency of synchronized ERSNA discharge (synchronized to the cardiac cycle) was greater in NS rats than in healthy rats. However, compared with healthy rats, there were no differences in duration or amplitude of synchronized ERSNA discharge. Thus, in the basal conscious state, the central defect in cardiac baroreflex regulation of ERSNA in NS rats was not manifest as changes in the duration or amplitude of synchronized ERSNA discharge. The magnitude of synchronized ERSNA discharge was increased by virtue of the increase in frequency with close synchronization to the cardiac cycle in the presence of normal arterial baroreflex regulation of ERSNA.

Increased efferent renal sympathetic nerve activity (ERSNA) has been identified as an important mediator of the abnormal acute and chronic renal sodium handling observed in nephrotic syndrome (NS) (1,2). Examination of aortic and cardiac baroreflex regulation of ERSNA in NS demonstrated that cardiac baroreflex regulation of ERSNA is abnormal, with the defect located in the central, rather than the peripheral, portion of the reflex, whereas aortic baroreflex regulation of ERSNA is normal (3). Whether afferent vagal nerve activity was increased by cardiac baroreceptor stimulation with intravenous volume expansion in anesthetized rats (3) or conscious (4) rats or by direct electrical stimulation of the afferent vagus nerve in anesthetized rats (4), there was lesser inhibition of ERSNA in NS rats compared with control rats. This defect was specific in that reductions in heart rate and mean arterial pressure were similar in NS and healthy rats. Basal ERSNA has been examined in conscious, chronically instrumented NS rats with arterial and cardiac baroreflexes intact (5). Sympathetic peak detection analysis of ERSNA was used to measure the synchronized bursts (peaks) that are characteristic of postganglionic ERSNA. This enabled a description of the number of active fibers, which is related to peak amplitude, the firing frequency of the active fibers, which is related to the peak-to-peak interval, and the extent of firing synchrony of the active fibers, which is related to the peak duration (6). NS rats had increased heart rate compared with control rats, so that the frequency of synchronized ERSNA discharge (synchronized to the cardiac cycle) was greater in NS rats than in healthy rats. However, compared with healthy rats, there were no differences in duration or amplitude of synchronized ERSNA discharge. Thus, in the basal conscious state, the central defect in cardiac baroreflex regulation of ERSNA in NS rats was not manifest as changes in the duration or amplitude of synchronized ERSNA discharge. The magnitude of synchronized ERSNA discharge was increased by virtue of the increase in frequency with close synchronization to the cardiac cycle in the presence of normal arterial baroreflex regulation of ERSNA.

Correspondence to Dr. Gerald F. DiBona, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242.
1046-6673/0808-1232$03.00/0
Journal of the American Society of Nephrology
Copyright © 1997 by the American Society of Nephrology
baroreceptors, i.e., random-order sequential denervation of the aortic and cardiac baroreceptors and stimulation of central portions of the aortic depressor and vagus nerves. The responses in NS rats that have normal aortic baroreflex regulation of ERSNA but a central defect in the cardiac baroreflex regulation of ERSNA were compared with those in congestive heart failure (CHF) rats that have peripheral defects in both aortic and cardiac baroreflex regulation of ERSNA (7,8).

Materials and Methods
Adult, male Sprague-Dawley rats (200 to 250 g at the time of purchase) allowed free access to normal sodium rat pellet diet (Teklad, Madison, WI) and tap water, were used for all experiments. All experiments were conducted in accord with National Institutes of Health Guide for the Care and Use of Laboratory Animals and the guidelines of the University of Iowa Animal Care and Use Committee.

Anesthesia
Rats were anesthetized with methohexitol (short duration, model preparation) or pentobarbital (long duration, acute experimental protocol), 50 mg/kg intraperitoneally. An oral endotracheal tube was inserted, and mechanical ventilation with room air was instituted. Muscle movements were prevented by repeated doses of gallamine (4 to 5 mg/kg intravenously).

Model Preparation
Using techniques described previously and validated for this laboratory, Adriamycin was administered to produce NS (1,2,4,5) and left coronary ligation was performed to produce chronic CHF (1,2,5,7–10). After recovery from anesthesia, rats were returned to individual metabolism cages with free access to normal sodium rat pellet diet and tap water. All subsequent studies were performed between 3 and 4 wk thereafter, at a time when ongoing renal sodium retention and edema formation were present. Age-matched control rats (CONTROL) were studied concurrently. Previous studies have demonstrated that cardiac index is decreased and fails to increase with progressive volume loading-induced increases in cardiac filling pressure in CHF rats (7) compared with both CONTROL (7) and NS (3) rats in which these measurements are normal. The dose of Adriamycin required for cardiotoxicity in the rat is substantially greater than the Adriamycin dose used herein to produce NS (11,12).

Procedures

Catheterization. Catheters were placed in the right femoral artery and femoral vein. The right femoral artery catheter was connected to an electronic pressure transducer (Statham P23Db) for the measurement of pulsatile and mean arterial pressure (MAP) and heart rate (HR). The right femoral vein catheter was connected to an infusion pump set to deliver 0.9% NaCl at 0.05 ml/min for the duration of the experiment.

Renal Sympathetic Nerve Activity Electrode. The left kidney was exposed through a left flank incision via a retroperitoneal approach. With the use of a dissecting microscope, a renal nerve branch from the aortocrenal ganglion was isolated and carefully dissected free. The renal nerve branch was placed on a recording electrode, and ERSNA was amplified (20,000×) and filtered (low, 30 Hz; high, 3000 Hz) via a Grass HIP511 high impedance probe (Quincy, MA), which led to a Grass P511 bandpass amplifier. The amplified and filtered neurogram was channeled to a Tektronix 5113 oscilloscope (Beaverton, OR) and Grass 7D polygraph for visual evaluation and to an audio amplifier/loudspeaker (Grass AM8) for aural evaluation. The quality of the ERSNA signal was assessed by its pulse synchronous rhythmicity; the signal-to-noise ratio was between 3:1 and 5:1. A further assessment was made during an intravenous injection of phenylephrine (3 μg/kg); as MAP increased, ERSNA decreased. When an optimal ERSNA signal was observed, the recording electrode was fixed to the nerve preparation with a silicone cement (Wacker Sil-Gel, Munich, Germany). The electrode cable was sutured to the back muscles and tunneled to the back of the neck where it was exteriorized. The left flank incision was closed.

Vagus and Aortic Depressor Nerve Isolation, Section, and Stimulation
Previous studies have characterized aortic (but not carotid) and cardiac baroreflex regulation of ERSNA in CONTROL, CHF, and NS (2,7) rats, whereas the study presented here was specifically designed to focus on these baroreceptor stations, leaving carotid baroreceptors intact. Through a cervical midline incision, the vagus (VAG) and aortic depressor nerves (ADN) were isolated bilaterally. For section, the nerves were doubly ligated and cut between the two ligatures. VAG nerve section (VAGX) was verified by noting the loss of the normal decrease in MAP, HR, and ERSNA after the intravenous administration of 50 μg/kg 2-methyl serotonin (13). ADN section (ADNX) was verified by noting an attenuation in the magnitude of the normal decrease in HR and ERSNA (measured prior to aortic depressor nerve section) after the intravenous administration of 3 μg/kg phenylephrine; intact carotid baroreceptor function accounted for the residual decreases in HR and ERSNA, because they were absent in rats subjected to combined carotid and aortic baroreceptor denervation (sinoaortic denervation) (3,4,7,14).

For stimulation of either ADN or VAG, a bipolar electrode was placed on the central portion of one of the cut nerves. The bipolar electrode was connected to a Grass S88 stimulator that was set to deliver square wave pulses of 4-V intensity, 2.0-ms duration, and 8-Hz frequency. For either nerve, these stimulation parameters produce a substantial inhibition of ERSNA (4,15–19).

In these experiments, activation of the cardiac baroreflex was performed using vagal stimulation instead of volume loading for several reasons. First, when using volume loading in NS and CHF, it is important to demonstrate that the stimulus to cardiac baroreceptors is similar in the CONTROL and in the NS or CHF rats. In our previous studies (3,7), we achieved this by measuring left ventricular end diastolic pressure (LVEDP). By measuring LVEDP, we could control for the possibility that, even though the volume load might be identical, the alterations in circulatory dynamics and Starling’s forces that exist in NS and CHF could lead to differences in distribution of a volume load, with different levels of LVEDP being achieved. To minimize the extent of invasive instrumentation but still ensure a quantitatively similar stimulus to the afferent limb of the cardiac baroreceptor reflex, we chose vagal stimulation, which permits precise selection of stimulation parameters. Second, based on our previous experience with consecutive ADNX and VAGX (8) and the well-known ability of these two reflex pathways to engage in compensatory responses for each other (20,21), it was deemed important to randomize the order of the denervations and stimulations. The use of a volume loading maneuver instead of vagal stimulation would not have allowed this random intervention approach because of the necessity of either waiting for all of the volume to be excreted (predicted to be greatly delayed in both CHF and NS) or attempting to restore volume status to the control prevolume loading state via hemorrhage (predicted to be difficult in both CHF and NS). Third, our previous
work (3) using changes in arterial pressure to examine arterial baroreflex control of ERSNA and volume loading to examine cardiac baroreflex control of ERSNA already demonstrated that cardiac, but not arterial, baroreflexes were impaired in NS. Fourth, our previous studies using vagal stimulation (4) provided results identical to those with volume loading (3) in terms of assessment of cardiopulmonary baroreflex regulation of renal sympathetic nerve activity in NS (using measurements of integrated voltage), further supporting the view that vagal stimulation reliably produces results and supports conclusions that are similar to those obtained using volume loading.

With respect to the possibility that afferents from organs other than the heart are activated by vagal stimulation, the findings noted above, in which similar results were obtained with vagal stimulation and volume loading, mitigates concerns in this area.

The issue of the specificity of the electrical stimulation parameters for different populations of afferent vagal fibers has been examined previously in the cat (16-19). Using a combination of strength-duration curves, measurement of nerve conduction velocity, and frequency-response curves for both the VAG and ADN, stimulation parameters of 4 V, 2-ms duration, and 8 Hz were shown to uniformly activate only unmyelinated C fibers. After VAG or ADN stimulation, the renal responses of decreased renal vascular resistance (renal vasodilation) or diuresis/naturation were blocked by cooling of the VAG or ADN (i.e., a reflex) or by renal denervation (i.e., due to decreased ERSNA).

**Experimental Protocol**

Two hours were allowed to elapse after surgical preparation. Then, during continuous measurement of MAP, HR, and ERSNA, one of two experimental protocols was undertaken. Protocol A consisted of four 5-min periods: control, bilateral ADNX, bilateral VAGX, and afferent VAG stimulation (VAGSTIM). Protocol B consisted of four 5-min periods: control, VAGX, ADNX, and afferent ADN stimulation (ADNSTIM). These two protocols randomized the order of baroreceptor afferent nerve section and applied afferent baroreceptor nerve stimulation after denervation of both aortic and cardiac baroreceptors to minimize the interaction between these two baroreflex pathways. Because previous studies (10) demonstrated immediate increases in ERSNA after either VAGX or ADNX (sinoaortic denervation) in both CONTROL and CHF rats that waned with time, 30 min was allowed for stabilization and development of steady state after each nerve section before the initiation of data collection. After initiation of each nerve stimulation, 2 min was allowed for stabilization before beginning data collection. These considerations assured steady-state measurements for the application of sympathetic peak detection analysis to the data set from each 5-min period. Before sacrifice of the CHF rats, LVEDP was measured by passing a catheter retrogradely from the right carotid artery across the aortic valve into the left ventricle. Rats were then sacrificed, and postmortem ERSNA was recorded for 30 min; this value was subtracted from all experimental values of ERSNA. At autopsy, pleural spaces and the peritoneal cavity were inspected for evidence of fluid collection. In the CONTROL and NS rats, bladder urine was taken for qualitative measurement of proteinuria using trichloroacetic acid precipitation (0 = lack of turbidity, 4+ = dense precipitate). The heart and kidneys were removed, blotted, and weighed.

**Analysis**

The amplified and filtered neurogram was full-wave rectified, integrated (Grass 7P3 resistance-capacitance integrator, 20-ms time constant) and stored as ERSNA on videotape (Vetter 4000A PCM, Rebersburg, PA) along with the raw neurogram, MAP, and HR (Grass 7P4 Tachograph) signals for later off-line analysis.

**Sympathetic Peak Detection Analysis.** The steady-state ERSNA displayed positive deflections that were proportional to the frequency discharge in the original neurogram and generally occurred with each cardiac cycle. Individual nerve bursts, still observable in the ERSNA record, were smoothed by subsequent filtering at 35 Hz. This smoothed ERSNA was used for analysis of synchronized discharge characteristics. This signal, along with pulsatile arterial pressure, was sampled at 200 Hz using an analog-to-digital converter (Lab-PC+) and standard data acquisition software (LabVIEW version 3.1.1, National Instruments, Austin, TX). HR was calculated by triggering from the systolic arterial pressure wave. The amplitude (height) and frequency of synchronized sympathetic discharges or peaks was determined using the Sympathetic Peak Detection Program (5,6,22-27). This involves the detection of each synchronized sympathetic discharge or peak in ERSNA (i.e., coordinated output from the central nervous system). A peak in synchronized ERSNA is defined as a significant increase followed by a significant decrease with a nadir on each side. The minimum acceptable peak height (threshold) was set at >25% of the average maximum peak height in the data series. Thus, at least 25% of possible nerve fibers were active together to be classified as a synchronized peak in ERSNA. Because peak height depends on the number of active fibers (25), this choice indicates that a sufficient number of fibers are active so as to generate a peak, the height of which is >25% of the peak generated by the maximum number of fibers active in the data series, i.e., the maximum peak height. This threshold was set at the beginning of the analysis for each experiment and not subsequently altered. The characteristic shape of a peak was used to ensure that high frequency noise or other artifacts were not falsely identified as peaks. This was achieved by averaging over two time scales, one fast (20 to 26 ms) and one slow (55 to 65 ms). The crossing of these two averages was used to define a peak. This peak detection profile (threshold, two time-scale averaging) detects >95% of the peaks (i.e., the number of peaks whose discharge was less than the threshold setting is <5% of the total peaks) and does so with a false positive/negative rate of <5% (18). With detection of individual peaks, the time interval until the subsequent peak and the height of each individual peak was calculated.

The rate of peaks occurring (peak frequency), the height of those peaks (peak height), and mean voltage from the original ERSNA signal (ERSNA) were averaged over 1-s periods and saved, together with MAP and HR. These 1-s averages were averaged over the duration of each period and appear in Tables 2 and 3. The mean peak height of the peaks occurring in the control period (absolute value) of each experiment was set to 100%, and the peaks in subsequent periods in that experiment were normalized to that value. Percent occurrence histograms of peak heights for the periods in protocols A and B are shown in Figures 1 and 2, respectively.

**Statistical Analyses**

Statistical analysis was conducted with multiway ANOVA and Scheffe’s test for pairwise comparisons among means (28). Statistical significance was taken at a value of $P < 0.05$. Data in text, tables, and figures are mean ± SEM.

**Results**

Table 1 shows the data for body weight, heart, kidney (both kidneys), organ weight to body weight ratio, and LVEDP in the CONTROL, NS, and CHF rats. All rats gained weight over the month period between the time of purchase and the time of the
Figure 1. Percent occurrence histogram of peak heights in protocol A. Panel A, CONTROL rats; Panel B, congestive heart failure (CHF) rats; Panel C, nephrotic syndrome (NS) rats. Filled circles, control period; open circles, aortic depressor nerve section (ADNX) period; filled squares, vagus nerve section (VAGX) period; open squares, vagus nerve stimulation (VAGSTIM) period. *P < 0.05 for VAGSTIM versus CONTROL, ADNX, and VAGX.

Figure 2. Percent occurrence histogram of peak heights in protocol B. Panel A, CONTROL rats; Panel B, CHF rats; Panel C, NS rats. Filled circles, control period; open circles, VAGX period; filled squares, ADNX period; open squares, aortic depressor nerve stimulation (ADNSTIM) period. *P < 0.05 for ADNSTIM versus CONTROL, VAGX, and ADNX.
In the intact state, intravenous administration of 3 \( \mu g/kg \) phenylephrine decreased ERSNA by 91 ± 6%; after sectioning of the ADN with intact carotid baroreceptor function, ERSNA was decreased by 51 ± 4% \((P < 0.05)\). In the intact state, intravenous administration of 50 \( \mu g/kg \) 2-methyl-serotonin decreased ERSNA by 70 ± 4%; after sectioning of the VAG, ERSNA was decreased by 3 ± 2% \((P < 0.05)\).

Table 2 shows the mean data from protocol A. In CONTROL rats, although there were immediate but transient changes in MAP, HR, and ERSNA after both ADNX and VAGX as previously noted \((8)\), in the presence of intact carotid baroreceptor function these changes were no longer present after the 30-min stabilization period following each nerve section. VAGSTIM significantly decreased MAP, HR, ERSNA, and peak height. In CHF rats, the results were similar to those seen in CONTROL rats, with VAGSTIM producing a significant decrease in MAP, HR, ERSNA, and peak height. VAGSTIM significantly decreased ERSNA and peak height by 17 and 24% in CONTROL rats and by 22 and 34% in CHF rats, respectively. In NS rats, although the responses to ADNX and VAGX were similar to CONTROL and CHF rats, VAGSTIM, while significantly decreasing MAP and HR, did not significantly decrease either ERSNA \((-5\%)\) or peak height \((-1\%)\).

Table 3 shows the mean data from protocol B. In CONTROL rats, although there were immediate but transient changes in MAP, HR, and ERSNA after both VAGX and ADNX as previously noted \((8)\), in the presence of intact carotid baroreceptor function these changes were no longer present.
Table 3. Summary of data for protocol B

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>VAGX</th>
<th>ADNX</th>
<th>ADNSTIM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control (n = 9)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>115 ± 4</td>
<td>117 ± 3</td>
<td>118 ± 4</td>
<td>101 ± 5b</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>326 ± 13</td>
<td>328 ± 11</td>
<td>326 ± 14</td>
<td>305 ± 14b</td>
</tr>
<tr>
<td>ERSNA (mV)</td>
<td>39.9 ± 2.4</td>
<td>39.6 ± 2.5</td>
<td>40.2 ± 2.9</td>
<td>34.0 ± 2.6b</td>
</tr>
<tr>
<td>peak height (mV)</td>
<td>58.8 ± 4.6</td>
<td>59.6 ± 4.4</td>
<td>58.5 ± 4.0</td>
<td>47.7 ± 3.9b</td>
</tr>
<tr>
<td>peak frequency (Hz)</td>
<td>5.97 ± 0.23</td>
<td>5.85 ± 0.14</td>
<td>5.94 ± 0.19</td>
<td>6.65 ± 0.26</td>
</tr>
<tr>
<td><strong>CHF (n = 8)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>122 ± 5</td>
<td>126 ± 7</td>
<td>129 ± 8</td>
<td>107 ± 9b</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>285 ± 8</td>
<td>285 ± 10</td>
<td>282 ± 13</td>
<td>270 ± 9b</td>
</tr>
<tr>
<td>ERSNA (mV)</td>
<td>41.5 ± 1.7</td>
<td>41.3 ± 2.2</td>
<td>41.4 ± 2.1</td>
<td>33.2 ± 2.8b</td>
</tr>
<tr>
<td>peak height (mV)</td>
<td>59.9 ± 3.0</td>
<td>61.0 ± 4.3</td>
<td>60.2 ± 3.7</td>
<td>47.0 ± 3.6b</td>
</tr>
<tr>
<td>peak frequency (Hz)</td>
<td>5.72 ± 0.20</td>
<td>5.66 ± 0.27</td>
<td>5.82 ± 0.21</td>
<td>6.71 ± 0.36</td>
</tr>
<tr>
<td><strong>NS (n = 8)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>132 ± 3</td>
<td>135 ± 4</td>
<td>139 ± 4</td>
<td>118 ± 3b</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>345 ± 11</td>
<td>342 ± 11</td>
<td>345 ± 14</td>
<td>320 ± 15b</td>
</tr>
<tr>
<td>ERSNA (mV)</td>
<td>38.3 ± 2.6</td>
<td>38.5 ± 2.7</td>
<td>40.7 ± 2.7</td>
<td>31.9 ± 2.8b</td>
</tr>
<tr>
<td>peak height (mV)</td>
<td>56.2 ± 3.4</td>
<td>55.1 ± 3.9</td>
<td>55.4 ± 3.7</td>
<td>43.2 ± 3.7b</td>
</tr>
<tr>
<td>peak frequency (Hz)</td>
<td>5.82 ± 0.10</td>
<td>6.28 ± 0.29</td>
<td>6.66 ± 0.42</td>
<td>6.96 ± 0.25</td>
</tr>
</tbody>
</table>

a Data are mean ± SEM. For actual in vivo microvolts, ERSNA and peak height millivolt values may be corrected for 20,000 amplification by dividing by 20 (for example, 47.0 mV = 2.35 μV). ADNSTIM, aortic depressor nerve stimulation. Other abbreviations as in Tables 1 and 2.

b P < 0.05 versus CONTROL, ADNX, and VAGX periods within rat group.

Discussion

Afferent stimulation of either ADN or VAG decreased MAP, HR, ERSNA, and peak height in CONTROL and CHF rats. This was associated with a shift in the percent occurrence histogram of peak heights of synchronized ERSNA discharge to an increased percent occurrence of peaks of smaller height, reflecting lesser numbers of active renal sympathetic nerve fibers (25). Although aortic and cardiac baroreflex regulation of ERSNA is abnormal in CHF rats, the defects are in the periphery at the level of the aortic and cardiac baroreceptors (7). Thus, electrical stimulation of the afferent baroreceptor nerves bypasses these peripheral defects and elicits normal responses, indicating that the central portions of aortic and cardiac baroreflex regulation of ERSNA are normal in CHF (7).

In NS rats, afferent stimulation of the ADN produced responses similar to those seen in CONTROL and CHF rats. Because aortic baroreflex regulation of ERSNA is normal in NS rats (3), this was an expected finding. However, afferent stimulation of the VAG in NS rats, while producing a decrease in MAP and HR, did not result in significant changes in ERSNA and peak height. This was further reflected in the percent occurrence histogram of peak heights of synchronized ERSNA discharge, which was unaffected by afferent stimulation of the VAG. Thus, these results indicate the presence of a central defect in cardiac baroreflex regulation of ERSNA in NS. This defect is specific for ERSNA, because the depressor and bradycardia responses to afferent stimulation of the VAG in NS were unaffected.
In previous studies (4) in which both carotid and aortic baroreceptors were denervated (sinoaortic denervation), afferent VAG stimulation at these settings produced greater decreases in ERSNA in CONTROL rats (70 to 80%); the reductions in ERSNA in NS rats of 50 to 60% were significantly less. It is likely that the lesser degree of renal sympathoinhibition after afferent VAG stimulation observed in the present study was accounted for by the presence of intact carotid baroreceptor function. With intact carotid baroreflex regulation of ERSNA, the decrease in MAP could result in reflex increases in ERSNA, which would tend to offset the sympathoinhibitory influence of afferent VAG stimulation. This experimental design was chosen to more closely relate to the studies in which the individual functions of both aortic (but not carotid) and cardiac baroreceptor pathways regulating ERSNA were evaluated (3,7). As observed previously in sinoaortic denervated rats (4), the results presented here confirm a lesser degree of renal sympathoinhibition during afferent VAGX in NS rats compared with CONTROL or CHF rats, even in the presence of intact carotid baroreceptor function.

In protocols A (Table 2) and B (Table 3), in all groups of rats during all periods, the peak frequency is slightly greater than the HR. This is accounted for by the fact that some cardiac cycles contain more than one peak. A decrease in ERSNA could occur via a decrease in peak frequency with little change in peak height. However, that is not the case with either VAGSTIM or ADNSTIM because, if anything, peak frequency was increased, possibly due to intact carotid baroreflex regulation of ERSNA (23). Thus, the decreases in ERSNA observed with VAGSTIM and ADNSTIM are dependent on decreases in peak height rather than on changes in peak frequency. These results confirm previous observations (3,4). Studies in anesthetized NS rats that used simultaneous measurements of afferent vagal nerve activity and ERSNA during cardiac baroreceptor stimulation with volume expansion (increased right atrial pressure) demonstrated that increases in afferent vagal nerve activity were normal, but the simultaneously measured decreases in ERSNA were attenuated (3). This was most pronounced when the compensatory influence of the arterial baroreceptors was removed by sinoaortic denervation. In sinoaortic denervated NS rats, inhibition of ERSNA was attenuated during volume expansion (conscious rats) or electrical stimulation of the afferent VAG (anesthetized rats) (4). These latter studies (4) also provided evidence for specificity of the defect for ERSNA because MAP and HR responses to both interventions were not affected in NS.

In the presence of intact carotid baroreceptor function, afferent stimulation of either ADN or VAG decreased ERSNA and peak height, but not peak frequency, in CONTROL rats. This was further supported by the percent occurrence histograms of peak heights of synchronized ERSNA discharges, which disclosed an increase in the percent occurrence of smaller peaks. Because peak height is a reflection of the number of active fibers (25), the observed decrease in ERSNA is due to a reduction in the number of active fibers as opposed to the same number of active fibers discharging at a reduced frequency. Thus, afferent stimulation of ADN or VAG elicits responses in central portions of their respective reflex pathways so as to reduce the number of postganglionic renal sympathetic nerve fibers that are actively discharging.

It is known that peak frequency is under sinoaortic baroreflex control (23), i.e., sinoaortic baroreflex activation decreases ERSNA by decreasing peak frequency without influencing peak height, whereas sinoaortic baroreflex deactivation (sinoaortic denervation) increases ERSNA by increasing peak frequency without influencing peak height. However, ADNSTIM could be considered to simulate aortic baroreflex activation, which might be expected to decrease ERSNA by decreasing peak frequency without influencing peak height. However, this was not observed. It is likely that the increase in peak frequency observed in the study presented here reflects intact carotid baroreceptor function in which the decreased MAP caused carotid baroreflex deactivation with increased peak frequency. This influence of intact carotid baroreceptor function prevailed over that of simulated aortic baroreflex activation via ADNSTIM.

Because afferent VAG activity responses to cardiac baroreceptor stimulation are normal in NS, the defect likely lies at or beyond the first synapse in the cardiac baroreflex pathway. One view of the organization of this pathway is as follows. Cardiac baroreceptor vagal afferent fibers (first-order neurons with cell bodies in ganglia of X cranial nerve) terminate in the nucleus tractus solitarius, a synapse using the excitatory transmitter substance L-glutamate. Second-order neurons project from the nucleus tractus solitarius to inhibitory γ-aminobutyric acid-containing neurons in the region near the nucleus ambiguus. These inhibitory γ-aminobutyric acid-containing neurons project to pacemaker neurons in the rostral ventrolateral medulla. The pacemaker neurons in the rostral ventrolateral medulla project to sympathetic preganglionic neurons in the intermediolateral cell column of the thoracolumbar spinal cord (L-glutamate). From the intermediolateral cell column, preganglionic renal sympathetic neurons emerge from the spinal cord and synapse (acetylcholine) in prevertebral and paravertebral ganglia, giving rise to the postganglionic renal sympathetic neurons, which constitute the intrinsic innervation of the kidney (norepinephrine) (reviewed in references 20 and 21).

Thus, the presence of polysynaptic pathway(s) with different neurotransmitter substances allows for the existence of multiple possible sites for this defect. Additional levels of complexity include: (1) the need to account for the specificity of the defect for ERSNA in that cardiac baroreflex inhibition of sympathetic neural outflow pathways that participate in the depressor and bradycardia responses to afferent vagus nerve stimulation are normal in NS; and (2) the existence of a large number of both descending and ascending inputs, potentially excitatory as well as inhibitory, into the various intermediate points in the cardiac baroreflex pathway (20,21).

The central location of the defect in cardiac baroreflex function in NS and its specificity for ERSNA suggest a mechanism involving discrete nuclei or receptors capable of being acted on by hormonal substances, or both. In this regard, the renin-angiotensin system is a candidate mechanism. NS is
characterized by increased activity of the renin-angiotensin-aldosterone system as part of a general neurohormonal activation, likely in response to a perceived (although difficult to measure) underfilling of the circulation. In healthy rats, alterations in endogenous angiotensin II, achieved by manipulations in dietary sodium intake, influence the arterial baroreflex regulation of ERSNA in a pressure-independent manner so that increased angiotensin II results in chronically elevated ERSNA. In this study, in healthy rats (29) and in CHF rats with increased activity of the renin-angiotensin system, intracerebroventricular (as well as intravenous) administration of an angiotensin II AT₁ receptor antagonist (losartan) decreased the heightened level of ERSNA and improved arterial baroreflex regulation of ERSNA toward normal (10). That this mechanism may apply to the central defect in cardiac baroreflex regulation of ERSNA in NS is suggested by preliminary studies using intravenous losartan in conscious NS rats (30,31).

In summary, the defect in cardiac baroreflex function in NS is specific for ERSNA, is central rather than peripheral, and affects the number of active renal sympathetic nerve fibers rather than their firing frequency.

Acknowledgments

This study was supported by Grants DK 15843, HL44546, and HL 55006 from National Institutes of Health and grants from the Department of Veterans Affairs.

References