Reflex effects on components of synchronized renal sympathetic nerve activity

GERALD F. DIBONA AND SUSAN Y. JONES
Department of Internal Medicine, University of Iowa College of Medicine, and Veterans Affairs Medical Center, Iowa City, Iowa 52242

DiBona, Gerald F., and Susan Y. Jones. Reflex effects on components of synchronized renal sympathetic nerve activity. Am. J. Physiol. 275 (Renal Physiol. 44): F441–F446, 1998.—The effects of peripheral thermal receptor stimulation (tail in hot water, n = 8, anesthetized) and cardiac baroreceptor stimulation (volume loading, n = 8, conscious) on components of synchronized renal sympathetic nerve activity (RSNA) were examined in rats. The peak height and peak frequency of synchronized RSNA were determined. The renal sympathoinhibitory response to peripheral thermal receptor stimulation was associated with an increase in the peak height. The renal sympathetic excitatory response to cardiac baroreceptor stimulation was associated with a decrease in the peak height. Although heart rate was significantly increased with peripheral thermal receptor stimulation and significantly decreased with cardiac baroreceptor stimulation, peak frequency was unchanged. As peak height reflects the number of active fibers, reflex increases and decreases in synchronized RSNA are mediated by parallel increases and decreases in the number of active renal nerve fibers rather than changes in the centrally based rhythm or peak frequency. The increase in the number of active renal nerve fibers produced by peripheral thermal receptor stimulation reflects the engagement of a unique group of silent renal sympathetic nerve fibers with a characteristic response pattern to stimulation of arterial baroreceptors, peripheral and central chemoreceptors, and peripheral thermal receptors.

Analysis of synchronized RSNA in the basal state and during certain reflex interventions under normal and some pathophysiological conditions in a variety of species indicates that changes in integrated RSNA are accompanied mainly by parallel changes in peak height, with lesser changes in peak frequency (1–3, 5, 8, 9, 12). From these observations, it could be suggested that when integrated RSNA decreases, actively discharging renal nerve fibers become silent, and that when integrated RSNA increases, previously silent renal nerve fibers begin discharging.

Using single fiber recordings in postganglionic renal sympathetic nerves, peripheral thermal receptor stimulation was shown to activate a group of previously silent renal nerve fibers that exhibit a unique pattern of responses to reflex stimuli, consistent with functional specificity (6). This group of fibers responded to stimulation of peripheral thermal receptors but not to stimulation of arterial baroreceptors or peripheral or central chemoreceptors. It was speculated that analysis of synchronized RSNA during peripheral thermal receptor stimulation would indicate that the increased integrated RSNA is associated with a parallel increase in peak height with little change in peak frequency.

In the current study, peripheral thermal receptor stimulation was achieved by application of heat to the rat's tail (6, 10, 22) in anesthetized rats. Synchronized RSNA was analyzed during peripheral thermal receptor stimulation and, for comparison, during cardiac baroreceptor stimulation (volume loading) in conscious rats, which is known to decrease integrated RSNA (8).

METHODS

Animals

Adult male Sprague-Dawley rats, 250–300 g, were allowed free access to normal sodium rat pellet diet (Teklad) and tap water drinking fluid for all experiments. All experiments were conducted in accordance with the NIH 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) or pentobarbital (long duration), 50 mg/kg ip.

Procedures

Catheterization. Catheters were inserted in a femoral vein for drug and solution infusion, in a femoral artery for measurement of mean (MAP) and pulsatile (PAP) arterial pressure and heart rate (HR), and into the right atrium via the right jugular vein for measurement of mean right atrial pressure (MRAP). All catheters were tunneled to the back of the neck and exteriorized. The femoral arterial and right
atrial catheters were filled with heparinized solution of 5% dextrose in water and plugged with stainless steel pins. The femoral vein catheter was connected to an infusion pump set to deliver 5% dextrose in water at 50 µl/min for the duration of the surgical preparation.

RSNA 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 aorticorenal ganglion was isolated and carefully dissected free. The renal nerve branch was then placed on a recording electrode. RSNA was amplified (20,000–30,000×) and filtered (low, 30 Hz; high, 3,000 Hz) via a Grass model HIP511 high-impedance probe, which led to a Grass model P511 band-pass amplifier. The amplified and filtered neurogram signal was channeled to a Tektronix model 5113 oscilloscope and Grass model 7D polygraph for visual evaluation, to an audio amplifier/loudspeaker (Grass model AM 8) for auditory evaluation, and to a rectifying resistance capacitance voltage integrator using a 20-ms time constant (Grass model 7P3). This rectified and integrated RSNA signal (integrated RSNA) was subsequently filtered at 0.1 Hz to obtain average integrated RSNA and at 35 Hz to obtain a pulsatile voltage signal where individual bursts are smoothed for the application of the Sympathetic Peak Detection Program. The quality of the RSNA neurogram signal was assessed by its pulse synchronous rhythmicity; signal-to-noise ratio ranged between 3:1 and 5:1. A further assessment was made during an intravenous injection of norepinephrine (3 µg); as MAP increased, RSNA decreased. When an optimal RSNA neurogram signal was observed, the recording electrode was fixed to the nerve preparation with a silicone cement (Wacker Sil-Gel). The renal nerve was cut distal to the recording electrode to ensure that afferent renal nerve activity was not recorded. The electrode cable was sutured to the back muscles and tunneled to the back of the neck, where it was exteriorized.

The group of rats prepared for the peripheral thermal receptor stimulation protocol (n = 8) was a different group of rats from that prepared for the cardiac baroreceptor stimulation protocol (n = 8). For the peripheral thermal receptor stimulation protocol, anesthesia was maintained and the rat was allowed to recover from surgery for 1 h. For the cardiac baroreceptor stimulation protocol, the rat was returned to its home cage and allowed to recover from anesthesia and surgery with continued infusion of 5% dextrose in water.

**PERIPHERAL THERMAL RECEPTOR STIMULATION.** At the conclusion of the 1-h postsurgical recovery period and while still anesthetized, the rats were subjected to the acute experimental protocol (n = 8). The femoral arterial catheter was connected to a Statham P23Db electronic pressure transducer coupled to a Grass model 7 polygraph. HR was determined with a Grass model 7P44 tachograph driven by the PAP wave form. The femoral vein infusion of 5% dextrose in water was continued, and the RSNA electrode was connected to a Grass HIP511 high-impedance probe. Thirty minutes later, the quality of the RSNA neurogram was again examined both for pulse synchronous rhythmicity and inhibition of RSNA during increases in MAP following intravenous norepinephrine. If RSNA quality was acceptable, then the experiment commenced. MAP, PAP, HR, MRAP, and RSNA were continuously recorded during a 10-min control period. Then, 0.9% NaCl was infused at 12.5 ml·kg⁻¹·min⁻¹ into the femoral vein to produce an increase in MRAP of at least 3.0 mmHg while recording was continued (8). Then, the rat was killed with an overdose of anesthetic, and postmortem activity, reflecting background noise, was measured for 30 min; this value was subtracted from all experimental values of RSNA.

**RESULTS**

Peripheral Thermal Receptor Stimulation

Baseline data are shown in Table 1. Figure 1 shows the neurogram and the integrated RSNA signal in a
Table 1. Baseline data in the two groups of rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cardiac Baroreceptor Stimulation</th>
<th>Peripheral Thermal Receptor Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>400 ± 13</td>
<td>377 ± 5</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>125 ± 2</td>
<td>117 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>409 ± 12</td>
<td>340 ± 21</td>
</tr>
<tr>
<td>MRAP, mmHg</td>
<td>−0.02 ± 0.5</td>
<td>ND</td>
</tr>
<tr>
<td>Average integrated RSNA, mV (0.1 Hz filtered)</td>
<td>29.8 ± 1.9</td>
<td>23.7 ± 1.8</td>
</tr>
<tr>
<td>Mean integrated RSNA, mV (35 Hz filtered)</td>
<td>30.4 ± 3.4</td>
<td>24.8 ± 2.7</td>
</tr>
<tr>
<td>Peak frequency, Hz</td>
<td>6.47 ± 0.17</td>
<td>6.59 ± 0.20</td>
</tr>
<tr>
<td>Peak height, mV</td>
<td>43.9 ± 4.1</td>
<td>37.7 ± 4.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of experiments. Time constant of the voltage integrator for RSNA was 20 ms. MAP, mean arterial pressure; HR, heart rate; MRAP, mean right atrial pressure; RSNA, renal sympathetic nerve activity; ND, not determined.

A single rat before and after immersion of the rat's tail in hot water. Following immersion of the rat's tail in hot water, there is a marked increase in the amplitude of the synchronized sympathetic bursts with relatively little change in their frequency (5.6 vs. 5.5 Hz). Figure 2 shows the responses of MAP, HR, mean integrated RSNA, peak height, and peak frequency to immersion of the rat's tail in hot water. The data are calculated as percentage of control, and the mean values for the group (n = 8) are shown. Immersion of the rat's tail in hot water elicited rapid responses, consisting of parallel significant increases of ~100% in both mean integrated RSNA and peak height, which were followed 5–15 s later by a significant increase of ~30% in MAP. The increase in HR evolved slowly to a maximum significant increase of ~15–20%, which plateaued at 80 s. This was also the time at which mean integrated RSNA, peak height, and MAP had decreased from their maxima to sustained plateaus that were 20%, 20%, and 10–15% above baseline control, respectively. Peak frequency exhibited an early but short-lived decrease (which did not achieve statistical significance) and returned to baseline control level by 60 s.

When peak height was plotted against mean integrated RSNA (both as % control), there was a significant positive linear correlation with a mean slope of 0.96. The values for the correlation coefficient, \( r \), ranged between 0.81 and 0.95 with values for \( P < 0.001 \) in each rat. When peak frequency was plotted against mean integrated RSNA (both as % control), there was a negative linear correlation that was not significant; the mean slope was −0.11. The values for the correlation coefficient, \( r \), ranged between 0.15 and 0.23 with values for \( P > 0.05 \) in each rat.

### Cardiac Baroreceptor Stimulation

Baseline data are shown in Table 1. During acute volume loading, the increase in MRAP averaged 3–4 mmHg. As observed previously (4, 20), MAP and HR decreased during acute volume loading with the maximum decreases of 6 ± 1 mmHg and 38 ± 8 beats/min, respectively, which were observed at ~150 s after initiation of volume loading. Using the 1-s averages, the cardiac baroreflex was plotted as average integrated RSNA (efferent output) against MRAP (afferent input). Average integrated RSNA decreased as MRAP increased. The ratio of the percentage change in RSNA to the change in MRAP (“gain” of the cardiac baroreflex) was taken as the slope of the linear regression of average integrated RSNA upon MRAP. For the group (n = 8), gain was −2.68 ± 0.21% RSNA/mmHg; the values for the correlation coefficient, \( r \), ranged between 0.35 and 0.60 with values for \( P < 0.001 \) in each rat.

When the data for peak height were expressed as percentage control and plotted against MRAP, a negative linear relationship was observed. The slope of the linear regression for the group was −2.78 ± 0.28% peak height/mmHg; the \( r \) values ranged between 0.37 and 0.62 with values for \( P < 0.001 \) in each rat. The plot of peak frequency against MRAP yielded a relationship that had a slope for the group of 0.06 ± 0.05 Hz/mmHg; the \( r \) values ranged between 0.01 and 0.15, none of which was significant.

The similarity of the slopes of the relationships of average integrated RSNA and peak height versus MRAP during acute volume loading suggested that the decrease in peak height was largely responsible for the decrease in average integrated RSNA. This is more clearly evident in Fig. 3, where the 1-s average values for peak height (mV), peak frequency (Hz), mean integrated RSNA (mV) (derived from Sympathetic Peak Detection Program), and MRAP (mmHg) are plotted against time, beginning 1 min prior to and continuing...
The result is that peripheral thermal receptor stimulation increases RSNA (mean integrated RSNA), and analysis of synchronized RSNA shows this to be associated with a parallel increase in peak height with no significant change in peak frequency. As peak height reflects the number of active renal nerve fibers, this indicates that peripheral thermal receptor stimulation elicits activity in previously silent renal nerve fibers.

In a previous study, single postganglionic renal sympathetic units were identified by their response to electrical stimulation of their preganglionic input, the splanchnic nerve (6). A large fraction of such identified single renal units were spontaneously active and exhibited an expected pattern of response to stimulation of arterial baroreceptors, peripheral and central chemoreceptors, and peripheral thermal receptors. However, a portion of these single renal units lacked spontaneous activity and, although unresponsive to stimulation of arterial baroreceptors and peripheral and central chemoreceptors, responded to peripheral thermal receptor stimulation. These findings indicated that postganglionic renal sympathetic nerve fibers are not a functionally uniform population and suggested the existence of functionally specific subgroups of renal nerve fibers.

The findings in the current study lend further support to this view. A reflex maneuver that activates previously silent renal nerve fibers would be expected to increase integrated RSNA and result in synchronized RSNA characterized by increases in peak height with little change in peak frequency. As shown in Figs. 1 and 2, these are the effects produced by peripheral thermal receptor stimulation.

Peripheral thermal receptor stimulation elicits an immediate increase in RSNA that is sufficient to produce renal vasoconstriction. Thus, while the group of single renal nerve fibers selectively activated by peripheral thermal receptor stimulation (6) exhibited a pat-
tern of reflex responses that was different from the expected pattern of "vasomotor" neurons (7), the increase in integrated RSNA (as observed in multifiber recordings) elicited by this stimulus results in renal vasoconstriction (22). This suggests that with peripheral thermal receptor stimulation, the effector responses to activation of functionally specific smaller groups of renal nerve fibers (single fiber recordings) might be readily masked or overwhelmed by the effector responses to activation of larger groups of renal nerve fibers (multifiber recordings).

Although reflex maneuvers that decrease RSNA could do so by decreasing peak height and/or peak frequency, the decrease in RSNA produced by cardiac baroreceptor stimulation is accompanied by a parallel decrease in peak height without a change in peak frequency. Using a similar protocol in borderline hypertensive rats, we previously observed that the decrease in RSNA produced by cardiac baroreceptor stimulation was accompanied by a parallel decrease in peak height (8). The current findings extend that observation to normotensive rats. These results indicate that cardiac baroreflex activation causes previously discharging renal nerve fibers to become silent.

The peripheral thermal receptor stimulus used herein was previously shown to activate a unique group of functionally specific renal nerve fibers (6). In those studies, the use of single renal nerve fiber recordings required the use of anesthesia. It was the intent of the current studies to analyze synchronized RSNA in a multifiber renal sympathetic nerve preparation during this same peripheral thermal receptor stimulus. Initial pilot studies in conscious rats disclosed that this stimulus produced an avoidance response whereby the rat attempted to remove the tail from the hot water by moving away from it. This necessitated the use of anesthesia for these experiments.

However, it is apparent that anesthesia did not significantly influence the major conclusion, i.e., regardless of whether RSNA was increased in anesthetized rats by peripheral thermal receptor stimulation or decreased in conscious rats by acute volume loading, the change in RSNA was largely dependent on an increase in peak height rather than an increase in peak frequency of synchronized RSNA.

Initially, Malpas and Ninomiya (16) reported that decreases in RSNA produced by graded steady-state stimulation of arterial baroreceptors in anesthetized cats were accompanied by parallel decreases in peak frequency with little change in peak height. However, later studies by Malpas et al. (12) using slower ramp increases in arterial pressure to stimulate arterial baroreceptors in conscious rabbits demonstrated that decreases in RSNA were accompanied by parallel decreases in both peak frequency and peak height. The reasons for this difference in results remains unexplained, but possible contributing factors include the following: anesthetized vs. conscious state, steady-state vs. ramp increases in arterial pressure for stimulation of arterial baroreceptors, and cat vs. rabbit.

Other investigators have examined multifiber and single fiber renal sympathetic responses to various reflex interventions. In the anesthetized cat (19, 23), it was found that alterations in arterial pressure produced changes in integrated voltage of multifiber RSNA and discharge frequency of single fiber activity that were in the same direction. Similar responses were found following stimulation of intestinal receptors with bradykinin (23). There are several important differences between these observations and the results reported herein. First, the processing and analysis of the nerve activity did not permit an evaluation of the separate contributions of increasing peak height and increasing peak frequency to the observed increase in integrated voltage of multifiber RSNA (19, 23). Second, it is possible that the findings are dependent on the reflex interventions being examined, as there is no a priori reason to believe that stimulation of cardiac baroreceptors and peripheral thermal receptors (herein) and stimulation of arterial baroreceptors and intestinal nociceptors (19, 23) would produce similar patterns of response of peak height and peak frequency in RSNA. Third, the difference in species, rat vs. cat, may be significant.
In summary, in the rat, cardiac baroreceptor stimulation to decrease RSNA and peripheral thermal receptor stimulation to increase RSNA resulted in changes in RSNA that were largely dependent on increases in peak height, representing an increase in the number of active renal sympathetic nerve fibers rather than an increase in the peak frequency of synchronized RSNA.

**Perspectives**

The view that reflex changes in RSNA are reflected in parallel changes in peak height of synchronized RSNA has implications for the detection and identification of functionally specific groups of renal nerve fibers. Decreases in peak height imply the silencing of a previously active group of renal nerve fibers and would be associated with the absence of the renal functional response coupled to their activation. The converse would apply to the situation of increases in peak height. The detection of change in renal function coupled to the situation of increases in peak height of synchronized RSNA would apply to the situation of increases in peak height. The converse response coupled to their activation. The converse role of efferent renal nerve activity on renal sensory receptors. Am. J. Physiol. 253 (Renal Fluid Electrolyte Physiol. 22): F767–F777, 1987.