Neural regulation of kidney function by the somatosensory system in normotensive and hypertensive rats

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Zhang, Tao, Chunlong Huang, and Edward J. Johns. Neural regulation of kidney function by the somatosensory system in normotensive and hypertensive rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1749–R1757, 1997.—This investigation examined the renal sympathetic nerve and renal excretory responses to somatosensory stimulation in normotensive and stroke-prone spontaneously hypertensive rats (SHRSP). Somatosensory activation was achieved by either subcutaneous capsaicin administration or exposure of the airways tract to irritant fumes from acetic acid in chloralose-urethan-anesthetized animals. In Wistar rats, blood pressure increased between 10 and 20% (P < 0.001–0.01), renal perfusion pressure was maintained unchanged, renal hemodynamics were unaltered, and urine flow and sodium excretion were decreased by 25 to 50% (P < 0.001–0.05). In the SHRSP, the somatosensory-induced increases in blood pressure were slightly larger (~15–20% P < 0.05) than those of the Wistar rats, whereas the excretory responses were one-half those of the normotensive animals (P < 0.05). The somatosensory challenges reflexly increased integrated renal sympathetic nerve activity in both normotensive and hypertensive rats. The power spectral analysis demonstrated that the increases in percentage power at heart rate frequency and total power were two to three times more (P < 0.05) in the Wistar rats compared with the SHRSP. The reduced ability of the SHRSP to modulate the energy in the renal sympathetic nerve signal at heart rate frequency might explain in part the attenuated functional responses to the somatosensory challenges.

power spectral analysis; sympathetic nerves; sodium excretion

MODULATION OF KIDNEY function represents a fundamental means whereby extracellular fluid volume and consequently cardiovascular homeostasis are maintained. A major regulatory mechanism of renal function is that exerted by the renal sympathetic nerves (8). The renal innervation is almost exclusively adrenergic, with nerve terminals being evident along both the vascular and tubular elements of the kidney (1), whereas physiological studies have demonstrated that direct electrical stimulation of the renal nerves at high rates reduces renal blood flow, glomerular filtration rate, and sodium and water excretion (3). Of greater significance was the early report of LaGrange and co-workers (10), who observed that, at low levels of renal sympathetic nerve stimulation, which had minimal effects on renal hemodynamics, large falls in fluid excretion still occurred. Subsequent studies revealed that this effect was a consequence of a direct action of the renal sympathetic nerves at the epithelial cells of the proximal tubule and thick ascending limb of the loop of Henle, at which site they stimulated sodium reabsorption (2, 9). Thus, via this action, the renal sympathetic nerves may have a major impact on fluid reabsorption and hence the level of extracellular fluid volume and blood pressure.

Renal sympathetic nerve activity is determined primarily by the cardiovascular baroreceptors. Thus decreases in perfusion pressure at the carotid sinus/aortic arch baroreceptors will reflexly raise renal sympathetic nerve activity (22) and result in a renal sympathetic nerve-dependent sodium retention (7). Conversely, volume expansion or mechanical stimulation of the atra results in a suppression of renal sympathetic nerve activity and a concomitant renal sympathetic nerve-mediated diuresis and natriuresis (19). The higher centers may contribute, as Koepke and DiBona (16) have found air-jet stress in conscious rats to cause a sympathetic nerve-dependent impact on kidney excretory function. A further element is the somatosensory system, and studies from this laboratory have shown that electrical stimulation of the brachial plexus (5) or stimulation of the subcutaneous sensory receptors (25) can elevate renal sympathetic nerve activity and lead to a renal sympathetic nerve-dependent antidiuresis and antinatriuresis and renin release (11).

There is now a consensus that activity within the sympathetic nervous system is elevated in hypertensive states (21). Indeed, in single fiber renal sympathetic nerve recordings, Lundin et al. (18) showed that the rate of firing of action potentials was much higher in the spontaneously hypertensive rats (SHR) compared with the normotensive controls. Moreover, the influence of the air-jet stress to cause a renal sympathetic nerve-mediated antinatriuresis and antidiuresis was much more evident in the SHR compared with the normotensive controls (16). By contrast, an earlier report from this laboratory (4) found that somatosensory stimulation using electrical activation of the brachial nerves in the anesthetized rat caused a renal sympathetic nerve-dependent sodium and water retention in normotensive rats, but in stroke-prone SHR (SHRSP), the excretory responses were very much blunted. The reasons underlying this blunted response in the SHRSP, in which renal sympathetic nerve activity was probably elevated, were unclear, but raised the question whether it was a defect within the central nervous system whereby the renal sympathetic nerve activity response might be reduced or altered in the hypertensive model.

This issue was addressed in the present study by comparing not only the magnitude but also the pattern of the renal sympathetic nerve activity responses together with the functional correlates to two different somatosensory challenges in normotensive and hypertensive rats. This was done by means of administration of capsaicin subcutaneously, to activate nociceptors within the skin, and by stimulating receptors within
the airways tract using irritant fumes and measuring renal function and subjecting renal sympathetic nerve activity to fast Fourier transformation to examine in detail changes in power spectral parameters that occurred, particularly at heart rate frequency, which is the point at which most energy in the signal is contained.

METHODS

All surgical techniques were carried out under the auspices of the United Kingdom Government project License PPL 40/1367 and personal investigator license PIL 40/00371 to E. J. Johns, PIL 40/04186 to T. Zhang, and PIL 40/02632 to C. Huang. Male Wistar rats, 308 ± 5 g, and SHRSP, 295 ± 8 g, were initially anesthetized with fluothane in O2-N2O, the femoral vein was immediately cannulated, and chloralose-urethane was given at a dose of 12 mg chloralose-180 mg urethane iv over 45 min followed by supplementary doses every 30 min. The animals were tracheotomized and breathed spontaneously. The left carotid and right femoral arteries were cannulated for measurement of systemic blood pressure and renal perfusion pressure (RPP) using pressure transducers (Spectramed, Statham, Oxnard, CA) and an amplifier (Grayden Electronics, Birmingham, UK).

Renal Function

The left kidney was exposed through a flank incision, and its artery was carefully cleared of connective tissue at the aorta/renal artery junction so that an electromagnetic flowmeter probe (Carolina Medical Electronics, King, NC) could be placed around it for the measurement of renal blood flow. At this site there is minimal risk of injury to the renal sympathetic nerves. Damage to the renal nerves became evident as a high urine flow and sodium excretion, and when this occurred the experiment was not taken further. The left ureter was cannulated to enable collection of urine samples. A thread was placed around the aorta rostral to the renal artery, which, when tightened against the plastic holder, resulted in the aorta being constricted such that RPP could be maintained unchanged in the face of varying systemic pressure (4). Normal saline (150 mM NaCl) was infused at 3 ml/h as soon as the femoral vein had been cannulated and was continued throughout the experiment. A 2-ml bolus of insulin (15 mg/ml) in saline was given as a primer immediately after the surgery had been completed, which was followed by an infusion of saline containing 15 mg/ml insulin at 3 ml/h throughout the rest of the experiment (4). The animals were allowed 2 h to stabilize before the experimental procedures were begun.

Experimental protocol. A series of six 15-min clearance periods were undertaken in four groups of rats, two Wistar and two SHRSP. Two clearances were taken to establish control levels, during the third period the experimental challenge was instigated and, immediately, the fourth clearance was taken. The animals were then allowed 30 min to return toward basal conditions before the recovery periods were taken.

Capsaicin challenge. Capsaicin was given subcutaneously as three 0.1-mg injections. The first injection was given at the start of the third clearance period, and the remaining doses of capsaicin were given at 105-s intervals.

Acetic acid challenge. Once the basal clearance had been taken, a tube containing acetic acid was placed 2–3 cm from the end of the tracheal cannula to stimulate the airways tract irritant receptors for a period of 5 min.

RPP was regulated at the level existing before each challenge to prevent any change in systemic blood pressure from influencing kidney function. Groups of Wistar and SHRSP were exposed to each of these experimental maneuvers. Plasma samples were taken at the beginning and end of each pair of clearance periods. Arterial blood samples (0.4 ml) were withdrawn from the carotid cannula, immediately centrifuged for 2 min (6,000 revolutions/min), and plasma was removed. The remaining packed red blood cells were resuspended in an equal volume of heparinized saline and reinfused into the animal within 5 min. The clearance period was started ~5 min after the reinfusion of the blood sample when the cardiovascular variables had settled. The urine produced during each clearance period was measured gravimetrically. Plasma and urine samples were assayed for inulin, and glomerular filtration rate was calculated as the clearance of inulin and electrolytes were measured by flame photometry (Corning model 410 C, Halstead, UK).

Renal Sympathetic Nerve Recordings

The bladder was cannulated to allow urine to drain, the left kidney was exposed via a retroperitoneal incision, and its ureter was cannulated. The renal sympathetic nerves were carefully dissected, deaned, and placed on bipolar stainless steel (Medwire, New York, NY) electrodes, and once a good pulsatile signal could be observed, in terms of the visual display on the oscilloscope and the sound from the audio-amplifier, they were sealed in place with Wacker Sil gel 604 (Wacker, Munich, Germany). The animals were allowed 2 h to recover from the surgery.

Protocols. Four groups of rats were used, two Wistar and two SHRSP subjected to either the capsaicin or acetic acid challenge. Blood pressure, heart rate, and integrated nerve activity were recorded continuously and displayed on a computer throughout the experiment. Each data collection period involved recording a 3.5-min period of blood pressure and renal sympathetic nerve activity, which was then stored on the hard disk of a computer.

Capsaicin challenge. As soon as the basal level was recorded, the nociceptors were activated by giving capsaicin (0.15 ml sc of a 1 mg/ml solution made up in absolute alcohol) into each forepaw separated by a 105-s period, and during this time a further 3.5-min collection period was carried out. Preliminary trials with the same volume of alcohol had no effect on blood pressure or renal sympathetic nerve activity.

Acetic acid challenge. Once basal levels were recorded, the airways tract irritant receptors were activated by placing a probe of acetic acid 2–3 cm from the end of the tracheal cannula for the 3.5-min data collection period.

Data analysis in the time domain. The renal sympathetic nerve activity was amplified by means of an optically isolated amplifier (Grayden Electronics) with a gain of 100,000 and high- and low-pass filters set at 0.1 and 1 kHz, respectively, to remove low- and high-frequency noise. Both blood pressure and renal sympathetic nerve activity were displayed on a dual-channel oscilloscope and stored on video tape after digitization with a pulse code modulator. The renal sympathetic nerve signal was also passed to an audio amplifier to allow further monitoring. At the same time, signals from the blood pressure and renal sympathetic nerve channels were relayed to a computer and digitized by means of an analog-to-digital converter (National Instrument, NB-M10–16H, Austin, TX).

A data acquisition program, written in LabVIEW language, was used for online analysis and, with the use of a sampling frequency of 100 Hz, generated the mean blood pressure, heart rate, and renal sympathetic nerve activity, which was rectified, integrated, and displayed every 1 s. A mean value...
was estimated over the 3.5-min collection period for each variable. Thirty minutes after the animals were killed, background noise activity was measured in the renal nerves, and the values were subtracted from the results. If the integrated value of the renal sympathetic nerve activity under basal conditions at the start of the study was <2 mV/s, the data were not analyzed further or included in the study.

Data analysis in the frequency domain. During the final minute of the recording periods when the signals were generally stable, a sample from the amplifier output was taken at high frequency (1 kHz) for blood pressure and renal sympathetic nerve activity, which was stored on the hard disk for off-line processing. The off-line analysis consisted of subjected the blood pressure and renal sympathetic nerve signals to a fast Fourier transformation, which separates the different frequencies present and generates the amount of energy (power) contained at each frequency to produce a power spectrum over a frequency range determined by the operator. This was done by taking the time series for each channel, which was then block averaged over 10 successive data points to obtain 6,100 mean values. To minimize aliasing, the signal was initially divided into three 0.5-min, 50% overlapping segments. Thereafter, each of the three segments was further divided in half and $2^{10}$ points from each of the six subsegments were passed through a Hanning window to minimize “end-leakage,” which may result from a finite length of data collection and possible lack of symmetry (23). The power spectrum was derived and the relative amount of energy in the renal nerve and blood pressure signals at each frequency, from 0 to 10 Hz in 0.1-Hz increments, was calculated. The total power in the spectrum was taken as the area under the curve from 0 to 10 Hz. The power at heart rate frequency was determined by establishing the frequency of the maximum peak in the blood pressure spectrum, and the power in the area of the renal sympathetic nerve spectra, which coincided with the heart rate frequency, ±0.1 Hz, was taken as the absolute power at heart rate frequency. The percentage power was calculated as a proportion of the total power. Cross-correlation analysis between the two signals, blood pressure and renal nerve activity, was performed to generate phase relationships. The phase difference was derived from the measurement of the real part and imaginary part of the cross-power spectrum of the two signals, which generates the phase lag between two signals, with a difference of 0°, demonstrating the phase relation between the two signals and a difference of 180°, which corresponds to a reciprocal relationship between the two signals. The phase difference only indicates the phase relationship between two signals. However, a change in the heart rate frequency could alter the time difference between the two signals, therefore, the time difference was also calculated (5).

Statistics

All data represent the average value calculated from the actual values recorded in individual rats and are expressed as means ± SE. In the functional studies, the effect of each challenge was taken as the difference between the value obtained in the third clearance period, when either capsaicin or acetic acid was given, and the average value of the two control clearances. In the renal sympathetic nerve recording studies, the response to each challenge was calculated as the difference between the value immediately before and that obtained during the administration of either capsaicin or the acetic acid fumes. Percentage changes quoted in the text were not used as a basis for statistical comparisons. Statistical analysis was performed using analysis of variance comparing basal and experimental values within groups, whereas between-group comparisons were followed by a Bonferroni-Dunn post hoc test. Significance was taken when $P < 0.05$.

RESULTS

Renal Functional Responses

Administration of the capsaicin subcutaneously to the Wistar rats (Table 1) resulted in a significant increase in blood pressure of some 8% ($P < 0.001$), and while RPP was maintained constant neither renal blood flow nor glomerular filtration rate changed. At the same time there were significant (all $P < 0.001$) reductions in urine flow of 41%, absolute sodium excretion of 62%, and fractional sodium excretions of 57% (Fig. 1A). Although not shown, both hemodynamic and renal functional variables returned toward control levels during the recovery periods. Exposure of the airways tract to the acetic acid (Table 1) significantly ($P < 0.01$) increased blood pressure, the magnitude of which was similar to that observed with the capsaicin challenge, and while renal perfusion pressure was kept at control levels neither renal blood flow nor glomerular filtration rate was altered. During this challenge, urine flow and absolute and fractional sodium excretions

| Table 1. Hemodynamic and renal functional responses to capsaicin and acetic acid in Wistar and SHRSP rats |
|-----------------------------------------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|
| MBP, mmHg | RPP, mmHg | RBF, ml·kg$^{-1}$·min$^{-1}$ | GFR, ml·kg$^{-1}$·min$^{-1}$ | UV, µl·kg$^{-1}$·min$^{-1}$ | UNaV, µmol·kg$^{-1}$·min$^{-1}$ | FE Na$_{\text{a}}$, % |
| Wistar  |
| Baseline (n = 8) | 115 ± 4 | 113 ± 4 | 17.9 ± 2.8 | 3.75 ± 0.42 | 31.0 ± 5.7 | 4.77 ± 1.3 | 1.08 ± 0.32 |
| Capsaicin | 124 ± 4 | 110 ± 5 | 17.4 ± 2.6 | 4.52 ± 0.60 | 18.4 ± 3.2* | 1.82 ± 0.48* | 0.46 ± 0.15* |
| Baseline (n = 6) | 116 ± 5 | 105 ± 7 | 13.4 ± 1.3 | 3.51 ± 0.73 | 23.3 ± 5.2 | 3.34 ± 0.49 | 0.70 ± 0.15 |
| Acetic acid | 125 ± 6† | 104 ± 7 | 12.6 ± 1.2 | 2.86 ± 0.64 | 18.7 ± 5.0 | 2.61 ± 0.60 | 0.40 ± 0.15† |
| SHRSP |
| Baseline (n = 13) | 167 ± 9 | 160 ± 10 | 23.5 ± 2.5 | 3.55 ± 0.4 | 30.6 ± 3.4 | 5.18 ± 0.76 | 1.15 ± 0.19 |
| Capsaicin | 195 ± 12 | 155 ± 9 | 21.2 ± 3.9 | 3.23 ± 0.5 | 22.7 ± 1.9 | 3.17 ± 0.40 | 0.84 ± 0.14 |
| Baseline (n = 5) | 161 ± 10 | 150 ± 17 | 19.3 ± 3.5 | 2.57 ± 0.49 | 25.0 ± 2.9 | 3.03 ± 0.98 | 0.67 ± 0.18 |
| Acetic acid | 187 ± 11† | 154 ± 17 | 17.8 ± 3.0† | 2.96 ± 0.60 | 25.4 ± 5.0 | 2.70 ± 0.85† | 0.54 ± 0.12† |

Values are means ± SE (n = no. of rats). Baseline represents mean values of 2 clearances obtained just before either capsaicin or acetic acid challenge. Capsaicin or acetic acid is mean value of clearance obtained after administration of compound. MBP, mean blood pressure; RPP, renal perfusion; RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine flow rate; UNaV, absolute sodium excretion; FE Na$_{\text{a}}$, fractional sodium excretion; SHRSP, stroke-prone spontaneously hypertensive rats. * $P < 0.001$; † $P < 0.01$; ‡ $P < 0.05$ comparison between baseline level and the experimental challenge.
were significantly \((P < 0.05-0.01)\) reduced by 20, 22, and 43\%, respectively (Table 1 and Fig. 1B). The magnitudes of these excretory changes were slightly smaller than those obtained as a result of the capsaicin challenge.

The blood pressure of the SHRSP was significantly \((P < 0.001)\) higher than that of the Wistar rats, but the control levels of the renal hemodynamic and excretory variables were very comparable (Table 1). In the SHRSP, capsaicin administration significantly \((P < 0.001)\) increased blood pressure by 16\%, which was larger \((P < 0.05)\) than the response observed in the Wistar group of rats (Table 1). Renal perfusion pressure was successfully stabilized during the capsaicin period, and although neither renal blood flow nor glomerular filtration rate changed, urine flow and absolute and fractional sodium excretions were significantly \((P < 0.05-0.01)\) decreased by 26, 39, and 27\%, respectively (Fig. 1A). The magnitudes of these excretory responses to capsaicin in the SHRSP were all significantly smaller \((P < 0.05)\) than those obtained in the Wistar rats (Fig. 1A).

Exposure of the SHRSP to the acetic acid challenge resulted in an increase in blood pressure of 17\% \((P < 0.01)\), which was also larger than that obtained in the Wistar rats exposed to acetic acid fumes (Table 1, \(P < 0.05\)). RPP was regulated at control levels under these conditions while renal blood flow decreased by 5\% \((P < 0.05)\) and glomerular filtration rate did not change. Absolute and fractional sodium excretions were significantly reduced by 11 \((P < 0.01)\) and 16\% \((P < 0.05)\), respectively, during the activation of the airways tract receptors. The magnitudes of the responses to the acetic acid challenge in absolute sodium excretion were significantly \((P < 0.05)\) smaller in the SHRSP compared with the Wistars (Fig. 1B). Under these conditions, although the responses in urine flow and fractional sodium excretion were less in the hypertensive animals, they did not quite reach statistical significance (Fig. 1B).

Renal Sympathetic Nerve Activity Responses

Figure 2A shows the initial blood pressure and integrated renal nerve responses in one animal to the exposure to the acetic acid challenge. There was a rapid increase in both blood pressure and integrated renal sympathetic nerve activity once the sensory receptors were activated, and, although initially there were fluctuations, they quickly settled back to stable but higher levels. The subsequent processing of the renal nerve signal produced a power spectrum and an example from one Wistar rat and one SHRSP before and during the acetic acid challenge shown in Fig. 2B. It can be seen that there was a major peak corresponding to the heart rate frequency and lesser peaks, one of which would coincide with the respiration frequency. During exposure to the acetic acid there were increases in the sizes of the renal sympathetic nerve spectral peaks coincident with the heart rate frequency. The grouped data from the Wistar rats are given in Table 2 and the percentage changes in Fig. 3A. The acetic acid challenge did not affect heart rate \((406 \pm 12 \text{ vs. } 408 \pm 9 \text{ beats/min})\) but significantly \((P < 0.01)\) increased both blood pressure and integrated renal sympathetic nerve activity by 16 and 31\%, respectively (Table 2). At this time, total power and the percentage power at the heart rate frequency rose between two- and threefold \((P < 0.01-0.001)\), respectively, while phase and time differences between blood pressure and the renal sympathetic nerve activity at heart rate frequency increased by 43 and 31\% (both \(P < 0.01\)), respectively (Table 2). The administration of capsaicin subcutaneously to the Wistar rats also caused increases in blood pressure of
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20% (P < 0.01), heart rate (400 ± 15 vs. 424 ± 15 beats/min, P < 0.01), and integrated nerve activity of 30% (P < 0.01). Under these conditions there were marked increases in total power of some 120% (P < 0.01), percentage power at the heart rate frequency of ~170% (P < 0.01), but no change in either phase or time differences (Table 2). These responses to capsaicin are displayed graphically in Fig. 3B. The magnitudes and patterns of the hemodynamic and renal nerve activity responses to the two different somatosensory challenges were very comparable in the Wistar rats.

The basal values of blood pressure (143 ± 6 vs. 99 ± 6 mmHg), total power (4.8 ± 1.2 vs. 7.3 ± 1.7 W), and percentage power at the heart rate frequency (20 ± 5 vs. 32 ± 6%) were higher, whereas the phase difference was lower in the SHRSP compared with the Wistar rats (1.04 ± 0.10 vs. 1.6 ± 0.2 radians). Exposure of the SHRSP to acetic acid (Table 2) had no effect on heart rate (303 ± 15 vs. 305 ± 17 beats/min), increased blood pressure by 18% (P < 0.001), and increased integrated renal sympathetic nerve activity by 10% (P < 0.01). The power spectrum showed that during this challenge total power in the renal nerve signal increased by 100% (P < 0.05) and the percentage power at the heart rate frequency by 58%, whereas there were significant (P < 0.05) decreases in both phase and time differences of 37 and 38%, respectively (Table 2). Although the magnitudes of the increases in blood pressure and integrated renal sympathetic nerve activity were larger in the SHRSP than Wistar rats (both P < 0.05), the increases in total power and percentage power at heart rate frequency were smaller (P < 0.05), as shown in Fig. 3A. The effect of the capsaicin subcutaneously in the SHRSP (Table 2) was to increase blood pressure by 20% (P < 0.001), heart rate (from 309 ± 11 to 321 ± 10 beats/min, P < 0.001), and integrated renal sympathetic nerve activity by 22% (P < 0.01). At this time, total power increased by 75% (P < 0.01), the percentage power at heart rate frequency by 44% (P < 0.001), and phase and time differences by 18 and 12% (both P < 0.01), respectively. The magnitude of these responses to capsaicin was significantly (P < 0.05–0.001) smaller than those obtained in the Wistar rats, as shown in Fig. 3B.

DISCUSSION

This investigation examined potential reasons for the differences in the renal nerve-mediated functional responses consequent to reflex activation of the renal sympathetic nerves by stimulation of the somatosensory system in normotensive and hypertensive rats (4,

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Values are means ± SE (n = no. of rats). RSNA, integrated renal sympathetic nerve activity; % power HR, percentage power at heart rate frequency; PD, phase difference; TD, time difference. Baseline represents value obtained at start of the study. *P < 0.001; †P < 0.01; ‡P < 0.05.
In these earlier reports (4, 5, 11), the renal sympathetic nerves were reflexly activated using electrical stimulation of the brachial plexus nerves in a way that ensured depolarization of the high-threshold sensory fibers. These fibers have a diverse origin, from muscle, joint, and skin sensory receptors, all of which could have contributed to different degrees and ways to the overall response. To overcome this limitation, two alternative options were taken; the first was to stimulate skin sensory receptors by infusing capsaicin subcutaneously (13), and the second was to expose the airways tract to irritant fumes. In this way, more well-defined somatosensory activation was likely to be achieved. There is a body of evidence showing that pain thresholds and blood pressure responses are elevated in the hypertensive states. Indeed, pain perception, as assessed by tooth pulp stimulation, is raised in borderline hypertensive patients compared with normal individuals (25), while in genetically hypertensive rats both pain sensitivity (27) and blood pressure responses are enhanced (26). Clearly, these differences in nociceptor sensitivity potentially complicate the interpretation of the data generated by the current study. Nonetheless, it was apparent that, although the blood pressure responses to the somatosensory challenge were greater in the hypertensive rats, the renal function and the pattern of renal nerve responses were not.

The renal function studies were undertaken at constant renal perfusion pressure, because pressure at the kidney itself can directly determine the level of sodium and water excretion (24). The data showed that both capsaicin and the irritant fumes led to a modest increase in systemic blood pressure, which probably reflected a generalized activation of the sympathetic nervous system by the somatosensory system (15). The concomitant reductions in urine flow and sodium excretions are probably mediated by the renal nerves, because in a previous study (14) it was shown that the capsaicin-induced reduction in fluid output was abolished after section of the renal nerves. Furthermore, because the antinatriuresis and antidiuresis occurred when renal hemodynamics did not alter, it can be taken that the excretory responses were due to a direct action at the level of the proximal tubule and thick ascending limb of the loop of Henle to stimulate the reabsorptive processes (2, 9). It was striking that in the hypertensive rats, although the capsaicin administration induced an increase in blood pressure somewhat larger in absolute terms than in the normotensive rats, the reductions in sodium excretion and urine flow were quite small, being only about one-half those obtained in the normotensive rats. These depressed excretory responses were similar to those observed in the group of SHRSP exposed to the irritant fumes, that is, attenuated antinatriuretic and antidiuretic responses to activation of a different set of somatosensory receptors. The question arises how fluid excretion might have changed in the two rat strains had perfusion pressure been allowed to rise with arterial pressure. Although the fluid handling would have been subjected to different factors, it is likely that differences between the strains would have been evident, with one contributory factor being the renal sympathetic nerves.

The possibility exists that the attenuated renal excretory responses in the hypertensive rats could arise because of a defect in the generation of sympathetic nerve activity to the kidney. This was addressed by examining the renal nerve activity in detail by subjecting it to power spectral analysis (5, 6, 25) and comparing how the parameters of the signal responded during reflex activation, because this approach has the potential of giving greater insight into how the pattern of nerve activity is organized. After administration of both the capsaicin and the irritant fumes, there was an increase in blood pressure and integrated renal sympathetic nerve activity, which was larger in absolute terms in the SHRSP compared with the Wistar rats but was a consequence of the higher baselines. Any rise in integrated renal sympathetic nerve activity will be associated with a rise in total power because, effec-
tively, both parameters represent the same energy change, one expressed in the time domain and one in the frequency domain. It was evident from the power spectrum that a major component of the energy was associated with a large peak at the heart rate frequency (6–8 Hz), with lesser inconsistent peaks at 1–2 Hz, and were coincident with the respiration frequency. This large proportion of the power at the heart rate frequency is the parameter that is most likely to have an impact in determining kidney function. The power spectral data derived for the Wistar animals showed that the total power and the percentage power at heart rate frequency was increased two- to fourfold by the capsaicin and noxious fume challenges. What this demonstrated was that not only did the total energy in the signal rise but that much of the increased energy in the signal occurred at heart rate frequency. Importantly, there was a large and dynamic response in this parameter, which was generated by the arterial (carotid sinus and aortic arch) baroreceptor-mediated control of the renal sympathetic nerve signal. It was of interest to compare these responses in the power spectral patterns with those obtained previously using electrical stimulation of the brachial nerves (5), in which total power was also increased but the percentage power at the heart rate frequency was markedly reduced, being shifted to the stimulation frequency of the brachial nerves. The point that needs to be emphasized is that, in this earlier report, there were large dynamic responses in the power at the heart rate frequency comparable to those found in the present study in the normotensive rats.

Both the phase and time differences were increased by the capsaicin and irritant fumes, which can be interpreted as meaning that, although the blood pressure was raised by the somatosensory challenge, the increase in renal sympathetic nerve activity that occurred as the blood pressure decreased at the arterial baroreceptors took place further into the blood pressure wave, i.e., further down the systolic/diastolic pressure slope. This would indicate that the arterial baroreflex control of the sympathetic outflow was operating in a way to normalize blood pressure. The role of the arterial baroreflex was also reflected in the time differences, which were independent of any changes in heart rate and tended to lengthen during the somatosensory activation, again indicative of a normal arterial baroreflex coming into play.

The hypertensive rats not only had a higher blood pressure but also a raised integrated renal sympathetic nerve activity if basal levels of all SHRSP groups were used and compared with the Wistar rats. This supports the report from Lundin et al. (18), using single fiber measurements, which showed that the absolute frequency of action potentials was higher in the SHR compared with the Wistar, and indeed, the SHRSP is a substrain of the SHR. The problem with the whole nerve recordings is that differences in the level of activity recorded could be due to the effectiveness and efficiency of the dissection. However, this particular argument cannot be applied to the power spectral analysis, which reflects the pattern of activity in the signal irrespective of absolute levels. This analysis revealed more obvious and marked differences, in that both total power and the percentage power associated with the heart rate frequency were higher in the SHRSP than Wistar, which supports our earlier reports (6, 28) and is further evidence of a raised renal sympathetic nerve traffic. A final point was that the phase and time differences were shorter in the SHRSP compared with the normotensive animals. This feature probably reflected the fact that the arterial baroreceptor reflex is set at a higher pressure; that is, the reflex increase in renal sympathetic nerve activity begins at a higher point along the systolic to diastolic pressure wave and is similar to that reported earlier (6, 28).

It was striking that in the SHRSP the capsaicin-induced increases in total power and percentage power at heart rate frequency were only between one-half to one-third those obtained in the Wistar rats. A very similar blunted neural response in the SHRSP was evident when the somatosensory system was challenged with the airways irritant fumes. This would suggest that in the SHRSP the central drive to generate renal sympathetic nerve activity was raised under basal conditions, relatively rigidly fixed, and less able to be modulated by the somatosensory inputs. Interestingly, these findings are consistent with our earlier observations with the SHRSP (6) using brachial nerve stimulation, in which the power at heart rate frequency was less able to be changed compared with the normotensive control. Together, these pieces of evidence can be taken to show that normally the renal sympathetic nerve traffic to the kidney may not only increase and decrease in magnitude, but, depending on the sensory input from a number of systems of the body, the pattern of activity held within the signal may be changed in that a greater amount of energy arrives associated with the heart rate frequency. By contrast, in the hypertensive situation the increase in energy occurs in a way that less change takes place at the heart rate frequency. This may reflect: some change or deficit of the mechanisms within the central nervous system that organize sympathetic outflow in the SHRSP and could contribute to the different neural control of peripheral organs.

The question arises whether the differing responses in the renal sympathetic nerve power spectral pattern in the hypertensive and normotensive rats to the somatosensory-induced challenges could help in any way to explain the blunted renal functional responses observed in the hypertensive rats. There is a body of evidence, using isolated vessels as well as sympathetic nerve stimulation in vivo (17, 20), demonstrating that larger sympathetically mediated vasoconstrictions could be achieved using high-frequency bursts of impulses rather than a continuous train containing the same number of depolarizing impulses. Furthermore, Harderi (12), using a range of isolated vessels, found that the high-frequency bursts of sympathetic activation caused a greater release of norepinephrine from the tissue than that obtained with the continuous stimulation with the same number of depolarizing pulses. Thus...
there is a relationship between the bursting activity and the amount of norepinephrine released and hence the magnitude of the postsynaptic response. Applying this view to the present study, one can argue that an important characteristic of the renal sympathetic nerve signal during reflex activation is the marked increase in the amount of energy associated with the bursting activity at the heart rate frequency. Thus in the SHRSP, the increase in energy occurring at the heart rate frequency in response to the somatosensory challenges was smaller than in the Wistar and therefore could result in relatively less norepinephrine being released and hence a reduced excretory response being obtained. This argument would be sustained by our earlier observations using electrical stimulation of the brachial nerves, where, in the Wistar rats, a single peak at the stimulation frequency became evident, whereas that at heart rate frequency virtually disappeared (5). By contrast, in the SHRSP, the heart rate frequency persisted and was accompanied by a second prominent peak at stimulus frequency (6), with the overall consequence that, although integrated renal sympathetic nerve activity rose, the pattern of the signal became developed into a more continuous mode; indeed, under these circumstances, the renal nerve-mediated antinatriuresis and antidiuresis were blunted (4).

Perspectives

This study showed that stimulation of the somatosensory system using both subcutaneous capsaicin and exposure to noxious fumes increased blood pressure, with minimal changes in renal hemodynamics and reduced water and sodium excretions. Importantly, the magnitude of the antinatriuretic and antidiuretic responses was much smaller in the hypertensive compared with the normotensive rats. The reasons for this blunted neural control of kidney function are not clear. However, the most important observation was that the renal sympathetic nerve power spectral patterns showed that somatosensory activation increased power at the heart rate frequency to a much greater extent in the Wistar than SHRSP. A strong possibility exists that this insensitivity and inability of the hypertensive rats to appropriately modulate the pattern of activity within the renal sympathetic nerve signal in response to the somatosensory challenge could, in part, explain the reduced functional response. This means that the normal neural regulation of fluid balance does not occur in the hypertensive animals, with the result that the cardiovascular system may be inappropriately stressed in the short term, which in the longer term could contribute to the vascular structural changes that occur as hypertension becomes established. Nonetheless, the question remains as to the cause of this very different patterning of renal sympathetic nerve activity in response to the somatosensory challenge. It suggests that the centers integrating sensory information from all systems of the body may be set with different biases in the hypertensive state. Whether this is so remains to be investigated.

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