Relationship between renal sympathetic nerve activity and renal blood flow during natural behavior in rats

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THE KIDNEY receives a major fraction of the cardiac output. Therefore, it is believed that any changes in renal blood flow (RBF) may exert a primary influence on the regulation of systemic arterial pressure ($P_a$; Refs. 4, 24). For example, exercise or daily activity brings into play $P_a$ regulation. Exercise causes an increase in muscle blood flow resulting in an increased cardiac performance to maintain $P_a$ (13, 24). At the same time, there is a reduction in renal and splanchnic blood flow during exercise, and blood flow is diverted to the contracting muscle area (24). Rowell (24) demonstrated that there was a significant inverse linear relationship between RBF and heart rate (HR) ranging from 80 beats/min at rest to 200 beats/min during exercise in humans. This suggested that normal activity causes changes in RBF that may play a significant role in maintaining $P_a$ at relatively unchanged levels during daily life (16). However, the mechanisms underlying the regulation of RBF in response to everyday activity have not yet been completely elucidated.

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The kidney receives a rich supply of sympathetic nerves, and it is increasingly perceived that renal sympathetic nerve activity (RSNA) plays a role in the regulation of renin release and tubular sodium reabsorption (4, 5). However, the significance of RSNA in the control of RBF has been difficult to determine. One reason underlying this difficulty may be attributed to the use of anesthetic agents that have a variety of different and time-dependent effects on the basal level of RSNA and its reflex control (5). However, a few attempts have been made to measure directly RSNA and RBF in conscious animals, but the results are inconsistent. In the dog, Kirchheim and colleagues (14) found that common carotid artery occlusion to unload carotid baroreceptors increased RSNA by 62% but produced little, if any, change in RBF. Moreover, the exposure of conscious dogs to auditory stimuli, which elicited a 500% increase in RSNA, caused a 40% decrease in RBF (10). Therefore, these observations suggested that renal vasoconstriction occurred only when RSNA was increased severalfold. Contrary to this view, there is evidence showing that RBF can be influenced by more modest changes in the behavioral state and psychological stress in conscious rats (8) and monkeys (6). Recently, Barrett et al. (1) have succeeded in measuring RSNA and RBF in conscious rabbits and found that the renal vasculature was sensitive to episodic increases in RSNA. The reasons underlying the above inconsistent results observed in dog, rat, monkey, and rabbits are not clear. Therefore, it is still uncertain as to the degree of RSNA required to have an impact on the regulation of RBF during daily activity.

The purpose of the present study was to determine whether a relationship existed between RSNA and RBF during different levels of normal daily activity. To achieve this aim, RSNA and RBF to the same kidney were measured simultaneously during different natural behaviors. The rat’s behavior was classified as rapid eye movement (REM) sleep, non-REM (NREM) sleep, quiet awake, moving, and grooming states, which it was hoped would allow a more direct insight into the relationship between changes in RSNA and changes in RBF across the entire range of physical activity.

MATERIALS AND METHODS

Animals. Wistar rats weighing between 250 and 298 g were used for all experiments. Rats were housed individually in a temperature-controlled (24°C), humidity-controlled (60%), and light-controlled (12:12-h light-dark cycle, light 0700–1900) room. The animals were allowed standard laboratory rat chow and water ad libitum and
handled every day. Rats were assigned randomly to two groups: 1) animals with the renal nerves intact in which RSNA and RBF to the same kidney were measured (intact rats, n = 8), and 2) rats in which the renal nerves were sectioned and RBF to the denervated kidney was measured (renal-denervated rats, n = 8).

All procedures were undertaken in accordance with the “Guiding Principles in the Care and Use of Animals in the Field of Physiological Sciences” published by the Physiological Society of Japan (21a) and with the prior approval of the Animal Care Committee of Nara Women’s University.

Surgical procedures. Both intact and renal-denervated rats were operated on in two stages. All procedures were performed aseptically in the operating room.

During the first surgical procedure, the electroencephalogram (EEG), electrocardiogram (ECG), and electromyogram (EMG) electrodes were implanted. The rat was anesthetized with pentobarbital sodium (45 mg/kg ip) and placed in a stereotaxic head holder. EEG electrodes were implanted over the frontal cortex (anteroposterior +2 mm, mediolateral −2 mm from bregma), the parietal cortex (anteroposterior −3 mm, mediolateral −2 mm from bregma), and over the cerebellum (1.5 mm posterior to lambda). Three stainless steel miniature screws (1.0-mm diameter), which served as electrodes, were screwed into the skull and secured with dental cement. Bipolar EMG electrodes were implanted in the bilateral trapezius muscle. A bipolar EMG electrode was also implanted in the biceps femoris muscle of the left and right hindlimbs to identify the exact point of the onset of moving behavior. The bipolar ECG electrode was implanted under the skin at the manubrium of the sternum and xiphoid process. The electrodes were exteriorized between the ears and passed through the center of a circled-cut Ducron sheet, fixed into place, and sutured to the skin. After surgery, the animals were housed individually in transparent plastic cages and allowed standard laboratory rat chow and water ad libitum.

At least 5 days after the first surgery, in the intact rats we implanted the electrode for the measurement of RSNA (18) and a Doppler flow cuff for the measurement of RBF. The left kidney, renal artery, and renal vein were exposed retroperitoneally through a left flank incision. The renal artery was dissected from the vein and connective tissue, and a Doppler flow cuff (Iowa Doppler Products, Iowa City, IA) was placed around the renal artery. Care was taken to preserve the nerves running along side the artery, and the flow cuff was positioned as close to the abdominal aorta as possible. The sympathetic nerves were dissected from the connective tissue with the aid of a dissecting microscope (M651, Leica, Heerbrugg, Switzerland). About 3 mm of the sympathetic nerve close to the kidney was carefully isolated from the connective tissue. A piece of laboratory film (~3 × ~4 mm, Paraffin, American National Can, Greenwich, CT) was placed under the isolated nerve. The tips of the two electrodes were hooked onto the nerve by placing the electrode between the renal nerve and the sheet. The third electrode tip was placed between the sheet and the tissue and served as a grounding electrode. The renal nerve activity was amplified using a differential amplifier, displayed on an oscilloscope and made audible with an audio amplifier. Subsequently, the exposed nerve and the wires of the electrode were embedded in a two-component silicone rubber (932, Wacher-Chemie, Munich, Germany). Once the gel had hardened, the silicone rubber was cut to a size of ~5 × ~5 mm and fixed to the surrounding tissue using glue containing α-cyanoacylate (Aralonpha. Tohwa Gousei Kagaku, Tokyo).

In the renally denervated rats, the renal artery and vein were carefully isolated, stripped of all visible nerves with the aid of the dissecting microscope, and painted with 10% phenol in 95% ethanol. The ureters were stripped of the brous coat near the renal pelvis of the kidney and painted with the phenol solution. The capsule of the kidney and any other nerves or connective tissue near the renal hilum were also stripped and painted with the phenol solution. The Doppler flow cuff was then placed around the renal artery. Thereafter in both groups, the catheters for the measurement of Ppa and central venous pressure (Pcv) were also implanted as described in our previous report (18). Briefly, the arterial catheter was implanted into the abdominal aorta via the tail artery. The tail artery on the ventral side was isolated, and polyethylene tubing (SP8; OD 0.5 mm, ID 0.2 mm, Natsume, Tokyo) was advanced into the tail artery so that the tip was ~1 cm above the aortic bifurcation. The venous catheter was implanted via the right internal jugular vein. The electrode, the probe, and catheters were also exteriorized between the ears and protected by the plastic tube, which ran from the rat to the swivel device on the top of the cage.

On completion of each surgery, antibiotics were given intraperitoneally (Fradiomycin, Mochida-Seiyaku, Tokyo). The animals were housed individually in transparent plastic cages. The animals were allowed standard laboratory rat chow and water ad libitum. Arterial and venous catheters were filled with heparin sodium solution (1,000 IU/100 ml) and were flushed every day.

Measurements. The EEG, ECG, EMG, and RSNA signals were amplified by a differential amplifier (MK-2, Biotech, Kyoto) and filtered at 0.16–50, 100–2,000, 0.16–150, and 150–2,000 Hz, respectively. The Doppler flow cuff was connected to a pulsed Doppler flowmeter (model PDV-20, Crystal Biotech, Northborough, MA). Arterial and central venous pressures were measured by connecting the catheters to a pressure transducer (DX-100, Nihon Kohden, Tokyo). The EEG was Fourier analyzed in 4-s epochs using a data-acquisition program for the computer. The power spectra were averaged in three frequency bands: delta (0.5–4 Hz), theta (4–9 Hz), and sigma (10–14 Hz). The root mean square value of EMG was calculated simultaneously. The amplified RSNA was integrated using a voltage integrator (AD-600G, Nihon Kohden). The area of integrated nerve discharge was calculated simultaneously by means of the computer using an analog-to-digital conversion at 1-ms intervals.

Physiological data were displayed continuously on an oscilloscope and were recorded simultaneously using a thermal head paper recorder and a magnetic tape recorder and sampled for analog-to-digital conversion at 1-ms intervals. The mean values of the data were calculated simultaneously and continuously displayed on the computer every 6 s and stored on a hard disk.

Experiments protocols. Recordings were carried out in a sound-attenuated, temperature (24°C)- and humidity (60%)-controlled chamber (Tabai-Espec, Osaka) not less than 3 days after the second surgery. The recording session was carried out between 10:00 AM and 3:00 PM after 1 h rest when all leads had been connected to the measuring instruments. Each recording session lasted 1–2 h and was repeated two to three times per day. The animals were monitored visually by the investigator through a small acrylic window of the chamber throughout the recording session. The momentary active behavior of each rat was noted at every second.

After the end of the final recording session of each day, the background noise of RSNA was determined when nerve activity was eliminated by increasing Ppa with an intravenous infusion of phenylephrine (10 μg). The background noise was then subtracted from the integrated RSNA data.

At the end of the entire procedure, the animal was killed with an anesthetic overdose, and the kidneys were removed and stored frozen (~40°C) to measure the tissue norepinephrine concentration. The norepinephrine concentration was measured using high-performance liquid chromatography coupled with trihydroxyindole fluorometry (20).

Data analysis. Behavioral states were scored by standard criteria on the basis of EEG and EMG as well as behavioral observations noted at the time of data collection. The animal’s behavior was classified as REM, NREM, quiet awake, moving, and grooming states. REM sleep was characterized by body rotation, irregular breathing, and muscle twitches in different parts of the body; the EEG was desynchronized and displayed low-voltage and high-frequency waves, and the predominant EEG power density occurred within the theta frequency
band, with a high value of the theta/delta ratio with a dramatic suppression of EMG. During NREM sleep, the animal lay immobile with eyes closed; the EEG was synchronized and displayed high-voltage and low-frequency waves and high-power density values in the delta-frequency band. Quiet awake was identified by a low-amplitude EEG, and the animal maintained a lying position with its eyes open. Moving and grooming behavior was identified by the visual observation taken during data acquisition. Moving behavior included any body movement except grooming, eating, and drinking, for example, stretching, exploring, and rearing.

To quantify the RSNA response, percent changes from baseline were calculated by taking the mean of the values during NREM period as 100% RSNA. Renal vascular conductance (RVC) was calculated by dividing RBF by renal perfusion pressure (P a = Pcv).

Statistical analysis. Statistical analysis was performed using ANOVA for repeated measures. When the F-values were significant (P < 0.05), individual comparisons were made using the Fisher’s least significant difference test (25). Dependency between two variables was quantified by a least-squares linear regression (25). Values were reported as means ± SE.

RESULTS
Typical recordings of EEG, EMG, RSNA, integrated RSNA, RBF, and P a during the transition between NREM and REM and between quiet awake to a moving state are shown in Fig. 1, A and B, respectively. RSNA decreased immediately after the onset of REM sleep, which was associated with an increase in RBF. In contrast, when walking commenced, RSNA and EMGs increased immediately while RBF decreased.

The group means of changes in RSNA across the different behavioral states are shown in Fig. 2. RSNA was lowest in REM sleep; it rose gradually in proportion to the increase in physical activity level. The mean value of change in RSNA relative to the NREM level was -29.0 ± 3.2% in REM sleep, 10.9 ± 1.8% in quiet awake, 29.4 ± 2.9% in moving state, and 65.3 ± 3.9% during grooming.

Renal denervation was verified by a marked reduction in the norepinephrine concentration of renal tissue, which in the intact rats was 165.9 ± 8.4 ng/g (wet tissue), whereas that in renal-denervated rats was 12.6 ± 8.3 ng/g (P < 0.05 vs. intact rats). The average changes in renal perfusion pressure (P a = Pcv), RBF, and RVC across the behavioral states in the intact and renal-denervated rats are shown in Fig. 3. In the intact rats, it was apparent that renal perfusion pressure was significantly higher during REM (111.0 ± 1.4 mmHg, P < 0.05) than NREM sleep (108.6 ± 1.1 mmHg), but there was a graded increase in P a during the transition from NREM sleep to quiet awake (109.5 ± 1.3 mmHg) and into moving (111.5 ± 0.7 mmHg, P < 0.05) and grooming (117.7 ± 0.7 mmHg, P < 0.05). A similar pattern was observed with the renal perfusion pressure in the renal-denervated group, that is, it was lowest in NREM sleep (105.2 ± 0.5 mmHg) compared with REM sleep (110.7 ± 0.7 mmHg, P < 0.05), quiet awake (112.2 ± 0.7 mmHg, P < 0.05), moving state (110.5 ± 0.5 mmHg, P < 0.05), and grooming state (117.5 ± 0.5 mmHg, P < 0.05). There was no significant difference in the renal perfusion pressure between intact and renally denervated rats at each corresponding behavioral state (Fig. 3).

In the intact rats, RBF was highest during REM sleep (9.69 ± 0.12 kHz), which increased by 4.8 ± 0.7% (P < 0.05) relative to NREM sleep (9.28 ± 0.10 kHz), while it decreased significantly to 8.78 ± 0.07 kHz (by -5.4 ± 0.6%) and 8.64 ± 0.07 kHz (by -6.6 ± 0.6%) relative to REM sleep during moving and grooming states, respectively (Fig. 3). In the renally denervated rats, RBF did not change significantly during REM sleep, quiet awake, and moving states compared with NREM sleep; however, it increased significantly (P < 0.05) to 9.96 ± 0.10 kHz (by 4.8 ± 0.8%) relative to NREM sleep (9.48 ± 0.08 kHz) during grooming. RBF in intact rats during both moving and grooming states was significantly (P < 0.05) lower than that in renally denervated rats (Fig. 3).

RVC, calculated from renal perfusion pressure and RBF, decreased gradually in the order of REM sleep, NREM sleep, quiet awake, moving, and grooming state in intact rats (Fig. 3). There were significant (P < 0.05) reductions of RVC by 8.4 ± 0.6 Hz/mmHg (-9.0 ± 0.8%) and 11.2 ± 0.6 Hz/mmHg (-12.3 ± 0.7%) during moving and grooming state relative to NREM sleep, respectively. In the renally denervated rats, RVC also decreased significantly by 4.3 ± 1.0 Hz/mmHg (-4.3 ± 0.8%) and 6.2 ± 1.0 Hz/mmHg (-7.1 ± 0.9%) relative to NREM sleep during moving and grooming, respectively; however, the magnitude of reductions of RVC in the renally denervated rats during both moving and grooming states was significantly attenuated compared with those of intact rats.

The relationships between RSNA, RBF, as well as RVC across the five behavioral states were analyzed using mean values for each episode. A significant (P < 0.05) inverse linear correlation was found to exist between the percent changes in RSNA and RBF relative to the NREM level across the entire range of behavioral states (Fig. 4). A significant (P < 0.05) inverse linear correlation was also found to exist between the percent changes in RSNA and RVC relative to the NREM level across the entire range of behavioral states (Fig. 5).

DISCUSSION
The major achievement of the present study was the evaluation of the relationship between RSNA and RBF as well as RVC in the same kidney across all behavioral states. The present study demonstrated that RSNA increased progressively and was associated with an increase in physical activity level in the order REM, NREM, quiet awake, moving, and grooming states, which corresponded inversely to the alterations in RBF. There was a significant inverse linear relationship between RSNA and RBF across the entire range of natural behavioral states. Moreover, we demonstrated that renal denervation abolished the alterations in RBF induced by the changes in behavioral states. Together, these observations suggested that the dynamic responses in RSNA induced by natural behavioral activities had a significant impact on RBF.

We categorized the rat’s behavior into five different states based on the visual observation, EEG, and EMG. Among the five behavioral states observed, NREM sleep occurred most frequently, and because this state was one when the hemodynamics were very stable it was taken as a reference point. In contrast to NREM sleep, the other behavioral states, including moving and REM sleep, did not persist for periods longer than ~200 s. Therefore, the data analyzed in the present study were obtained over a 12- to 200-s period (~90 s on average) once the behavioral state had changed to a different level. Therefore, the following discussion will be limited to the responses that
occurred within the ~90 s after the onset of the behavioral change.

The present study describes the relationship between the changes in RSNA and RBF to the same kidney during natural behavioral changes ranging from REM sleep to grooming in the same animal. This is a sound foundation for understanding the state-dependent changes in RSNA and RBF for which only fragmentary information has been available previously. The present observation that the magnitude of the increase in RSNA was related proportionally to the physical activity level is in good agreement with previous reports. REM sleep resulted in a suppression of RSNA by an average of ~29%

Fig. 1. A: typical recording from an individual rat of electroencephalogram (EEG), electromyogram (EMG; cervical, right hindlimb, left hindlimb), renal sympathetic nerve activity (RSNA), integrated RSNA, renal blood flow (RBF), and systemic arterial pressure (P<sub>a</sub>) during a transition from non-rapid eye movement (NREM) sleep to rapid eye movement (REM) sleep. B: typical recording of EEG, EMG (cervical, right hindlimb, left hindlimb), RSNA, integrated RSNA, RBF, and Pa during a transition from NREM sleep to Moving (Mov) through a short period of quiet awake (Quiet) state. Data are presented at 2 different recording speeds.
relative to the NREM sleep, which was the lowest value found for the five behavioral states (Fig. 2). REM sleep was characterized by a low EMG activity indicative of the low physical activity. Futuro-Neto and Coote (7) have reported in decerebrate cats challenged with physostigmine to induce pseudo-REM sleep that RSNA decreased by 42.5% relative to the NREM sleep, which is in good agreement with the present observations. It is well established that exercise and spontaneous locomotion is associated with an increase in RSNA in rabbits and cats (17, 21). In the present study, RSNA was increased significantly during moving and then to the highest level, of some 65% relative to the NREM sleep, during grooming. Therefore, natural daily activity changes RSNA over the range from 29 to 65% relative to the NREM sleep in rats. These moderate or mild changes in RSNA were well correlated with the degree of change in RBF. Interestingly, RBF also responded in a state-dependent manner, in that it decreased as the physical activity level rose. The mean RBF was highest during REM sleep and lowest during grooming (Fig. 3), which was consistent with previous reports. Cianci et al. (2) have reported that splanchnic blood flow increased significantly during REM sleep relative to the level recorded during NREM sleep in rabbits, while Grady and Bullivant (8), using the rat, have reported that RBF decreased at times when there was an increase in physical activity. In humans, Grimby (9) observed a high correlation between the decreases in RBF during graded exercise and the percentage of maximum oxygen consumption. These reports support the present observations that the alteration in RBF closely corresponded to the increase in physical activity. When the RSNA and RBF observations are taken together, the present study provides the evidence that the
magnitude of the changes in RSNA as well as the RBF could be related to the level of physical activity, and moreover, the state-dependent alterations of RSNA are mirrored by the changes in RBF.

The functional significance of RSNA in regulating RBF in response to daily activity seems to be evident from the present study and supports previous reports. First, in Fig. 4, there was a significant \( (r = 0.61, P < 0.05) \) inverse linear relationship between percent changes in RSNA (range −97.9 to 236.6%) and RBF (range −17.3 to 35.0%) while the \( r \) value between RSNA and RBF was relatively low. It has been well established that small increases in RSNA may have effects on renin release and sodium reabsorption but with minimal changes in RBF (4, 5). Even though the \( r \) value between RSNA and RBF was low, it was statistically significant (Fig. 4), suggesting that the changes in RSNA induced by natural behavioral change could significantly influence the changes in RBF. Second, the present study demonstrated that renal denervation abolished the decrease in RBF observed during moving and grooming in the intact kidney. This is in agreement with the previous reports. Mueller et al. (19) demonstrated in conscious rabbits that the decrease in RBF (range −10 to 17%) in the innervated kidney was evident at all workloads and was intensity dependent; however, there was no significant change in RBF (range 0 to −3.1%) in the denervated kidney at the onset of exercise. Hohimer and Smith (11) demonstrated in the conscious baboon that 4 min of dynamic leg exercise decreased RBF through the innervated kidney by 15% while RBF through the surgically denervated kidney fell only 1%. Furthermore, temporal denervation of the kidney by infusion of Xylocaine markedly attenuated the vasoconstrictor response to mild disturbances and normal daily activities in rats (8). These results suggest that elimination of RSNA either surgically or pharmacologically prevented the kidney from decreasing its blood flow. Third, in Fig. 3, RVC was calculated from the pressure gradient \( (P_a - P_e) \) and RBF using the conventional analog of Ohm’s law, and again the renal denervation attenuated the reduction of RVC during moving and grooming. Moreover, there was a significant inverse relationship between RSNA and RCV (Fig. 5). These findings indicate that RSNA contributes significantly to the reduction of RVC, namely the increase in RSNA may exert a vasoconstrictor influence on the renal vasculature such that RBF is decreased. Taking all the above evidence together, it is safe to conclude that RSNA seems to regulate RBF by exerting a direct vasoconstrictor action on the renal vasculature during different levels of normal daily activity in intact rats.

Perspectives

The observation that the unidirectional changes in \( P_a \), RSNA, and HR occurred during transition from NREM, quiet awake, moving, and grooming states, is consistent with a previous report (15) and may address an important issue of central modulation of baroreflex control of sympathetic nerve activity. The unidirectional changes in \( P_a \), RSNA, and HR cannot be explained by a single baroreflex relationship in that state-related modulation of the baroreflex may be crucial in permitting simultaneous increases in \( P_a \), RSNA, and HR during the transition from NREM to grooming. The neural activity of the higher central regions that are responsible for the behavioral state control, including the periaqueductal gray, amygdala, locus ceruleus, and dorsal raphe, has been shown to change in a state-dependent manner. For instance, the amygdala has a strong influence on arousal state, with evidence showing unit activity to be altered discretely in response to the changes in sleep-wakefulness cycle (22). Similarly, the activities of the noradrenergic neurons of the locus ceruleus have been correlated with changes in sympathetic outflow and behavioral states (23). Indeed, functional connections have been shown to exist between the rostral ventrolateral medulla, which is one of the major nuclei responsible for the baroreflex control of sympathetic outflow, and the central regions, including the periaqueductal gray, amygdala, locus ceruleus, and dorsal raphe (3). However, the detailed mechanisms underlying central modulation of baroreflex control of sympathetic outflow are not fully understood and deserve further study.

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