Relationship between renal sympathetic nerve activity and arterial pressure during REM sleep in rats

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Submitted 23 January 2002; accepted in final form 4 October 2002

Miki, Kenju, Makiko Kato, and Suzuko Kajii. Relationship between renal sympathetic nerve activity and arterial pressure during REM sleep in rats. Am J Physiol Regul Integr Comp Physiol 284: R467–R473, 2003. First published October 10, 2002; 10.1152/ajpregu.00045.2002.—The relationship between renal sympathetic nerve activity (RSNA) and systemic arterial pressure obtained during rapid eye movement (REM) sleep was compared with that obtained in other sleep and awake states. Electrodes for the measurements of RSNA, electrocardiogram, electromyogram, and electroencephalogram and a catheter for the measurement of systemic arterial pressure were implanted while the animals were under aseptic conditions at least 5 days before the experiment. During the transition from non-REM (NREM) to REM sleep, RSNA and heart rate (HR) decreased immediately by 46 ± 2% (P < 0.05) and 22 ± 3 beats/min (P < 0.05), respectively, over 3 s after the onset of REM sleep. Meanwhile, systemic arterial pressure increased gradually after the onset of REM sleep, which was apparently independent of the changes in RSNA. During REM sleep, the relationships between RSNA/HR and systemic arterial pressure were dissociated compared with that obtained during the other behavioral states. These data indicate that the interdependency between systemic arterial pressure and RSNA during REM sleep is likely to be modified compared with other behavioral states.

electroencephalogram; heart rate; behavioral states; rapid eye movement

RAPID EYE MOVEMENT (REM) sleep results in specific changes in cardiovascular status compared with non-REM (NREM) sleep (22). In humans, systemic arterial pressure initially remains either unchanged or increases during the transition from NREM sleep to REM sleep and thereafter increases further and becomes unstable with a wide range of fluctuation (12, 24). Changes in sympathetic nerve activity have been thought to be the main cause for the cardiovascular perturbations occurring during REM sleep (22, 24); however, the functional role of sympathetic nerve activity in regulating systemic arterial pressure during REM sleep remains unclear.

Direct measurement of sympathetic nerve activity during REM sleep has been performed in humans and cats, but the findings of the changes in sympathetic nerve activity during REM sleep are not consistent at present. Sympathetic nerve outflow to the muscle vasculature during REM sleep has been measured in humans using microneurography, and it was found to consistently increase during REM sleep (7, 19, 24). By contrast, Baust et al. (3) using the cat was the first to show a reduction of renal sympathetic nerve activity (RSNA) during REM sleep, which was also reported by Iwamura et al. (8) and Futuro-Neto and Coote (6). The reasons underlying the discrepancy between human and cat observations have not been clarified. Species difference has been pointed out as one possible reason for the difference because systemic arterial pressure decreased consistently in cats (1, 8, 11) while it increased in humans (24). Another possible explanation for the divergent findings may be a regional difference in the sympathetic outflow, which has been observed under various physiological states, including hypoxia, hypercapnia, and spinal cord cooling in mainly acutely prepared animals (23); however, only one study by Futuro-Neto and Coote (6) demonstrated the existence of regional differences of sympathetic outflow during REM sleep in acutely prepared cats. Because it is impossible to measure RSNA during REM sleep in humans, a species other than cats might be suitable for the study of sympathetic regulation of systemic arterial pressure during REM sleep (2, 11). Indeed, in contrast to the cat, the rat has been reported to increase systemic arterial pressure during REM sleep (4, 22), which was similar to that in humans. As yet, no attempt has been made to measure sympathetic nerve activity during REM sleep in the rat.

The present study was designed to examine the responses of sympathetic nerve activity and cardiovascular function during REM sleep in nonanesthetized freely moving rats. Furthermore, the study was designed to evaluate the functional relationship between RSNA and systemic arterial pressure across the sleep and awake states to determine whether the functional influence of RSNA on systemic arterial
pressure during REM sleep was different from other behavioral states.

METHODS

Experiments were performed on 10 Wistar rats, weighing 276 ± 3 g. Rats were housed individually in a temperature-controlled (24°C) and light-controlled (12:12-h light-dark cycle, light 700–1900) room. The animals were allowed standard laboratory rat chow and water ad libitum and handled every day. All procedures were in accordance with the Guiding Principles in the Care and Use of Animals in the Fields of Physiological Sciences published by the Physiological Society of Japan and with the prior approval of the Animal Care Committee of Nara Women’s University. Any animal exhibiting pain or distress was euthanized with an overdose of pentobarbital sodium intravenously.

Surgery. The animals were subjected to two separate surgical procedures that were performed aseptically in the operating room. During the first surgical procedure, the electroencephalogram (EEG), electrocardiogram (ECG), and electromyogram (EMG) electrodes were implanted. The rats were anesthetized with pentobarbital sodium (45 mg/kg ip) and then placed in a stereotaxic head holder. Three screw electrodes, connected to Teflon-coated stainless steel wires for the registration of EEG, were inserted into the skull over the frontal cortex (anteroposterior +2 mm, mediolateral −2 mm from bregma), the parietal cortex (anteroposterior −3 mm, mediolateral −2 mm from bregma), and the cerebellum (1.5 mm posterior to lambda) and secured in place with dental cement. The bipolar EMG and ECG electrodes were implanted under the skin at the manubrium of sternum and xiphoid process in the bilateral trapezius muscles, respectively. The electrodes were exteriorized between the ears and protected by segmental plastic tubes.

At least 5 days after the first surgery, the second surgery was carried out, which involved implantation of the electrodes for the measurement of RSNA and the catheter for the measurement of systemic arterial pressure (14). The left kidney was exposed retroperitoneally through a left flank incision. Approximately 3 mm of the renal sympathetic nerve was carefully isolated, and a bipolar stainless steel wire electrode (AS633, Cooner, Chatsworth) was hooked onto the renal nerve. The renal nerve activity was amplified by a differential amplifier displayed on an oscilloscope and through an audio amplifier. After recordings of the nerve activity were assessed as satisfactory, the wires of the electrode and the isolated nerve were embedded in a two-component silicone gel (932, Wacker-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-cyanoacrylate (Aralonha, Tohwa Gousei Kagaku, Tokyo). Thereafter, the incision was closed. The arterial catheter was implanted into the abdominal aorta via the tail artery. Both electrode and catheter were exteriorized between the ears and protected by a segmental plastic tube.

On completion of each surgery, antibiotics were given intraperitoneally (Fradimycin, Mochida-Seiyaku, Tokyo). The animals were housed individually in transparent plastic cages and then acclimated to the recording environment over the recovery periods. The arterial catheter was filled with a heparin sodium solution (1,000 IU/ml saline) and was flushed every day.

Measurements. Recordings were carried out in a sound-attenuated, temperature-controlled (24°C), and humidity-controlled (60%) chamber (Tabai-Espec, Osaka, Japan) not less than 4 days after the second surgery. The experimental session was carried out between 10:00 AM and 4:00 PM after a 1-h rest when all electrodes and catheters had been connected to the measuring instruments. Each recording session lasted 1.5–2 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, and the active behavior of each rat was noted at every second.

EEG, ECG, EMG, and RSNA signals were amplified with a differential amplifier (MK-2, Biotex, Kyoto, Japan) and filtered at 0.16–50, 100–2,000, 0.16–150, and 150–2,000 Hz, respectively. Arterial pressure was measured by connecting the catheter to the pressure transducer (DX-30, Nihon Kohden, Tokyo). Physiological data were recorded simultaneously on thermal head paper recorder (ORP 1200, Yokogawa-Denki, Tokyo) and a magnetic tape recorder (RX-8016, TEAC, Tokyo) and sampled at 1,000 Hz using the 12-bit analog-to-digital (A/D) converter of the computer. The EEG was Fourier analyzed continuously and simultaneously every 4 s using a data-acquisition program (Visual Designer 4.0, Intelligent Instrumentation, Tucson, AZ) for personal computer. EEG power density values were averaged in three frequency bands: delta (0.5–4.0 Hz), theta (6.0–9.0 Hz), and sigma (10.0–14.0 Hz) bands. The amplified RSNA signal was led to a voltage integrator and then sampled using an A/D converter (at 1,000 Hz), and the area of the integrated nerve discharges was calculated simultaneously by a computer. The root mean square value for the EMG was calculated simultaneously. Heart rate (HR) was determined with a cardiotachometer (AT-601G, Nihon Kohden) triggered by the ECG. The mean values of systemic arterial pressure, HR, integrated RSNA, root mean square value of EMG, the average power density of delta, theta, sigma bands, and the ratio of the average power density of delta to theta band were calculated and stored on the computer disk every 6 s.

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 systemic arterial pressure with an intravenous infusion of phenylephrine (10 μg). The background noise was then subtracted from the integrated RSNA data.

Data analysis. Behavioral states were scored by standard criteria on the basis of EEG and ECG as well as behavioral observations noted at the time of data collection. The animals’ behavior was classified as REM, NREM, quiet awake, moving, and grooming states (Fig. 1). REM sleep was characterized by body relaxation, irregular breathing, and muscle twitches in different parts of the body. The EEG was desynchronized and displayed low-voltage and high-frequency waves; 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 low-voltage and high-frequency waves, high-power density values in the delta-frequency band, and the EMG was low. 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 of the response were calculated by taking the mean of these values during the NREM period as 100% RSNA on each day.

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. Correlations between two variables were analyzed by a least-squares linear regression, and 95% confidence bands for the linear regression line were calculated \((21)\). Values were reported as means ± SE.

RESULTS

Transition between NREM and REM sleep. Figure 2 illustrates a typical recording of ECG, EEG, EMG, systemic arterial pressure, HR, RSNA, and integrated RSNA during a transition between NREM and REM sleep. The integrated RSNA and HR decreased within the first 5–10 s after onset of REM sleep while systemic arterial pressure increased gradually over the first 30–60 s of the REM sleep period.

Changes in mean values of systemic arterial pressure, HR, and RSNA during the transition from NREM to REM sleep for 134 episodes in 10 rats are shown in Fig. 3. Systemic arterial pressure increased gradually after onset of REM sleep, which became significant at 30 s until the end of the REM sleep period. HR and RSNA decreased immediately after onset of REM sleep, and the changes became significant within 3 s, decreasing by \(22 ± 3\) beats/min \((P < 0.05)\) and \(46.0 ± 2.1\% \((P < 0.05)\) at 3 s after onset of REM sleep, respectively. The reductions in HR and RSNA were maintained throughout the REM sleep period.

On termination of REM sleep, the rat exhibited two different behavioral states; we observed 32 episodes of moving and 103 episodes of NREM sleep during the recording sessions for the 10 rats (Fig. 4). With the transition from REM sleep to NREM sleep, systemic arterial pressure immediately decreased toward the pre-REM level and even below the pre-REM level, but this difference did not reach statistical significance. HR and RSNA returned to the pre-REM level preceded by an overshoot increase with a peak value of \(432 ± 3\) beats/min \((9 s, P < 0.05)\) and \(159 ± 8\% \((3 s, P < 0.05)\), respectively, in the NREM group.

During the transition from REM to a moving state, systemic arterial pressure decreased immediately toward the pre-REM level. There was a temporal but significant reduction of systemic arterial pressure that occurred between 15 and 21 s after the end of the REM period compared with the pre-REM level in the moving group. HR and RSNA increased immediately after the end of the REM period and increased significantly relative to the pre-REM level, which was preceded by an overshoot increase to \(447 ± 8\) beats/min \((21 s, P < 0.05)\) and \(160 ± 15\% \((3 s, P < 0.05)\), respectively, in the moving group.

Relationship between systemic arterial pressure, HR, and RSNA across the behavioral states. Table 1 summarizes the mean values in systemic arterial pressure, HR, and RSNA across the range of behavioral states. Mean values for systemic arterial pressure, HR, and RSNA were highest in the grooming state. There were graded reductions in systemic arterial pressure, HR, and RSNA associated with the decreases in physical activity in order as follows: grooming, moving, quiet awake, and NREM sleep. However, systemic arterial pressure increased significantly to the level of moving in REM sleep while HR and RSNA were lowest in this state.

The relationships between the changes in RSNA vs. systemic arterial pressure and HR vs. systemic arterial pressure are shown in Figs. 5 and 6, respectively. There was a significant linear relationship between RSNA and systemic arterial pressure across the states of grooming, moving, quiet awake, and NREM sleep (Fig. 5). The relationship between RSNA and systemic arterial pressure obtained during REM sleep was out of the range of the 95% confidence bands of the linear regression line. There was also a significant linear relationship between HR and systemic arterial pressure among states of grooming, moving, quiet awake, and NREM sleep but not during REM sleep (Fig. 6).

DISCUSSION

We have demonstrated in this study that REM sleep resulted in an immediate and sustained reduction of RSNA followed by a gradual increase in systemic arterial pressure. Because the reduction of RSNA preceded
the increase in systemic arterial pressure, the reduction of RSNA was not directly related to the increase in systemic arterial pressure during the transition to REM sleep. We also demonstrated that the relationship between RSNA and systemic arterial pressure obtained during REM sleep was out of the range of 95% confidence band of the linear regression line generated from data obtained in other awake and sleep states. This suggested that the functional relationship between RSNA and systemic arterial pressure obtained during REM sleep seems to be unique compared with that obtained during other states in rats.

It is generally agreed that a rise in physical activity and/or arousal level causes simultaneous increases in both RSNA and systemic arterial pressure. This may be referred to as the “exercise pressor reflex” (16) or “fight-or-flight reflex” (9). A linear relationship was obtained between RSNA and systemic arterial pressure during natural behavioral states, including NREM, quiet awake, moving, and grooming but not REM sleep (Fig. 5). Although the mechanisms underlying the simultaneous rise in systemic arterial pressure and RSNA associated with the increases in physical and/or arousal levels are beyond the scope of the present investigation, the interrelationship between systemic arterial pressure and RSNA obtained from NREM, quiet awake, moving, and grooming seems to be subjected to the same control systems. However, it was evident from the present study that the relationship between RSNA and systemic arterial pressure during REM sleep was out of the range of 95% confidence band of the linear regression line obtained during other natural behavioral states (NREM, quiet awake, moving, and grooming). The present study also demonstrated that systemic arterial pressure increased gradually during REM sleep despite the step reduction of RSNA that occurred at the same time. Because the reduction of RSNA preceded the increase in systemic arterial pressure at the onset of REM sleep (Fig. 3), it is likely that the trigger for the reduction of RSNA cannot be attributed to the changes in systemic arterial pressure via the baroreflex, assuming that acute resetting of baroreflex control of RSNA may not happen during the transition from NREM to REM sleep. These data suggest that the systems of neural regulation of systemic arterial pressure during REM sleep are different from other behavioral states. It is therefore likely that the interrelationship between RSNA and systemic arterial pressure during REM sleep is unique compared with other behavioral states in rats.

The mechanisms underlying the increase in systemic arterial pressure and decrease in RSNA during REM sleep are not evident. However, it is noteworthy that systemic arterial pressure increased gradually during REM sleep while there was a concomitant sharp reduction of RSNA and HR (Fig. 3). It has been reported that cardiac output is decreased during REM sleep in hu-
mans (15) and rats (25), which would be consistent with the reduction of HR shown in Fig. 3. Therefore, an increase in total peripheral vascular resistance could be responsible, in part, for the increase in systemic arterial pressure during REM sleep. It would seem reasonable that any vasoconstriction that occurred during REM sleep would be caused by neurogenic factors rather than humoral factors, because during the transition from REM to the post-REM state, systemic arterial pressure decreased within the first 3 s to a level slightly below that of the pre-REM period. It is generally accepted that a rapid vasodilatation of this nature can be attributed to neurogenic factors rather than the humoral factors (20). Consequently, it would be expected that sympathetic nerve activity would be increased during REM sleep. Indeed, in humans, it has been reported consistently that sympathetic nerve traffic to the muscle vascular bed was raised during REM sleep. Somers et al. (24) demonstrated that muscle sympathetic nerve activity rose by ~215% while blood pressure reached levels similar to those during wakefulness. Importantly, in the present study, systemic arterial pressure rose to levels comparable to those recorded in the moving state, which would be consistent with the observation of systemic arterial pressure during REM sleep in humans (24). Based on these pieces of evidence, one possible explanation for the increase in systemic arterial pressure during REM sleep could be vasoconstriction of the muscle bed caused by a raised muscle sympathetic nerve activity.

Fig. 3. Changes in $P_a$, HR, and RSNA during the transition from NREM sleep to REM sleep. Heavy continuous lines represent mean values of 134 episodes in 10 rats. Hatched area above and below mean lines represents ± SE. Dotted lines represent the averaged level obtained during NREM (pre-REM) period. *Significant differences ($P < 0.05$) from the mean value of the NREM (pre-REM) period.

Fig. 4. Changes in $P_a$, HR, and RSNA during the transition from NREM sleep to post-REM states. Heavy continuous lines represent mean values of 103 episodes in 10 rats that were in NREM sleep during post-REM period. Light continuous lines represent mean values of 32 episodes in 10 rats that were in moving state during post-REM period. Hatched area above and below mean lines represents ± SE. Dotted lines represent the averaged level of pre-REM (NREM) period shown in Fig. 3. *Significantly different ($P < 0.05$) from mean values of pre-REM period shown in Fig. 3 in NREM group. †Significantly different ($P < 0.05$) from mean values of pre-REM period shown in Fig. 3 in moving group.
These observations impinge on the question of whether REM sleep results in differential responses in sympathetic outflow between the kidney, possibly the heart, and muscle. Futuro-Neto and Coote (6) have reported the existence of regional differences of sympathetic outflow during REM sleep. They demonstrated that there were reductions in the activities of the greater splanchnic nerve, inferior cardiac nerve, lumbar sympathetic chain, and renal nerve, while there was an increase in sympathetic fibers to the gastrocnemius muscle during desynchronized sleeplike periods induced by physostigmine in decerebrated cats. The observation of an increase in sympathetic nerve activity to the gastrocnemius observed by these investigators (6) agreed well with the reports of an increase in muscle sympathetic nerve activity during REM sleep in humans (7, 19, 24). The decrease in renal and inferior cardiac sympathetic nerve activity reported by Futuro-Neto and Coote (6) was comparable to that reported in intact cats (3) and the present observations in rats. This view is compatible with the changes in regional blood flow reported during REM sleep in cats, that is, distinct and profound increases in blood flow to the kidney and mesentery and decreased blood flow to the muscle (5). Together with the above results, it may be concluded that 1) the discrepancy between the observed changes in RSNA in cats (3, 8) and rats (Figs. 2–4) and muscle sympathetic nerve activity in humans (7, 19, 24) may be attributed to the regional difference in the sympathetic outflow (23), not to the species difference; and 2) the increase in systemic arterial

<table>
<thead>
<tr>
<th>No. of Episodes</th>
<th>REM 160</th>
<th>NREM 142</th>
<th>Quiet Awake 60</th>
<th>Moving 103</th>
<th>Grooming 76</th>
</tr>
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<tbody>
<tr>
<td>Duration, s</td>
<td>98 ± 6</td>
<td>118 ± 1</td>
<td>66 ± 8</td>
<td>32 ± 2</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>Systemic arterial pressure, mmHg</td>
<td>105.0 ± 0.8*</td>
<td>97.4 ± 0.8</td>
<td>102.6 ± 1.4*</td>
<td>106.9 ± 0.8*</td>
<td>109.0 ± 1.1*</td>
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<tr>
<td>Heart rate, mmHg</td>
<td>385.9 ± 2.4*</td>
<td>403.1 ± 2.7</td>
<td>419.0 ± 4.4*</td>
<td>459.0 ± 3.0*</td>
<td>470.7 ± 2.8*</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>54.0 ± 1.8*</td>
<td>100</td>
<td>119.5 ± 3.8*</td>
<td>216.5 ± 8.8*</td>
<td>231.0 ± 12.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. NREM, non-rapid eye movement; REM, rapid eye movement. Mean value of integrated renal sympathetic nerve activity (RSNA) during NREM sleep was referred to as 100%. *Significant difference (P < 0.05) with respect to NREM.

Fig. 5. Relationship between RSNA and Pa across changes in behavioral states. Values are means ± SE. Classification of the behavioral states is as in Fig. 1. A significant (P < 0.05) linear relationship between RSNA and Pa (y = 0.073x + 91.8) was obtained between the NREM, quiet awake, moving, and grooming states, but there was a dissociation in the relationship between RSNA and Pa obtained during REM.

Fig. 6. Relationship between HR and Pa across changes in behavioral states. Values are means ± SE. Classification of the behavioral states is as in Fig. 1. A significant (P < 0.05) linear relationship between HR and Pa (y = 0.156x + 35.7) was obtained between the NREM, quiet awake, moving, and grooming states. However, the relationship between HR and Pa obtained during REM sleep was dissociated from the linear regression line.
pressure observed during REM sleep is likely to be caused by the increased resistance of the muscle vascular bed, which is able to overcome the decreased vascular resistance of the visceral organs.

Perspectives

Epidemiological studies have revealed that REM sleep state is an indicator or trigger for the onset of acute cardiovascular accidents such as sudden cardiac death, myocardial infarction, and ischemic stroke (13, 17, 18). We observed in the present study that there was a large increase and overshoot in RSNA and HR during the transition from REM to NREM/moving states in rats (Fig. 4), while systemic arterial pressure was a large increase and overshoot in RSNA and HR during the transition from REM to NREM/moving states in rats (Fig. 4), while systemic arterial pressure undershoot the NREM control level. It is noteworthy that systemic arterial pressures undershoot the NREM control level when the rats were awake and moving at the end of REM sleep. This suggests that the heart needs to accelerate immediately because of an exaggerated increase in sympathetic outflow to maintain systemic arterial pressure within a certain range. Consequently, the elevated sympathetic nerve outflow to the heart may potentially trigger the onset of cardiac arrhythmias and infarcts in patients who have chronic cardiovascular diseases, especially when they wake up and move after the end of REM sleep.

We thank Dr. E. J. Johns (Dept. of Physiology, University College Cork, Ireland) for critical reading of the manuscript. This study was supported in part by the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and a grant from the Kitsuen Kagaku Foundation of Japan.

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