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Nocturnal Oscillations in Plasma Renin Activity and REM-NREM Sleep Cycles in Humans: A Common Regulatory Mechanism?

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Summary: To establish the strength of the relationship between the nocturnal oscillations in plasma renin activity (PRA) and the sleep stage patterns, 42 PRA profiles from blood collected at 10-min intervals and the concomitant polygraphic sleep recordings were analyzed. In all cases, PRA curves exactly reflected the pattern of sleep stage distribution. When sleep cycles were complete, PRA levels oscillated at a regular 100-min period, with a strong spectral density. Declining PRA levels always coincided with REM sleep phases and increasing levels with NREM sleep phases. More precisely, peak levels corresponded to the transition from deep sleep stages toward lighter ones. The start of the rises in PRA generally marked the transition from REM sleep to stage 2. For incomplete sleep cycles, PRA curves reflected all disturbances and irregularities in the sleep structure. Spontaneous and provoked awakenings blunted the rise in PRA normally associated with NREM sleep, which indicates that disturbing sleep modifies the renin release from the kidneys. These results suggest that a common mechanism within the central nervous system controls both PRA oscillations and the REM-NREM sleep alternation. **Key Words:** Sleep—Plasma renin activity—Ultradian rhythm.

The renin-angiotensin system is involved in the control of arterial blood pressure and plays an important part in essential hypertension. The active hormone, angiotensin II, is a potent vasopressor, rapidly eliminated from the bloodstream, so that plasma renin activity (PRA) is commonly measured as an index of the activity of the system. Extending the results of Mullen et al. (1), we have shown that during night sleep, PRA displays sustained oscillations of about 100-min periodicity, which are related to the REM-NREM sleep cycles (2). Damped and irregular fluctuations are observed during daytime in awake subjects. These night-day differences suggest that the ultradian PRA rhythm is strongly linked to sleep (3).

This article focuses on nocturnal PRA profiles and presents a detailed analysis of

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their temporal relationships to the sleep cycles. Night experiments were generally performed in subjects receiving their usual Na diet, but, to clarify the relationship to the sleep cycles, we occasionally used a low-Na diet, which is known to increase renin release (4).

METHODS

Subjects and procedures

Forty-two nocturnal PRA profiles from blood collected at 10-min intervals and the concomitant polygraphic recordings of sleep were analyzed. Subjects were healthy male volunteers, aged 21–26 years, with no evidence of any disease and taking no medication. They had normal routines of work, meals, and sleep. Before their final enlistment, they took part in an experimental session to familiarize themselves with the new environment and with catheter insertion. Informed written consent was obtained from all subjects.

Seventeen subjects underwent two night studies at a 2-week interval. They received their usual Na diet (individual 24-h urinary Na excretion was between 104 and 205 mEq/day). About a month later, six of the subjects took part in a third night study after 3 days of a low-Na diet (individual 24-h urinary Na excretion was between 24 and 75 mEq/day). Lights were switched off at 2300 h and turned again either at 0700 h (11 subjects) or at 0800 h (six subjects). In addition, in order to define the effect of provoked awakenings, two subjects underwent a third night study when they were awakened for 40 min (lights on) after the third complete sleep cycle, interrupting a 20–25-min REM sleep phase. The experiments then lasted from 2300 to 0900 h. These subjects were also on a low-Na diet for 3 days.

The studies were performed in sound-proof, air-conditioned sleep chambers. Before the subjects entered the sleep chamber, electrodes were attached for the following uninterrupted electrophysiological recordings: two electroencephalograms, two electro-oculograms, one electromyogram, and one electrocardiogram. Sleep stages were scored from the polygraphic recordings according to established criteria (5). At 2100 h, a catheter was inserted into an antecubital vein. Blood was removed continuously using a peristaltic pump and collected in an adjoining room at 10-min intervals from 2300 to 0800 h. A maximum of 200 ml of blood was removed, and this produced no significant changes in hematocrit.

Plasma sample measurements

Blood samples were immediately centrifuged at 4°C and the plasma stored at −20°C. PRA was measured by RIA of angiotensin I generated after the incubation of plasma (6). All samples from one night were analyzed in the same assay. The intra-assay coefficient of variation for duplicate samples was 4.6%.

Relationship between PRA oscillations and sleep stages

The method used consisted in analyzing, oscillation by oscillation, the relationship between the dominant sleep stages (NREM and REM sleep) and the rising and falling trends of PRA oscillations. Differences in proportions were tested with χ^2 tests. In a second step, the relationships between the peak and trough levels of each PRA oscillation and the corresponding dominant sleep stages were examined.

Data analysis

Spectral analysis (BMDP1T and BMDP2T programs) was used to detect the predominant periodicities of the PRA oscillations. First, the data were filtered using a three-point moving average. Then, a difference filter, defined as $x_{dt} = x_t - x_{(t-1)}$, was used to remove low-frequency components. The spectral density function was determined with the Fast-Fourier transformation method, and a Blackman and Tukey smoothing window was used (7). The spectral window bandwidth was 0.01 cycle/min, and the frequency spacing was 0.0019 cycle/min. The spectral density indicates the variability fraction, expressed as harmonic components with given periods, and thus reveals the strength of the oscillatory pattern.

The strongest periods were used to fit a sine wave to the nocturnal patterns. Mean amplitude was evaluated by calculating plasma changes between the peaks closest to the apogees of the fitted curve and the troughs closest to the following nadir.

Paired *t* tests were used to assess the statistical significance of differences between the mean values observed during each of the two nights from the six subjects on their usual Na diet (N1 and N2) and those from the same subjects on a low-Na diet (N3). Individual PRA curves, illustrated in Figs. 1, 2, and 4, were smoothed using the moving averages method over a three-point span.

RESULTS

Regular REM-NREM sleep cycles

Figure 1 shows the nocturnal PRA oscillations and their relationship to regular REM-NREM sleep cycles, in one subject on a normal-Na diet and in one on a low-Na diet. Typical sleep stage patterns with the regular occurrence of REM sleep were accompanied by PRA oscillations of about 100-min periodicity, with strong spectral densities. The mean amplitude of the oscillations, expressed as a percentage of the means, was about 50%. In the six subjects on a low-Na diet, mean nocturnal PRA levels were significantly higher ($p < 0.01$) and the oscillations were significantly amplified ($p < 0.05$), which clarified the relationships with the sleep cycles (Table 1).

A close relationship was found between PRA oscillations and REM-NREM sleep alternation. In the 42 curves analyzed, NREM sleep was linked with increasing PRA levels, and REM sleep occurred as PRA was declining or at a nadir. Of the 181 NREM sleep phases recorded, all but eight occurred as PRA levels were rising. All but five of the 161 REM sleep phases occurred as PRA was declining. The frequencies observed differed significantly according to the sleep stage ($\chi^2 = 292.5$; $p < 0.001$). More precisely, the transition from REM sleep to stage 2 coincided with the start of the rise in PRA levels. In about 10% of the cases (18 observations), REM sleep continued while PRA levels were rising. The peak levels corresponded to the transition from deep and quiet sleep states toward lighter and more activated states. Exceptionally (eight observations), the decline began during sleep stage 2 without any stage transition; a large number of transient activation phases were then detected on the electrophysiological recordings. PRA levels always rose when sleep began.

Irregular REM-NREM sleep cycles

When the pattern of sleep stage distribution was not the classical one, PRA profiles clearly reflected the irregularities in the sleep structure. Figure 2 illustrates such irregular sleep stage patterns and the concomitant PRA profiles. In particular, one subject

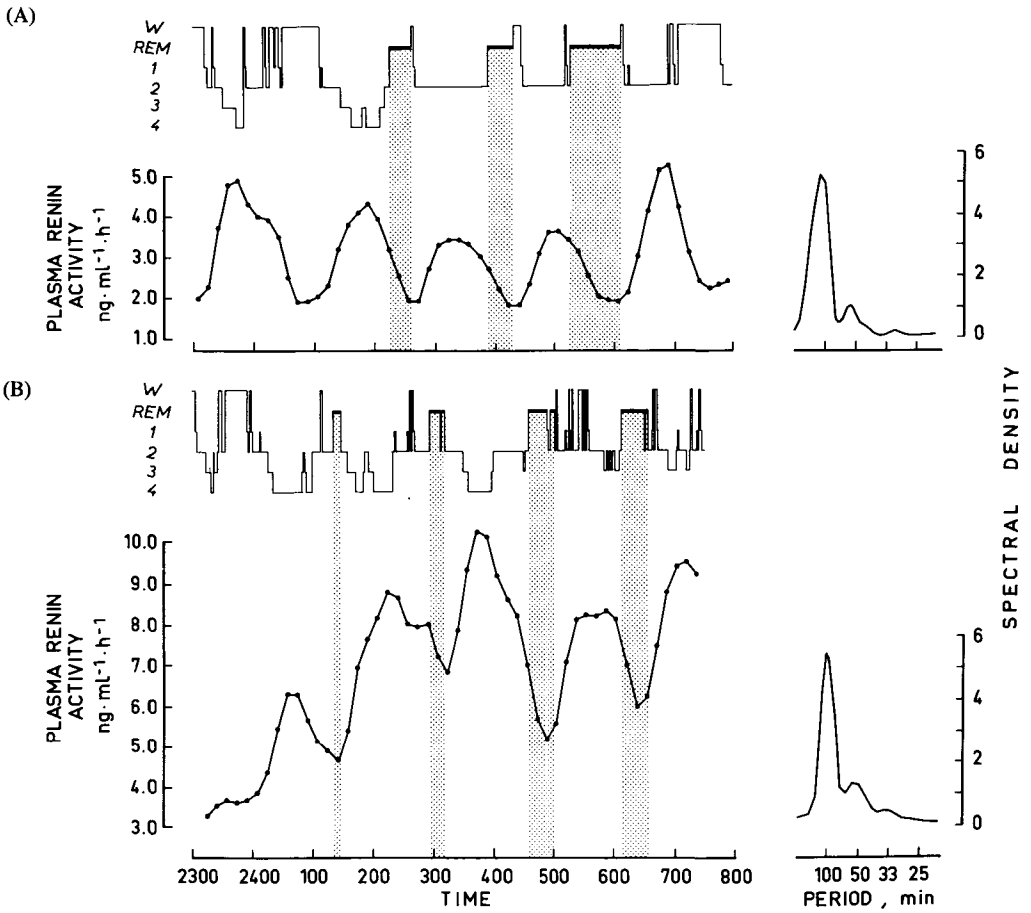

FIG. 1. Nocturnal PRA oscillations and their relationships with regular REM-NREM sleep cycles, in one subject on a normal Na diet (A) and in one on a low-Na diet (B). REM phases lie within the shaded areas. The spectral density gives the strength of the oscillatory patterns.

who had shorter REM-NREM sleep cycles also had shorter PRA cycles (Fig. 2A). During prolonged sleep stage 2, small fluctuations occurred in the PRA levels which only oscillated at the end of the night when REM-NREM sleep cycles were observed (Fig. 2B). Finally, damped fluctuations without any predominant periodicity—similar to those observed during the daytime in awake subjects—occurred in one subject who slept badly (Fig. 2C).

Effect of awakenings

Spontaneous awakenings occurring in the descending portion of the oscillations apparently did not affect the PRA time course (see example in Fig. 1A). However, they did interrupt the rise in PRA levels. Figure 3 illustrates the mean PRA oscillations when REM sleep was interrupted by provoked awakenings. Awakenings at 0700 h interrupted a 25–30-min REM sleep phase in eight subjects. These profiles were used to average point-by-point PRA levels during the last incomplete sleep cycle and during the nearest complete sleep cycle on the same night. Figure 3 shows that awakenings blunted the rise in PRA normally associated with the transition from REM to NREM sleep, which

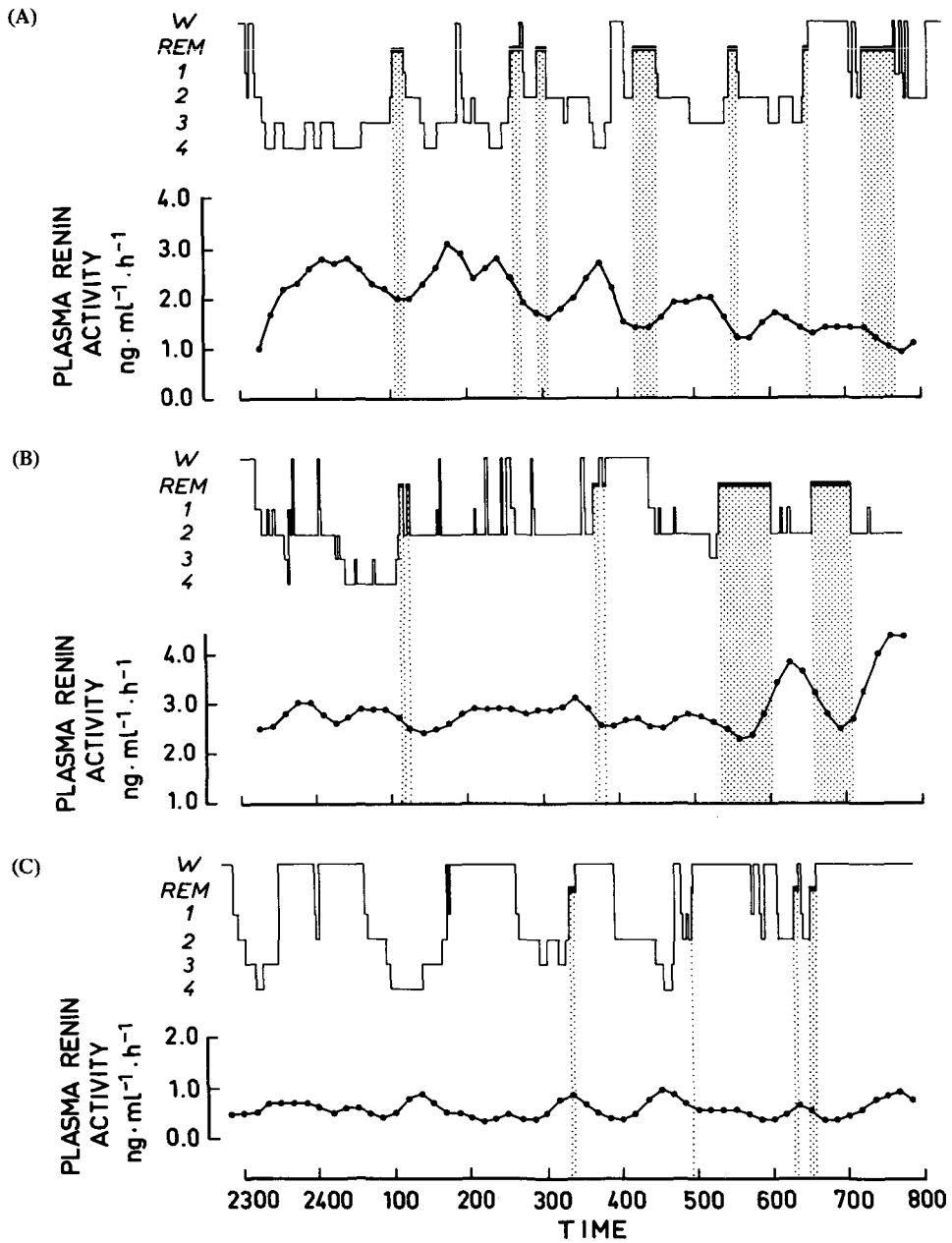

FIG. 2. Examples of individual PRA profiles accompanying irregular patterns of sleep stage distribution. A: Short REM-NREM sleep cycles. B: Prolonged sleep stage 2. C: Frequent waking periods.

makes it clear that disturbing the sleep structure modifies the renin release from the kidneys.

Finally, in Fig. 4, concomitant PRA and sleep stage patterns are shown that illustrate the strong concordance of the two phenomena. This curve, which summarizes our findings, was obtained from one of the two subjects on a low-Na diet who was awakened for 40 min after the third complete sleep cycle. REM sleep had then lasted 20 min,

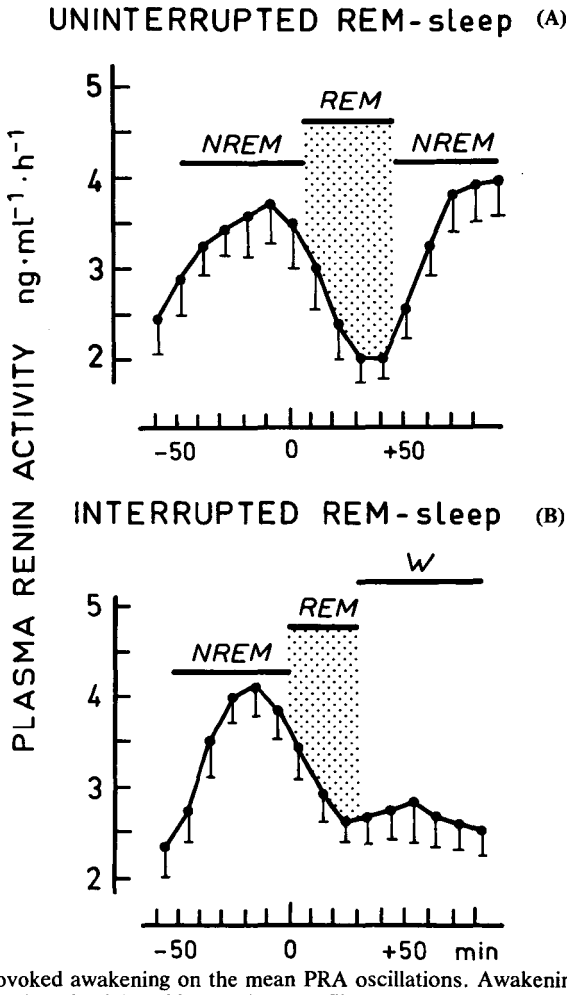

FIG. 3. Effect of provoked awakening on the mean PRA oscillations. Awakenings at 0700 h interrupted a 25–30-min REM sleep phase in eight subjects. These profiles were used to average point-by-point PRA levels during a complete sleep cycle (A) and during the last incomplete sleep cycle on the same night (B).

and signs of the transition towards sleep stage 2 were visible on the electroencephalograms. PRA fluctuations, amplified by sodium depletion, followed the sleep stage pattern step by step: an initial increase associated with sleep onset, slightly depressed by spontaneous brief awakenings, then two major increases associated with slow-wave-sleep (SWS) phases, the second broken by intermittent awakenings. The start of each decline corresponded to the transition from stage 4 to stages 3 and 2. Afterward, a slight increase, associated with a short SWS phase, was observed, followed by a major decrease due to the provoked awakening. Finally, at the end of the night, the PRA pattern was similar to that observed when sleep began.

DISCUSSION

The results of this study establish the strength of the relationship between PRA nocturnal oscillations and sleep stage alternation. NREM sleep phases coincided with

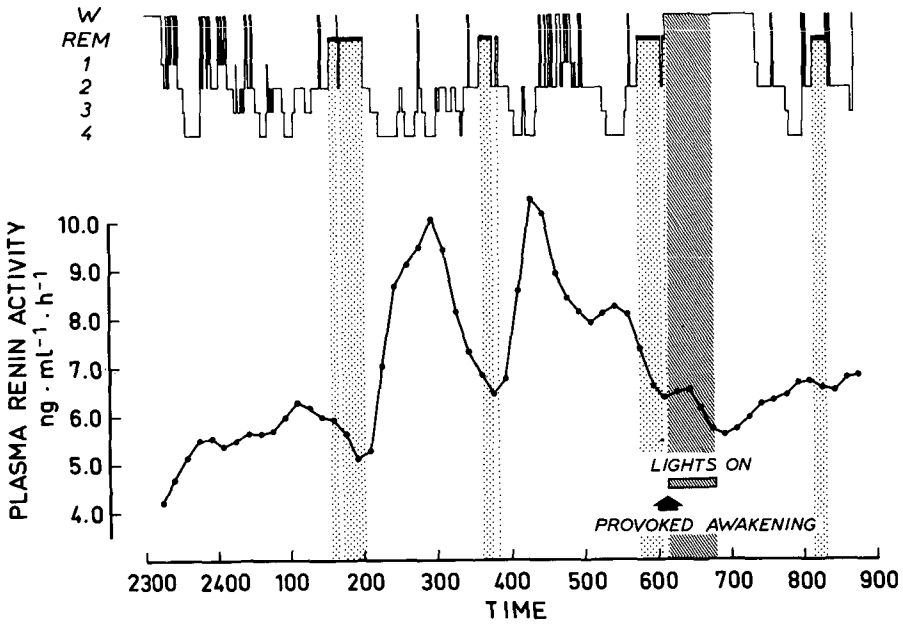

FIG. 4. Nocturnal PRA and sleep stage patterns in one Na-depleted subject who was awakened for 40 min after the third REM sleep phase. This provoked awakening blunted the PRA rise normally associated with the transition from REM to NREM sleep. This figure is typical of the strong concordance between these phenomena.

increasing PRA levels, whereas REM sleep occurred after PRA levels had decreased, indicating reduced renin release or even none. The beginning of the decline corresponded to the transition from deep sleep stages toward lighter ones; the start of the rise in PRA levels generally marked the transition from REM sleep to stage 2. Spontaneous and provoked awakenings blunted the rise associated with NREM sleep. So, PRA curves exactly reflect the sleep stage patterns. When sleep cycles are complete, PRA levels oscillate at a regular 100-min periodicity, with strong spectral density. For irregular sleep cycles, PRA curves reflect all disturbances and irregularities in the sleep structure.

Na balance only modulates the mean PRA levels. A low-Na diet enhances renin secretion and amplifies the nocturnal oscillations, which clarifies the temporal relationships with the sleep cycles. Under these conditions, the nocturnal PRA profiles provide

Table 1. *Effect of a low-Na diet on the nocturnal PRA patterns in six subjects*

Nights	Mean nocturnal PRA levels (ng/ml/h)	Mean amplitude of the oscillations
N1: Spontaneous Na diet	2.20 ± 0.43	1.20 ± 0.26
N2: Spontaneous Na diet	2.05 ± 0.39	1.12 ± 0.20
N3: low-Na diet	6.47 ± 1.1[a]	3.03 ± 0.55[b]

[a] $p < 0.01$.
[b] $p < 0.05$.
These are significant differences between the mean levels on the two nights for six subjects on spontaneous Na diet (N1 and N2) and on the third night for the same subjects on a low-Na diet (N3).

a new picture of the interactions between intrarenal mechanisms [the macula densa is sensitive to changes in Na load (4)] and extrarenal mechanisms [which involve the renal sympathetic nerves (8,9)] regulating renin release from the kidneys.

The renin-angiotensin system is the first endocrine system shown to be strongly linked to sleep stage alternation. Any explanation for this concomitance can, for the moment, be but speculative. PRA oscillations might be related to the hemodynamic changes accompanying the regular recurrence of REM sleep. Heart rate, recorded in the present study, shows the classic overnight decrease and the superimposed increases—both in heart rate and heart rate variability—that are well-known REM sleep phenomena (10,11). Periodic changes in blood pressure (10,11) and an increased sensitivity of the baroreceptor reflex during REM sleep (12) have also been described. Animal studies have revealed that stimulating various areas in the brain produces increases in PRA associated with increases in blood pressure. However, there is pharmacological evidence that the renin response can be separated from the blood pressure response (13). So, it is not clear whether the renin-angiotensin system is involved in such hemodynamic changes or whether the oscillations might be elicited by a common process in the central nervous system controlling both the alternation of the sleep cycles and renin secretion from the juxtaglomerular cells.

The strong concordance between these phenomena suggests that such a common mechanism does exist. Studies on humans (14,15) and on animals (16,17) have presented evidence that the serotoninergic neurons stimulate renal renin secretion; however, there is debate as to whether the resulting increase in PRA is mediated via descending neurons from the sympathetic nervous system (18). Recent studies have challenged this concept (19) and have partially characterized a renin-releasing peptide that originates both in the blood (20) and in the hypothalamus (21). Inside the raphe nuclei, serotoninergic neurons exhibit a high discharge during waking, a decreased activity during SWS, and almost no activity during REM sleep (22). Both the serotoninergic discharge and the sympathetic nervous outflow lead one to expect higher renin release during the waking periods. On the contrary, our results show that renin release is reduced or blocked during intrasleep waking periods. Therefore, no simple explanation for the concomitance of the two phenomena can as yet be put forward. The study of the mechanisms involved in the physiological regulation of renin release may help to indentify the complex processes underlying the organization of sleep (23). The perspective offered here provides a framework into which other observations and new findings can be fitted. In particular, it would be of interest to investigate the possible roles played in sleep regulation by the brain renin-angiotensin system (24,25) and by hypothalamic renin-releasing peptide (21).

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