Modulation of Episodic Renin Release During Sleep in Humans

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We previously described a strong concordance between nocturnal oscillations in plasma renin activity and sleep cycles. To examine whether modifying renal renin content or release influences the response to central stimuli linked to sleep stage alternation, plasma renin activity was measured every 10 minutes from 11:00 PM to 8:00 AM in three groups of six subjects. The first group received one 40 mg dose of the diuretic furosemide; the second group underwent the night experiment after 3 days on a low sodium diet; the third group received one 100 mg dose of the β-blocker atenolol. Each subject underwent a control night when a placebo was given. The nocturnal curves were analyzed with a pulse detection program. For the control nights, 74 of the 83 sleep cycles were associated with significant plasma renin activity oscillations; non-rapid eye movement sleep occurred in the ascending portions and rapid eye movement sleep in the declining portions of the oscillations. These oscillations persisted in the three groups of subjects during the experimental nights and the relation with the sleep stages was not disturbed. Acute stimulation by furosemide amplified the oscillations and led to a general upward trend of the nocturnal profiles. Similarly, a low sodium diet, which led to a slow increase in renal renin content, provoked large oscillations with high initial levels. However, in both cases the mean relative amplitude of the oscillations, expressed as a percentage of the nocturnal means, was similar to that of the control nights and approximated 60%. Atenolol reduced the increases associated with non-rapid eye movement sleep. Small oscillations were detected in 16 of the 23 sleep cycles recorded. These results give evidence of the strength of the sleep-related processes generating the oscillations, which are amplified or depressed by factors known to control renin release. These factors create a baseline environment that modulates the expression of central stimuli associated to the transition from rapid eye movement sleep or waking period toward deep sleep stages. (Hypertension 1990;15:370–375)

During the past few years, substantial advances have been made in the identification of the multiple factors regulating renin release. There is increasing evidence that complex interactions exist among the different mechanisms involved. Reviews that have been concerned with tentative classification point out the major role of intrarenal mechanisms, of sympathetic stimulation, and of several humoral factors, all interacting with mutual facilitation or inhibition.1–3

There is also evidence that the central nervous system participates in controlling renin secretion.4,5 In this respect, the finding in humans of a 100 minute ultradian rhythm in plasma renin activity (PRA) strongly linked to the rapid eye movement (REM) and NonREM (NREM) sleep cycles was of particular interest. Extending the results of Mullen et al,6 we described nocturnal oscillations in PRA strongly linked to the REM-NREM sleep cycles.7,8 Increasing PRA levels mark the transition from REM sleep to NREM sleep, and declining levels coincide with the transition from deep sleep stages toward lighter ones. PRA curves exactly reflect the pattern of sleep stage distribution. This strong concordance between renin release and quantifiable changes in central nervous system activity stresses the importance of the central control of renin release.

A further study established that the relations between PRA oscillations and sleep stage alternation persist in moderate hypertensive patients, but low renin levels frequently observed in these patients were accompanied by small oscillations (personal observations). On the contrary, in patients given an angiotensin converting enzyme (ACE) inhibitor in
single or repeated doses, the mean PRA levels were increased, and the nocturnal oscillations were strongly amplified. These results suggest that both the sleep-related mechanisms and some peripheral mechanisms influence the nocturnal PRA profiles.

The aim of this study was to determine whether the relation between PRA oscillations and the sleep stage pattern was influenced by drugs or situations known to alter renal renin content or release. Furosemide, which provokes a rapid stimulation of renal renin content, was administered, and its effect on the nocturnal oscillations and on their relations to the sleep stages was tested. The influence of the $\beta$-blocker atenolol, known to reduce renin release, was studied to determine whether $\beta$-adrenergic receptors are involved in the ultradian PRA rhythm. Finally, the nocturnal profiles observed after one dose of these drugs were compared with those observed after a low sodium diet, which slowly increases renal renin content. The results illustrate the modulatory role of different mechanisms on the nocturnal PRA oscillations linked to sleep stage alternation.

Methods

Eighteen normal men (21–28 years old) were studied in three experimental series. Subjects were healthy volunteers with no evidence of any disease and were taking no medication. 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. The experiment was approved by the Strasbourg Hospital Ethics Committee.

The subjects underwent two randomized, night studies with 2-week intervals between them. Each of the subjects participated in one control night when a placebo was given and in one experimental night. During the experimental night, six subjects received a single 40 mg dose of the diuretic furosemide (Lasilix, Laboratoires Hoechst, Puteaux, France) at 10:00 PM; six other subjects received a single 100 mg dose of the $\beta$-blocker atenolol (Tenormine, ICI-Pharma, Cergy, France) at 10:00 PM. In both experimental series, the subjects were on their usual sodium diet (individual 24-hour urinary sodium excretion ranged between 105 and 186 meq/day). In the third experimental series, six subjects were studied after 3 days on a low sodium diet (individual 24-hour urinary sodium excretion ranged between 24 and 65 meq/day).

The studies were performed in a soundproof, air-conditioned sleep chamber. Before the subjects entered the sleep chamber, electrodes were attached for the following uninterrupted electrophysiological recordings: two electroencephalograms, two electrooculograms, one electromyogram, and one electrocardiogram. Sleep stages were scored from the polygraphic recordings according to established criteria. At 9:00 PM, a catheter was inserted into an antecubital vein. Blood was removed continuously with a peristaltic pump and collected in an adjoining room at 10-minute intervals from 11:00 PM to 8:00 AM. A maximum of 200 ml blood was removed, and this produced no significant changes in hematocrit. Light was switched off at 11:00 PM, and the subjects were awakened at 8:00 AM.

Plasma Sample Measurements

Blood samples were immediately centrifuged at 4°C and the plasma stored at −20°C. PRA was measured by radioimmunoassay of angiotensin I (Ang I) generated after the incubation of plasma. All samples from one night were analyzed in the same assay. The intra-assay coefficient of variation (CV) for duplicate samples was 4.0% for levels between 10 and 20 ng Ang I/ml/hr; 6.0% for levels between 2 and 10 ng Ang I/ml/hr; 10.0% for levels between 1 and 2 ng Ang I/ml/hr; and 30% for levels less than 1 ng Ang I/ml/hr. The interassay CV was 16.6% in the normal concentration range. The detection limit was 0.18 ng Ang I/ml/hr; it is defined as the smallest value that can be distinguished from zero. This value was calculated from the 95% confidence limits at the zero point of the standard curve.

Relations Between Plasma Renin Activity Oscillations and Sleep Stages

For quantitative detection and characterization of PRA oscillations, the nocturnal data were analyzed with the pulse analysis program ULTRA. This program takes into account the detection limit of the analytical procedure and the variable precision of the assay for the various ranges of concentrations. It was used at a threshold three times the CV for each concentration range. All changes in levels that did not meet the criteria for significance were eliminated, thus giving a clean profile. An oscillation was considered significant if both the increase and the decrease were significant. For each significant oscillation, the ascending portion, the declining portion, the total duration, and the absolute increment were calculated. A recent study compared various programs for pulse identification and found that the performance of ULTRA is similar to that of other programs. The relation between the dominant sleep stage and the ascending and declining portion of PRA oscillations was then analyzed. Each complete NREM-REM sleep cycle was considered. A sleep cycle was considered incomplete when the waking period lasted at least 20 minutes. Paired t tests were used to assess the statistical significance of differences between the mean values observed during the control night and the experimental night from each of the six subjects in the three experimental series. Individual PRA curves illustrated in Figures 2–4 were smoothed by using the moving-average method over a three-point span.

Results

Mean Nocturnal Profiles

Figure 1 shows the mean nocturnal PRA profiles in the three groups of six subjects who received either
furosemide or atenolol or who were on a low sodium diet for 3 days, together with the control profiles. Averaging the curves smoothed out the individual, nonsynchronized oscillations and revealed the overall trend during the night. As expected, the mean PRA levels were significantly lower in subjects given atenolol (0.96±0.20 ng Ang I/ml/hr vs. 2.64±0.31 ng Ang I/ml/hr during the control nights; p<0.01). Acute stimulation by furosemide led to a sharp ascending trend in the nocturnal profiles with increased mean levels (5.08±0.73 ng Ang I/ml/hr vs. 2.11±0.33 ng Ang I/ml/hr during the control nights; p<0.02). A low sodium diet for 3 days gave almost the same picture with high initial PRA levels (mean nocturnal levels: 5.57±0.65 ng Ang I/ml/hr vs. 2.14±0.28 ng Ang I/ml/hr during the control nights; p<0.01).

Subjects Receiving Furosemide

Figure 2 illustrates two individual PRA profiles with the corresponding sleep stage patterns in two subjects who received one 40 mg dose furosemide at 10:00 PM, together with the profiles obtained during the control night. As previously described in subjects under basal conditions,8 PRA levels oscillated during night sleep, and the oscillations were closely related to sleep stage alternation. In the 18 subjects on spontaneous sodium intake studied during a control night, 72 complete sleep cycles (NREM-REM) and 11 incomplete sleep cycles (NREM-Wake) were recorded (Table 1). All but nine were linked to significant PRA oscillations; NREM sleep occurred as PRA levels were increasing and REM sleep coincided with decreasing PRA levels. Only two of nine NREM sleep phases without any detectable PRA oscillations were associated with declining PRA levels, and one REM sleep phase occurred as PRA levels were increasing. The mean nocturnal levels ranged between 1.22 and 3.40 ng Ang I/ml/hr, and the mean absolute amplitude of the oscillations was 1.31±0.15 ng Ang I/ml/hr. The mean relative amplitude, expressed as a percentage of the nocturnal means, approximated 60%.

In the six subjects who received one 40 mg dose furosemide at 10:00 PM, frequent awakenings occurred because of the diuretic effect of the drug, leading to less regular REM-NREM sleep cycles and consequently to less regular PRA oscillations.8

![Figure 1](http://hyper.ahajournals.org/content/hyp/15/4/637/F1.large.jpg)

**Figure 1.** Line graph showing mean plasma renin activity profiles from three groups of six subjects who received either furosemide or atenolol or who were on a low sodium diet for 3 days, together with mean profiles observed during control nights. AI, angiotensin I.

![Figure 2](http://hyper.ahajournals.org/content/hyp/15/4/637/F2.large.jpg)

**Figure 2.** Line graphs showing individual nocturnal plasma renin activity profiles and corresponding sleep stage patterns for two subjects who received 40 mg furosemide at 10:00 PM together with profiles observed during control night. During rapid eye movement (REM) sleep, plasma renin activity profiles lie within shaded areas. W, waking period; AI, angiotensin I.
TABLE 1. Detection and Characterization of the Plasma Renin Activity Oscillations Associated With the Sleep Cycles

<table>
<thead>
<tr>
<th>Sleep cycles</th>
<th>Controls (n=18)</th>
<th>Furosemide (n=6)</th>
<th>Low sodium diet (n=6)</th>
<th>Atenolol (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete (NREM-REM)</td>
<td>72</td>
<td>21</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>Incomplete (NREM-W)</td>
<td>11</td>
<td>10</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>31</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>Significant associated oscillations</td>
<td>74</td>
<td>26</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>Mean absolute amplitude (±SEM)</td>
<td>1.31±0.15</td>
<td>2.97±0.33</td>
<td>3.25±0.46</td>
<td>0.78±0.14</td>
</tr>
<tr>
<td>Mean nocturnal levels (±SEM)</td>
<td>2.25±0.19</td>
<td>5.08±0.73</td>
<td>5.57±0.65</td>
<td>0.96±0.20</td>
</tr>
</tbody>
</table>

NREM, non-rapid eye movement sleep; REM, rapid eye movement sleep; W, waking period; Ang I, angiotensin I.

Twenty-one complete sleep cycles (NREM-REM) and 10 incomplete sleep cycles (NREM-Wake) were recorded (Table 1). Individual profiles revealed large oscillations superimposed on the general trend. The mean absolute amplitude of the oscillations was increased (2.97±0.33 ng Ang I/ml/hr). However, the mean relative amplitude, expressed as a percentage of the nocturnal mean, was unchanged at about 60%.

The relation between the ascending and declining portions of the oscillations and the dominant sleep stages persisted. Of the 31 sleep cycles recorded, 26 coincided with significant PRA oscillations. Twenty-one REM sleep phases were recorded, one of which corresponded to increasing PRA levels and three of which occurred when PRA levels did not significantly change. More often than in subjects on control nights, REM sleep continued after PRA levels had begun to rise (35% of the subjects receiving furosemide vs. 10% of the subjects during the control nights).

Subjects on a Low Sodium Diet

Comparison of the nocturnal PRA profiles after an acute stimulation of renin release by furosemide with the profiles observed after 3 days on a low sodium diet revealed no clear-cut differences. A typical profile with the corresponding sleep stage patterns in one subject on a low sodium diet and on a control night with ad libitum sodium intake is illustrated in Figure 3. After 3 days on a low sodium diet, the initial PRA levels were high (3.41±0.45 ng Ang I/ml/hr vs. 1.57±0.42 ng Ang I/ml/hr during the control nights; p<0.01). Averaging PRA values point by point during the last complete sleep cycles (NREM-REM) in the six subjects on a low sodium diet and in the same subjects during the control night more clearly revealed the effect of low sodium intake; the nocturnal oscillations were strongly amplified (mean absolute amplitude: 3.25±0.46 ng Ang I/ml/hr vs. 1.31±0.08 ng Ang I/ml/hr during the control nights).

Again, a strong relation between the PRA oscillations and the sleep stage patterns was found (Table 1).

Subjects Receiving Atenolol

Atenolol given at 10:00 PM progressively decreased the PRA levels and blunted the increases associated

FIGURE 3. Line graphs showing the effect of a low sodium diet on the nocturnal plasma renin activity profile in one subject. Mean profiles were obtained by averaging point-by-point plasma renin activity levels in six subjects during last complete sleep cycle on control nights and on experimental nights. W, waking period; REM, rapid eye movement sleep; NREM, non-rapid eye movement sleep; Ang I, angiotensin I.
with NREM sleep. Figure 4 illustrates typical individual curves in two subjects. Of the 23 sleep cycles recorded, 16 were accompanied by detectable oscillations. Their mean absolute amplitude was low (0.78±0.14 ng Ang I/ml/hr). The increases in PRA associated with NREM sleep were below the detection limit of the analytical method for seven sleep cycles, particularly toward the end of the night.

Discussion

In previous studies,7,8 we described nocturnal oscillations in PRA strongly linked to REM-NREM sleep alternation. The PRA curves exactly reflect the pattern of sleep stage distribution, with increased renin secretion when sleep becomes deeper and reduced or even abolished secretion during the transition toward lighter sleep stages or waking periods. The results from this study reveal that factors known to modify renal renin content or release only modulate the amplitude of the oscillations without disturbing their relations to sleep stages. The oscillations were not obscured, which reveals the dominant role of the sleep-related processes in the generation of PRA oscillations. Thus, it appears that the normal secretion of renin is not continuous during night sleep but rather remains intermittent even when renin release is modified by other factors. These results contrast with previous results on other hormonal systems (i.e., for cortisol release) whose spontaneous secretory episodes are overshadowed by increases provoked by diverse external stimuli.14

Administration of furosemide, quite apart from the rapid natriuresis, leads to vasodilation in the kidney, which is an important stimulant of renin synthesis. The rapidly enhanced renin release is indicated by sharp nocturnal upward trends of PRA with large superimposed oscillations. With few exceptions, the ascending phases coincided with NREM sleep while REM sleep occurred as PRA was decreasing.

Stimulating renal renin content by a low sodium diet provides almost the same picture. The initial PRA levels were then strongly enhanced, and the overall upward trend persisted. This experimental situation resembles the effect of long-term heat exposure15 when sodium losses through sweating are high, activating the renin-angiotensin-aldosterone system. In these conditions also, large oscillations of PRA were observed leading to a general upward trend, which could be interpreted as an effect of circadian rhythmicity or could simply reflect an accumulation of renin due to a low metabolic clearance rate. On the contrary, the β-blocker atenolol, which barely crosses the blood-brain barrier, reduced the increases associated with NREM sleep. It is commonly accepted that β-adrenergic receptors are involved in the release of renin,16 but it is still debated whether the sympathetic nervous system mediates centrally stimulated renin release.17,18 Our results then suggest that β-adrenergic receptors may play a role in transmitting the signals linked to sleep stage alternation that generate the ultradian rhythm.

Stimulating renin release increased the absolute amplitude of the nocturnal oscillations, but their relative amplitude, expressed as percentage of the mean, remained constant and approximated 60%. This result emerged from the present study in normal volunteers given furosemide and in subjects on a low sodium diet. Similar results were observed in a previous study in hypertensive patients in whom low renin levels were associated with small oscillations; in these patients, an ACE inhibitor in single or repeated doses led to large oscillations, which could be as high as 6 ng Ang I/ml/hr. However, in both untreated and treated patients, the relative amplitude of the oscillations remained unchanged and was also 60% of the nocturnal means.

These data demonstrate the strength of the sleep-regulatory mechanisms that give the intermittent signal for renal renin release, while other mechanisms intervene as oscillation-amplitude modulators. Mathematical models that are now being developed will help evaluate the relative importance of continuous versus episodic renin release during the nighttime and also during the daytime in the absence of REM-NREM sleep cycles.

In conclusion, the results add further evidence to suggest that, apart from the numerous processes that control renin secretion in an additive or antagonist manner, the activity of the juxtaglomerular cells depends on the complex processes governing sleep stage alternation. The common mechanisms have still to be identified. A possible role for serotonin, which is involved both in sleep19,20 and in renin secretion,21-23 can be postulated. However, the low renin release...
during waking periods, when serotonin discharge is high, does not support this hypothesis. A possible role for the renin-releasing peptide recently characterized by Van de Kar et al 25 must await further investigation. However, it may well prove difficult to isolate one single factor from the multiple processes underlying sleep stage alternation. 24

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References

KEY WORDS • plasma renin activity • diuretic therapy • sympatholytics • sleep • diet
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