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ANTIDIURETIC HORMONE: EPISODIC NOCTURNAL SECRETION IN ADULT MEN--ETC(U)
JUL 75 R T RUBIN , R E POLAND , F RAVESSOUD N00014-73-C-0127

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ANTIDIURETIC HORMONE: EPISODIC NOCTURNAL SECRETION IN ADULT MEN

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Abstract

Nocturnal release of antidiuretic hormone (ADH) in eight young adult men occurred in a pulsatile, episodic fashion with no consistency from night to night in the same subject. There was no discernible relationship between ADH release and plasma sodium levels, sleep stages, or time of night.

Many recent human sleep-endocrine studies have revealed that the anterior pituitary hormones are secreted in a pulsatile, episodic fashion, each having a specific nocturnal release pattern in normal adults (1-3). Daytime release of these hormones also is episodic, so that considered together these data strongly suggest important "open loop" central nervous system control mechanisms regulating secretion of the hypothalamic releasing and inhibiting factors (4). Investigation of posterior pituitary hormone release during sleep is a logical extension of these studies. The recent development of a precise, sensitive radioimmunoassay for human antidiuretic hormone (ADH; arginine vasopressin) (5) permitted the measurement of this hormone in normal young adult men during all-night sleep and dreaming.

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• Methods

Subjects were eight healthy male volunteers, ages 18 to 32, on no drugs or medications. They were studied for four consecutive nights in the sleep laboratory, according to an established protocol detailed elsewhere (6,7). The subjects were allowed normal meals and activity during the day, but nothing by mouth after 7 p.m. They were recumbent in bed at 10 p.m. and stayed awake until lights out at 11 p.m.

Continuous electroencephalographic, electrooculographic, and electromyographic recordings were made each night during the hours of sleep (11 p.m. to 7 a.m.). Each 20 second epoch of recording was scored as awake; stage 1, 2, 3, 4; or rapid eye movement sleep (REM) by established criteria (8). The first and second nights were for adaptation to the laboratory. During the third and fourth nights, blood was sampled every 20 minutes between 11 p.m. and 7 a.m. from an adjoining room through a long tubing connected to an indwelling venous catheter (9,10). Each sample was collected into EDTA, centrifuged, and the plasma immediately separated and frozen. Total blood withdrawal was kept below 250 ml. each night.

ADH was measured by a recently developed radioimmunoassay (5). This method has a reported intra-assay variability of 7% and an inter-assay variability of 15%. Because the Bentonite extraction was yielding variable recoveries, an extraction procedure reported earlier was used (11). One ml. of plasma was extracted with acetone, delipidated with a petroleum ether wash, blown to dryness under nitrogen, and then assayed. The entire set of samples from all eight subjects was run in a single assay.

Six plasma pool replicates (two each at three different dilutions) averaged 1.16 ± 0.11 $\mu\text{U/ml}$ (mean $\pm$ standard deviation), yielding an intra-assay coefficient of variation of 9%. The maximum variation between the duplicates themselves was 25% and occurred with the highest pool dilution. The minimum detectable concentration of ADH was 0.5 $\mu\text{U/ml}$.

Results

The pattern of nocturnal secretion of ADH was different for each subject, and also was inconstant between the two study nights for the same subject. ADH appeared to be released in a pulsatile, episodic manner, similar to the patterns of release of the anterior pituitary hormones (1). With the 20 minute sampling schedule, two to four major increases in ADH concentration generally were evident during the eight hour period (Figures 1 and 2). During these episodes (marked in the figures by asterisks), plasma ADH concentrations increased 100% to 300%, which represents a change many times greater than the variability within the assay procedure itself (9%). Thus, the large episodic increases in plasma ADH concentration evident in Figures 1 and 2 appear to reflect a true pulsatile secretion of this hormone, whereas the minor fluctuations in ADH may represent only intra-assay variability.

Plasma sodium levels in the eight subjects were quite constant throughout the night, averaging 135 meq/l and having a maximum coefficient of variation of 4%. The small, apparently random fluctuations in sodium bore no discernible relationship to the episodic changes in ADH levels.

Sleep patterns in the eight subjects were normal. The first laboratory adaptation night showed the usual lower amount

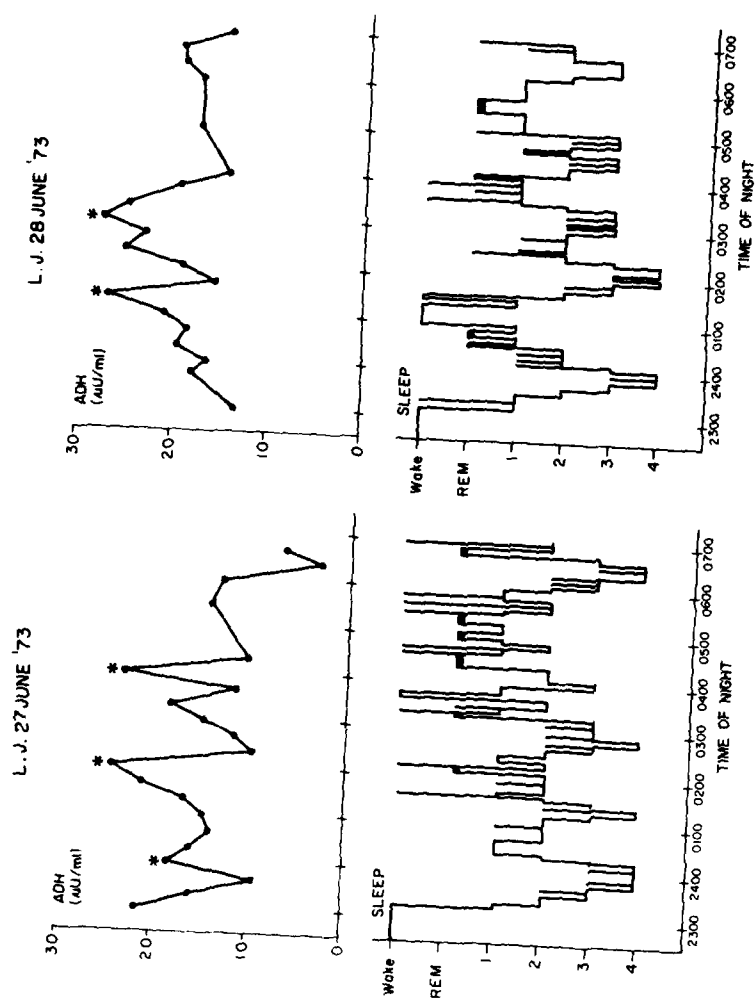


FIGURE 1
Subject L. J. sampled every 20 min. during sleep on two nights.

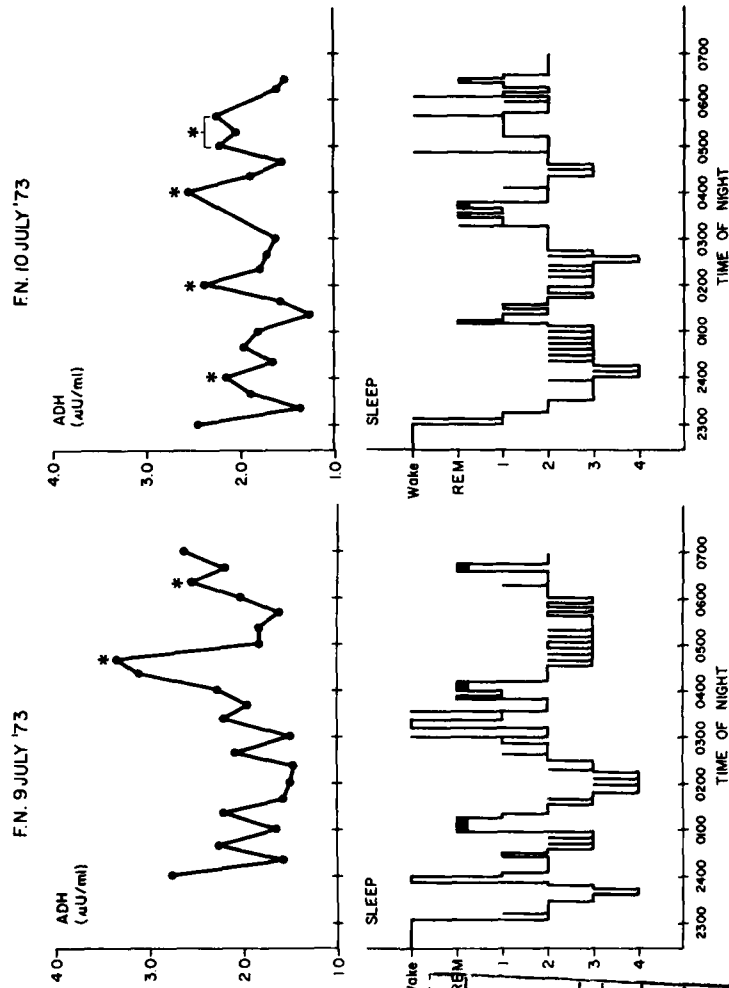


FIGURE 2
Subject F.N. sampled every 20 min. during sleep on two nights.

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of REM sleep, compared to the subsequent three nights. All sleep parameters were stable across nights 2-4 of the study.

Table 1 presents the average values of ADH by stages of sleep for each of the eight subjects. Because there were very few data points for stage 1 sleep, these data were combined with awake in the sleep analyses. Stages 3 and 4 also were combined because of few data points for stage 4 sleep.

A two-way analysis of variance indicated highly significant differences in mean ADH values between the eight subjects ($F = 8.27$; $df = 7,21$; $p = <.0005$), whereas the differences in ADH values between sleep stages averaged across the subjects were clearly non-significant ($F = 0.902$; $df = 3,21$; $p = N.S.$). A

TABLE 1
Average ADH Values by Sleep Stage for Each Subject

Subject	<u>Awake + I</u>	<u>II</u>	<u>III + IV</u>	<u>REM</u>	<u>Contrast Score</u>
C.A.	0.9	0.9	0.8	0.9	-0.4
F.N.	2.1	2.0	1.5	1.8	-2.2
C.C.	2.8	3.1	5.9	3.1	12.4
J.S.	1.8	1.9	2.0	1.5	1.2
L.J.	1.8	1.8	1.4	1.5	-1.3
K.W.	1.2	1.1	1.0	1.3	-1.0
D.H.	1.4	2.6	2.8	2.8	5.4
J.L.	<u>1.1</u>	<u>2.1</u>	<u>1.6</u>	<u>1.7</u>	<u>2.4</u>
Mean (μ U/ml)	1.64	1.94	2.13	1.83	2.06
Standard Deviation	0.62	0.72	1.64	0.75	4.84
Contrast Coefficient	-4	+1	+4	-1	

non-parametric test, Kendall's rank-order coefficient of concordance (12), also was used to compare the ADH levels for the four categories of sleep staging across the eight subjects; it too was clearly non-significant ($W=.05$). Finally, a multivariate parametric test biased toward significance was utilized by constructing a posterior contrast designed to maximize the mean differences between the sleep stages. The contrast coefficients are shown at the bottom of Table 1. Each subject's score for each sleep stage was multiplied by the contrast coefficient for that stage. These scores were summed for each subject, as shown in the right-hand column of Table 1. Under the null hypothesis of no differences between sleep stages, the mean of the contrast scores for the eight subjects should not be significantly different from zero. The zero- μ t value = 1.21 and degrees of freedom = 7; this also is clearly non-significant. Thus, baseline levels of ADH do not appear to change throughout the night, nor do the secretory episodes appear to be significantly related to sleep stages, at least in normal young adult men.

Discussion

The radioimmunoassay used in this study is sensitive (0.3-0.5 μ U/ml) and specific for ADH. The reported intra-assay variability, based on ten replicate samples, is 7% (5); the coefficient of variation of our single assay, based on six replicates, was 9%. The antiserum shows extremely low cross-reactivity with oxytocin. Recovery of ADH from serum with the Bentonite extraction procedure averages 80%; however, at the time our samples were assayed the Bentonite extraction was yielding inconstant results. Therefore, an acetone extraction

procedure described for a different assay system was used (11); recoveries for this extraction were consistently 70%. The ADH values reported herein are uncorrected for recovery.

This radioimmunoassay has been used to measure ADH in several groups of subjects (5). It clearly distinguishes levels in normals, both dehydrated and with ad lib water, and in subjects with pituitary diabetes insipidus, nephrogenic diabetes insipidus, and inappropriate ADH secretion. Normal male and female volunteers after an overnight (12 hour) dehydration averaged 7.2 $\mu\text{U/ml}$ (range 4.2-11.5). With a similar dehydration our subjects had generally lower levels, although some secretory peaks reached 4 to 7 $\mu\text{U/ml}$. The differences in overall levels may be partially explained by the lower recovery of the acetone extraction procedure used in our study and by some destruction of ADH by vasopressinase in the plasma during the few minutes between blood sampling and freezing of the plasma. Since all samples were handled identically, these two factors should have had a uniform effect and should not have contributed to the considerable variability in ADH levels seen in our subjects.

The results of this study imply that ADH, like the anterior pituitary hormones, is secreted in several episodes during the night. In our subjects these episodes were characterized by 100% to 300% increases in plasma ADH concentration, which clearly exceeded the intra-assay variation of 9%. The episodic increases in plasma ADH generally were short-lived, which is consistent with the reported half-life of 9 minutes for injected Pitressin (5).

In view of the diversity of neural connections to the hypothalamic ADH-secreting magnocellular neurons and the plethora of stimuli that release ADH (13), the observed lack of association between sleep staging and episodic ADH release is of interest. Several lines of evidence indicate that certain brain stem nuclei are involved in the orderly transition between the stages of sleep (14). The changes in EEG frequency and amplitude that are specifically related to sleep staging have been recorded from limbic structures (hippocampus) in man (15). Electrical stimulation of these brain stem and limbic areas involved in sleep (including hippocampus) in the unanesthetized monkey can provoke ADH release (13), suggesting that there are neural connections between these areas and the supraoptic and paraventricular nuclei. Also, in the monkey, magnocellular neuroendocrine cells which are continuously active during waking develop a rhythmic, periodic discharge during slow-wave sleep, with an accelerated discharge during brief episodes of higher voltage EEG activity (16). However, the relationship of these slow-wave sleep-related bursts of magnocellular neuronal firing to ADH release in the monkey is not yet known. In the human, even less is known about the activity of these hypothalamic neurons during sleep. Our data suggest that regardless of the pattern of magnocellular firing in relation to sleep staging in the human, the episodic secretion of ADH is not related to slow-wave or other stages of sleep.

In an earlier study of renal activity during sleep, it was noted that urine volume decreased and urine osmolality increased in conjunction with REM sleep (17). This inverse change in urine volume and osmolality was regarded as an alteration in the

water resorptive mechanism of the kidney, which was hypothesized to be a result of REM-related ADH release. Our finding of no relationship between sleep stages and plasma ADH levels measured directly stands in contrast to the hypothesis of this earlier study. Several reasons may exist for this difference. First, our subjects were young men, whereas the subjects of the earlier report were ages 45 to 74; age-related differences in ADH release patterns have not yet been investigated. Second, it has not been shown that young men have the same REM-related decrease in urine volume that older men have. Third, chemical and neural factors other than ADH conceivably might influence urine volume within a short time, possibly including the intrarenal antagonism of ADH by prostaglandins (18) or sympathetically mediated changes in overall or regional renal blood flow during REM sleep. Generalized sympathetic activation appears to be a regular autonomic correlate of REM sleep, but the possible renal effects of such periodic sympathetic discharge remain unexplored.

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