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Decreased Delta-Sleep and Plasma Delta-Sleep-Inducing Peptide in Patients with Cushing Syndrome

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Abstract

To evaluate the sleep disturbances of patients with Cushing syndrome and to examine the relationship between the sleep disturbances and plasma levels of delta-sleep-inducing peptide-like immunoreactivity (DSIP-LI), we performed three polysomnographic/endocrinological studies in patients with Cushing syndrome. In study 1, polysomnography was studied in 12 patients and 12 matched normal volunteers. In addition, DSIP-LI was measured every 30 min for 24 h in 9 patients with Cushing syndrome and 12 normal volunteers. The percentage of time spent in delta sleep (stages 3 and 4) was significantly reduced in patients with Cushing syndrome (5.8 ± 1.4%; mean ± SEM) compared to normal volunteers (14.0 ± 2.5%) ($p < 0.01$). REM sleep indices, however, were not significantly different between the two groups. There was a significant negative correlation between amount of delta sleep and 08.00 h DSIP-LI ($r = -0.43$, $p < 0.05$), which is against the notion of a causal relationship between DSIP-LI and delta sleep. The circadian rhythm of plasma DSIP-LI was found to be similar in Cushing patients and normal volunteers. In study 2, we measured plasma levels of delta-sleep-inducing peptide-like immunoreactivity (DSIP-LI) at 08.00 h in 65 patients with Cushing syndrome and 49 normal volunteers. The 08.00 h DSIP-LI concentrations of 797 ± 57 pmol/l (mean ± SEM) in the patients with Cushing syndrome were significantly reduced compared to the level of 1,062 ± 99 pmol/l found in the normal volunteers ($p < 0.05$). In study 3, plasma was drawn simultaneously from the petrosal sinuses and peripheral veins of Cushing patients. No central-to-peripheral gradient for plasma DSIP-LI was noted and neither peripheral, nor central plasma DSIP-LI was affected by administration of intravenous ovine CRH. We conclude that patients with Cushing syndrome have less delta sleep and lower plasma concentrations of DSIP-LI than normal controls, however a causal relationship between the two appears to be unlikely. The pituitary does not appear to be the site of synthesis of plasma DSIP, whose source remains unknown.

Key Words

Delta sleep in Cushing syndrome
Cushing syndrome
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Introduction

Patients with both endogenous and exogenous Cushing syndrome have been noted to have sleep disturbances [1], although the actual sleep abnormalities reported have varied widely. As early as 1972, Krieger and Glick [2, 3] and Krieger [4] found reduction of sleep EEG stages III and IV (delta sleep; slow-wave sleep) in patients with endogenous hypercortisolism and suggested that this was due to a 'central mechanism' and not merely to elevated cortisol. More recently, Shipley et al. [5, 6] studied 11 patients with Cushing disease and found shorter rapid eye movement (REM) latency and increased REM density than in normal volunteers, but no differences in delta sleep. Administration of exogenous glucocorticoids has also been found to affect sleep. For example, Gillin et al. [7] found that oral prednisone (60 mg given 6 h before sleep onset) significantly reduced REM sleep, increased REM latency and stage 2 sleep, but did not affect delta sleep. Fehm et al. [8] found that 1 mg of dexamethasone given immediately before sleep onset, led to a reduction in REM and stage 4 sleep, while infusion of 100 mg of hydrocortisone throughout the night reduced REM sleep and increased stage 4 sleep. On the other hand, decreased adrenal steroid production, studied by withdrawal of cortisol replacement in patients with adrenal insufficiency as well as metyrapone administration to healthy volunteers, increased delta sleep [9].

In an attempt to study the relationship between endogenous hypercortisolism and sleep stage and to resolve the discrepancies between the findings of Krieger and Glick [2, 3] and Krieger [4] and those of Shipley et al. [5, 6], we performed polysomnography on 12 patients with Cushing syndrome and 12 age- and sex-matched normal volunteers. We were also interested in examining the role of delta-sleep-inducing peptide (DSIP), a nonapeptide which has been found to have sleep-inducing and -maintaining properties in several species, including humans [10–13], on the sleep parameters of patients with Cushing syndrome and normal volunteers. DSIP-like immunoreactivity (DSIP-LI) has been found in thyrotropic cells in mice [14] and corticotropic/melanotropic cells in humans and pigs [15, 16], where it appeared to be colocalized with ACTH and corticotropin-like intermediate-lobe peptide (CLIP). We, therefore, hypothesized that levels of this hormone may be altered in patients with Cushing syndrome and may be related to their sleep disturbances. To test this hypothesis, DSIP-LI was measured every 30 min for 24 h in 9 patients with Cushing syndrome and 12 normal volunteers. In addition, we initiated a second, larger study in which we measured plasma levels of DSIP-LI at 08.00 h in 65 patients with Cushing syndrome and 40 normal volunteers. To study whether DSIP is synthesized in the pituitary in humans, we implemented a third study in which we measured DSIP-LI in plasma from the inferior petrosal sinuses which receive the venous drainage of the pituitary, and compared the inferior petrosal sinus levels with those of plasma drawn simultaneously from a peripheral vein.

Subjects and Methods

Twenty-Four Hour Sampling and Polysomnography (Study 1)

Patient Selection

Twelve healthy volunteers (8 females and 4 males) and 12 patients with Cushing syndrome (8 females, 4 males; 9 with Cushing disease and 3 with ectopic ACTH syndrome) were admitted to participate in the polysomnographic portion of the study (study 1). The volunteers were matched with patients with Cushing syndrome for gender and age resulting in similar ages of both the group with Cushing syndrome (34.9 ± 7.9 years, mean ± SD, range 24–50) and the normal volunteers (33.7 ± 8.9 years, mean ± SD, range 22–48). The protocol was approved by the NICHD institutional review board and all subjects gave informed consent prior to participating in the study. Normal volunteers were admitted to the inpatient ward for 5 days. Patients with Cushing syndrome underwent this study during their inpatient diagnostic stay. Both normal volunteers and patients with Cushing syndrome were excluded if they had taken any medications known to affect sleep or the hypothalamic-pituitary-adrenal (HPA) axis during the preceding 3 days. Normal volunteers who had a history of psychiatric illnesses based on the Schedule for Affective Disorders-Lifetime Version (SADS-LA) interview [17] or the Atypical Depression Diagnostic Scale [18] were also excluded. Four patients with Cushing syndrome had atypical depression, attributed to their disease state [Dorn et al., submitted]. All of the patients with Cushing syndrome had clinical, biochemical and pathological confirmation of Cushing syndrome.

Study Design

Both normal volunteers and patients with Cushing syndrome underwent an adaptation night for the sleep EEG during which they were attached to the EEG leads but no sleep was recorded (day 1). The polysomnographic study began at 08.00 h on the following day (day 2). An indwelling catheter was placed in an antecubital vein at least 1 h prior to starting the study. Blood samples were taken from an indwelling catheter every 30 min from 08.00 h (day 2) until 08.00 h on the following day (day 3). Three of the patients with Cushing syndrome did not complete the blood drawing portion of the study due to difficulty with venous access. Participants stayed in their rooms during the study, but their movements were not restricted. Lights were turned off at 22.30 h and turned on at 07.00 h.

Continuous polygraphic recording took place from 08.00 h on day 2 until 08.00 h on the following day (day 3). Sleep stages were identified from electroencephalogram (EEG), vertical and horizontal electrooculogram (EOG), and electromyogram (EMG). The following sleep parameters were calculated for each subject for the whole

night: (a) total sleep time and total time spent in the respective sleep stages (wakefulness, stages 1, 2, 3, 4, and REM) and amount and duration of movement; (b) percentage of time spent in different sleep stages (with reference to total sleep time); (c) latency of the different sleep stages (with reference to sleep onset). The percentage cumulative sum of REM and delta sleep was calculated as the percentage of those parameters out of the total REM and delta sleep, respectively, at each time point for each individual. The cumulative sum of these parameters was then averaged for both the group of patients with Cushing syndrome and the normal volunteers and their slope and area under the curve (AUC) were compared between both groups. All participants also completed a self-report questionnaire on their sleep each morning upon awakening for 4 days starting with the day prior to the adapt night. Questions were selected form the reports of Edinger and Stout [19]. Questions such as difficulty sleeping and difficulty staying asleep were rated on a scale of 1–5, with 1 being 'extremely difficult' and 5 being 'very easy', and the question of quality of sleep was rated on a scale of 1 to 5, with 1 being 'very poor' and 5 being 'excellent'. Participants were also asked to rate how much their sleep was disturbed by the EEG equipment and blood drawing on the night of the study on a scale of 1–5 with 1 being 'a very great deal' and 5 being 'not at all'. The mean score of the four days was computed for each patient.

Morning Plasma DSIP-LI Measurements (Study 2)

Forty-nine normal volunteers (25 females and 24 males, age 35.6 ± 9.6 years, mean ± SD; range 22–62) and 65 patients with Cushing syndrome (55 females, 10 males; age 40.3 ± 12.3 years, mean ± SD; range 22–62; 45 with Cushing disease and 15 with ectopic ACTH syndrome, 5 with adrenal adenomas) had plasma drawn for DSIP-LI between 08.00 h and 10.00 h (study 2). With the exception of 6 of the patients in the ectopic ACTH syndrome group, all patients had their tumor identified at surgery and/or had biochemical cure of their hypercortisolism following surgery. Six patients with suspected ectopic ACTH syndrome have not had their ACTH-secreting tumor identified. However, they were categorized as having ectopic ACTH syndrome based on high urinary free cortisol excretion and lack of central-to-peripheral gradient at petrosal sinus sampling [20].

Petrosal Sinus Sampling (Study 3)

Petrosal sinus sampling was performed on 7 patients with Cushing syndrome (6 females and 1 male; 5 with Cushing disease, 1 with ectopic ACTH syndrome and 1 with an adrenal adenoma; age 40.3 ± 12.4 years, mean ± SD, range 29–64) as previously described [21]. Briefly, after sedative administration and systemic anticoagulation with heparin, catheters were advanced to the inferior petrosal sinuses under fluoroscopic guidance, and their positions verified by contrast injections. Between 09.00 and 11.00 h, blood was obtained simultaneously from each petrosal sinus and a peripheral vein 1 min before and 3 min after i.v. administration of ovine CRH (1 μg/kg) and analyzed for DSIP-LI and ACTH as described below. All patients were hypercortisolemic as determined by elevated urinary free cortisol measurement (>360 nmol/day) at the time of sampling. The protocol for the use of ovine CRH with petrosal sinus sampling was approved by the NICHD institutional review board, and informed consent was obtained from all subjects before the procedure.

Hormonal Analyses

Blood was collected from an indwelling catheter, transferred to a prechilled tube containing EDTA and refrigerated until centrifugation (within 6 h). The supernatant was frozen at −20°C until assay for DSIP-LI, cortisol and ACTH. Becuase DSIP levels were found to decrease with time [22], the assay for DSIP-LI was always initiated between 2 and 9 days after collection. DSIP-LI was measured by radioimmunoassay (RIA) as previously described [23] using antiserum K-7914, which recognizes the nonapeptide and its phosphorylated and precursor forms. Cortisol was measured by RIA [24] and ACTH by immunoradiometric assay [25] as previously described.

Statistical Analyses

To obtain a smoother pattern of the diurnal rhythm of plasma DSIP-LI (study 1), a running average of DSIP-LI for each hour was obtained by taking the mean of the value 30 min before the hour, on the hour and 30 min after the hour. Differences in the 24-hour variations in DSIP-LI plasma levels of patients with Cushing syndrome and normal volunteers were statistically assessed by a two-way repeated-measures analysis of variance (ANOVA) (SuperANOVA, Abacus Concepts, Inc., Berkeley, Calif.) that included the between factors 'group' (normal volunteers vs. patients with Cushing syndrome) and the within factor 'time of day'. Unpaired Student t tests were used to compare sleep parameters as well as plasma DSIP-LI levels between the patients with Cushing syndrome and normal volunteers. Simple linear regressions (Statview, Brainpower Inc., Calabasas, Calif.) were used to test associations between mean plasma levels of DSIP-LI, cortisol and ACTH and percentage of delta sleep out of total sleep time.

Results

Twenty-Four-Hour Sampling and Polysomnography (Study 1)

The results of polysomnographic recordings in patients with Cushing syndrome and normal volunteers are summarized in table 1. Percentage of stage 3 and stage 4, and delta sleep (combined stages 3 and 4) were significantly reduced in patients with Cushing syndrome ($p < 0.05$ for stage 3 and for stage 4; $p < 0.01$ for delta sleep). The percentage of delta sleep in patients with Cushing syndrome (5.8%) was approximately one-third that of normal volunteers (14.0%). Patients with both Cushing syndrome and atypical depression (n = 3), had a similar percentage of delta sleep (5.9 ± 4.5%) as the group of patients with Cushing syndrome without depression (n = 9) (5.7 ± 1.5%; NS). Additionally, patients with ectopic ACTH syndrome (n = 3) had a similar percentage of delta sleep (7.1 ± 1.0%) as the group with Cushing disease (n = 9) (5.3 ± 1.9%; NS). The percentage of stage 2 sleep was also significantly increased in patients with Cushing syndrome compared to normal volunteers ($p < 0.05$). REM sleep indices, REM latency times and total sleep latency times were not significantly different between the two groups although there was a trend toward increased sleep latency ($p < 0.1$) in the Cushing syndrome group. In both groups, daytime polygraphic recordings demonstrated awake po-

lysomnography and did not demonstrate daytime sleeping or any pathological events. The cumulative percentage of time spent in delta and REM sleep throughout the night (when corrected for sleep latency) was similar for both the group of patients with Cushing syndrome and the normal volunteers (data not shown).

Table 2 summarizes the results of sleep surveys in which the participants were asked to rate their sleep during the study. There were no significant differences between the two groups on any item demonstrating that both groups had a similar subjective assessment of their sleep. Both groups were disturbed by the recording and blood drawing procedures to a similar extent.

The mean hourly plasma DSIP-LI levels from 08.00 to 07.00 h the following day is depicted in figure 1 for both groups. The diurnal rhythm of plasma DSIP-LI in normal volunteers was previously reported [22, 26, 27], with high levels of the hormone in the day and low levels at night. Cushing patients exhibited a similar diurnal pattern of plasma DSIP-LI as normal volunteers. Analysis of variance did not show significant differences in the plasma levels of DSIP-LI between the two groups ($F = 0.61$; d.f. = 1,17, p = NS). Thus, although the mean level at each time was lower for the Cushing syndrome group, the considerable patient-to-patient variation prevented statistical significance to be observed.

To examine whether the decreased DSIP-LI and the decreased percentage of delta sleep were related, we correlated the 08.00 h level of plasma DSIP-LI drawn the morning preceding and following the sleep study as well as the average DSIP-LI for 24 h in the 12 normal volunteers and 9 patients with Cushing syndrome who completed both the polysomnography and blood-drawing portions of the study. When both groups were combined, there was a negative correlation between 8 a.m. plasma DSIP-LI values on the preceding day and percentage of delta sleep ($r = -0.43$, $p < 0.05$) and between 8 a.m. plasma DSIP-LI levels on the subsequent day and percentage of delta sleep throughout the night ($r = -0.42$, $p < 0.05$). There was also a negative correlation between peak DSIP-LI levels during the 24 h sampling and percentage of delta sleep ($r = -0.45$, $p < 0.05$). The correlations between average daily plasma DSIP-LI and percentage of delta sleep were not statistically significant. When the above correlations were performed on the two groups (patients with Cushing syndrome and normal volunteers) separately, the correlations were not significant. Correlations between percentage delta sleep and 08.00 h values of plasma ACTH or cortisol on the preceding and subsequent day compared to the sleep study and between percentage delta sleep and the average daily

Table 1. Sleep parameters in normal volunteers and patients with Cushing syndrome

	Normals	Cushing
Total sleep, min	290±26	259±28
NREM, %	87.8±2.2	89.70±1.4
REM, %	12.2±2.2	10.3±1.4
Sleep latency, min	61.4±17.9	117±26.4
REM latency, min	103±30.7	109±26.6
REM density, eye movements/min	1.98±0.33	1.63±0.19
Stage I, %	9.7±2.0	9.5±1.9
Stage II, %	63.8±3.4	74.0*±2.7
Stage III, %	9.6±1.7	4.7*±1.0
Stage IV, %	4.4±1.4	1.1*±0.58
Delta, %	14.0±2.5	5.8**±1.4
Waking sleep after sleep onset, min	97.9±22.7	82.2±16.7
Early morning wakening, min	64.7±15.6	54.9±16.3
Sleep efficiency, %	53.0±4.6	4.0±5.2

* $p < 0.05$ versus normal volunteers; ** $p < 0.01$ versus normal volunteers.

Table 2. Subjective assessment of sleep in normal volunteers and patients with Cushing syndrome

Subjective assessment		Normals	Cushing
Number of times awoke	Average	2.25	1.35
	SEM	0.19	0.15
Difficulty sleeping	Average	3.35	3.42
	SEM	0.28	0.38
Difficulty staying asleep	Average	3.18	3.18
	SEM	0.27	0.35
Quality of sleep	Average	3.13	3.24
	SEM	0.36	0.26
Sleep disturbance	Average	2.23	3.61
	SEM	0.34	0.40

The questions of difficulty sleeping and difficulty staying asleep were rated on a scale of 1–5, with 1 being 'extremely difficult' and 5 being 'very easy', and the question of quality of sleep was rated on a scale of 1–5, with 1 being 'very poor' and 5 being 'excellent'. Participants were also asked to rate how much their sleep was disturbed by the EEG equipment and blood drawing on the night of the study on a scale of 1–5 with 1 being 'a very great deal' and 5 being 'not at all'. There were no significant differences between normal volunteers and Cushing syndrome patients for any of the subjective assessments.

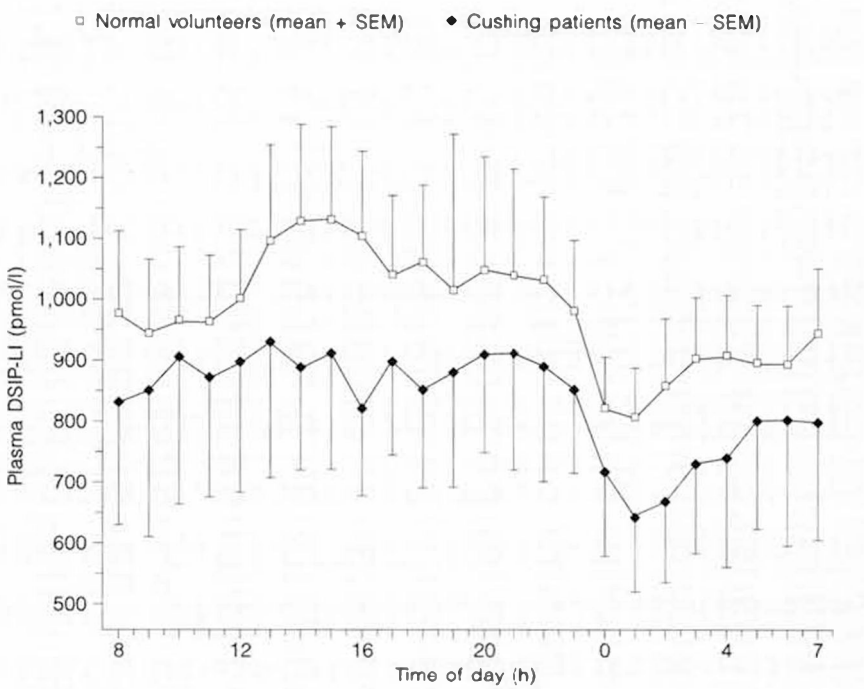

Fig. 1. Diurnal rhythm of plasma DSIP-LI in normal volunteers and patients with Cushing syndrome. Blood was drawn every 30 min from 08.00 to 08.00 h the following morning. Values 30 min before and after the hour were averaged with the value at the hour to give a running hourly average. Data represent the mean + SEM for the normal volunteers and the mean − SEM for the Cushing group at each time point. ANOVA did not show a significant variance between the two groups ($F = 0.61$; d.f. = 1,17, p = NS).

cortisol or ACTH level were all nonsignificant (data not shown) for both the two groups separately or combined.

Morning Plasma DSIP-LI Measurements (Study 2)

Since a significantly decreased percentage of delta sleep was found in the Cushing syndrome group, we were interested in examining whether this decrease was due to low levels of the putative delta-sleep-inducing hormone, DSIP, in a larger number of patients with Cushing syndrome and controls. We, therefore, measured morning plasma DSIP-LI levels in 65 patients with Cushing syndrome and found levels of 797 ± 57 pmol/l (mean ± SEM) to be significantly reduced compared to the levels of 1,062 ± 99 pmol/l found in 49 normal volunteers ($p < 0.05$). The scatterplot of the DSIP-LI concentrations in the two groups (fig. 2) shows considerable variation of levels in both groups as well as the decreased mean of patients with Cushing syndrome. Plasma DSIP-LI levels were not affected by the sex of the individual and were not altered by the etiology of the Cushing syndrome (table 3). The 08.00 h DSIP-LI levels seen in the 24 h sampling (fig. 1) were similar to those of the single measurement values for each group (fig. 2).

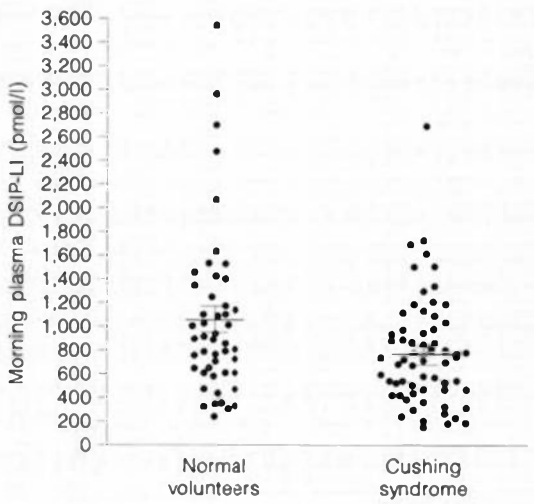

Fig. 2. Scatterplot of morning plasma DSIP-LI of 49 normal volunteers and 65 patients with Cushing syndrome. The mean ± SEM is depicted with horizontal lines.

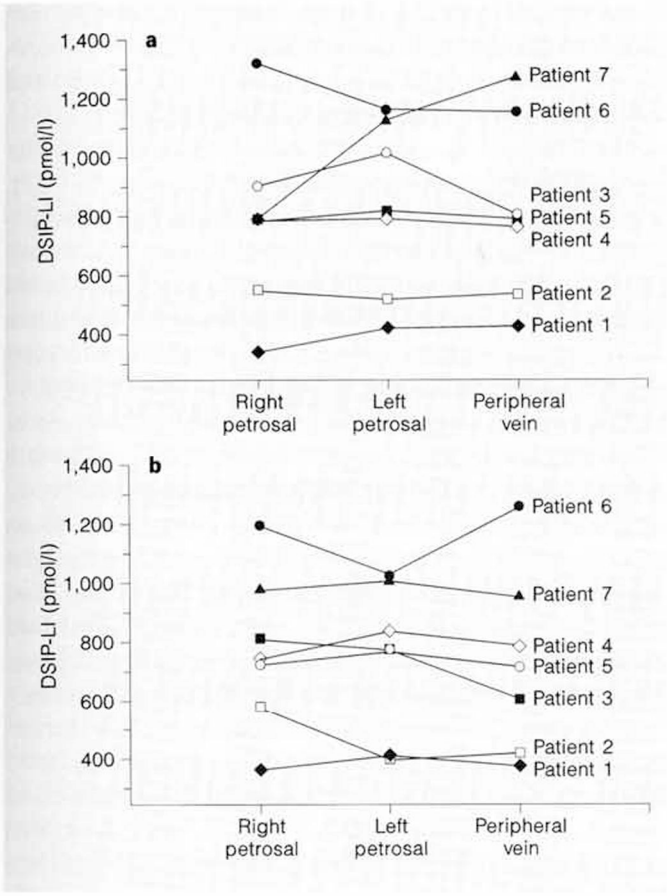

Fig. 3. Concentrations of plasma DSIP-LI in the petrosal sinuses and peripheral vein of patients with Cushing syndrome before (**a**) and after (**b**) intravenous administration of ovine CRH (1 μg/kg). Patients 1–5 had Cushing disease, patient 6 had an adrenal adenoma and patient 7 had ectopic ACTH syndrome. There was no central-to-peripheral gradient of plasma DSIP-LI nor was there stimulation of plasma DSIP-LI after ovine CRH administration.

Table 3. Plasma DSIP-LI levels in normal volunteers and patients with Cushing syndrome

	Plasma DSIP-LI, pmol/l
Normal volunteers (49)	1,062 ± 99
Cushing syndrome (65)	797 ± 57*
Cushing disease (45)	844 ± 74
Ectopic ACTH syndrome (15)	717 ± 94
Adrenal adenoma (5)	599 ± 104
Normal volunteer males (24)	1,031 ± 166
Normal volunteer females (25)	1,089 ± 113

Morning plasma DSIP-LI was determined by radioimmunoassay.

* $p < 0.05$ versus normal volunteers.

Petrosal Sinus Sampling (Study 3)

To detetermine if the pituitary is an important source of circulating human DSIP, plasma DSIP-LI levels from the inferior petrosal sinuses which drain the pituitary [21] were compared to DSIP-LI levels obtained from a simultaneously drawn peripheral sample in patients with Cushing syndrome. In both baseline samples (fig. 3a) and in samples after intravenous administration of ovine CRH (1 μg/kg) (fig. 3b), neither central-to-peripheral nor side-to-side gradient of DSIP-LI was observed. The mean ± SEM for DSIP-LI before ovine CRH was 740 ± 118, 808 ± 113 and 806 ± 120 pmol/l in the right, left petrosal sinus and peripheral vein, respectively. The mean ± SEM for DSIP-LI after ovine CRH was 754 ± 101, 714 ± 99 and 714 ± 120 pmol/l in the right, left petrosal sinus and peripheral vein, respectively. DSIP-LI levels in neither of the petrosal sinuses nor the peripheral vein were affected by ovine CRH administration. The lack of any plasma DSIP-LI gradients, before or after ovine CRH administration was in marked contrast to the results obtained with plasma ACTH. Each of the 5 patients with Cushing disease had ACTH levels which showed a central-to-peripheral gradient, lateralization to the side of the tumor and response to ovine CRH (data not shown) similar to previous reports [20, 28].

Discussion

In 1974, Krieger and Glick [3] reported marked reductions in stages 3 and 4 sleep in 4 treated and 6 untreated patients with Cushing syndrome as well as in 4 'eu-corticoid' patients with hypothalamic tumors. An increase in stage 2 sleep was also noted in the patients with active Cushing disease. The authors postulated that the decreased delta sleep was due to elevated ACTH levels. The same laboratory [29] described one patient with Cushing syndrome secondary to an adrenal adenoma who had no delta sleep while actively hypercortisolemic. However, following cure by adrenalectomy, her delta sleep returned to normal 16 months postoperatively. The authors did not explain how both ACTH-dependent and ACTH-independent Cushing syndrome could cause reduced delta sleep.

Eighteen years later, Shipley et al. compared the sleep parameters of patients with Cushing syndrome to those of normal volunteers [5], as well as to those of patients with major depressive disorder [6]. The authors found that 32% of the patients with Cushing syndrome had sleep apnea [5]. Compared to 14 healthy controls, 11 patients with Cushing disease who did not have sleep apnea had a

longer total sleep latency but decreased REM latency. They also reported that more time was spent awake, REM density was increased and stage 4 sleep was decreased by a small, but significant amount. There was no significant difference in percentage of delta sleep between the two groups, but a very low percentage of delta sleep (8.7%) was observed. Most studies have found that approximately 15% of total sleep is spent in delta sleep in a population of similar age [30]. The authors noted, however, that a higher low-frequency filter setting was used in the normals (0.5 Hz) than the patients with Cushing syndrome (0.3 Hz), which could have resulted in reduced delta activity in the normal volunteers. To resolve the discrepancies between the studies by Krieger and Glick [2, 3] and Krieger [4] and Shipley et al. [5, 6], we carefully studied the sleep architecture in patients with Cushing syndrome with a constant filter setting (1.5 Hz).

In agreement with the study by Krieger and Glick [3], we found markedly decreased delta sleep in patients with Cushing syndrome. This was evident as a decrease in stage 3 and in stage 4 sleep. There was also a significant increase in stage 2 sleep which was similar to the percentage decrease in delta sleep. We did not observe the changes in REM and sleep latencies or REM density noted by Shipley et al. [5, 6]. As we did not record respiratory function, sleep apnea would not have been detected.

In both the normal volunteers and patients with Cushing syndrome, poor sleep efficiency was observed. This may have been due to the difficulty of sleeping in a hospital bed or to disruption of sleep by blood drawing. As both groups underwent similar study conditions and had similar sleep efficiencies, the sleep disruption most likely cannot account for the striking differences in delta sleep observed.

We were next interested in determining what hormone, if any, would be altered in the patients with Cushing syndrome that could explain the decreased percentage of delta sleep. The nonapeptide, DSIP, seemed a possible candidate, as there is much evidence that this putative delta-sleep-inducing hormone [10–13] is involved in the regulation of and is probably regulated itself by the HPA axis. For example, synthetic DSIP inhibited both the basal and CRH-induced release of ACTH from cultured mouse anterior pituitary cells [14]. CRH and arginine vasopressin, on the other hand, two hormones which normally stimulate the release of ACTH, inhibited DSIP release from anterior pituitary cells [14]. Furthermore, the finding of DSIP-LI in corticotrope/melanotrope cells suggested that this hormone may be expressed in abundance in the corticotropic tumors of patients with Cushing disease. Additionally, since ectopic ACTH-secreting tumors are dedifferentiated neoplasms, it is possible that they express neuropeptides other than ACTH. For these reasons, we elected to study DSIP in patients with Cushing syndrome.

DSIP has been previously thought to be a sleep-inducing peptide, specifically stimulating delta sleep [10–13], although some studies have not confirmed that administration of this peptide does indeed increase delta sleep [31–33]. We have recently shown a distinct diurnal rhythm for plasma DSIP-LI, with highest levels in the late afternoon and evening and decreased levels after turning off the lights and allowing sleep to occur [22]. Plasma DSIP-LI levels were found to be substantially lower in REM sleep and somewhat lower in delta sleep. The fact that hormonal levels were strongly correlated with body temperature, but did not rise before, during or after an episode of delta sleep, suggests that the circulating hormone may not be delta sleep-promoting. Banks and Kastin [34] demonstrated that CSF and plasma DSIP-LI do correlate, and that DSIP crosses the blood-brain barrier. Recently, a 77 amino acid peptide (DSIP-immunoreactive peptide; DIP) which cross-reacts with DSIP antiserum but does not contain the amino acid structure of DSIP, has been isolated from porcine brain and shown to contain a leucine-zipper motif [35]. It is not clear if this peptide has any sleep-promoting properties, nor is it clear if the antibody used in this current study recognizes DIP.

Single draw a.m. values of plasma DSIP-LI were found to be significantly lower in patients with Cushing syndrome compared to normal volunteers (fig. 2). However, there was a wide interindividual variation and a large number of patients belonging to each group was studied to demonstrate statistical significance. The 24-hour profile of plasma DSIP-LI in patients with Cushing syndrome was also compared to that of normal volunteers (fig. 1) and found to be similar. Thus both normal volunteers [22] and patients with Cushing syndrome exhibit a distinct diurnal rhythm for plasma DSIP-LI, and these data also suggest that this diurnal rhythm is not dependent on cortisol since the Cushing patients had no diurnal cortisol rhythm (data not shown).

We then correlated plasma DSIP-LI levels with percentage of delta sleep. When values from the patients with Cushing syndrome were combined with those from normal volunteers, there was a significant negative correlation between percentage delta sleep and 8 a.m. plasma DSIP-LI levels on the morning preceding and following the night of polysomnography as well as between percentage delta sleep and peak plasma DSIP-LI levels during the 24-hour period of sampling. On the other hand, there was

no correlation between percentage of delta sleep and ACTH or cortisol levels. Although Cushing patients were found to have both decreased delta sleep and decreased DSIP-LI, the small difference of DSIP-LI between the two groups, its similar diurnal rhythm and the fact that plasma DSIP-LI was not positively correlated with percentage of delta sleep suggests that the two observed phenomena may not be causally related. Alternatively, the decreased plasma DSIP-LI levels could lead to alterations in other hormones, which could then affect delta sleep in a manner not examined in this study.

Petrosal sinus sampling allowed us to examine whether the human pituitary was indeed a major source of DSIP synthesis. Unlike ACTH, we did not find a central-to-peripheral gradient for plasma DSIP-LI in any patient studied. Although a peptide with a long plasma half-life might undergo recirculation from peripheral to petrosal blood and thus obscure a central to peripheral gradient, the reported half-life of DSIP is 5–8 min [11], sufficiently short not to allow this phenomenon to occur. Rather, it appears that most circulating DSIP-LI does not originate from the pituitary in the patients studied. As there have been reports that administration of intravenous ovine CRH increased DSIP levels in normal volunteers but decreased DSIP levels in patients with depression [36], our findings that ovine CRH did not affect either peripheral or petrosal plasma DSIP-LI levels are noteworthy. The fact that previous studies have stored plasma containing DSIP for various time intervals prior to assay and we have shown that plasma DSIP-LI decays with storage [22] may explain these different findings. The lack of stimulation by ovine CRH, the lack of central-to-peripheral gradient and the lack of finding elevated plasma DSIP-LI levels in patients with corticotroph tumors (Cushing disease) are all consistent with the belief that a site other than the corticotroph cell is the major site of DSIP synthesis in humans.

Previous studies have found that disordered sleep is reported by over 50% of the patients with Cushing syndrome [1]. Additionally, patients with Cushing syndrome report daytime fatigue and cognitive impairment [1, 37, 38], which might be related to their poor nighttime sleep. It is noteworthy that in our sample, Cushing patients did not report more subjective sleep disturbances than the matched volunteers. The significantly decreased delta sleep in the patients with Cushing syndrome did not directly correlate with plasma DSIP-LI, cortisol or ACTH levels. Decreased delta sleep may be due to another centrally acting neuropeptide, such as growth-hormone releasing hormone [39–41] or other as yet undiscovered naturally occurring sleep peptide(s) which may be altered in Cushing syndrome.

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