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Hypothalamic–Pituitary–Adrenal Axis Activity and Sleep in Posttraumatic Stress Disorder

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Alterations of the hypothalamic–pituitary–adrenal (HPA) axis and sleep disturbances have been described separately in post-traumatic stress disorder (PTSD). It is not known if HPA alterations and sleep disturbances are associated in PTSD. This study examined sleep and HPA activity in 20 male medication-free subjects with PTSD and 16 matched healthy controls. Two nights of polysomnography were obtained and 24-h urinary cortisol was collected during day 2. Subjects self-administered a low-dose (0.5 mg) salivary dexamethasone test at home. Compared with controls, PTSD subjects had higher 24-h urinary μg cortisol/g creatinine (mean ± SD 40 ± 17 *vs* 28 ± 12, $p = 0.03$) but not significantly higher 24-h urinary cortisol (mean ± SD 52 ± 15 μg/day *vs* 43 ± 23, $p = 0.19$). PTSD subjects showed a trend towards less cortisol suppression after dexamethasone (73% ± 18 *vs* 83% ± 10, $p = 0.06$). In the combined sample, delta sleep was significantly and negatively correlated with 24-h urinary cortisol ($r = -0.36$, $p = 0.04$), and with 24-h urinary cortisol/g creatinine on a trend level ($r = -0.34$, $p = 0.06$). Our results suggest that increased cortisol is negatively associated with delta sleep. This may contribute to sleep abnormalities in conditions associated with elevated cortisol, possibly including PTSD. Future studies should explore the temporal relationship between HPA activity, sleep disturbances, and psychopathology after a traumatic event.
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INTRODUCTION

Sleep disturbances are among the most common symptoms of post-traumatic stress disorder (PTSD) and are represented by nightmares (in the re-experiencing cluster) and difficulty in falling and staying asleep (in the hyperarousal cluster) as specified in DSM-IV criteria (Neylan *et al*, 1998; Pillar *et al*, 2000). Hence, it is surprising that only two studies have shown that PTSD, a putative hyperarousal disorder, is associated with decreased visually scored delta sleep (Glaubman *et al*, 1990; Kramer and Kinney, 1988). This contrasts with a larger number of studies of PTSD that have found no differences in visually scored delta sleep (Mellman *et al*, 1995a–c, 1997; Ross *et al*, 1994a, b; Woodward *et al*, 1996a–c). One possible explanation for failing to find reduced delta sleep in PTSD relates to the poor sensitivity of visual sleep staging, or sleep macroarchitecture, in quantifying delta sleep. Indeed, the only two studies that applied quantitative measures of delta sleep have found decreased delta sleep in patients with PTSD (Neylan *et al*, 2003; Woodward *et al*, 2000).

In healthy subjects, delta sleep activity peaks in the first half of the night and is temporally associated with the nadir of cortisol output (Steiger, 2002). Delta sleep is believed to be the primary marker for sleep homeostasis and the restorative function of sleep (Borbely and Achermann, 2000). Multiple studies have shown that increased hypothalamic corticotropin-releasing factor (CRF) release is associated with disturbed sleep and particularly with decreased delta sleep activity (reviewed in Neylan *et al*, 2003). In turn, treatment with a CRF receptor antagonist increased delta sleep in depressed patients (Held *et al*, 2004).

While there are consistent results of elevated CRF in the cerebrospinal fluid in PTSD patients (Baker *et al*, 1999; Bremner *et al*, 1997), studies using 24-h urinary cortisol and the dexamethasone suppression test to characterize HPA activity have reported variable results. In PTSD, 24-h urinary cortisol was increased (De Bellis *et al*, 1999; Lemieux and Coe, 1995; Maes *et al*, 1998; Pitman and Orr, 1990), unchanged (Baker *et al*, 1999; Rasmusson *et al*, 2001;

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Young and Breslau, 2004) or decreased (Thaller *et al*, 1999; Yehuda *et al*, 1995, 1990) compared to healthy controls. Also, cortisol suppression after dexamethasone was reported to be diminished (Atmaca *et al*, 2002; Thaller *et al*, 1999), unchanged (Dinan *et al*, 1990; Halbreich *et al*, 1989; Lindley *et al*, 2004; Lipschitz *et al*, 2003), or to be enhanced (Stein *et al*, 1997; Yehuda *et al*, 2002, 1993). Some of these inconsistencies might be related to differences among studies regarding the study population, age, and gender differences, use of psychotropic medication, comorbid substance abuse, and dose of dexamethasone (for reviews, see Rasmusson *et al*, 2003; Yehuda, 2002).

We have previously shown that PTSD subjects had a diminished adrenocorticotropin (ACTH) response and a less pronounced decrease of delta sleep to an indirect CRF challenge with metyrapone (Neylan *et al*, 2003). In that study, PTSD subjects had significantly less delta sleep, but no significant differences in total sleep time, sleep maintenance, REM latency, or REM density compared to control subjects. By blocking the last step of cortisol synthesis, metyrapone acutely reduces cortisol levels, attenuates cortisol-mediated feedback inhibition at the pituitary, hypothalamus, and hippocampus, while increasing the release of hypothalamic CRF. Therefore, an attenuated increase of ACTH and a diminished decrease of delta sleep after metyrapone in subjects with PTSD could be explained by chronic increased CRF activity and downregulated CRF receptors.

To extend our previous findings with metyrapone that suggested increased CRF in PTSD, in the current study we examined the relation between delta sleep and 24 h urinary cortisol in 20 male medication-free subjects with PTSD without any substance abuse in the last 2 years and 16 age- and sex-matched controls. Since previous literature in PTSD reported both 24-h urinary cortisol and 24-h urinary cortisol per g creatinine (Glover and Poland, 2002; Lemieux and Coe, 1995; Rasmusson *et al*, 2001), we examined both parameters. Subjects also self-administered a low-dose (0.5 mg) salivary dexamethasone suppression test at home to specifically address the issue of possible enhanced negative feedback inhibition in PTSD (Yehuda, 2002).

We hypothesized that (1) there would be a negative correlation between delta sleep and 24-h urinary cortisol and 24-h urinary cortisol/g creatinine, respectively and (2) that subjects with PTSD would have less 24-h urinary cortisol, less 24-h urinary cortisol/g creatinine, and an increased negative feedback inhibition of salivary cortisol after 0.5 mg of dexamethasone compared to controls.

MATERIALS AND METHODS

Participants

Medically healthy male subjects between 20 and 60 years were recruited from web-based and newspaper advertisement and from the San Francisco Veterans Affairs Medical Center PTSD Outpatient Program. For this study, only men were included to eliminate the possible confound of gender, which has been demonstrated both in HPA activity (Heuser *et al*, 1994; Otte *et al*, 2005; Seeman *et al*, 1995) and sleep (Armitage and Hoffmann, 2001). All subjects provided written informed consent. The study protocol and consent form was approved by the Committee on Human Research at the University of California, San Francisco (UCSF). In all, 20 PTSD subjects and 16 controls completed two nights of polysomnography on the General Clinical Research Center at UCSF. Subjects spent the three days on the General Clinical Research Center at UCSF. They were allowed to have one cup of coffee in the morning, but were not allowed to nap during the day.

Patients were included when they were positive for current chronic PTSD, as indexed by the Clinician Administered PTSD Scale (CAPS) criteria (Blake *et al*, 1995). Subjects filled out the Beck Depression Inventory (BDI) (Beck *et al*, 1961) and the Impact of Event Scale-Revised (IES-R) (Weiss and Marmar, 1996) each morning after the sleep recordings.

Subjects were excluded if they met the criteria for alcohol or substance abuse within the past 2 years, schizophrenia, schizoaffective disorder, bipolar disorder, and obsessive compulsive disorder as assessed by the Structured Clinical Interview for DSM-IV (SCID; First *et al*, 1996). Medical exclusion criteria included any history of central nervous system disease, or use of any medication affecting the brain. All subjects were free of any psychotropic medications and had not been hospitalized for at least 2 months prior to participation according to self-report.

All subjects in this study also participated in our previous study with metyrapone (Neylan *et al*, 2003).

Sleep Recording

Ambulatory polysomnography (Oxford MR95 digital recorder) was used to monitor two nights of sleep. Subjects adhered to a stable sleep–wake schedule at their habitual times. The first night of polysomnography was used as an adaptation night and was not utilized in the analyses. The parameters recorded included an electroencephalogram (EEG) at leads C3 and C4, right and left electrooculograms (EOG), submental electromyogram (EMG), and an electrocardiogram (EKG) in accordance with standardized guidelines. An oximeter (Respironics Cricket) was used to screen for obstructive sleep apnea (OSA). The cutoff criterion for apnea was 10 desaturation events per hour in bed, which has been shown to have a sensitivity of 98% and a specificity of 48% in detecting OSA (Series *et al*, 1993). Subjects who screened positive for OSA were excluded. All sleep was imported into Pass Plus (Delta Software) analytic software and visually scored in 30-s epochs (Rechtschaffen and Kales, 1968). Delta sleep activity was analyzed by period amplitude analysis (PAA) using the Pass Plus (Delta Software) analytic software. Integrated amplitude of 0.3–4.0 Hz activity per 30 s epoch was analyzed by NREM cycles on all epochs of NREM sleep across the night following the technique described by Feinberg *et al* (1987, 1991) and (Travis *et al*, 1991). Epochs scored as wake were not included in these analyses. Movement artifact was visually monitored and recorded and not included in the analyses.

Neuroendocrine Measures

A 24-h urinary collection was obtained on the second day of sleep recordings for integrated measures of cortisol and creatinine release. Samples were immediately frozen after

the collection and were analyzed at the General Clinical Research Center's endocrine core lab at the Brigham & Women's hospital in Boston, MA. Urinary cortisol was measured using a competitive binding immunoenzymatic assay. Urinary creatinine was measured in parallel to control for adequate sampling. Only samples with creatinine values within the normal range (0.8–2.0 g/24 h) were used. Results are expressed as 24-h μg cortisol and as 24-h μg cortisol/g creatinine.

Subjects self-administered the low-dose (0.5 mg) salivary dexamethasone suppression either at least 1 week before or after the sleep recordings in their home environment to ensure that dexamethasone does not interfere with sleep measures. Subjects were instructed to maintain a stable sleep–wake schedule during the dexamethasone suppression test, to perform this procedure in the middle of their working week, to awaken at the same clock time on the pre- and post-dexamethasone saliva collection days, and to abstain from activities such as brushing or flossing their teeth, smoking, exercising, and ingesting food or drink prior to or during the collection. On day 1, salivary cortisol was collected 1 h after wake time and dexamethasone was given 15 h after wake time. The following morning, salivary cortisol was collected again 1 h after wake time. Samples were collected at home using Salivettes (Sarstedt Inc, Newton, NC), returned by mail, and deep-frozen (−78°C) until assay. We relied on the subjects to inform us about the time of sampling. As dexamethasone bioavailability may affect cortisol levels (O'Sullivan *et al*, 1997), we measured dexamethasone levels for covariance analysis and to check compliance with the protocol. Salivary cortisol and dexamethasone were assayed using radioimmunoassay as previously described (Goenjian *et al*, 1996).

Statistical Analyses

Differences in demographic characteristics between PTSD subjects and controls were compared using *t*-tests for continuous variables and χ^2 tests for dichotomous variables. Mean 24-h urinary cortisol concentration and salivary cortisol suppression after dexamethasone was compared between groups with univariate ANOVA. The effect of dexamethasone on cortisol was also analyzed by a repeated-measures ANCOVA with condition (dexamethasone) as a within-subjects factor and group membership as a between-subjects factor covarying for dexamethasone levels. The relationships among measures of PTSD and depression, sleep and HPA activity were analyzed by two-tailed Pearson correlations. All values are expressed as mean and standard deviation. As a nominal level of significance, $\alpha = 0.05$ was accepted.

RESULTS

There were no significant differences between PTSD subjects and controls regarding age, body mass index (BMI), smoking, urinary creatinine, urinary volume, and salivary dexamethasone concentrations (Tables 1 and 2). As expected, participants with PTSD had higher scores on CAPS, IES-R, BDI, and a higher rate of current and lifetime depression (Table 2).

Table 1 Demographic and Psychometric Data in PTSD Subjects and Healthy Controls

	PTSD ($n = 20$)	Controls ($n = 17$)	*p*-value
Demographics			
Age	49 (7)	48 (10)	0.66
BMI	27 (5)	25 (3)	0.13
Smokers[a]	5	1	0.19
Psychopathology			
CAPS	65 (18)	2 (<1)	<0.001
IES-R	20 (11)	2 (3)	<0.001
BDI	12 (7)	4 (5)	<0.001
Current depression	4	0	0.04
Lifetime depression	14	0	<0.001

Data are mean (SD) unless otherwise indicated. BMI = Body Mass Index; CAPS = Clinician Administered PTSD Scale; IES-R = Impact of Event Scale-Revised; BDI = Beck Depression Inventory.
[a]Number.

24-h Urinary Cortisol

Of the 36 participants, six subjects (three PTSD subjects, three controls) had a creatinine value outside the normal range (0.8–2 g/24 h), suggesting either an incomplete collection or overcollection (creatinine values outside the normal range varied between 0.5 g/24 h and 2.5 g/24 h). These subjects were excluded for the analyses.

Mean 24-h urinary cortisol did not differ significantly between groups (Table 2). PTSD subjects had greater 24-h urinary cortisol/g creatinine compared with controls (Table 2 and Figure 1). There was a strong correlation between 24-h urinary cortisol/g creatinine and 24-h urinary cortisol ($r = 0.83$, $p < 0.001$). PTSD subjects with and without current depression did not differ in either 24-h urinary cortisol/g creatinine or 24-h urinary cortisol. Repeating the analyses without smokers did not change the results.

Low-Dose (0.5 mg) Salivary Dexamethasone Suppression Test

Three control subjects did not return their samples of the dexamethasone test. We excluded two participants (one PTSD subject, one control) who showed an implausible and strong increase of their cortisol values after dexamethasone, suggesting a possible collection error, for example, contamination of the saliva with food. The values for cortisol suppression after dexamethasone for these subjects were more than 3 SD below the mean cortisol suppression of the sample. Thus, 19 PTSD subjects and 12 controls were included in the analyses of the dexamethasone test.

In a repeated-measure ANOVA for log-transformed salivary cortisol values, there was neither a main effect for group ($F = 2.5$, $p = 0.12$) nor a group × condition interaction ($F = 2.2$, $p = 0.15$). As expected, there was a strong effect of condition (dexamethasone) on cortisol ($F = 162.8$, $p < 0.001$). Adding dexamethasone concentration as a

Table 2 Endocrine and Sleep Data in PTSD Subjects and Controls

	PTSD ($N=20$)[a]	Controls ($N=16$)[b]	Effect size[c]	*p*-value
Neuroendocrinology				
24 h cortisol (μg)	52 (15)	43 (23)	0.47	0.19
24 h cortisol/creatinine (μg/g)	40 (17)	28 (12)	0.80	0.03[a]
Urinary creatinine (g/24 h)	1.4 (0.4)	1.4 (0.4)	0	0.52
Urinary volume (l/24 h)	2.0 (0.9)	1.8 (0.8)	0.23	0.43
Pre-DEX salivary cortisol (ng/dl)	1180 (680)	1060 (410)	0.22	[d]
Post-DEX salivary cortisol (ng/dl)	260 (140)	165 (90)	0.77	[d]
% Cortisol suppression	73 (18)	83 (10)	0.65	0.06
Dexamethasone concentration	37 (35)	61 (62)	0.51	0.18
Sleep				
Delta sleep (min)	13 (19)	38 (41)	0.80	0.02[a]
Delta sleep (% of total sleep)	3 (5)	10 (10)	0.90	0.02[a]
Delta integrated amplitude	127 441 (40 259)	156 557 (42 763)	0.70	0.04[a]

Data are mean (SD). DEX = dexamethasone.
[a]PTSD: $n=17$ for urinary cortisol, $n=19$ for dexamethasone test.
[b]Controls: $n=14$ for urinary cortisol, $n=12$ for dexamethasone test.
[c]Effect size expressed as Cohen's *d*.
[d]No significant main effect for group ($F=2.5$, $p=0.12$) nor a group × time interaction ($F=2.2$, $p=0.15$) in a repeated-measures ANOVA.

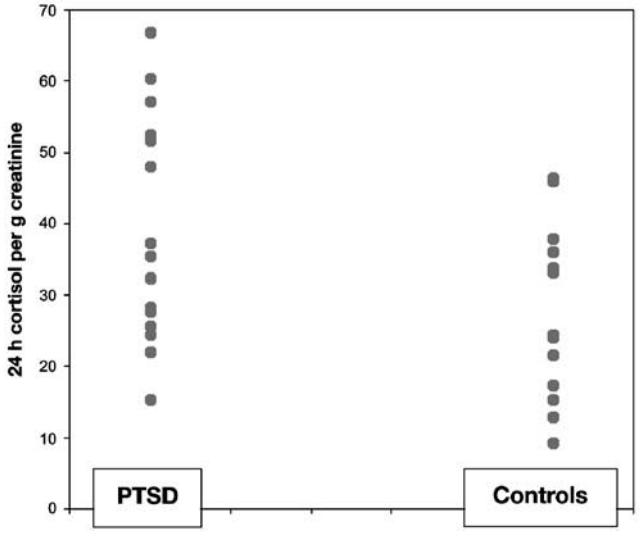

Figure 1 Individual 24-h urinary cortisol/creatinine values in participants with PTSD ($n=20$) and matched healthy controls ($n=16$). Values are expressed as urinary secretion of μg cortisol/g creatinine over 24 h.

covariate in the analyses or using raw values did not change the results. However, there was a trend for less salivary cortisol suppression after dexamethasone in PTSD subjects compared with controls (Table 2 and Figure 2). There were no differences in pre-DEX cortisol levels (Table 2). Again, PTSD patients with and without current depression did not differ in their post-dexamethasone cortisol levels or percent suppression (both *p*-values >0.50). Also, repeating the analyses without smokers did not change the results.

Delta Sleep

PTSD subjects spent fewer minutes in delta sleep than controls and had smaller percentage of delta sleep (Table 2). These results have been presented and are described in greater detail elsewhere (Neylan *et al*, 2003).

Association between Delta Sleep and HPA Axis

In the combined sample of PTSD and control subjects, time spent in delta sleep was significantly and negatively correlated with 24-h urinary cortisol ($r=-0.36$, $p=0.04$) and with 24-h urinary cortisol/g creatinine on a trend level ($r=-0.34$, $p=0.06$). We also examined the correlation between 24-h urinary cortisol and measures of delta sleep separately in both groups. The magnitude of the negative correlations between 24-h urinary cortisol and time spent in delta sleep did not differ between groups (PTSD subjects $r=-0.26$, controls $r=-0.33$, $p=0.83$). If analyzed separately for each group, these correlations between urinary cortisol and delta sleep were not significant, presumably related to the decrease in statistical power.

DISCUSSION

We confirmed our first hypothesis, finding higher levels of 24-h urinary cortisol associated with less delta sleep. Contrary to our hypotheses, we found increased 24-h urinary cortisol/g creatinine in subjects with PTSD. We did not observe a significant main effect for group or a group × time interaction in the dexamethasone suppression test. However, PTSD subjects had a trend for less rather than more cortisol suppression after dexamethasone.

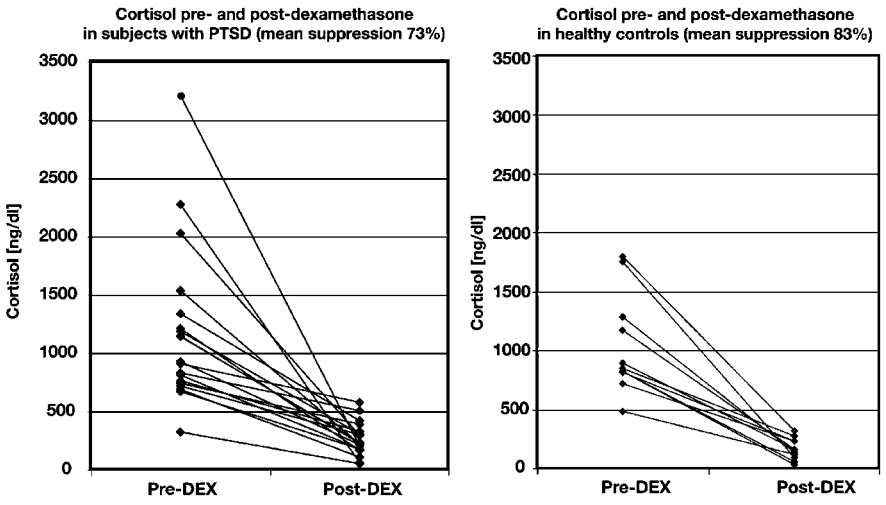

Figure 2 Individual salivary cortisol values before and after 0.5 mg dexamethasone in participants with PTSD ($n = 19$) and matched healthy controls ($n = 12$). Saliva samples were taken 1 h after wakening on the first day for pre-DEX cortisol values. Dexamethasone (0.5 mg) was administered 15 h after wakening on the first day and 1 h after wakening on the second day. Saliva was collected to determine post-DEX cortisol values.

The negative relation between 24-h urinary cortisol and delta sleep that we found in our sample is likely to be driven by hypothalamic CRF. The evidence supporting the role of increased hypothalamic CRF being associated with decreased delta sleep includes the following: (1) hypercortisolemic depression is associated with increased hypothalamic CRF and decreased delta sleep (Steiger, 2002), (2) there is a strong inverse relationship between delta sleep and pulsatile cortisol release (Born and Fehm, 1998; Vgontzas *et al*, 1999), (3) exogenous cortisol infusion, which reduces CRF in the hypothalamus, increases delta sleep (Bohlhalter *et al*, 1997; Friess *et al*, 2004, 1994), (4) metyrapone administration, which leads to an increase in hypothalamic CRF, causes a decrease in delta sleep (Jahn *et al*, 2003; Neylan *et al*, 2003), and (5) rats with genetically reduced hypothalamic CRF spent more time in delta sleep than genetically intact rats (Opp, 1997).

The mechanism by which CRF might decrease delta sleep is not known. One candidate explanation is that an increase in hypothalamic CRF release affects other brain areas involved in sleep or arousal. This possibility is supported by studies showing that not only extrahypothalamic CRF neurons but also neurons from the hypothalamus (Aston-Jones *et al*, 1986; Valentino *et al*, 1992) project to the locus ceruleus, which may be a point of integration between neurohormonal and neurotransmitter CRF systems (Koob, 1999). It is possible that stimulation of the locus ceruleus by way of CRF neurons from the hypothalamus is of sufficient magnitude to affect delta sleep. However, other constituents that parallel cortisol release from the adrenal gland, such as dehydroepiandrosterone (DHEA), could be involved in sleep regulation. Consistent with this hypothesis, DHEA has recently been associated with sleep disturbances and symptom load in PTSD (Rasmusson *et al*, 2004; Sondergaard *et al*, 2002).

Our findings of less delta sleep, greater 24-h urinary cortisol/g creatinine, and a trend for less cortisol suppression after dexamethasone are consistent with increased hypothalamic CRF release in PTSD subjects compared with controls. Two studies have found higher levels of CRF in the cerebrospinal fluid in PTSD (Baker *et al*, 1999; Bremner *et al*, 1997). In our previous study, subjects with PTSD had a diminished ACTH response to an indirect CRF challenge with metyrapone, also suggesting increased CRF activity in PTSD subjects at baseline (Neylan *et al*, 2003).

However, findings regarding the HPA axis have been mixed in PTSD (for reviews, see Rasmusson *et al*, 2003; Yehuda, 2002). Our sample of PTSD patients showed rather increased HPA activity and decreased delta sleep (see also Neylan *et al*, 2003). We cannot rule out the possibility that there are biological subgroups of PTSD and that, perhaps for a variety of random factors, we selected a subgroup with worse sleep disturbance and increased HPA activity. Similarly, in depression altered HPA axis activity is found in only about 50–60% of patients (Wolkowitz *et al*, 2001). In our study, comorbid depression cannot explain the results because PTSD patients with and without depression did not differ on any HPA measure. It is also possible that the focus on middle-aged male subjects who were free from psychotropic medication and comorbid substance abuse, who were recruited to a study focused on sleep disturbances, may explain how our results differ from earlier studies.

In our study, the magnitude of the effect size of group on urinary cortisol differed depending on whether cortisol was expressed as 24-h urinary cortisol or 24-h urinary cortisol/g creatinine (Table 2). In theory, these estimates should produce nearly identical estimates in a complete 24 h collection, and they did not raise the possibility of random errors in the collection of the urine samples. However, urinary volume and creatinine were not significantly different between groups. Our data support the possibility that urinary cortisol methodology may not provide the most accurate assessment of hormonal release due to subjects' problems in adhering to the protocol, as has been previously suggested (Yehuda *et al*, 2003). The findings also suggest that observations about the relationship between cortisol and sleep should be confirmed using a more comprehensive methodology such as 24-h plasma cortisol circadian rhythm analysis.

Previous studies have used the salivary low-dose dexamethasone test in traumatized adults and adolescents, but some of them did not specifically examine PTSD (Goenjian *et al*, 1996; Heim *et al*, 1998; Kellner *et al*, 1997). Further, these studies either had no control group (Goenjian *et al*, 1996; Kellner *et al*, 1997) or no healthy control group (Heim *et al*, 1998). Our results are in agreement with recent studies in adolescents (Lipschitz *et al*, 2003) and adults (Lindley *et al*, 2004), which also failed to find an enhanced negative feedback inhibition in PTSD in a low-dose salivary dexamethasone test. Interestingly, in our study, dexamethasone concentrations were somewhat lower in patients compared with healthy controls (Table 2). This might have contributed to our results and could indicate higher cortisol in patients over time, since dexamethasone half-life is shortened with increasing cortisol levels, presumably due to induction of metabolizing enzymes (Holsboer *et al*, 1986; Stokes *et al*, 2002). However, it should be noted that other studies found increased cortisol suppression in PTSD subjects using the low-dose dexamethasone suppression test in plasma (Stein *et al*, 1997; Yehuda *et al*, 1993).

Several limitations must be kept in mind when appraising our findings. First of all, we only found increased cortisol in PTSD when relating it to urinary creatinine output and only a trend for less cortisol suppression, indicating rather subtle HPA alterations. Although we made every effort to ensure adequate collection of saliva and urine samples, we relied on participants' self-report in terms of adhering to the protocol. Our small sample size might have reduced our power to detect significant differences in all HPA measures. In fact, we would have needed 72 subjects in each group to have 80% power on a significance level of 0.05 to detect a significant between-group difference of the effect size we found for 24-h urinary cortisol. In addition, we would have needed 32 subjects in each group to find a significant group difference in percent cortisol suppression after dexamethasone. Further, we examined only men and therefore we do not know if our results are generalizable to women. Finally, we do not have direct evidence that increased CRF is responsible for the HPA findings, decreased delta sleep, or symptoms of PTSD and depression. Once a CRF antagonist becomes available in humans, these hypotheses should be further tested.

In summary, we found decreased delta sleep, increased 24-h urinary cortisol/g creatinine, and a trend for less cortisol suppression after dexamethasone in patients with PTSD. Greater 24-h urinary cortisol/g creatinine was associated with less delta sleep. Future longitudinal studies should focus on cause and effect relationships among psychopathology, HPA alterations, and sleep disturbances in PTSD.

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