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FULL-LENGTH ORIGINAL RESEARCH

Basal hypercortisolism and trauma in patients with psychogenic nonepileptic seizures

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Summary

Purpose: Several studies have indicated that psychogenic nonepileptic seizures (PNES) are associated with psychological trauma, but only a few studies have examined the associations with neurobiologic stress systems, such as the hypothalamus–pituitary–adrenal (HPA) axis and its end-product cortisol. We tested several relevant HPA-axis functions in patients with PNES and related them to trauma history.

Methods: Cortisol awakening curve, basal diurnal cortisol, and negative cortisol feedback (using a 1 mg dexamethasone suppression test) were examined in 18 patients with PNES and 19 matched healthy controls (HCs) using saliva cortisol sampling on two consecutive days at 19 time points. Concomitant sympathetic nervous system (SNS) activity was assessed by analyzing saliva α-amylase (sAA).

Results: Patients with PNES showed significantly increased basal diurnal cortisol levels compared to HCs. This effect was driven mainly by patients reporting sexual trauma who showed a trend toward higher cortisol levels as compared to patients without a sexual trauma report. Importantly, the increased basal diurnal cortisol levels in patients were not explained by depression, medication, or smoking, or by current seizures or group differences in SNS activity.

Discussion: This is the first study showing that basal hypercortisolism in patients with PNES is independent of the acute occurrence of seizures. In addition, basal hypercortisolism was more pronounced in traumatized patients with PNES as compared to nontraumatized patients with PNES. These findings suggest that HPA-axis activity provides a significant neurobiologic marker for PNES.

KEY WORDS: Psychogenic nonepileptic seizures, HPA Axis, Salivary cortisol, Trauma, Alpha-amylase.

Several studies have indicated that psychogenic nonepileptic seizures (PNES) are associated with a history of psychological trauma, such as sexual and physical abuse (for reviews see, e.g., Fiszman et al., 2004; Sharpe & Faye, 2006; Roelofs & Spinhoven, 2007). Only few studies have investigated the association of PNES with neurobiologic stress systems, such as the hypothalamus–pituitary–adrenal (HPA) axis with cortisol as its end-product. The majority of these studies focused on the effects of seizure-like activity on cortisol levels and found mostly increased cortisol levels in patients with PNES (as well as in confirmed epilepsy patients) related to seizures (e.g., Mehta et al., 1994; Tunca et al., 2000). So far, only two studies have investigated basal activity of the HPA axis in PNES, and the results are conflicting. Tunca et al. (1996) did not find increased basal cortisol levels in a sample of 25 patients with conversion disorder (including 20 patients with PNES) compared to healthy controls (HCs) but did find decreased cortisol suppression after dexamethasone administration. In contrast, in a sample of eight patients with PNES, Tunca et al. (2000) later observed increased morning serum cortisol levels at baseline (an average time interval of 18 h had elapsed since the last seizure). In addition, we found no indications for increased stress-induced cortisol levels in patients with PNES but, in line with previous notions of increased basal activity of physiologic stress-related systems in patients with PNES, we found decreased levels of basal heart rate variability (Bakvis et al., 2009), often taken as an indication of hyperarousal (see, e.g., Thayer & Brosschot, 2005, for a review). In summary, previous accounts of HPA-axis activity in patients with PNES have shown mixed results, and none of the previous studies has investigated the relationship between interpersonal trauma and HPA-axis activity in patients with PNES.

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In an attempt to establish a neurobiologic marker associated with PNES, the present study was designed to test several relevant HPA-axis functions, including cortisol awakening response (CAR), basal diurnal cortisol, and negative cortisol feedback (using a dexamethasone suppression test, DST) in patients with PNES and to relate eventual findings to the occurrence of seizures and trauma history. In contrast to previous studies on basal HPA-axis activity in patients with PNES, we used a stress-free noninvasive method for measuring cortisol (saliva instead of blood: Kirschbaum et al., 1993; Kirschbaum & Hellhammer, 1994), we tested only those patients whose diagnosis was based on an ictal electroencephalography (EEG)–video registration of a typical seizure, and we controlled for current depression, use of psychotropic medication, smoking, and menstrual cycle. In addition, we checked whether eventual alterations in HPA-axis activity were accompanied by concomitant group differences in activation of the sympathetic nervous system (SNS) through salivary α-amylase (sAA), which is indicative of acute stress (for reviews see Granger et al., 2007; Rohleder & Nater, 2009).

Based on Tunca et al. (1996, 2000), we predicted that we would find increased HPA-axis activity (as evidenced by increased basal diurnal cortisol levels, increased CAR, and increased post-DST cortisol) in patients with PNES compared to HCs. Second, in accordance with an extensive body of literature linking negative life experiences to long-lasting increases in HPA-axis activity in both animals (e.g. Sapolsky et al., 1997; Anisman et al., 1998) and humans (for reviews see, e.g., Yehuda, 2006; Gunnar & Quevedo, 2008), and, in particular, in accordance with previous findings suggesting that sexual trauma was related to increased threat vigilance in patients with PNES (Bakvis et al., 2009), we hypothesized that the expected increased HPA-axis activity in patients with PNES would be related to sexual trauma.

Methods

Participants

From March 2005 until April 2007, 20 patients with PNES, who had been admitted to a tertiary epilepsy center, were recruited by the attending neurologists. Inclusion criteria were: (1) diagnosis of PNES based on an ictal video-EEG recording of a typical seizure, (2) PNES characterized by complete or partial loss of consciousness (specified as an ictal diminished or loss of adequate responsiveness or postictal memory impairment of the ictal event), (3) the occurrence of at least two seizures in the year prior to the experiment, (4) no history of concomitant epileptic seizures, (5) no comorbid neurologic disease diagnosis, and (6) no diagnosis of endocrine disorder(s). Two of the 20 patients had to be excluded because of the occurrence of several seizures during the test days and consequently missing values. The remaining 18 patient (7 male, 11 female) had a mean age of 31.6 [standard deviation (SD) 10.8) years. Demographic data, use of contraceptives, use of psychotropic medication, smoking status, current comorbid DSM-IV axis I diagnoses (assessed using the MINI: Mini-International Neuropsychiatric Interview Sheehan et al., 1998; Van Vliet & de Beurs, 2007), self-reported interpersonal traumatic experiences, seizure characteristics, and the occurrence of seizures an hour preceding sampling on test days are provided in Table 1. The healthy control (HC) group was recruited through advertisements in local newspapers. Inclusion criteria were (1) no psychiatric diagnosis, (2) no medical disease diagnosis, (3) no neurologic disease diagnosis, and (4) no use of medication.

One of the 20 HCs who participated in this study was removed from analyses post hoc, due to extremely high cortisol levels indicative of endocrinopathy and was advised to contact her physician for assessment. The remaining 19 HCs (10 male, 9 female) had a mean age of 35.1 (SD 13.5) years. Patients and HCs did not differ with respect to age, gender, and use of contraceptives (Table 1). Slightly more patients with PNES smoked and as expected, more patients with PNES used psychotropic medication. Furthermore, PNES patients reported higher rates of sexual trauma and overall interpersonal trauma compared to the control group (see Table 1 for statistics).

Measures

Cortisol and α-amylase

Saliva samples for cortisol assessments were obtained using Salivette collection devices with a cotton roll (Sarstedt, Rommelsdorf, Germany). In total, 19 samples were taken over two consecutive days. The salivary samples on day 1 were taken at the time of awakening and 15, 30, 45, and 60 min afterwards (cortisol awakening response, CAR) and at 10:00 h, 12:00 h, 14:00 h 16:00 h, 18:00 h, 20:00 h, and 22:00 h (basal diurnal cortisol). Participants were instructed to take a tablet of dexamethasone (1 mg) at 23:00 h on day 1. The following day, participants collected salivary samples again at the time of awakening and 15, 30, 45, and 60 min afterwards and at 16:00 h and 22:00 h (post-DST cortisol). Saliva samples were stored at −20°C before assaying. Biochemical analysis of free cortisol in saliva was performed using a competitive electrochemiluminescence immunoassay (ECLIA, Elecsys 2010, Roche Diagnostics, Basel, Switzerland), as described elsewhere (Van Aken et al., 2003).

To check whether eventual alterations in HPA-axis activity were accompanied by concomitant group differences in SNS activation, α-amylase levels were also analyzed from these saliva samples. α-Amylase levels have been shown to reflect SNS activity (for reviews see Granger et al., 2007; Rohleder & Nater, 2009). Biochemical analysis of sAA was performed using a kinetic maltotrioside method (CNP-G3; DiaSys Diagnostic Systems, Holzheim, Germany) at

Table 1. Demographic and clinical characteristics for 18 patients with PNES and 19 healthy controls

Variable	Patients (n = 18)	Controls (n = 19)	Statistics
Mean age (SD) in years	31.6 (10.8)	35.1 (13.5)	$t(35) = 0.84$, p = 0.400
Number of women	11	9	$X^2(1) = 0.70$, p = 0.402
Using contraceptives	6	8	$X^2(1) = 0.30$, p = 0.582
Smokers	6	1	$X^2(1) = 4.75$, p = 0.029[a]
Taking psychotropic medication	9	0	$X^2(1) = 12.55$, p < 0.001[b]
Paroxetine	4		
Risperidone	1		
Fluoxetine	2		
Oxazepam	1		
Sertraline	1		
Temazepam	1		
Valproic acid	1		
Flurazepam	1		
Citalopram	1		
Participants reporting interpersonal trauma	11	5	$X^2(1) = 4.56$, p = 0.033[a]
Sexual	7	2	$X^2(1) = 4.04$, p = 0.044[a]
Emotional	8	4	$X^2(1) = 2.31$, p = 0.129
Physical	6	3	$X^2(1) = 1.55$, p = 0.214
Current comorbid psychopathology	11	0	
None	7	19	
Mood disorder	3		
Anxiety disorders			
Panic disorder	3		
Agoraphobia	3		
Social phobia	3		
Generalized anxiety disorder	3		
Obsessive compulsive disorder	1		
Somatoform disorders			
Pain disorder	1		
Somatization disorder	1		
Hypochondrias	1		
Mean age (SD) at onset seizures	26.6 (12.3)	–	
Disease duration in years (SD)	5.3 (5.5)	–	
Number of patients reporting seizures 1 h Preceding sampling			
Day 1			
Cortisol awakening response	0	–	
Basal diurnal cortisol	3	–	
Day 2	1	–	

[a]p < 0.05; [b]p < 0.001.
PNES, psychogenic nonepileptic seizures; SD, standard deviation.

405 nm for serum on a Vitalab Selectra (Merck, Darmstadt, Germany) after dilution of saliva with saline (25 μL saliva plus 10 mL saline). The detection limit for the method in serum (or diluted saliva) was 2 U/L. The intraassay variability coefficient was 1.6% at 411 U/L (n = 10); the interassay variability coefficient was smaller than 2.8% in the range of 117–652 U/L (n = 30).

Emotional, physical, and sexual trauma

Emotional, physical, and sexual traumas were measured by means of the Traumatic Experiences Checklist (TEC), a 26-item self-reported questionnaire with good reliability and validity (Nijenhuis et al., 2002). The scores for the presence of both emotional trauma (emotional neglect and emotional abuse in various settings) and sexual trauma (sexual harassment and sexual abuse in various settings) are based on six items. The scores for the presence of physical abuse in various settings are based on three items. For all three types of interpersonal trauma a dichotomous score (*yes/no)* was calculated.

Procedure

Candidate participants were invited for an initial informative session and subsequently to select dates appropriate for testing. Because of the influence of estradiol on the HPA axis (Van Veen et al., 2008), women using oral contraceptives had to be tested in their gap-week. Women not on oral contraceptives had to be tested in the follicular phase of their menstrual cycle and were, therefore, instructed to record one menstrual cycle and to contact the researcher when the second menstruation had started to plan the definite dates for testing. After the test days were planned and

participants had provided informed consent, all participants were administered a semistructured diagnostic interview by author P.B. and two trained psychology master students to screen for DSM-IV axis I disorders (American Psychiatric Association, 1994; assessed using the MINI: Mini-International Neuropsychiatric Interview, Sheehan et al., 1998; Van Vliet & de Beurs, 2007). In addition, the trauma questionnaire was administered. Next, participants were informed about the necessity of strictly following the procedures and the time schedule for saliva sampling to obtain valuable data. They were instructed to contact the principal investigator to postpone the test in case of a febrile illness within 3 days before the test. In addition, they were asked not to perform strenuous physical exercise and to avoid stressful situations as much as possible on these 2 days. Finally they had to write down their activities and the occurrence of seizures during the hour before saliva sampling on the test days. For each saliva sample, participants were asked to place the cotton wad from a Salivette saliva collection tube in their mouth until the cotton roll was saturated, and to subsequently keep the tube containing the wad prelabeled with date and time in the refrigerator. For the awakening samples subjects were instructed to start saliva sampling immediately at awakening. Subjects were instructed to complete the early morning sampling before breakfast and possible medicine intake to avoid contamination of saliva with food or drinks. They were asked not to brush their teeth before completing the saliva sample 60 min after awakening. In addition, participants were instructed not to eat, drink, or smoke 15 min before sampling. All instructions were given both verbally and in writing.

Participants received financial incentives for their participation in this study. The protocol was conducted in accordance with the declaration of Helsinki and approved by the medical ethical committee of the Leiden University Medical Centre (LUMC).

Statistics

Outliers in cortisol were defined as values that deviated >2.58 standard deviations (SDs; i.e., the 99th percentile) from the group mean per assessment. For patients 0.9% and for HCs 1.1% of the total amount of cortisol samples was removed. Missing data including outliers (patients 1.2%; HCs 2.5%) were interpolated linearly by using the participant's preceding and following salivary cortisol values, and modeling the average curve from the participants' group over these values for that point in time. Subsequently, to normalize distributions, cortisol levels were subjected to natural log transformation before analyses. Separate repeated-measures analyses of variance (ANOVA rm) were conducted for the CAR, for the basal diurnal cortisol, and for post-DST cortisol, each with time (salivary time points) as within-subject factor and group (patients, HCs) as between-subject factor. For the CAR and post-DST cortisol we controlled for the time of awakening (ToA) on days 1 and 2, respectively, by adding this variable to the analysis as a covariate. In the case of significant group effects, we controlled for depression, psychotropic medication, and smoking by repeating the analysis for (1) a subgroup of patients without a current depression; (2) a subgroup of patients not on psychotropic medication; (3) nonsmoking participants; and (4) nonsmoking, nondepressed participants not on psychotropic medication. To test whether group differences in cortisol could be attributed to seizures in patients, analyses were repeated for those patients not reporting seizures one hour before saliva sampling. Finally, to investigate whether cortisol effects were particularly pronounced for those patients who reported sexual trauma, we conducted an additional three-group analysis with post hoc least significant difference (LSD) analyses comparing PNES patients with and without sexual trauma reports and HCs without sexual trauma reports.

α-Amylase was investigated to test whether possible group differences in cortisol levels were accompanied by concomitant group differences in SNS activation. Outliers in basal diurnal α-amylase were defined as values that deviated >2.58 SD from the group mean per assessment. For patients 2.4% and for HCs 3.0% of the samples were removed. Missing data including outliers (patients 3.2%; HCs 3.0%) were interpolated linearly. To normalize distributions, α-amylase levels were subjected to natural log transformation before analyses. An ANOVA rm was conducted for basal diurnal α-amylase, with time (salivary time points) as within-subject factor, and group (patients, HCs) as between-subject factor.

All statistical analyses described employed a two-tailed alpha of 0.05. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, U.S.A.) 16.0 for Windows.

Results

Cortisol awakening response (CAR)

To investigate possible group effects in the CAR, we conducted a two-way ANOVA rm for the morning cortisol levels, with time (five assessment points from awakening until 60 min after awakening) as within-subject factor and group (patients, HCs) as between-subject factor. Results showed a significant effect for time [$F(4,32) = 6.13$, $p = 0.001$] but not for group [$F(1,35) = 1.21$, $p = 0.279$], or time × group [$F(4,32) = 2.14$, $p = 0.099$]. These effects for group did not alter when adding ToA on day 1 (ToA1) as a covariate to this analysis (Group [$F(1,33) = 1.04$, $p = 0.315$]; time × group [$F(4,30) = 2.02$, $p = 0.116$]; ToA1 [$F(1,33) = 0.15$, $p = 0.704$]). Therefore, groups did not differ significantly with respect to the CAR.

Post dexamethasone suppression test (DST) cortisol

To investigate possible group effects in negative cortisol feedback by the DST, a two-way ANOVA rm was

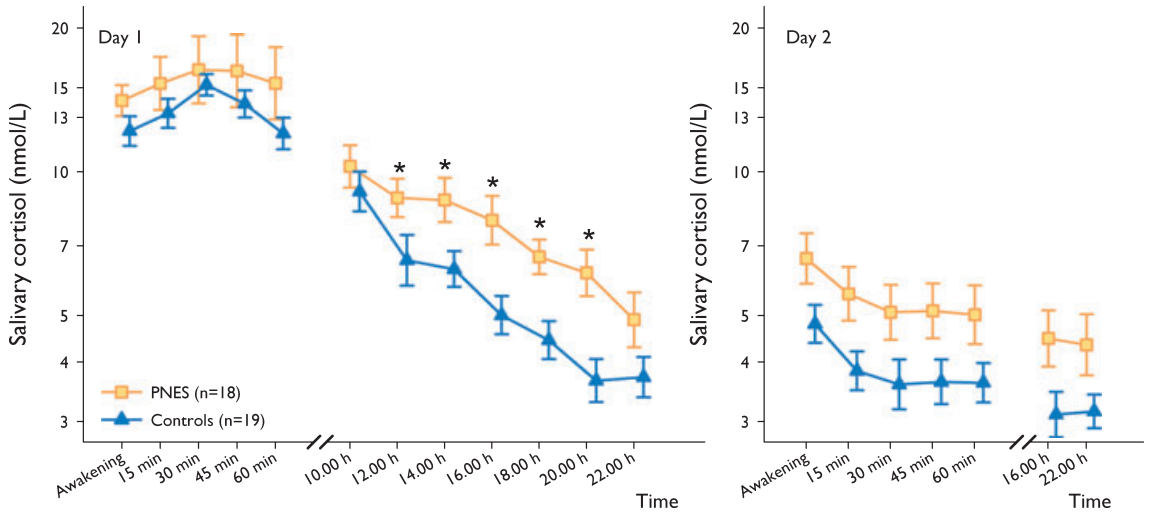

Figure 1.
The left panel shows the basal salivary cortisol concentrations of day 1 on a logarithmic scale. The right panel shows the post–dexamethasone suppression test (DST) salivary cortisol concentrations of day 2 on a logarithmic scale. Groups did not differ significantly on salivary cortisol awakening response (CAR; at awakening, +15 min., +30 min., +45 min., +60 min.). Patients with psychogenic nonepileptic seizures (PNES) had higher basal diurnal cortisol levels (10:00 h, 12:00 h, 14:00 h, 16:00 h, 18:00 h, 20:00 h, and 22:00 h see *). Post-DST cortisol levels (awakening, +15 min., +30 min., +45 min., +60 min., 4 p.m., and 10 p.m.) were increased in patients with PNES, but this group effect disappeared when controlling for smoking and psychotropic medication. [Correction made after online publication 6 Nov 2009: images for figures 1 and 2 switched]

conducted for the post-DST cortisol levels, with time (seven assessment points from awakening until 60 min after awakening and at 16:00 h and 22:00 h) as within-subject factor and group (patients, HCs) as between-subject factor. Results showed significant main effects for both time [$F(6,30) = 8.59$, $p < 0.001$] and group [$F(1,35) = 4.90$, $p = 0.033$ – see Fig. 1]. The time × group interaction was not significant [$F(6,30) = 0.16$, $p = 0.984$]. These results for group did not change when adding ToA on day 2 (ToA2) as a covariate to this analysis (group [$F(1,33) = 5.41$, $p = 0.026$]; time × group [$F(6,28) = 0.15$, $p = 0.987$]; ToA2 [$F(1,33) = 0.49$, $p = 0.488$]). ToA2 was, therefore, excluded from the subsequent analyses. This main effect for group remained significant when excluding patients with depression (remaining *N* patients = 15; *N* controls = 19; group [$F(1,35) = 4.90$, $p = 0.033$]); remained a statistical trend when excluding patients taking psychotropic medication (*N* patients = 9; *N* controls = 19; group [$F(1,26) = 3.86$, $p = 0.060$]); but did not remain significant when excluding smoking participants (*N* patients = 12; *N* controls = 18; group [$F(1,28) = 1.01$, $p = 0.325$]). Therefore, we cannot conclude that post-DST cortisol levels are specifically effected for patients with PNES.

Basal diurnal cortisol

To investigate possible group effects in basal diurnal cortisol levels, we conducted a two-way ANOVA rm for the salivary basal diurnal cortisol, with time (seven assessment points from 10:00 h until 22:00 h) as within-subject factor, and group (patients, HCs) as between-subject factor. Results showed significant main effects for time [$F(6,30) = 22.60$, $p < 0.001$] and Group[1] [$F(1,35) = 8.89$, $p = 0.005$] and most importantly a significant time × group interaction [$F(6,30) = 3.07$, $p = 0.018$ – see Fig. 1]. The time × group effect for basal diurnal cortisol remained significant when repeating these analyses for (1) a subgroup of patients without a current depression (*N* patients = 15; time × group [$F(6,27) = 3.16$, $p = 0.018$]); (2) a subgroup of patients not taking psychotropic medication (*N* patients = 9; *N* controls = 19; time × group [$F(6,21) = 2.82$, $p = 0.036$]); (3) a subgroup without smoking participants (*N* patients = 12; *N* controls = 18; time × group [$F(6,23) = 3.24$, $p = 0.019$]). In addition, this effect remained a statistical trend, even when repeating this analysis for the small subgroup of nondepressed, nonsmoking participants who were not using psychotropic medication (*N* patients = 6; *N* controls = 18; time × group [$F(6,17) = 2.34$, $p = 0.079$]).

Post hoc F testing indicated, that PNES patients displayed higher basal cortisol at 12:00 h [$F(1,35) = 5.88$, $p = 0.021$], 14:00 h [$F(1,35) = 6.21$, $p = 0.018$], 16:00 h [$F(1,35) =$

[1]We found the same group effect when conducting an ANOVA with area under the curve (AUC) with respect to ground [(AUCg); $F(1,35) = 10.37$, $p = 0.003$); for more details see Pruessner et al., 2003, formula 2]

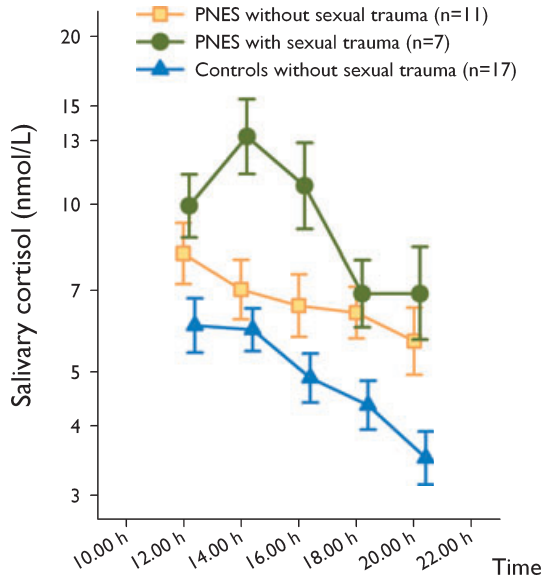

Figure 2.
Basal diurnal salivary cortisol concentrations of day 1 on a logarithmic scale. Healthy controls (n = 17) differed significantly from both patients with (n = 7) and without (n = 11) sexual trauma on basal diurnal cortisol levels. In addition, patients with sexual trauma showed increased cortisol levels as compared to patients who did not report sex trauma. [Correction made after online publication 6 Nov 2009: images for figures 1 and 2 switched]
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9.85, p = 0.003], 18:00 h [$F(1,35) = 10.29$, p = 0.003] and 20:00 h [$F(1,35) = 11.63$, p = 0.002]; (other p > 0.106).

To check whether these increased basal diurnal cortisol levels in patients with PNES were not related to current seizures, we repeated the latter tests for the 15 patients not reporting seizures one hour prior to sampling. Results showed that the group effects remained significant at all time points: 12:00 h [$F(1,32) = 6.06$, p = 0.019], 14:00 h [$F(1,32) = 5.60$, p = 0.024], 16:00 h [$F(1,32) = 8.38$, p = 0.007], 18:00 h [$F(1,32) = 8.73$, p = 0.006] and 20:00 h [$F(1,32) = 9.75$, p = 0.004]; (other p > 0.098), indicating that the higher basal diurnal cortisol in patients with PNES was not attributable to current seizures.

To test our second hypothesis that this effect would be particularly pronounced in PNES patients who experienced sexual trauma, an additional analysis for the five basal diurnal cortisol sample points on which groups differed (12:00 h, 14:00 h, 16:00 h, 18:00 h, 20:00 h) was conducted. A two way ANOVA rm, with time (five assessment points) as within-subject factor and group [patients with sexual trauma (n = 7), patients without sexual trauma (n = 11), and HCs without sexual trauma (n = 17; see Table 1)] as between-subject factor showed a main effect for group [$F(2,32) = 9.11$, p = 0.001], and a statistical trend toward significance for time × group [$F(8,60) = 1.90$, p = 0.077; see Fig. 2]. Post hoc LSD analyses indicated that the HC group had significantly lower basal diurnal cortisol rates as compared to patients with sexual trauma (p < 0.001); as well as patients without sexual trauma (p = 0.021). Furthermore, post hoc LSD analyses indicated a trend toward significance (p = 0.067) for the difference in basal diurnal cortisol between patients with and without a history of sexual trauma.

Therefore, patients with PNES displayed heightened basal HPA-axis activity compared to HCs, and this effect was particularly pronounced in those patients reporting sexual trauma.

α-Amylase

In order to investigate whether the group effects in basal cortisol levels were associated with concomitant group effects in SNS activity, we conducted a two-way ANOVA rm for saliva basal diurnal α-amylase levels, with time (seven assessment points from 10:00 h until 22:00 h) as within-subject factor, and group (patients, HCs) as between-subject factors. Results showed that there was a significant main effect for time [$F(6,30) = 5.45$, p = 0.001], but that there were no significant effects for group (all p > 0.211), indicating that patients' increased HPA-axis activity was not accompanied by concomitant group differences in SNS activation.

Discussion

The main purpose of the present study was to investigate HPA-axis activity in patients with PNES compared to age- and gender-matched healthy controls (HCs).

Patients' basal cortisol levels were augmented in the afternoon and evening. Previous reports of increased HPA-axis activity in patients with PNES also indicated higher levels of afternoon and evening cortisol (Tunca et al., 2000). In the latter study, however, the increased cortisol levels were related to seizures. Here, we show that the increased basal cortisol levels in patients with PNES occurred independent of the acute presence of seizures. In addition, we found that the increased basal cortisol levels could not be explained by factors such as increased physical activity or acute psychological stress, as indicated by the absence of concomitant group differences in sAA, which is predictive of SNS activity (see Granger et al., 2007 and Rohleder & Nater, 2009, for reviews). Finally, the enhanced basal HPA-axis activity could not be attributed to current depression, use of psychotropic medication, or smoking. Based on these findings, it seems justified to conclude that our patients with PNES showed basal diurnal hypercortisolism.

There were no significant group differences in CAR, but in line with previous findings (Tunca et al.,1996) our patients with PNES seemed to show somewhat increased post-DST cortisol. Tunca et al. (1996) did not, however,

control for psychotropic medication and smoking, and when we adjusted for these factors our post-DST effects disappeared.

Our second aim was to test whether the increased HPA-axis activity would be particularly pronounced in traumatized patients with PNES. Based on previous findings of increased threat vigilance in PNES patients who reported sexual trauma (Bakvis et al., 2009), we predicted higher cortisol levels in PNES patients with self-reported sexual trauma compared to patients without self-reported sexual trauma. Interestingly, the PNES patients in the present study reported significantly more sexual trauma compared to the HCs, but no group differences were found in the reports of emotional and physical trauma history, which may be interpreted as supporting the critical role of sexual trauma in PNES. Cortisol analyses indeed specified that whereas both PNES patients with and without a history of sexual trauma showed increased basal cortisol levels compared to HCs, these findings were more pronounced for the traumatized patients ($p = 0.067$). This result is in line with a previous finding in patients with depression showing increased cortisol levels only in traumatized depressed patients compared to nontraumatized depressed patients (Heim et al., 2000b).

Relating our findings of increased HPA-axis activity in patients with PNES to other relevant stress-related disorders suggests that PNES show little overlap with posttraumatic stress disorder (PTSD).[2] In a systematic review and meta-analysis on basal cortisol levels in adult patients with PTSD, Meewisse et al. (2007) reported lower basal afternoon cortisol in female PTSD patients, particular in those patients reporting sexual or physical trauma. In addition, cortisol hypersuppression following DST (for a review see De Kloet et al., 2006) and increased noradrenergic activity (e.g., Southwick et al., 1999; Yehuda, 2001) have been reported in most studies in patients with PTSD. Similar signs of hypocortisolism have also been observed in stress-related bodily disorders, such as burnout, chronic fatigue, fibromyalgia, and chronic pelvic pain (see Heim et al., 2000a for a review). Our findings of *hyper*cortisolism in PNES patients may more resemble previous findings in patients with a primary dissociative disorder. Increased basal 24-h urine cortisol was found in 46 patients with dissociative identity disorder (DID) compared to HCs (Simeon et al., 2007). Post-DST cortisol was also increased in these DID patients as well as in nine patients with depersonalization disorder (Simeon et al., 2001). Based on this scarce evidence, one might hypothesize that the HPA-axis hyperactivity in PNES patients shares more overlap with dissociative disorder than with PTSD. This notion is in accordance with previous suggestions that PNES might share some common underlying mechanism with dissociative disorder (Kuyk et al., 1996; Roelofs et al., 2002; Kihlstrom, 2005; Brown et al., 2007).

Some strengths and limitations of the present study should be considered. A strength of the present study is that all patients were diagnosed using the gold standard: an ictal video-EEG registration of a typical seizure in order to confirm the absence of epileptiform activity, making PNES diagnosis maximally reliable (e.g., Reuber & Elger, 2003). Another strong point of this study is that relevant factors affecting the HPA axis, such as age, gender, menstrual cycle, contraceptives, smoking, current depression, and psychotropic medication were controlled for. On the other hand, although our sample size ($n = 18$) was comparable to that of other clinical studies, we had only a small subgroup of nondepressed, nonsmoking, and unmedicated patients ($n = 6$). Therefore, this study needs to be replicated in a larger sample of patients with PNES. This might also offer an opportunity to further differentiate subgroups of PNES patients with respect to seizure-type or with respect to early childhood versus adulthood trauma. To further specification, it would be interesting to also include a control group of traumatized healthy participants. Another limitation of the study is that interpersonal trauma rates were based on a self-report questionnaire and were not verified using independent sources.

In conclusion, the results of the present study imply that PNES are associated with basal hypercortisolism, which was particularly pronounced in traumatized patients with PNES. The increased basal cortisol levels were not related to current seizures, and there were no concomitant group differences in SNS activity. It, therefore, seems unlikely that the increased HPA-axis activity was merely due to group differences in current physical or acute psychological stress factors, but rather posits a relevant neurobiologic marker for this stress-related disorder.

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We confirm to have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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[2]In Table 1, PTSD is not mentioned in the list of current comorbid psychopathology, indicating that none of the PNES patients fulfilled PTSD diagnostic criteria.

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