Altered cortisol awakening response in posttraumatic stress disorder

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\textbf{Summary} An altered function of the hypothalamic-pituitary-adrenal axis is assumed to be characteristic for Posttraumatic Stress Disorder (PTSD), although there is inconsistent empirical evidence. Only few studies examined the awakening cortisol response and a daytime profile in PTSD. Salivary cortisol levels were measured at seven intervals from awakening until 8 PM in trauma-exposed subjects with ($N=29$) and without PTSD ($N=19$) and in 15 non-exposed controls. While the three groups did not differ with respect to their first cortisol level immediately after awakening, the expected cortisol increase to awakening 15–60 min later was significantly lower in PTSD patients compared to non-PTSD subjects and healthy controls. This effect remained stable when trauma-exposed subjects with comorbid major depression were excluded from the analysis. A significant negative correlation between the overall cortisol secretion (AUC\textsubscript{G}) and overall PTSD symptomatology and hyperarousal symptoms was found. The findings are discussed in light of the hypothesis of a counterregulation of hyperarousal symptoms and chronic stress in PTSD.

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1. Introduction

Alterations in the activity of the Hypothalamic-Pituitary-Adrenal (HPA) axis are thought to be an important factor in the development of stress-related disorders. In posttraumatic stress disorder a more sensitive negative feedback inhibition, for example, reflected by an increased number and sensitivity of glucocorticoid receptors (Yehuda et al., 1993), is assumed to result in diminished baseline cortisol levels (Yehuda et al., 1996). However, neuroendocrinological studies in PTSD patients yielded contradictory results showing either decreased baseline levels of cortisol (Yehuda et al., 1993), no differences between PTSD patients and controls (Young and Breslau, 2004) or even higher cortisol levels in PTSD patients (Lemieux and Coe, 1995). This conflicting evidence might be related to the fact that the studies were not comparable with respect to sample characteristics that affect HPA axis activity, such as trauma...
characteristics, gender, daily activities, nicotine and alcohol abuse or medication status (Rasmusson et al., 2003).

So far, only few studies measured the circadian rhythm of cortisol secretion in PTSD, although the increase in salivary cortisol to awakening provides a simple and reliable means of assessing the dynamic activity of the HPA axis (Prüssner et al., 1997). Examining the circadian rhythm of cortisol secretion in PTSD patients, Yehuda et al. (1996) reported normal cortisol levels in the morning and lower than normal levels in the later evening. In a recent study, the time since trauma exposure but not PTSD diagnosis itself was found to be positively associated with an increase in saliva cortisol after awakening (Young et al., 2004). Rohleder et al. (2004) examined the awakening cortisol response and a short daytime profile in a small sample of Bosnian refugees and healthy controls. They reported lower daytime cortisol levels and no typical cortisol increase after awakening in PTSD patients compared to the controls. Based on these findings, the present study investigated the awakening and daytime profile of a larger and more heterogeneous sample of trauma-exposed subjects with and without PTSD and the relationship of the cortisol response and PTSD symptoms.

2. Methods and materials

2.1. Subjects

Participants of the study were 31 trauma-exposed persons with PTSD, 23 trauma-exposed subjects without PTSD and 19 healthy controls, who were matched for age, sex and level of education. All participants were Caucasian. From this original sample two subjects with terminal illness as traumatic event and eight subjects showing incongruent data from the self-report diaries and the electronic monitoring device for the sampling of cortisol were excluded from the analyses. Thus, a total sample of 63 subjects (29 PTSD, 19 non-PTSD and 15 healthy controls) remained for the statistical analyses.

The PTSD diagnosis was based on the diagnostic criteria of DSM-IV TR (APA, 2000). The experienced traumatic events included severe accidents (N=30), violent crime (N=12) and sexual assault or rape (N=3). The two trauma-exposed groups were not significantly different with respect to the types of trauma they experienced and the time span since trauma onset. All participants completed the German Version of the Structured Clinical Interview of DSM-IV (SCID-I; First et al. 1997; German Version: Wittchen et al. 1997) to assess psychiatric disorders other than PTSD. Ten PTSD and 2 non-PTSD subjects had a comorbid major depression. None of the participants met diagnostic criteria for current alcohol or drug abuse/dependence or psychotic disorders. The healthy controls did not meet any diagnostic criteria for mental disorder. The 12 subjects diagnosed with major depression used antidepressant medication, another five subjects took hypertension-reducing medication. So far, no influences of these medications on cortisol secretion have been found. As the exclusion of the five subjects with hypertension from the statistical analyses did not reveal a different pattern of results, these patients remained in the final sample. Patients diagnosed with major depression and treated with antidepressant medication were excluded in a reanalysis of the cortisol profile. Seven female subjects were on contraceptive medications, however, these subjects were equally distributed among the three groups ($\chi^2(2) = 1.00; p=0.61$). All subjects gave written informed consent and the study was approved by the local ethics committee.

2.2. Measures

All trauma-exposed subjects completed the German Version of the Impact of Event Scale-Revised (IES-R; Maercker and Schützwohl, 1998), the German version of the Center for Epidemiological Studies Depression Scale (CES-D; Hautzinger and Bailer, 1991) and the Assessment of General Stress Susceptibility (FSR; Schulz, Jansen and Schlotz, 2005). The latter has been validated in a sample of 975 subjects and has been shown to be a valid and reliable instrument for the assessment of stress reactivity. The questionnaire consists of 29 items, forming an overall stress reactivity score as well as six subscales (stress reactivity related to work overload, work failure, social conflicts, social evaluation, the anticipatory (pre stress) and recovery phase (post-stress) of stress).

2.2.1. HPA axis activity

Saliva samples were collected into Salivette tubes (Sarstedt, Nümbrecht, Germany), immediately after as well as 30, 45 and 60 min after awakening (awakening response) and at 1100, 1500 and 2000 h (short daytime profile). Participants were instructed not to smoke, drink caffeine, eat or brush their teeth in the sampling period. This instruction was given verbally as well as through
detailed written information accompanying the sampling tubes. In addition, subjects were asked to complete a diary during the sampling period, which among other information assessed their sleep duration the night before the sampling procedure. Free cortisol levels in saliva were measured using a commercially available chemiluminescence assay (IBL, Hamburg, Germany). Self-reports of wake-up times as well as an electronic monitoring device (MEMS Track Cap, Aardex, Switzerland) were employed to control for any influence of sampling time. Eight subjects (2 PTSD, 2 non-PTSD, 4 HC) were excluded from the analysis because their self-reported and electronically monitored sampling times were not congruent.

3. Statistical analyses

Differences in PTSD symptomatology, depressive symptoms as well as stress reactivity were calculated by one-way analyses of variance (ANOVAs) with Bonferroni-corrected post-hoc tests. Group differences in the awakening cortisol response between PTSD patients, trauma-exposed subjects without PTSD and healthy controls were calculated by a one-way repeated measures ANOVA with four within-group levels (baseline immediately after awakening as well as 30, 45 and 60 min after awakening, i.e. awakening profile). A repeated measures ANOVA with three within-group levels (1100, 1500 and 2000 h) was performed to compute differences in the subsequent circadian profile of free cortisol levels (short daytime profile). When repeated-measures ANOVAs revealed significant interactions, one-way ANOVAs at the different sample points and Bonferroni-corrected post-hoc tests were performed. As comorbid depression was shown to have a significant influence on cortisol secretion, the ANOVAs were again computed for trauma-exposed subjects without.

In addition to the awakening and day time profile, the area under curve with respect to baseline cortisol levels (AUCG) was calculated by a one-way ANOVA with Bonferroni-corrected post-hoc tests. As the individual difference between the sample point 1 h after awakening and 1100 h enters the calculation of the AUC, different wake-up times might influence the AUC significantly. Therefore, wake-up times were included as a covariate in the statistical analysis of the AUC. In addition, the AUC was correlated with PTSD symptomatology using Pearson correlations.

To control for possible effects of age, sex, intake of contraceptive medication, number of cigarettes smoked on a usual day or differing wake-up times, these variables were included as covariates in the ANOVAs. For differences in sleep duration the night before saliva sampling, a one-way ANOVA with Bonferroni-corrected post-hoc tests was carried out.

4. Results

4.1. Clinical data

PTSD patients and trauma-exposed subjects without PTSD were significantly different in the overall sum of PTSD symptoms and the symptom clusters re-experiencing, avoidance and hyperarousal (Table 1). In addition, PTSD subjects showed significantly more depressive symptoms ($F(2,60) = 12.36; p < .001$) and higher stress reactivity related to work overload ($F(2,58) = 6.24; p < .05$), social conflicts ($F(2,58) = 9.71; p < .001$), social evaluation ($F(2,58) = 8.39; p < .001$), the pre-stress phase ($F(2,58) = 8.11; p < .001$) as well as overall stress reactivity ($F(2,58) = 9.18; p < .001$) compared to healthy controls (all $p < .01$) and nonPTSD subjects (all $p < .05$). Subjects did not differ significantly in their stress reactivity with regard to work failure ($F(2,58) = 1.24; ns$) and post-stress situations (post-stress phase: $F(2,58) = 2.70; ns$).

5. Cortisol response

PTSD patients showed a significantly lower cortisol awakening response than non-PTSD subjects and healthy controls (group effect: $F(2,60) = 8.23; p < .001$). A significant group by sampling time interaction ($F(6,180) = 3.28, p < .05; \epsilon = 0.72$) revealed that the three groups did not differ with regard to baseline cortisol levels immediately after awakening ($F(2,60) = 0.82; ns$), but that the PTSD patients exhibited a reduced increase of cortisol secretion.
30 min \((F(2,60)=8.73; p<.001;\) PTSD vs. HC: \(p<.05;\) PTSD vs. non-PTSD: \(p<.01),\) 45 min \((F(2,60)=6.96; p<.01;\) PTSD vs. HC: \(p<.05;\) PTSD vs. non-PTSD: \(p<.01)\) and 60 min \((F(2,60)=4.90; p<.05;\) PTSD vs. HC: \(p<.001)\) after awakening (Fig. 1). However, the typical cortisol increase after awakening was not completely absent in PTSD patients. Paired comparisons showed a trend towards significance for the cortisol increase 30 min after awakening in PTSD patients \((t=2.00; p=.06)\) but a significant increase in non-PTSD subjects \((t=−4.49; p<.001)\) and healthy controls \((t=−6.17; p<.001)\). The cortisol increase was significantly smaller in PTSD patients than in trauma-exposed subjects without PTSD and healthy controls \((F(2,60)=7.17 p<.01;\) PTSD vs. HC: \(p<.01;\) PTSD vs. non-PTSD: \(p<.01)\).

The reduced awakening response in PTSD patients compared to both other groups remained when trauma-exposed subjects with comorbid depression were excluded (Fig. 1(b); group effect: \(F(2,48)=5.04, p<.01;\) PTSD vs. HC: \(p=.06;\) PTSD vs. non-PTSD: \(p<.05;\) group \(\times\) time interaction: \(F(6,144)=3.32; p<.05)\). Single comparisons showed that non-depressed PTSD patients exhibited a significantly reduced increase of cortisol secretion 30 min after awakening \((F(2,48)=7.83; p<.01;\) PTSD vs. HC: \(p<.05;\) PTSD vs. non-PTSD: \(p<.001)\) and 45 min after awakening \((F(2,48)=4.01, p<.05;\) PTSD vs. HC: \(p=.09;\) PTSD vs. non-PTSD: \(p<.05)\).

Analysis of the short daytime profile revealed no significant difference in the cortisol secretion between PTSD patients and trauma-exposed subjects without PTSD and healthy controls \((F(2,60)=2.45; p=.10)\).

### Table 1

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>PTSD (N=29)</th>
<th>NPTSD (N=19)</th>
<th>HC (N=15)</th>
<th>F-Value/p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>47.0 (10.8)</td>
<td>48.3 (12.4)</td>
<td>40.5 (13.7)</td>
<td>(F(2,60)=1.95;) ns</td>
<td></td>
</tr>
<tr>
<td>M (SD); range</td>
<td>27-65</td>
<td>19-71</td>
<td>20-67</td>
<td></td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>13/16</td>
<td>10/9</td>
<td>8/7</td>
<td>(\chi^2=.41;) ns</td>
</tr>
<tr>
<td>PTSD symptoms</td>
<td>Overall score</td>
<td>66.00 (23.47)</td>
<td>17.47 (13.40)</td>
<td>(F(1,46)=66.62; p&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>Re-experiencing</td>
<td>3.23 (1.26)</td>
<td>1.09 (0.83)</td>
<td>(F(1,46)=42.79; p&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>Avoidance</td>
<td>2.51 (1.24)</td>
<td>0.73 (0.75)</td>
<td>(F(1,46)=36.36; p&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>Hyperarousal</td>
<td>3.45 (1.25)</td>
<td>0.73 (0.62)</td>
<td>(F(1,46)=84.71; p&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>Depression (ADS)</td>
<td>1.22 (0.38)</td>
<td>0.89 (0.27)</td>
<td>(F(2,60)=12.36; p&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>Stress reactivity (FSR)</td>
<td>65.07 (8.95)</td>
<td>56.47 (9.37)</td>
<td>(F(2,58)=9.18; p&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>Wake-up time M (SD)</td>
<td>0705 h (0123 h)</td>
<td>0704 h (0120 h)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sleeping hours before sampling</td>
<td>7.23 (1.33)</td>
<td>7.34 (1.96)</td>
<td>(F(2,60)=.72;) ns</td>
</tr>
</tbody>
</table>

Figure 1 Salivary cortisol response to awakening (1= awakening, 2=30 min after awakening, 3=45 min after awakening, 4=60 min after awakening) and daytime profile \((5=1100 h, 6=1500 h and 7=2000 h)\) in (a) patients with posttraumatic stress disorder (PTSD; \(N=29\)), trauma-exposed persons without PTSD (NPTSD; \(N=19\)) and healthy controls (HC; \(N=15\)) and (b) trauma-exposed subjects with and without PTSD, comorbid depression excluded (PTSD: \(N=19\); NPTSD: \(N=19\)) and healthy controls (\(N=15\)).
The AUC was significantly different in the three groups \((F(2,60)=5.50;\ p<.01)\), with PTSD patients showing lower values than the nonPTSD subjects \((p<.01)\) and the healthy controls \((p=.08)\).

Among trauma-exposed subjects, the overall PTSD symptom score and the number of hyperarousal symptoms were significantly negatively correlated with the AUCG (overall symptom score: \(r=-.30;\ p<.05\); hyperarousal: \(r=-.40;\ p<.01\)). The correlation between avoidance and re-experiencing symptoms and the AUCG were not significant (re-experiencing: \(r=-.23\); avoidance: \(r=-.21\)).

### 6. Confounding variables

To control for possible confounding effects, the analyses where repeated with the covariates age, sex, intake of contraceptive medication, cigarette smoking and wake-up times. These covariates showed no significant impact on the reported group effects or interactions, neither with regard to the morning profile nor overall cortisol secretion (AUC). In addition, one-way ANOVAs of the wake-up-times and the sleeping hours during the night before cortisol sampling showed no significant differences between the three groups (wake-up times: \(F(2,60)=2.39;\ ns\); sleeping hours: \(F(2,60)=0.72;\ ns\)).

### 7. Discussion

In the line with the study by Rohleder et al. (2004), PTSD patients showed a significantly reduced cortisol increase 30–60 min after awakening (awakening response). This effect remained stable after excluding PTSD patients with comorbid major depression and on antidepressant medication and after controlling for possible confounding variables such as age, sex, intake of contraceptive medication, cigarette smoking and wake-up time. Bhagwagar, Hafizi and Cowen (2003) found significantly greater levels of waking salivary cortisol in recovered depressed patients compared to healthy controls. These contrasting results suggest different neurobiological mechanisms in both disorders despite their overlapping symptomatology.

In contrast to the blunted cortisol morning response in PTSD patients, baseline cortisol levels immediately after awakening were not significantly different between the three groups. Similarly, Young et al. (2004) found no differences between PTSD patients and trauma-exposed subjects without PTSD in cortisol levels after awakening. However, the two findings are not fully comparable as Young et al. collected only one morning sample and they instructed the subjects to collect that sample within 30 min of awakening. Taking into account our finding of a blunted awakening cortisol response, the one-point cortisol assessment at a certain time interval after awakening might have led to blurred values. In contrast to our results, Neylan and colleagues (2005) found no differences between PTSD patients and trauma-exposed subjects without PTSD in the time course of cortisol, i.e. no reduced cortisol increase after awakening in PTSD patients. However, in line with our findings and other previous studies (e.g. Yehuda et al., 1996), they observed reduced morning cortisol levels in PTSD patients, as indicated by the AUC of the morning cortisol profile. In our study, cortisol secretion during the day and evening was not affected in PTSD patients compared to both other groups, whereas Young et al. (2004) reported elevated cortisol evening levels in PTSD patients compared to nonPTSD subjects and healthy controls. Yet, separating PTSD patients with comorbid depression from those with ‘pure’ PTSD resulted in different results in Young et al.’s study: ‘pure’ PTSD patients no longer differed from nonPTSD subjects and healthy controls as it was the case in the present study when we excluded PTSD patients with comorbid major depression.

The finding of a reduced cortisol awakening response in PTSD patients as shown in the present study suggests some important and interesting implications for the development of PTSD and accompanying changes in neurobiology. Some authors (e.g. Prüßner et al., 1997) have suggested that the typical cortisol increase 30–60 min after awakening usually reflects an enhanced release of ACTH and a lower cortisol awakening response is assumed to be associated with higher glucocorticoid receptor sensitivity, a relatively robust finding in PTSD research (e.g. Yehuda et al., 1993).

In addition, the blunted cortisol awakening response does not seem to be specific for PTSD patients, but has recently also been reported for patients with chronic fatigue syndrome (Roberts et al., 2004) and patients with unilateral or bilateral lesions of the hippocampus (Buchanan et al., 2004; Wolf et al., 2005). There is strong evidence that the hippocampus is affected in PTSD patients (Bremner et al., 1997), however, it is not clear whether the observed hippocampal reduction in trauma-exposed subjects with PSTD is a predisposing factor or a result of the illness (Gilbertson et al., 2000). Longitudinal studies are needed to clarify the relationship between hippocampal damage and
dysregulation of the HPA axis in the development and maintenance of PTSD.

In the present study, the AUC was significantly negatively correlated with the overall PTSD symptoms in trauma-exposed subjects with and without PTSD. Interestingly, Aerni and colleagues (2004) found a significant treatment effect of low-dose cortisol administration on the intensity of re-experiencing and hyperarousal symptoms in two of three treated patients. Despite the low sample size and possible confounding effects of several variables (other medical treatment of the patients, differing symptom status and elapsed time since the traumatic event) the results point into the same direction. However, our study did not find a significant relationship between cortisol secretion and re-experiencing symptoms. The negative relationship between cortisol secretion and the presence of hyperarousal symptoms on the one side and the positive relationship between cortisol administration and reduction of hyperarousal symptoms on the other side might be interpreted in the light of etiological models that assume that hyperarousal symptoms and thus an over-stimulation of the stress response system are counterregulated by symptoms of emotional numbing and reduced cortisol levels (Foa et al., 1995). The heightened stress reactivity in PTSD patients might also be in favour of this interpretation and is even more interesting in the light of previous research that increased levels of perceived stress are associated with elevated waking cortisol concentrations in healthy volunteers (Wüst, Federenko, Hellhammer and Kirschbaum, 1999). However, to further understand this relationship and its etiological significance, studies are needed that measure the cortisol response to awakening and throughout the day in PTSD patients with various symptom patterns, in persons with high risk of developing PTSD (e.g. firefighters) and in healthy controls at different stress levels.

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References


