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Abstract

The aim of the study was to evaluate the association between post-traumatic disorder (PTSD) and hypothalamic–pituitary–adrenal (HPA) axis responses to the triggering trauma. A companion paper evaluates the adrenergic response and interactions between the two. We measured plasma and saliva cortisol, hourly urinary excretion of cortisol, plasma levels of adrenocorticotropin (ACTH), and the leukocyte glucocorticoid receptor (GR) density of 155 non-injured survivors of traumatic events (91 males and 64 females; 125 road traffic accidents, 19 terrorist attacks, 11 others). Measurements were taken during survivors’ admissions to an emergency room (ER) of a general hospital, and in the mornings, 10 d, 1 month, and 5 months later. Symptoms of peri-traumatic dissociation, PTSD, and depression were assessed on each follow-up session. The clinician-administered PTSD scale (CAPS) conferred a diagnosis of PTSD at 5 months. Survivors with (\(n = 31\)) and without (\(n = 124\)) PTSD at 5 months had similar levels of hormones at all times. Plasma cortisol levels decreased with time in both groups. Female subjects had lower ACTH levels than males. PTSD in females was associated with higher levels of ACTH. In unselected cohorts of trauma survivors, PTSD is not preceded by a detectable abnormality of peripheral HPA axis hormones.

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Introduction

The occurrence of post-traumatic stress disorder (PTSD) has been equated with an abnormal acquisition and/or insufficient extinction of a conditioned fear response (Pitman and Delahanty, 2005). Stress hormones modulate the acquisition and the extinction of conditioned fear responses (Joels et al., 2006; McGaugh, 1988). A significant proportion of this effect occurs shortly after an exposure to a stressor (Joels et al., 2006). Thus, stress hormones’ response to the traumatic events may contribute to the occurrence of PTSD (Pitman, 1989; Yehuda, 2002a).

Yehuda (2002b) proposed a two-factor model of the role of initial stress hormones in the aetiology of PTSD. The model combines immediate adrenergic response and delayed hypothalamic–pituitary–adrenal (HPA) axis response; the former enhancing emotional recall of the traumatic event, and the latter ‘terminating’ the adrenergic surge, via negative feedback, and thereby containing the related enhancement of emotional recall. The model predicts that PTSD follows an elevated adrenergic response to the traumatic event and/or a failure to mount adequate levels of circulating cortisol. This paper addresses the HPA axis component of this model. A companion paper (Videlock et al., 2007) addresses the adrenergic response.

Evidence of abnormal HPA axis functioning in PTSD comes from findings of lower resting levels of circulating cortisol, and from consistent evidence...
of heightened HPA axis response to challenge tests in chronic PTSD (for review Kloet et al., 2006; Yehuda, 2002a).

In acute PTSD, Delahanty et al. (2000) found lower 15 h urinary excretion of cortisol in injured survivors of motor vehicle accidents who subsequently developed acute (1 month) PTSD. Other studies of the early HPA axis response to traumatic events provided conflicting results. In injured children Delahanty et al. (2005) found a strong (r>0.5) positive correlation between 12 h urinary cortisol excretion upon admission to a trauma unit and PTSD symptoms 6 wk later. Bonne et al. (2003) and Heinrichs et al. (2005) showed similar levels of initial plasma cortisol in survivors with and without PTSD. Aardal-Eriksson et al. (2001) showed a negative correlation between morning saliva cortisol and concurrent PTSD symptoms in soldiers, 5 d after a traumatic exposure, and positive correlation between cortisol levels and PTSD symptoms, 2 and 9 months later.

These previous studies have evaluated small samples, male subjects, and used a single peripheral measure of the HPA axis activity. None has measured hormone levels immediately after exposure. The possibility of a progressive, time-dependent alteration of the HPA axis, such as the one found for startle responses (Shalev et al., 2000), has not been explored.

The current study evaluated male and female survivors. Biological measures were obtained within hours of the traumatic events. We used several corroborating measures of HPA axis activity.

The study’s main hypothesis was that PTSD is associated with lower initial levels of plasma cortisol. Alternatively, we explored the hypothesis that PTSD is associated with a time-dependent decrease in plasma cortisol levels (a ‘progressive sensitization’ hypothesis).

The study’s main outcome measure was PTSD at 5 months. Its main predictive variable was plasma cortisol. Saliva cortisol, urinary excretion of cortisol and plasma adrenocorticotropic (ACTH) levels provided auxiliary measure of HPA axis activity. Hypothesized response modifiers included gender, trauma intensity, lifetime exposure to traumatic events, and lymphocytes’ glucocorticoid receptors (GRs). The latter have been implied as moderators of the HPA axis hypersensitivity in PTSD (Yehuda et al., 1995).

Method

Participants

Adult survivors of traumatic events who were admitted to the emergency room (ER) of a general hospital for assessment and treatment following a traumatic incident were eligible for the study. Subjects were not included if, during the incident, they sustained physical injury requiring surgical procedure, had an intravenous line inserted, suffered head injury involving loss of consciousness or were intoxicated upon ER admission. Subjects were not included if the traumatic event was part of ongoing victimization (e.g. domestic violence). Pregnant women, subjects with a lifetime history of endocrine disorder and subjects with chronic PTSD were not included. The study was approved by the Committee on Research Involving Human Subjects (Helsinki Committee) of the Hebrew University – Hadassah Medical School.

Instruments

The Clinician-Administered PTSD Scale (CAPS; Blake et al., 1995) conferred a diagnosis of PTSD at 5 months. The CAPS evaluates the frequency and the intensity of the 17 DSM-IV PTSD criteria. It yields a categorical (present/absent) diagnosis of PTSD, and a continuous symptom severity score. A score of ≥2 on an item was considered as positive endorsement of the symptom. The Structured Clinical Interview for DSM-IV (First et al., 1995) evaluated current and lifetime DSM-IV Axis I disorders. Trained clinical psychologists administered the structured interviews.

The Impact of Events Scale – Revised (IES-R; Weiss, 1996) evaluated symptoms of intrusion, avoidance and hyperarousal at all time-points. The Trauma History Questionnaire (THQ; Green, 1996) evaluated lifetime exposure to traumatic events. This self-report questionnaire inquires about an exposure to any of 13 distinct traumatic events, and for each event, the presence of fear, helplessness, or horror. A traumatic event was defined by the presence of both exposure and emotional reaction (as per DSM-IV PTSD Criterion ‘A’). The Beck Depression Inventory (BDI; Beck et al., 1961) evaluated symptoms of depression. The Peritraumatic Dissociation Questionnaire (PDEQ; Marmar et al., 1997) evaluated the occurrence of dissociation symptoms during the traumatic event. An ad-hoc Trauma Severity Instrument was administered during ER admission. This instrument comprises 11 ‘yes/no’ items that document distinct traumatic occurrences (e.g. witnessing injury, witnessing death, having relatives injured) and nine items, scored 0–5, that evaluate emotional reactions experienced during or shortly after the event (e.g. pain, fear, perceived control).
Biological samples and their analyses

Plasma, saliva, urine, and lymphocyte preparation

Samples for hormonal analyses were collected following subjects’ consent in the ER and upon their arrival to the hospital on subsequent sessions. Blood samples were spun immediately in a cold centrifuge, and frozen for subsequent analysis. For GR analyses, 25 ml plasma were obtained and mononuclear cells were isolated using Ficoll Hypaque within 1 h following blood draw. Cells were centrifuged at 300 g at 4 °C, washed four times in ice-cold Hank’s buffer, and pelleted. An aliquot of the suspension was counted by a haemocytometer and the final pellet stored at −70 °C.

Saliva samples were collected, at the same time, in two salivettes, and preserved at −40 °C. Urine samples were collected (a) during 4 h in the ER (initial void and subsequent collection) and (b) at the beginning of each follow-up assessment session (single void). The time since last void was recorded for each urine collection. Hourly urine excretion was determined by multiplying the concentration by the volume and then dividing by the time since last void.

Laboratory tests were performed at James J. Peters Bronx Veterans Affairs (Dr Yehuda’s laboratory) according to previously published protocols (Goenjian et al., 1996; Yehuda et al., 1995). Biological samples were frozen and sent via courier, on dry ice. Samples were analysed upon arrival at the laboratory. The laboratory personnel were blind to subjects’ identities, time since trauma and diagnostic status.

Procedure

Eligible subjects received information about the study and were asked to participate in its ER component. Consenting subjects signed an informed consent for the ER portion of the study. In total, 270 subjects were included in that portion, and 182 of those agreed to receive a telephone call in view of further participation.

Subjects seen in the ER (n = 270) did not differ from 1436 consecutive trauma admissions to the same ER (Shalev et al., 2005) in age (31.2 ± 10.9 vs. 32.7 ± 11.0), gender distribution (58%; 53% males, χ² = 1.48, n.s.), subjective sense of danger during the event (72.8% vs. 76.3%, χ² = 0.81), and fear experienced during the event (87.8% vs. 91.5%, χ² = 2.2, n.s.).

A total of 163 (89.5%) of the 182 consenting subjects attended the first follow-up visit, at which time they signed an informed consent for the follow-up portion of the study. Exclusion criteria were found in six of these subjects, and two others were omitted for seemingly unreliable reporting of symptoms, leaving 155 valid subjects in the study. Follow-up assessments took place at Hadassah University Hospital Traumatic Stress Laboratory. Subjects received a $35 fee for their participation.

Statistical analysis

Analysis of variance (ANOVA) was used for group comparisons. Repeated-measures ANOVA evaluated the study’s second hypothesis. Pearson’s product-moment correlations were used to examine associations between continuous variables. Biological measures were square-root-transformed (Bland and Altman, 1996), although Table 1 shows untransformed (physiological) levels.

Outlying biological measures were defined as those whose square-root values were more than three times the interquartile range from the top of a box-whisker plot. Outliers were treated as missing values. There were eight outliers in the ER (one in plasma cortisol, two in urinary cortisol, and four in plasma ACTH) and one outlier at 10 d (saliva cortisol). Other missing biological values (Table 1) resulted from technical problems in conservation, preparation, transport, and laboratory processing.

Results

Sample characteristics, attrition and missing values

ER admission took place 1.91 ± 3.31 h after a traumatic event. Subsequent assessments were, respectively, 10.6 ± 3.9, 38.4 ± 3.0, and 159.3 ± 44.7 d later. Blood samples, during follow-up, were taken, respectively, at 10:06 ± 50 min, 09:58 ± 42 min and 10:04 ± 39 min of the first second and third follow-up visits. Traumatic events among participants included road traffic accidents (n = 125), terror attacks (n = 19), and other incidents (e.g. work accidents, home accidents, military incidents, traumatic loss; n = 11). Most of the traumatic events (84.3%) occurred during daytime (06:00–18:00 hours).

The study’s participants (n = 155, 59% males) did not differ from those seen in the ER (n = 270, 55% males) in age [t(260) = 0.048, p = 0.96], gender distribution [χ²(1) = 0.99, p = 0.32] trauma type [χ²(4) = 1.85, p = 0.76], ER pulse rate [t(191) = 1.24, p = 0.22], diastolic blood pressure [t(240) = 1.40, p = 0.16], systolic blood pressure [t(72) = 0.65, p = 0.52], and ER hormone levels (respectively for participants vs. non-participants, plasma cortisol: 13.42 ± 5.38 ng/dl vs.
13.76 ± 6.45 ng/dl; plasma norepinephrine: 310.96 ± 173.84 pg/ml vs. 307 ± 144.04 pg/ml; plasma ACTH: 21.24 ± 16.80 pg/ml vs. 22.56 ± 19.412 pg/ml; all t values <1).

Of 155 valid subjects seen in the ER, 137 were seen at 10 d, 124 at 1 month, and 149 (96%) at 5 months. Subjects who missed an assessment did not differ from full participants in demographics, psychometric and biological variables. A greater number of full participants reported having had a traumatic event in the past (64.0%; 43.2%, x^2 = 5.59, p < 0.02) and childhood trauma [30.6%; 13.6%, x^2(1) = 4.75, p < 0.03].

PTSD and comorbid disorders

Thirty-one subjects (20%, n=155) had PTSD at 5 months (PTSD group) and 124 did not (Non-PTSD group). The study groups had had similar gender distribution, age, and body mass index (BMI). PTSD was associated with higher levels of symptoms at all time-points (Table 2).

Comorbid disorders, at 5 months, comprised depressive episodes [n=24, including 16 (52%) of the PTSD group], anxiety disorders [n=11, including three (10%) of the PTSD group], and other disorders (n=2, one in the PTSD group). There was no difference in ER biological measures between subjects with and without major depression.

Test of the study’s first hypothesis (lower ER cortisol predicts PTSD)

Survivors with and without PTSD had similar levels of plasma cortisol, saliva cortisol, plasma ACTH, GR counts and urinary excretion of cortisol in the ER (all F values <1; Table 1). Figure 1 illustrates the distribution of ER plasma cortisol levels.
controlled for the effects of gender, age, BMI, time of blood draw, time from the event to ER assessment, trauma severity (objective and subjective), peritraumatic dissociation, number of previous traumatic events, and number of traumatic events during childhood. Smoking (yes/no) had no effect on stress hormone levels at any time-point.

Two-way ANOVAs using PTSD and trauma type (road traffic accidents, terror events, all others) as grouping factors yielded a non-significant group difference in ER plasma cortisol levels \[F(5, 131) = 1.25, p = 0.28\].

Pearson’s product-moment correlations between each of the ER biological measures and 5 months PTSD symptoms (separately, IES and CAPS total scores), as well as each of the three PTSD symptom clusters (intrusion, avoidance, and hyperarousal) were not statistically significant.

Test of the study’s second hypothesis
(time-dependent sensitization)

PTSD and non-PTSD subjects had similar endocrine measures at 10 d, 1 month, and 5 months (Table 1).

Plasma cortisol levels inter-correlated across the study (e.g. the correlation coefficients of 1-month values were \(r = 0.378\) for the ER, \(r = 0.647\) for 10 d, and \(r = 0.485\) for 5 months; all \(p\) values < 0.001).

One-month measures were omitted from the following repeated-measures ANOVA, because they greatly increased the number of missing observations. However, data analyses with 1 month’s values revealed similar results.

Repeated-measures ANOVAs for plasma cortisol showed a significant main effect of time [10 d and 5 months, \(F(1, 120) = 4.56, p = 0.03\)], a non-significant main effect of diagnosis \(F(1, 120) < 1\), and a non-significant time × diagnosis interaction \(F(1, 120) < 1\).

Repeated-measures ANOVAs for saliva cortisol (PTSD = 19, non-PTSD = 80) urinary cortisol (PTSD = 20, non-PTSD = 92) and plasma ACTH (PTSD = 21, non-PTSD = 100) equally yielded non-significant group effects.

Repeated-measures ANOVA for GR density related to PTSD (PTSD = 10, non-PTSD (n = 54)] showed non-significant main effects of time \(F(2, 124) < 1\) and diagnosis \(F(1, 52) = 1.62, p = 0.18\) and no interaction \(F(2, 124) < 1\).

Table 2. Mean level of demographics trauma characteristics and psychometrics

<table>
<thead>
<tr>
<th></th>
<th>PTSD</th>
<th>No PTSD</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Value</td>
</tr>
<tr>
<td>Age</td>
<td>31</td>
<td>31.2 ± 11.6</td>
</tr>
<tr>
<td>Gender [M (%)/F (%)]</td>
<td>31</td>
<td>16 (51 %)/15 (48 %)</td>
</tr>
<tr>
<td>BMI</td>
<td>31</td>
<td>40.2 ± 6.7</td>
</tr>
<tr>
<td>Trauma severity (\text{objective and subjective})</td>
<td>31</td>
<td>36.8 ± 13.1***</td>
</tr>
<tr>
<td>Prior traumatic events (n)</td>
<td>31</td>
<td>12 (38.7 %)**</td>
</tr>
<tr>
<td>Childhood trauma (n)</td>
<td>31</td>
<td>6 (19.4 %)</td>
</tr>
<tr>
<td>PTSD symptoms (\text{IES-R})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 d</td>
<td>24</td>
<td>64.1 ± 21.8***</td>
</tr>
<tr>
<td>1 month</td>
<td>23</td>
<td>59.2 ± 21.4***</td>
</tr>
<tr>
<td>5 months</td>
<td>31</td>
<td>56.2 ± 21.7***</td>
</tr>
<tr>
<td>Dissociation (\text{PDEQ})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 d</td>
<td>25</td>
<td>24.3 ± 6.8***</td>
</tr>
<tr>
<td>1 month</td>
<td>23</td>
<td>21.6 ± 6.5*</td>
</tr>
<tr>
<td>5 months</td>
<td>31</td>
<td>22.8 ± 6.8***</td>
</tr>
<tr>
<td>Depression (\text{BDI})</td>
<td></td>
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<tr>
<td>10 d</td>
<td>26</td>
<td>19.7 ± 9.4***</td>
</tr>
<tr>
<td>1 month</td>
<td>21</td>
<td>16.4 ± 10.7***</td>
</tr>
<tr>
<td>5 months</td>
<td>31</td>
<td>17.9 ± 12.3***</td>
</tr>
</tbody>
</table>

PTSD, Post-traumatic stress disorder; BMI, body mass index; IES-R, Impact of Events Scale – Revised; PDEQ, Peritraumatic Dissociation Questionnaire; BDI, Beck Depression Inventory.

\( *p < 0.05, **p < 0.01, ***p < 0.001. \)
Putative effects of gender were examined, for each biological variable, using gender diagnosis ANOVA. The only significant finding concerned plasma ACTH, for which ANOVAs showed a consistent gender diagnosis interaction in the ER \( F(1, 134) = 5.76, p = 0.002 \), at 1 month \( F(1, 109) = 11.2, p = 0.001 \), and at 5 months \( F(1, 141) = 4.32, p = 0.04 \). Women had lower levels of plasma ACTH at all time-points. In post-hoc analyses, women who developed PTSD had higher ACTH levels than women who did not develop PTSD in the ER \( F(1, 55) = 9.27, p = 0.005 \), at 1 month \( F(1, 46) = 16.0, p < 0.005 \) and at 5 months \( F(1, 57) = 3.96, p = 0.052 \). ER and 1-week ACTH levels in women correlated with 5-month PTSD (CAPS) symptoms \( (r = 0.33, p < 0.03) \).

In contrast, levels of ACTH in men did not differentiate those with and without PTSD [ER: \( F(1, 79) = 1.49, p = 0.225 \); 1 month: \( F(1, 63) = 1.50, p = 0.225 \); 5 months: \( F(1, 84) = 1.22, p = 0.27 \)]. There was no statistically significant correlation between ACTH levels and PTSD symptoms in men.

**Likelihood of Type II error**

Considering the current sample’s size and the observed group difference, the likelihood of a Type II error in the comparison of ER cortisol is 0.92, and in that of 5 months’ cortisol 0.82. However, extremely large samples would be required to increase the statistical power to 80%, at \( \alpha = 0.05 \) with the current group differences (e.g. over 1000 for ER plasma cortisol).

**Discussion**

The study’s main hypothesis of an association between ER cortisol levels and PTSD was not confirmed. Similarly, the study’s second hypothesis of a time-dependent differential change in HPA axis measures was not confirmed.

Our results are in line with the Bonne et al. (2003) and Yehuda et al. (1998) negative studies. They are at odds with Delahanty et al. (2000) – a study that comprised injured survivors and predicted acute (1-month) PTSD. In another study by Delahanty et al. (2005) the PTSD/non-PTSD differences in initial cortisol levels were not significant after controlling for injury severity. This study did not extend, to acute PTSD, previous findings of lower cortisol in chronic PTSD (reviewed in Yehuda, 2002a). However, most previous studies concerned survivors with years or decades-long PTSD. The latter may not be comparable with our subjects, some of whom are likely to recover in the years that follow their end-point assessment in this study.

The absence of group difference in ACTH levels is consistent with other negative studies of ACTH in PTSD (Kanter et al., 2001; Newport et al., 2004), and with a recent finding of similar cortisol production rates in PTSD patients and normal subjects (Wheler et al., 2009). Lower ACTH in women was found by Yehuda et al. (2004), and is consistent with the theory that adrenal glands in women are more sensitive to ACTH (Horrock et al., 1990). Finally, the lack of a difference in GR measures is in line with de Kloet et al.’s (2007) recent study of combat veterans, which showed that trauma exposure alone can change static (i.e. unprovoked) GR binding. A difference between PTSD and non-PTSD subjects was better captured, in that study, by the GR response to challenge.

Because HPA axis activity follows a diurnal cycle, the timing of ER sample collections constitutes a pertinent limitation of this study. Yehuda et al. (1996) have shown that the larger differences between subjects with and without PTSD occur during the morning. In this study, an analysis of hormone levels in subjects who were seen in the ER before 10:00 hours yielded non-significant group differences.

The relatively late hour of sample collection during follow-up is another limitation of this study, which
could have reduced the likelihood of finding group differences. A further limitation of the study was the short duration of urine collection, which was meant to capture the first hours following trauma, but did not enable a summation of the HPA axis’ diurnal activity. Previous studies of acute PTSD similarly used 12 or 15 h of urine collection (Delahanty et al., 2000, 2003).

Resting measures of peripheral HPA hormones may not reliably reflect the axis’ CNS activity (Baker et al., 2005). Future studies should evaluate CSF hormone levels, plasma corticotropin-releasing factor, or use provocation tests to explore the axis dynamics. Other response modifiers, such as personality type or history of child abuse may also be considered for future studies. Because this study did not separate these subgroups, a parsimonious reading of our results is that in unselected cohorts of trauma survivors PTSD is not preceded by a detectable abnormality of peripheral HPA axis hormones.

Recent studies have emphasized the role of extinction and consolidation in the aetiology of PTSD (Yehuda et al., 2006). These recent views should lead us to reconsider the role of the immediate response in the aetiology of PTSD. Thus, our previous rush to contribute much of the aetiology of PTSD to the very early responses deserves a second thought.

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Statement of Interest

None.

References


