Altered salivary alpha-amylase awakening response in Bosnian War refugees with posttraumatic stress disorder

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Summary In posttraumatic stress disorder (PTSD), chronic activation of the sympathetic nervous system (SNS) has been suggested. No study so far has investigated diurnal secretion patterns of salivary alpha-amylase (sAA) in PTSD, a promising candidate for non-invasive assessment of SNS activity. We compared sAA diurnal profiles between a group of Bosnian War refugees with PTSD and a healthy control group, and further analyzed for associations with psychiatric symptoms and glucocorticoid (GC) sensitivity of inflammatory regulation. PTSD patients showed a sAA awakening response profile that was opposite to those seen in healthy controls, i.e. an increase instead of a sharp decrease. Patterns of sAA secretion were further positively associated with psychiatric symptoms of PTSD. Finally, higher sAA awakening responses were associated with higher GC sensitivity of inflammatory cytokine production. These findings are in line with altered SNS function in PTSD, and lend further support for employing assessment of diurnal sAA profiles as non-invasive biomarkers in stress-related disease.

1. Introduction

Posttraumatic stress disorder (PTSD) develops in some but not all individuals that have been subjected to a traumatic event. PTSD involves alterations in different neurobiological structures and biochemical systems, yet it is characteristically associated with neuroendocrine dysregulations in the stress system, i.e. in the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). The majority of previous research has focused on neuroendocrine changes of the HPA axis. PTSD has been found to be associated with lower baseline concentration of cortisol in plasma, saliva, and urine (e.g. Boscarno, 1996; Heim et al., 2000; Mason et al., 1986; Rohleder et al., 2004a), stronger suppression of cortisol after administration of synthetic glucocorticoid (GC, de Kloet et al.,...
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2006; Newport et al., 2004; Yehuda et al., 2002), increased release of hypothalamic corticotropin releasing hormone (CRH, Baker et al., 1999; Bremner et al., 1997; Sautter et al., 2003), higher number of peripheral GC receptors in lymphocytes (Yehuda et al., 1993), and higher GC sensitivity of different target tissues (Grossman et al., 2006; Rohleder et al., 2004a). Nevertheless, contrasting findings (e.g. Lemieux and Coe, 1995; Liberzon et al., 1999; Maes et al., 1998; Pitman and Orr, 1990) prevent final conclusions, especially as there are reasons to believe that hypocortisolism may rather be related to trauma exposure and subgroups of PTSD and not to PTSD itself (for a systematic review see Meewisse et al., 2007). Taken together, previous findings point towards a chronically hyperactive and hypersensitive HPA axis, combined with a sustained condition of hypocortisolism in individuals diagnosed with PTSD.

In contrast to PTSD-related HPA axis dysfunctions, neuroendocrine correlates of SNS activation in PTSD are less well studied. Given the suppressive effect of cortisol on SNS activity (Munck et al., 1984), a consequence of chronic hypocortisolism might be that catecholamine levels are elevated in PTSD patients. Indeed, previous investigations point towards a hyperactive SNS in PTSD with increased levels of norepinephrine (NE) and epinephrine (E) in 24 h urine samples (Kosten et al., 1987; Yehuda et al., 1992), elevated diurnal plasma concentrations of NE (Yehuda et al., 1998), and increased norepinephrine in the cerebrospinal fluid (CSF; Geraci et al., 2001) in PTSD. However, just as many studies do not find differences in the sympathoadrenal function between individuals with or without PTSD (e.g. Blanchard et al., 1991; McFall et al., 1992; Pitman and Orr, 1990). Consequently, it remains unclear whether the SNS is indeed chronically activated in PTSD.

The relatively small number of studies examining the activity of the SNS in PTSD may be explained by the complexity, i.e. the stress or invasiveness of previously available means of biochemical SNS activation measurement (urine, blood, and CSF). Only recently, a potential marker derived from saliva has been proposed, which may constitute a convenient and non-invasive indicator of SNS activity, i.e. the salivary enzyme alpha-amylase (sAA, see reviews in Nater and Rohleder, 2009; Rohleder and Nater, 2009). Salivary alpha-amylase follows a characteristic daily secretion pattern showing a sharp decrease in the first hour after awakening followed by an increase in activity throughout the day (Nater et al., 2007; Rohleder et al., 2004b). It has been shown that sAA levels rise in response to both physical (e.g. Chaterton, 1996; Kivlighan and Granger, 2006; Skosnik, 2000) and psychological stress (e.g. Chaterton et al., 1997; Gordis, 2006; Nater et al., 2005; Rohleder et al., 2004b; Stroud et al., 2006). Further, it was found that the sAA release can be stimulated by beta-adrenergic agonists (Gallagher and Petersen, 1983), alpha-2-adrenergic receptor antagonists (Ehler et al., 2006), and inhibited by beta-adrenergic blockers (van Stegeren et al., 2006). Moreover, some studies also report correlations between changes in sAA secretion and stress-induced norepinephrine release (Chaterton, 1996; Rohleder et al., 2004b). Finally, Nater et al. (2006) reported that sAA secretion is positively correlated with heart rate variability (HRV) measures of sympathovagal balance. Altogether, hitherto existing findings suggest that sAA constitutes a promising candidate as a non-invasive biomarker for SNS activity. However, as summarized in a recent commentary, further evaluation might be warranted (Bosch et al., 2011). Recent studies have shown that the daily secretion pattern of sAA is altered in individuals reporting chronic stress (Nater et al., 2007), in young women experiencing chronic shame (Rohleder et al., 2008), in children with asthma experiencing chronic home life stress (Wolf et al., 2008), and in caregivers for cancer patients (Rohleder et al., 2009).

Given the interactive role of the HPA axis and the SNS in the development and maintenance of PTSD, an additional and easier obtainable SNS marker would spread more light on stress system pathophysiology in PTSD. We hypothesize that daily secretion patterns of sAA are altered in PTSD patients compared to healthy controls, based on alterations of daily sAA secretion in chronic stress conditions. In order to test this hypothesis we analyzed saliva samples of Bosnian War refugees with PTSD and healthy controls that were collected in the context of a previously published study (Rohleder et al., 2004a). This allows us to additionally link sAA secretion patterns in PTSD patients with other health relevant parameters measured in the previous study, such as inflammatory regulation.

2. Methods and materials
2.1. Study participants
For the current study we analyzed data from a group of Bosnian War refugees and healthy controls who had provided saliva samples in the context of a previous study (Rohleder et al., 2004a). From the original 25 participants, two PTSD patients and two healthy controls (laboratory staff members) were excluded from all analyses due to insufficient saliva volume. The final number of participants was n = 21 (n = 10 Bosnian War refugees with PTSD and n = 11 controls). Bosnian War refugees were recruited from the Psychosocial Center (PSC) of Refugees in Düsseldorf, Germany, a psychological treatment facility specialized for war-related trauma. With the exception of two participants, all Bosnian War refugees underwent psychotherapy while participating in the study. Bosnian War refugees were screened at the PSC for traumatic events using the Harvard Trauma Questionnaire (HTQ, Mollica et al., 1992). The traumatic events that were reported the most were the following: separation from members of the family (12), near-death experiences (11), deficient food or water supply (10), witnessing murder/s of strangers (10), lack of shelter (7), prison (5), as well as experiencing the killing of a family member/friend (5), torture (4), or combat (4). In case of a reported trauma, refugees were further screened for PTSD symptoms using the International Diagnostic Checklist, which is based on the DSM-IV criteria for PTSD (American Psychiatric Association, 1994). Twelve of the screened Bosnian War refugees fulfilled DSM-IV criteria for PTSD and were accordingly included in the PTSD group. These reported an average of 21.67 traumatic events (range 16–26). Five Bosnian War refugees who did not fulfill DSM-IV criteria for PTSD were included in the control group: on average, they reported 21 traumatic experiences (range 17–27). The remaining six controls, i.e. healthy and non-war refugees reported on average 1.2 traumatic experiences (range 0–3). All participants were free of somatic diseases,
medication intake, or substance abuse. All were fully informed orally and in writing (in German or Bosnian) about the aim, course, and methods of the study. Bosnian participants were informed by their psychotherapist, co-therapist and by a translator. Written informed consent was obtained from all participants. The study was conducted in accordance with the declaration of Helsinki. The study protocol was approved by the ethics committee of the University of Düsseldorf.

2.2. Procedures

The study’s procedure consisted of a first appointment at the Psychosocial Center of Refugees in Düsseldorf (Bosnian War refugees), or in the laboratory (controls), and of two days saliva home-sampling assessment. Upon arrival at the first appointment, participants were fully instructed about the study’s procedure. After providing informed consent, a registered nurse drew blood from the participants. Bosnian War refugees’ therapist was present during study procedures to provide support if necessary. Participants also filled out a battery of questionnaires. PTSD patients and control participants were then instructed and trained on how to collect saliva samples for the saliva home-sampling assessment (all described below). The saliva home-sampling assessment took place on two consecutive weekdays after the first appointment. Saliva samples were returned to the refugee’s therapist by the participant in person.

2.3. Sampling methods and biochemical analysis

2.3.1. Salivary alpha-amylase
Participants were instructed to gently chew on cotton swabs for 1 min and place them into a plastic tube thereafter (Salivettes, Sarstedt, Nümbrecht, Germany). On both days, sampling times were immediately, 30, 45, and 60 min after awakening, and at 1100, 1500, and 2000 h. Compliance was monitored using MEMS6 devices, which record the time each sample was taken (Aardex, Zug, Switzerland). Participants stored saliva samples at room temperature until returning the samples to the laboratory, after which they were immediately stored at −20°C, which has been shown not to affect sample integrity (for an extensive review see Rohleder and Nater, 2009). sAA was measured by an enzyme kinetic method as described previously (Rohleder and Nater, 2009). Inter- and intra-assay coefficients of variation (CV) were below 10%.

2.3.2. Glucocorticoid sensitivity of inflammatory cytokine production
All blood samples were collected between 0900 h and 1300 h and processed as previously described (Rohleder et al., 2004a). To determine in vitro GC sensitivity of peripheral inflammation, 5 mL of blood was drawn into heparinized syringes (Braun, Melsungen, Germany). In addition, 2.7 mL of blood was drawn into EDTA monovettes (Sarstedt, Nümbrecht, Germany) for a differential blood count. Heparinized blood was incubated with the bacterial endotoxin lipopolysaccharide (LPS, Escherichia coli, Sigma, Deisenhofen, Germany) and differing concentrations of dexamethasone (DEX) for 6 h at 37°C and 5% CO2. Plasma supernatant was harvested and stored at −80°C until IL-6 and TNF-α concentrations were measured using commercial enzyme-linked immunosorbent assays (ELISA; BD Pharmingen, San Diego, California), with intra-assay CVs between 2.0% and 3.9% and inter-assay CVs between 4.1% and 9.3%.

To quantify GC sensitivity of the inflammatory response, we computed the half maximal inhibitory concentration (IC50) indicating the concentration of DEX required to inhibit cytokine production by 50%. Cytokine concentrations were corrected for the respective number of monocytes (Berczi, 1998; Born et al., 1995).

2.3.3. Psychometric measurements
Symptoms related to the diagnostic criteria for PTSD were assessed by the psychiatric screening instrument Harvard Trauma Questionnaire (HTQ, Mollica et al., 1992). This semi-structured interview inquires about traumatic life events, the most traumatic event the respondent experienced in the course of refugee, the possibility of experienced head injury, PTSD diagnostic criteria based on DSM-IV and additional refugee-related symptom items. The HTQ is a cross-cultural instrument that has been used and validated in several different cultural contexts and languages (Mollica et al., 2001). In the present study, one of the authors (LJ) translated the HTQ from German into Bosnian; back-translation into German was provided by a trained translator at the PSC to ensure quality of translation.

The revised version of the Symptom Checklist 90 (SCL-90R, Derogatis and Cleary, 1977) was used to assess overall psychological distress as well as symptoms and intensity of psychological problems and psychopathologic symptomatology. The SCL-90R is a self-report questionnaire consisting of 90 items. For each symptom, the participant rates the experienced severity on a 5-point scale ranging from 0 ‘not at all’ to 4 ‘extremely’. The symptoms are categorized in nine dimensions representing various psychopathologies (e.g. somatization, depression, anxiety, psychoticism, etc.). A general stress index can be calculated to indicate overall psychological distress (Global Severity Index, GSI).

2.4. Statistical analyses

To test for normality of distribution and homogeneity of variance, Kolmogorov–Smirnov and Levene’s test were applied prior statistical analyses. Where appropriate (violation of sphericity assumption) Greenhouse–Geisser corrections were applied for repeated measures ANOVAs (Greenhouse and Junker, 1992; Vasey and Thayer, 1987). Salivary alpha-amylase values were averaged for the two sampling days before analysis. To estimate the sAA awakening response, we calculated the slope of the morning response for samples taken in the first hour. Student’s t-test and χ²-test were calculated to assess differences in demographic characteristics and in the slope of the sAA awakening response between groups. Pearson correlations were computed to test for bivariate associations between sAA secretion indices (i.e. slope of the sAA awakening response, the sAA wake-up concentration (+1 min), and the area under the curve with respect to the ground (AUC0) as a measure for total sAA daily output) and self-reports of psychiatric symptoms (i.e. sum score of HTQ and mean GSI score of the SCL-90R) as well as markers for GC sensitivity (i.e. IC50 of IL-6 and
TNF-α). Analysis of variance (ANOVAs) for repeated measures with four and seven within-group levels were computed for the sAA awakening response (+1 min, +30 min, +45 min, +60 min), and the sAA diurnal profile (1100 h, 1500 h, 2000 h), respectively, to analyze differences in the daily secretion patterns of sAA between PTSD patients and controls.

3. Results

3.1. Preliminary analyses

Groups did not differ significantly in terms of sex distribution (PTSD: 4 women, 6 men; controls: 8 women, 3 men; \( \chi^2 = 2.29; p = 0.13 \)), age (PTSD: 44.8 years ± 10.24 SD; range = 30–64; controls: 46.4 years ± 12.73 SD; range = 30–68; \( t(18) = -0.31; p = 0.76 \)), weight (PTSD: 76.7 kg ± 8.29 SD; controls: 74.27 kg ± 7.31 SD; \( t(19) = 0.71; p = 0.48 \), and smoking (\( t(19) = 0.3; p = 0.77 \)). Groups did not significantly differ in their wake-up times (PTSD: 6:49 ± 1:01 h SD; controls: 7:31 ± 0:59 h SD; \( F[1, 20] = 2.53; p = 0.129 \)). Wake-up times were further not correlated with the slope of the sAA awakening response (\( r = -0.166; p = 0.472 \)) and the sAA wake-up concentration (\( r = -0.154; p = 0.518 \)). By design, the groups significantly diverged in their mean HTQ scores (PTSD: 22.6 ± 3.2 SD; controls: 9.2 ± 9.3 SD; \( t(18) = 4.31; p = 0.001 \)). Further, groups significantly diverged in the mean GSI score of the SCL-90R (PTSD: 1.68 ± 0.65 SD; control participants: 0.47 ± 0.82 SD; \( t(16) = 3.48; p = 0.003 \)), as well as in all subscales (all \( p \leq 0.038 \)).

3.2. Salivary alpha-amylase awakening response and daily secretion pattern

To test whether PTSD patients show an altered sAA awakening response, we first calculated a repeated-measures ANOVA including the four morning samples and found a significant difference between patients and control participants (group-by-time interaction: \( F[2.13, 40.39] = 5.54; p = 0.007 \)). As shown in Fig. 1, this result was due to opposing sAA awakening response profiles, with healthy controls showing a sharp decrease after awakening, and PTSD patients showing an increase. This result was further supported by the significant difference in the slope of the sAA awakening response between groups (\( t(18) = 2.6; p = 0.018 \), and by a trend in the sAA wake-up concentration (\( t(19) = -1.96; p = 0.065 \)) and in the sAA concentration 45 min after awakening (\( t(19) = 1.87; p = 0.076 \)). Of note, Pearson correlation revealed a significant association between the slope of the sAA awakening response and the sAA wake-up concentration (\( r = -0.586; p = 0.007 \)). AUCG including only the four morning samples did not differ between groups (\( F[1, 20] = 0.83; p = 0.374 \). To test for the possible impact of the main covariates (i.e., age, sex, weight), each covariate was separately included in the analyses. None of the covariates significantly impacted the findings. We further computed a repeated measures ANOVA including all seven saliva sample time points to test for group-specific differences in the daily secretion pattern of sAA. No significant difference between the PTSD and the control group was found in their daily sAA secretion patterns (group-by-time interaction: \( F[4.14, 78.6] = 2.01; p = 0.099 \) and in their AUCG (\( F[1, 20] = 0.36; p = 0.555 \)). Together, these findings indicate distinct group-specific sAA awakening response profiles, with PTSD patients failing to show the sharp decrease in sAA after wake-up seen in healthy controls.

3.3. Associations between salivary alpha-amylase and self-reports of psychiatric symptoms

To test whether daily sAA secretion patterns are related to psychiatric symptoms associated with PTSD, we first included the sum score of the HTQ as a covariate in the repeated-measures ANOVA to control for the influence of the severity of the specific trauma. Although we were not able to rigorously test for mediation due to our small sample size, inclusion of the HTQ as a covariate increased the \( p \)-value of the repeated-measures ANOVA for the sAA awakening response profile to a non-significant level (group-by-time interaction: \( F[2.08, 35.32] = 1.3; p = 0.286 \)). We further computed Pearson correlations to test whether mean HTQ scores were associated with the slope of the sAA awakening response and the wake-up measure. As shown in Fig. 2, HTQ was significantly associated with the slope of the sAA awakening response (\( r = 0.67; p = 0.002 \)), indicating that higher scores in HTQ were related to higher, positive sAA awakening responses (i.e., increases instead of decreases). HTQ scores were further significantly related to sAA wake-up concentrations (\( r = -0.55; p = 0.012 \)), revealing a negative association between HTQ scores and the first sAA measures taken immediately after awakening. No significant correlations were found between HTQ scores and the slope of the sAA daily response (\( r = -0.13; p = 0.596 \)) or the AUCG (\( r = -0.058; p = 0.82 \)).

To test whether amylase secretion is related to symptoms of psychopathology, we included the mean GSI score of the SCL-90R as a covariate in the repeated-measures ANOVA. Inclusion of mean GSI resulted in an increased \( p \)-value of the repeated-measures ANOVA for the sAA awakening response profile to a non-significant level (group-by-time interaction: \( F[1.94, 29.04] = 1.26; p = 0.3 \)). Pearson correlations revealed...
no significant associations between mean GSI and the slope of the sAA awakening response ($r = 0.39; p = 0.12$), but was inversely related with sAA wake-up concentration ($r = -0.51; p = 0.03$). Further explorative analyses revealed a significant association specifically between the SCL-90R anxiety subscale and the sAA wake-up concentration ($r = -0.62; p = 0.006$). No significant correlations were found between SCL-90R scores and the slope of the sAA daily response ($r = -0.059; p = 0.823$) or the AUC$_C$ ($r = -0.018; p = 0.949$).

### 3.4. Associations between salivary alpha-amylase and inflammatory regulation

We have previously reported that the PTSD and the control group significantly differed in the sensitivity of the inflammatory cascade towards glucocorticoid inhibition (GC sensitivity, as indexed by the IC$_{50}$ of IL-6 and TNF-α) (Rohleder et al., 2004a). Because GC sensitivity might be modulated by the SNS (for review see Rohleder, 2011), and sAA alterations found here might point to a dysregulation of SNS activity, we tested whether GC sensitivity was associated with the daily pattern of amylase secretion. We separately included IC$_{50}$’s of IL-6 and TNF-α as covariates in the repeated-measures ANOVA. Inclusion of the IC$_{50}$ of IL-6 increased the $p$-value of the awakening response to a non-significant level ($F[2.25, 38.21] = 2.47; p = 0.09$). Pearson correlations between the IC$_{50}$ of IL-6 and the slope of the sAA awakening response revealed a significant inverse association ($r = -0.67; p = 0.002$) (Fig. 3). No significant correlations were found between the IC$_{50}$ of IL-6 and the slope of the sAA daily response ($r = 0.094; p = 0.704$) or the AUC$_C$ ($r = -0.271; p = 0.292$). The inclusion of the IC$_{50}$ of TNF-α as covariate did not change the significant $p$-value for the awakening response profile ($F[2.02, 34.25] = 3.36; p = 0.046$). Pearson correlations between the IC$_{50}$ of TNF-α and the slope of the sAA awakening response revealed a trend towards significance ($r = -0.44; p = 0.061$). While no significant correlation was found between the IC$_{50}$ of TNF-α and the slope of the sAA daily response ($r = -0.041; p = 0.869$), the IC$_{50}$ of TNF-α correlated negatively with the AUC$_C$ ($r = -0.496; p = 0.043$). Due the inverse association of the IC$_{50}$ with GC sensitivity, these results show that higher sAA awakening responses are associated with higher GC sensitivity of inflammatory cytokine production.

### 4. Discussion

The purpose of the present study was to characterize diurnal profiles of salivary alpha-amylase in PTSD, with the goals (1) to evaluate the usefulness of salivary alpha-amylase as an additional tool for studying stress related disorders, and (2) to better understand SNS alterations in PTSD. We found that PTSD patients showed sAA awakening response profiles opposite of those seen in healthy individuals, such that PTSD patients showed an increase instead of a sharp decrease in sAA values after awakening. Furthermore, we found sAA secretion patterns to be positively associated with self-reports of psychiatric symptoms of PTSD, more specifically with the severity of the specific trauma (HTQ) as well as psychological symptoms (GSI of the SCL-90R), particularly with regard to anxiety (SCL-90R subscale ‘anxiety’). Finally, we found associations between inflammatory regulation and sAA secretion, with higher sAA wake-up responses being associated with higher GC sensitivity of inflammatory cytokine production.

This was the first study to investigate daily secretion patterns of sAA in PTSD. Considering sAA as a non-invasive SNS marker as recently proposed (for an overview see Nater and Rohleder, 2009; Rohleder and Nater, 2009), our findings correspond to outcomes of studies reporting altered levels of other SNS biomarkers (i.e. NE, E, 3-methoxy-4-hydroxyphenylglycol, MHPG) found in CSF, urine, and plasma of PTSD patients (Geraciotti et al., 2001; Kosten et al., 1987; Yehuda et al., 1998, 1992; Young and Breslau, 2004). In contrast to investigations reporting significantly elevated daily sAA secretion profiles in participants reporting other forms of chronic stress (Nater et al., 2007; Rohleder et al., 2008, 2009; Wolf et al., 2008), our findings point to specific alterations of the wake-up response.
At least two questions arise from the current findings; i.e. what might be causing the alterations of the sAA awakening response profile in PTSD, and what the implications for individuals suffering from PTSD might be. In healthy individuals, as shown also in our controls and other studies, amylase concentrations decrease sharply in response to waking up, followed by a continuous increase throughout the day (Nater et al., 2007; Rohleder et al., 2004b; Strahler et al., 2010; Wolf et al., 2008). Our present findings suggest that the sAA awakening response shows the most pronounced alterations in PTSD. This finding is in line with the results of a recently published study, where an increase instead of the expected decrease in the sAA awakening profile has been reported for male competitive ballroom dancers (Strahler et al., 2010). This was interpreted as a consequence of long-term stress induced by repeated competitions. However, no study has examined the sAA awakening response in other stress-related diseases, such as depression. While it might be too early to speculate about mechanisms underlying the altered sAA awakening response, it is interesting that similar alterations of the wake-up response are found for another stress system with a strong diurnal rhythm, i.e. the HPA axis (Chida and Steptoe, 2009; Wessa et al., 2006). This could be mediated by behavioral consequences of PTSD, such as changes in sleep quality or duration, but it could also be related to changes in brain structures relevant for regulation of stress system activity, such as the hippocampus. Changes of the latter structure have been found associated with the HPA axis wake-up response (Buchanan et al., 2004). Regarding consequences of altered patterns, it might again be too early to speculate, but alterations of HPA axis diurnal rhythms have been found associated with negative health outcomes, such as short survival of breast cancer patients (Sephton et al., 2000), or subclinical depression (Mangold et al., 2011).

This is the first study investigating associations between daily sAA secretion patterns and self-reports of PTSD symptoms. Our findings support earlier but so far preliminary evidence suggesting associations between self-reports of PTSD with other biomarkers of SNS activity (Geracioti et al., 2001; Yehuda et al., 1992), or self-reports of trait and state anxiety and sAA in non-PTSD, i.e. healthy participants (Noto et al., 2005; Takai et al., 2004). The finding of positive associations between sAA secretion and self-reports of PTSD symptoms is in line with results by Yehuda et al. (1992), who found positive correlations between catecholamine concentrations in urine, and the severity of the intrusive symptom cluster. This finding has been replicated by Geracioti et al. (2001) who investigated catecholamines in the CSF of combat veterans with chronic PTSD. In conclusion, with this study we were able to show that sAA concentrations are associated with severity of trauma, and symptoms of psychopathology, as well as anxiety. Of note, correlations in the present study were found in the entire group of participants.

We were further interested to explore whether sAA alterations in PTSD were associated with health-relevant biological mechanisms. We had previously shown that glucocorticoid sensitivity of the inflammatory response pathway was increased in individuals diagnosed with PTSD (Rohleder et al., 2004a). This was interpreted as compensatory upregulation, probably related to inflammatory overactivity in PTSD (e.g. Spitzer et al., 2010). Because GC and noradrenergic signaling converge on inflammatory target cells (see Rohleder, 2011), we hypothesized a relationship between sAA alterations and GC sensitivity. In fact, the positive association found between the wake-up response and GC sensitivity of the inflammatory response system indicates that those participants with the highest deviation from a normal morning sAA response also showed the highest sensitivity of their monocytes to the anti-inflammatory GC signal. Although a higher GC sensitivity is usually interpreted as beneficial, in the context of PTSD, GC sensitivity has been interpreted as an inefficient compensatory response to low circulating cortisol, and thereby indicative of endocrine and immune dysregulation (Rohleder et al., 2010). Thus the association of sAA with GC sensitivity in this study is in line with the overall pattern of dysfunctional neuroendocrine regulation, and might suggest a pathway between SNS alterations and inflammatory disinhibition in PTSD.

4.1. Limitation

Due to some limitations, the current results need to be interpreted with some caution: The restrictions of our study include the relatively small sample size and the mixed control group consisting of traumatized and non-traumatized participants of varying ethnicities. Nevertheless, given the small sample size, we argue that the latter might rather be considered a strength than a weakness due to the following reasons: The fact that half of the control group has been traumatized but did not fulfill the complete picture of PTSD, gave us some hints that a trauma exposure alone might not be enough and the full symptom-picture of PTSD must be present. A further advantage of our approach was that we have an ethnic mixed control group, which minimizes the confounding influence of ethnicity on the outcome. What is more, given the cross-sectional design of our study, our results need to be interpreted with some caution. We show here that sAA levels covary with trauma severity as well as symptoms of psychopathology. Whether the sAA levels also vary within patients, for instance as a function of time elapsed since the trauma, remains to be shown. Moreover, the application of a non-standardized semi-structured interview for the use of PTSD diagnosis is a limitation. Finally, despite the fact that sAA has been shown to be related to different markers for SNS activation, several questions, for example related to the contribution of the parasympathetic nervous systems, are still being critically discussed (Bosch et al., 2011).

In the present study we set out to examine daily secretion patterns of salivary alpha-amylase in PTSD and found that sAA awakening response profiles are altered in PTSD, and that alterations are associated with psychiatric symptoms and efficiency of glucocorticoid inflammatory regulation. Our results provide further cautious support for altered SNS activity in PTSD. Furthermore, we were able to replicate and extend the literature on associations between self-reports of PTSD and biochemical disease markers. Finally, the present findings contribute to the growing literature on salivary alpha amylase and underscore its usefulness as a non-invasive, convenient and cost effective means of assessing SNS activity.
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Conflict of interest

None of the authors reported any potential conflicts of interest.

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References


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