JOURNAL OF CHILD AND ADOLESCENT PSYCHOPHARMACOLOGY
Volume 25, Number 4, 2015

Pp. 344–350
DOI: 10.1089/cap.2014.0034

Elevated Salivary Alpha Amylase in Adolescent Sexual Abuse Survivors with Posttraumatic Stress Disorder Symptoms

Brooks R. Keeshin, MD,[1] Jeffrey R. Strawn, MD,[2,3] Dorothee Out, PhD,[4,5] Douglas A. Granger, PhD,[4,6] and Frank W. Putnam, MD[7]

Abstract

Objective: Little is known regarding neuroendocrine responses in adolescent girls with posttraumatic stress disorder (PTSD) who have experienced sexual abuse. Therefore, we collected saliva samples three times daily for 3 days to assess concentrations of salivary alpha amylase (sAA) – a surrogate marker for autonomic nervous system (ANS) activity and, in particular, sympathetic activity – in sexually abused adolescent girls.

Methods: Twenty-four girls (mean age: 15 ± 1.4 years) who had experienced recent sexual abuse (i.e., sexual abuse occurred 1–6 months prior to study enrollment) and 12 healthy comparison subjects (mean age: 14.8 ± 1.3 years) completed a structured interview and assessments to ascertain symptoms of posttraumatic stress, then collected saliva at home upon awakening, 30 minutes after waking, and at 5 p.m. on three consecutive school days.

Results: For sexually abused girls, total PTSD symptoms were associated with higher overall morning levels of sAA ($r[20] = 0.51$, $p = 0.02$), a finding driven by intrusive symptoms ($r[20] = 0.43$, $p < 0.05$) and hyperarousal symptoms ($r[20] = 0.58$, $p = 0.01$). There were no significant differences in diurnal sAA secretion between the sexually abused girls and healthy comparison adolescents.

Conclusions: Overall morning concentrations of sAA in sexually abused girls are associated with overall PTSD severity as well as symptoms of hyperarousal and intrusive symptoms, possibly reflecting symptom-linked increases in ANS tone. These data raise the possibility that alterations in ANS activity are related to the pathophysiology of sexual abuse-related PTSD in adolescent girls, and may inform therapeutic interventions (e.g., antiadrenergic medications).

Introduction

Posttraumatic stress disorder (PTSD) is a common outcome among adolescents with a history of sexual abuse (Copeland et al. 2007). Moreover, the autonomic nervous system (ANS), a collection of central and peripheral neural networks, is one of several systems responsible for the homeostatic regulation of stress responses and is implicated in the pathophysiology of PTSD (Strawn and Geracioti 2008). Specifically, the catecholamine norepinephrine (NE) is elevated in the cerebrospinal fluid of combat veterans with chronic PTSD, and correlates with the severity of PTSD symptoms (Geracioti et al. 2001). However, studies of peripheral (e.g., plasma or urinary) NE have demonstrated mixed results, with some reports showing elevated levels of NE (Jensen et al. 1997), and other studies finding no difference between patients with and without PTSD (McFall et al. 1992). Similarly, 24 hour urine catecholamine studies have predominantly demonstrated elevated catecholamine excretion in patients with PTSD compared with healthy subjects (Yehuda et al. 1998), with some studies showing no differences (Mellman et al. 1995). In adults with PTSD, noradrenergic dysfunction (Strawn and Geracioti 2008) likely explains the utility of alpha$_1$ antagonists in the treatment of intrusive symptoms in PTSD.

Hypothalamic pituitary adrenal (HPA) axis change, such as a blunting of the cortisol awakening response, has been previously associated with increased severity of posttraumatic stress symptoms in abused adolescents (Keeshin et al. 2014). In addition, changes in ANS functioning relatively proximal to the event may predict trauma-related psychopathology. This is consistent with observations of elevated plasma NE levels in children who developed PTSD within

[1]Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah.
[2]Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati College of Medicine, Cincinnati, Ohio.
[3]Department of Pediatrics, Division of Child and Adolescent Psychiatry, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio.
[4]Institute for Interdisciplinary Salivary Bioscience Research, Arizona State University, Tempe, Arizona.
[5]Centre for Child and Family Studies, Leiden University, Leiden, the Netherlands.
[6]Johns Hopkins University School of Nursing, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland.
[7]Department of Psychiatry, University of North Carolina, Chapel Hill, North Carolina.

6 months of a motor vehicle accident (MVA) compared with MVA survivors without PTSD (Pervanidou et al. 2007). In pediatric survivors of sexual abuse (regardless of PTSD diagnosis) and in maltreated children with PTSD, 24 hour urinary catecholamine excretion is increased (De Bellis et al. 1994, 1999).

Salivary alpha amylase (sAA), an enzyme produced by the salivary glands, is widely used as a surrogate marker of ANS tone (Granger et al. 2007). In this regard, both sympathetic and parasympathetic nervous system activity contribute to the secretion of alpha amylase into saliva, although levels of sAA primarily reflect sympathetic activity (Bosch et al. 2011). As such, one recent study suggests that stress-related increases in sAA parallel similar increases in plasma NE concentrations (Nater et al. 2006). The secretion of sAA follows a specific diurnal pattern, with a decrease in sAA levels in the 30 minutes after awakening, followed by an increase throughout the remainder of the day. Alterations in the diurnal pattern of sAA secretion and in acute sAA stress reactivity abound in adults with chronic stress (Nater et al. 2006) and in adult refugees with chronic PTSD (Thoma et al. 2012). Young children exposed to chronic war violence exhibit elevated levels of afternoon sAA compared with controls (Feldman et al. 2013). In addition, in contrast to the control group, sAA and cortisol responses to social stress do not correlate among maltreated and traumatized adolescents, suggesting an asymmetric functioning of HPA and ANS activity (Gordis et al. 2008). However, to our knowledge, diurnal sAA secretion is uncharacterized in pediatric patients who are at risk for PTSD.

It is unclear whether developmental factors or prior morbidity and previous experiences of adversity explain the contrasting ANS findings between adults and children who have experienced trauma and have symptoms of posttraumatic stress, making it challenging to apply findings from the adult literature to pediatric populations. With this in mind, we hypothesized that diurnal concentrations of sAA would be higher in sexually abused girls than in healthy comparison girls, and that sAA concentrations would correlate with severity of symptoms of posttraumatic stress among sexually abused girls when measured between 1 and 6 months post sexual abuse.

Methods

Participants

This study was a prospectively planned secondary analysis of salivary analytes collected from abused teenage girls and control subjects (Keeshin et al. 2014). Sexually abused adolescents ($n = 24$) were recruited from an urban children's advocacy center. Eligibility criteria included not being pregnant, speaking English, and being a female between the ages of 12 and 17 years who had experienced forensically substantiated sexual abuse 1–6 months prior to study enrollment. Families of all patients who met these criteria were contacted via letter and follow-up phone call, and interested families participated in a secondary screen before being invited to participate in the study. Additional criteria covered during the secondary screen included verifying that the participants had a normal intelligence quotient (IQ); were postmenarchal; and had no history of major neurological or medical illness, significant head trauma, lifetime substance dependence, psychosis, mania, or PTSD. Additionally, age- and race-matched healthy comparison subjects were recruited from the community ($n = 12$) through the use of posters displayed in the hospital and recruitment emails sent to all hospital employees. All caregivers with adolescent girls who met the abovementioned criteria were informed of the details of the study and invited to participate. Written informed consent was obtained at the time of the initial study visit by a legal guardian as well as assent by the participant. Cincinnati Children's Hospital Medical Center Institutional Review Board approved the study protocol.

Demographic and psychometric measurements

Data were obtained from the adolescent and her caregiver(s), and included age, gender, race, family structure, school placement, and socioeconomic status. Additionally, caregivers also completed the Childhood Trust Events Survey (CTES) (Pearl 2000). Symptoms of PTSD were assessed with the Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA) (Ohan et al. 2002), depressive symptoms were assessed with the Reynolds Adolescent Depression Scale-2 (RADS-2) (Osman et al. 2010), dissociative symptoms were inventoried with the Adolescent Dissociative Experiences Scale (ADES) (Armstrong et al. 1997), and pubertal status was determined with the Duke Self-Staging Tanner Scale (Duke 1980).

CTES. CTES (Pearl, 2000) assesses previous accidental, community, and interpersonal adversities. The CTES is a 26 item measure that records whether or not different adversities occurred sometime in childhood. The possible responses (''yes,'' ''no,'' and ''don't know'') to each question are provided by the caregiver, and this instrument does not weight the frequency or duration of adverse experiences. The 26 items included in the measure are composed of 15 potentially traumatic events that could be life threating or result in serious injury, and 11 additional adversities commonly experienced by children.

CAPS-CA. CAPS-C, a structured interview that obtains detailed histories of specific traumatic events, and assesses both frequency and intensity of currently experienced symptoms, was used to assess PTSD symptoms. The instrument is aligned with *Diagnostic and Statistical Manual of Mental Disorders,* 4th ed., Text Revision (DSM-IV-TR) PTSD symptoms and has high reliability, ranging from 0.92 for frequency and 0.98 for intensity ratings and strong internal consistency with alphas ranging from 0.85 to 0.87 for the three symptom clusters of PTSD as described in DSM IV TR (American Psychiatric Association 2000).

RADS-2. RADS-2 is a 30 item self-report measure intended for children ≥ 11 years of age that examines different aspects of depression, including dysphoric mood, anhedonia, negative self-evaluation and, somatic complaints. The RADS-2 has an internal consistency of 0.92 and a test–retest reliability of 0.89.

ADES. ADES, a 30 item self-report, assesses both depersonalization and derealization dissociative symptoms. Symptom frequency is rated on a scale of 0 to 10, with 10 signifying that the symptom happens all the time. Prior studies have demonstrated that scores of 3.7 suggest clinically significant dissociation.

Saliva collection

Participants were instructed to collect saliva samples at waking, 30 minutes after awakening and between 4 and 6 p.m. for 3 school days. They were instructed to swallow, think about a favorite food, sublingually place the oral swab, and maintain the swab in that position for 2 minutes without chewing. After 2 minutes, the oral swab was removed from the mouth and placed in a prelabeled swab storage tube and frozen. Participants were provided detailed,

step-by-step instructions, practiced saliva collection during the research visit until they felt comfortable performing the process independently, and were provided contact information regarding whom to contact if there was a question regarding saliva collection. Saliva samples were collected at least 60 minutes after any consumption of food or strenuous physical activity. Samples were collected using 1 × 4 cm absorbent synthetic oral swabs (Salimetrics, State College, PA). When requested, families were contacted (via text messaging or phone calls) to ensure that the morning and afternoon samples were collected. All participants documented saliva collection times on a log sheet.

In addition, participants indicated the presence of one or more sleep difficulties the evening prior to the morning saliva collection (unable to fall asleep, waking up during the night, nightmares, constantly going to the bathroom, and other disturbances). Samples were stored in the participant's freezer until the research team retrieved all samples, at which point the samples were stored at −80°C until processing. Samples were assayed for sAA in singlet using a kinetic reaction assay, without modifications to the manufacturer's recommended protocol (Salimetrics, State College, PA). The assay utilized 10 μL of saliva, and the lower limit of detection was 0.4 U/mL, with an average interassay coefficient of variation < 10%. For each sample, we determined raw sAA levels (in U/mL) and salivary flow rate (mL/min).

Analytic strategy

For each of the 3 collection days, the sAA awakening response was calculated as the difference between the 30 minute postwaking value and the waking value. For the diurnal slope (i.e., the change in sAA occurring from the 30 minute postwaking to the afternoon sample), a linear regression was fitted for each day, predicting sAA levels from time since waking. Finally, we calculated averages for awakening levels, 30 minute postwaking levels, overall morning levels (i.e., first and second samples), and afternoon levels across collection days. All diurnal sAA measures were calculated using the raw, untransformed sAA levels.

We determined the association (Spearman rho) between raw sAA levels (in U/mL) and salivary flow rate (mL/min). In addition, we examined whether sleep duration and reported sleep problems were associated with diurnal sAA on each collection day using Mann–Whitney *U* tests and Spearman rho correlations. Then, for each participant, the diurnal measures were averaged across collection days. The average for each measure was based on 3 collection days for 100% (afternoon alpha amylase) and 78% (overall morning alpha amylase and diurnal change) of the sample. Reasons for missing data were insufficient specimen volume, extremely high sAA levels (> 1000 U/mL), or because the second sample was not collected 25–35 minutes after the first sample. A square root transformation was sufficient to correct for the skewed distribution of the diurnal slope, sAA levels at 30 minutes postwaking and afternoon sAA levels, as well as for prior adversities and prior potentially traumatic experiences. In addition, one outlier for 30 minutes postwaking and for afternoon sAA levels was winsorized (i.e., recoded to the next highest value in the distribution) (Tabachnick and Fidell 2000). A log transformation was applied to dissociative experiences. As three girls were on medications that could affect levels of sAA, we entered medication use as a covariate.

For the main analyses, analyses of (co)variance (ANCOVA) were used to test differences in trauma-related symptoms (overall PTSD severity of symptoms, as well as individual criterion subscales as delineated in the CAPS-CA; intrusive symptoms, avoidant symptoms, and hyperarousal symptoms), sleep duration and difficulties (the presence of one or more sleep disturbances the prior night), and diurnal sAA between the abused and control groups (controlling for medication use in the analyses for diurnal sAA). We also calculated partial correlations and ANCOVAs to examine the associations among PTSD, depressive, and dissociative symptoms with individual differences in diurnal sAA within the abused group, again controlling for medication use.

Results

Descriptive statistics

In Tables 1 and 2, means, medians, and standard deviations are reported separately for the abused girls and the girls from the control group. Problems with sleep (defined as reporting at least one sleep difficulty during the 3 study days) were more common in the abused group than in the healthy controls (88% vs. 50%, $\chi^2 = 6$, $p < 0.01$). Across all participants, there were no significant associations between sleep duration and diurnal sAA for any of the collection days ($p = 0.18$–0.98). Similarly, reported sleep problems were not associated with diurnal sAA on any of the collection days ($p = 0.11$–0.99), with the exception of the diurnal slope on day 3, which was significantly higher for girls who reported sleep problems during the preceding night than for girls who did not report sleep problems ($p = 0.04$). Spearman rho correlations showed that for all samples, salivary flow rate was not significantly associated with sAA levels (Spearman rho = −0.30–0.24, $p = 0.12$–0.97) and, therefore, sAA levels were not corrected for flow rate.

Table 1. Descriptive Statistics for Sexually Abused Girls and Healthy Comparison Girls

	Healthy comparison (n = 12)	*Sexually abused (n = 24)*
Age (years)	14.8 ± 1.3	15.0 ± 1.5
Ethnicity (%)		
White	50	38
Black	42	42
Other	8	21
Pubertal development		
Breast stage	3.9 ± 0.7	4.4 ± 0.6*
Genital stage	4.2 ± 0.4	4.3 ± 0.8
Months since abuse	-	3.2 ± 1.6
Prior adversities	0.7 ± .8	3.3 ± 1.9**
Prior potentially traumatic events[a]	0.4 ± .5	2.4 ± 2.1**
Depressive symptoms	37.7 ± 4.7	50.8 ± 10**
Dissociative experiences (log)	0.4 ± 0.2	0.9 ± 0.5**
PTSD symptom severity (CAPS-CA score)		
Intrusive recollection	1.0 ± 2.7	10.4 ± 7.7**
Avoidant/numbing	1.9 ± 2.4	15.8 ± 9.7**
Hyperarousal	3.6 ± 3.4	16.1 ± 7.8**
Total CAPS-CA score	6.5 ± 6.6	42.3 ± 22.5**

Means are reflected ± their standard deviations.

[a]Any trauma reported in healthy controls did not meet A2 criterion for DSM-IV-TR PTSD (i.e., did not involve intense fear, helplessness, horror, or agitation) and reported minimal to no trauma symptoms on the CAPS-CA.

*$p < 0.05$, **$p < 0.01$.

PTSD, posttraumatic stress disorder; CAPS-CA, Clinician-Administered PTSD Scale for Children and Adolescents; DSM-IV-TR, *Diagnostic and Statistical Manual of Mental Disorders,* 4th ed., Text Revision.

Adapted from Keeshin et al. 2014.

Table 2. Descriptive Statistics for Diurnal sAA (U/mL, Untransformed) for Abused Girls and Control Girls

	Control (n = *12*)			*Abused* (n = *23–24*)		
	Mean	*Median*	*SD*	*Mean*	*Median*	*SD*
Diurnal sAA						
Levels at awakening	75.82	62.35	43.82	70.50	60.95	44.27
Levels 30 postwaking	51.08	40.79	25.12	54.86	31.53	64.64
Morning levels	64.33	64.32	22.71	55.50	47.62	36.91
Levels in the afternoon	92.66	86.77	38.98	107.21	77.47	102.47
Awakening response	−26.51	−39.96	54.77	−22.99	−14.10	35.34
Diurnal slope	3.92	3.44	3.17	5.49	4.29	5.23

sAA, salivary alpha amylase.

Prior adversities were significantly more common among the abused girls than the healthy comparison participants (3.3 + 1.9 vs. 0.7 + 0.8, $p < 0.001$). Specific prior childhood adversities that were most common among the abused girls were unexpected deaths in the family (63%), caregiver depression or mental illness (54%), and experiencing bullying outside of the home (54%). In contrast, the most common adversities among the comparison subjects were medical procedures (25%) and unexpected deaths in the family (25%). None of the comparison subjects reported any significant current or prior distress related to their prior adversities and none of these experiences met criteria A1 or A2 criteria for PTSD (i.e., abuse or serious and life-threatening violence). Among the 24 sexually abused adolescents, all adolescents experienced their most recent episode of sexual abuse 1–6 months prior to participation in the study. Two of the girls in the study experienced episodes of sexual abuse prior to the 1–6 month window, and on average, sexually abused adolescents had experienced 2.4 lifetime potentially traumatic events, which was significantly more than the comparison subjects' average of 0.4 (Table 1). Age of onset of prior sexual abuse and potentially traumatic events were not collected as part of the study.

Differences in diurnal sAA between abused and healthy comparison subjects

ANCOVAs were conducted to examine differences in diurnal sAA between the abused group and the nonabused, healthy comparison girls; medication use was entered as a covariate. Compared with the healthy girls, survivors of sexual abuse did not differ in terms of absolute values of sAA at awakening (F [1, 33] = 0.03, $p = 0.86$) and 30 minutes after waking (F [1,33] = 0.39, $p = 0.54$), in overall morning levels (i.e., average of the first and second samples; F [1,32] = 0.17, $p = 0.68$) and in afternoon levels (F [1, 33] = 0.01, $p = 0.93$) (Table 2). In addition, there were no significant differences between the abused and control groups for the awakening response (F [1,32] = 0.01, $p = 0.93$) or the increase in sAA levels across the day (F [1,33] = 0.35, $p = 0.56$).

Associations between trauma-related symptoms and diurnal sAA in abused girls

Partial correlations among the diurnal sAA measures and PTSD, and depressive and dissociative symptoms in abused girls, controlling for medication use, are shown in Table 3. When we examined partial correlations between trauma-related symptoms and sAA levels at different time points across the day, the association with dissociative experiences was significant for afternoon levels (r [21] = 0.49, $p = 0.02$). PTSD symptoms were not related to sAA morning awaking response or change in sAA throughout the day. However, PTSD symptoms were associated with higher overall morning levels (r [21] = 0.51, $p < 0.01$) (Fig. 1), which appeared to be driven by hyperarousal (r [20] = 0.58, $p < 0.01$) and intrusive symptoms (r [20] = 0.43, $p < 0.05$).

Discussion

To our knowledge, this is the first study to assess the relationship between diurnal sAA and PTSD-related symptomatology, controlling for duration and type of most recent trauma. Here, we have observed that sAA concentrations, in a cohort of sexually abused adolescent girls, were directly related to the severity of PTSD symptoms. sAA concentrations were examined at three time points over 3 consecutive days, and were associated with symptom severity among the sexually abused girls, which may be an early indicator of ANS dysregulation, and relate to the pathoetiology of PTSD symptoms.

In our cohort of sexually abused adolescent girls, the severity of PTSD symptoms correlated with overall morning sAA concentrations. Importantly, this complements findings by Feldman and others that saw increases in afternoon sAA among war- exposed young children, and this contrasts with findings related to sAA in adults with chronic PTSD, wherein changes in the sAA awakening response but not in absolute morning values of sAA are observed (Thoma et al. 2012). Other markers of stress, especially cortisol, have also demonstrated different properties when measured in children and adults with PTSD, which may be a factor of

Table 3. Partial Correlations for the Abused Group Between Diurnal sAA Variables and Previous Traumatic Experiences, Depression, Dissociative Experiences and PTSD Symptoms, Controlling for Medication Use

	Morning levels	*Awakening response*	*Afternoon levels*
Months since abuse	0.12	0.09	0.31
Previous traumas	0.14	0.19	0.12
Depressive symptoms	0.40	0.01	0.35
Dissociative experiences	0.29	0.17	0.49*
PTSD symptoms			
Intrusive recollection	0.43*	−0.05	0.33
Avoidant/numbing	0.37	−0.15	0.23
hyperarousal	0.58*	−0.14	0.25
Total score	0.51*	−0.13	0.29

*$p < 0.05$. Degrees of freedom ranged between 20 and 21.
sAA, salivary alpha amylase; PTSD, posttraumatic stress disorder.

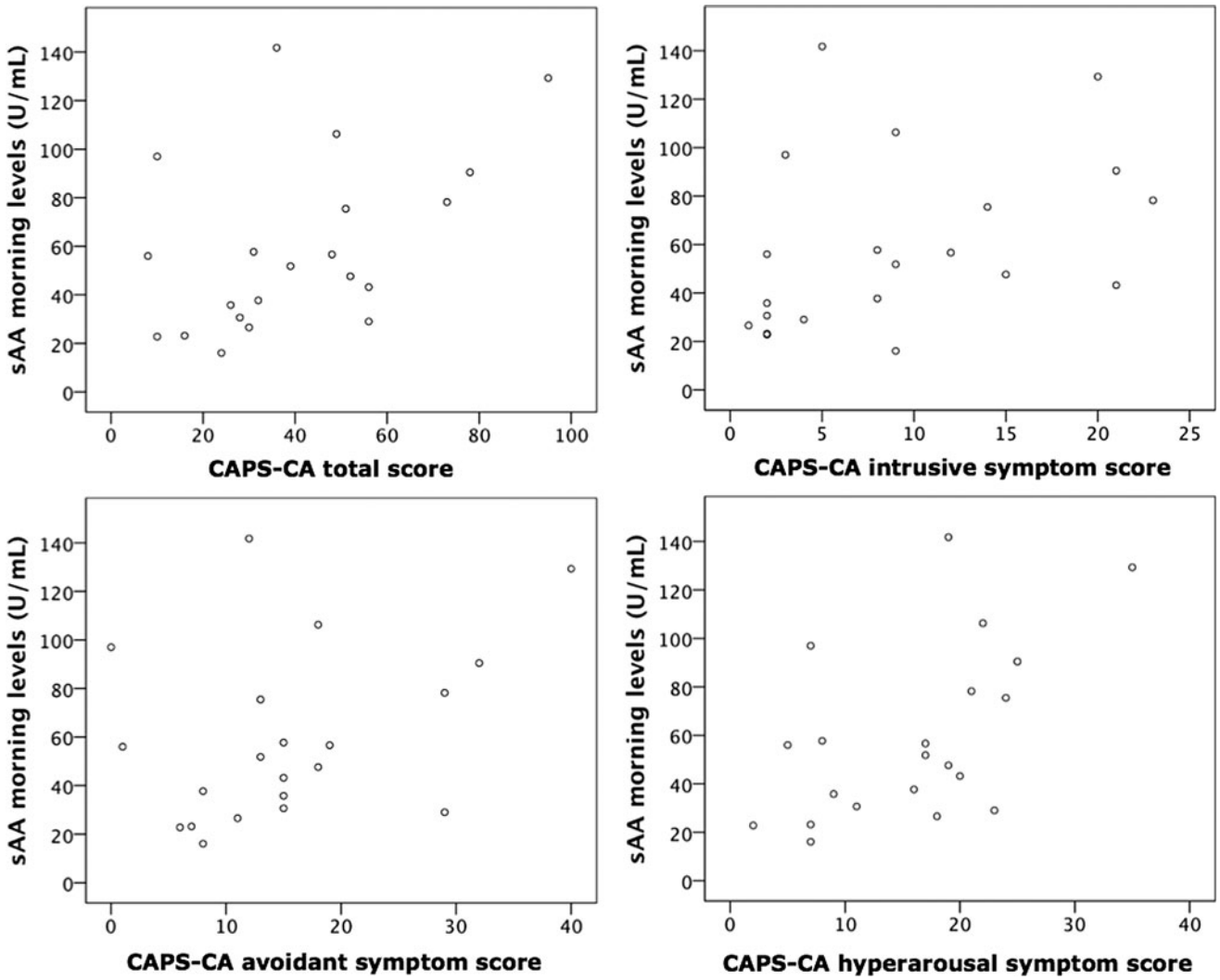

FIG. 1. Scatterplots of the Clinician Administered Posttraumatic Stress Scale for Children and Adolescents (CAPS-CA) score, and morning levels of salivary alpha amylase (sAA) (U/mL) in abused adolescent girls. Significant associations between morning sAA and CAPS-CA symptom severity were observed for total posttraumatic stress disorder (PTSD), intrusive, and hyperarousal symptoms, but not for avoidance symptoms. Girls who were treated with medications were excluded in these figures.

development and/or time since exposure to trauma (Trickett et al. 2010). In our study, we did not observe a significant difference in afternoon levels of sAA between high- and low-symptom girls, which is similar to the findings by Thoma and colleagues, suggesting the potential importance of collection time to the utility of sAA measurements in this population.

The results of studies evaluating nonsalivary peripheral NE have yielded conflicting results with regard to the prognostic value of NE as a biomarker for trauma-related psychopathology, and plasma concentrations of NE may poorly reflect central noradrenergic tone in PTSD (Geracioti et al. 2001). Our study demonstrates an association between severity of PTSD and overall morning sAA. This finding is similar to other findings among children with acute PTSD, in which morning plasma NE levels were elevated in children with PTSD compared to MVA survivors who did not develop PTSD (Pervanidou and Chrousos 2007), and in which there were no differences noted in afternoon NE levels between PTSD and non-PTSD traumatized children. In addition, the elevations in sAA are consistent with studies demonstrating increases in 24 hour catechalomine excretion in pediatric PTSD (De Bellis et al. 1999).

The findings associating dissociative symptoms and sAA are intriguing. Previous studies have demonstrated negative correlations between urinary NE and dissociative symptoms in adults without PTSD (Simeon et al. 2003) as well as in traumatized adults in the peritraumatic period (Delahanty et al. 2003). Our study demonstrates a positive correlation between current dissociative symptomatology and sAA concentrations among traumatized adolescents. This may represent an independent association between sAA and dissociation, or may represent syndromic overlap, with a strong correlation between ADES and CAPSCA total scores present among the abused girls in the current study (r [22] = 0.66, $p < 0.01$). These findings regarding dissociation may be of particular relevance, given the new dissociative subtype specifier of PTSD as described in *Diagnostic and Statistical Manual of Mental Disorders,* 5th ed. (DSM-V), based on the evidence that PTSD with dissociation is not only clinically different than nondissociative PTSD, but also has different underlying biological features (Lanius et al. 2010; American Psychiatric Association 2013).

Cognitive behavioral therapies are the first line treatment for traumatized children and adolescents (Keeshin and Strawn 2014);

however, not all benefit from such interventions. Pharmacologically, adrenergic modulating agents have demonstrated efficacy in reducing or ameliorating nighttime disturbances in adults with PTSD. Specifically, prazosin has demonstrated efficacy in the reduction of nighttime symptoms in multiple randomized control trials in adults (Kung et al. 2012) and in case reports in children (Strawn and Keeshin 2011). Other antiadrenergic agents commonly used in the pediatric population, such as guanfacine and clonidine, have demonstrated mixed results in the treatment of PTSD (Keeshin and Strawn 2014). Future research that incorporates proxies for ANS, such as sAA, may provide a more complete understanding of underlying pathophysiological changes associated with PTSD and treatment response, stratifying children who would most benefit from different treatment modalities as well as opening up new areas of clinical investigation.

Limitations

Although this is the first study of diurnal sAA in sexually abused adolescents, there are several limitations. The ANS operates in concert with other stress response systems that have been implicated in the pathophysiology of PTSD and changes in the hypothalamic pituitary adrenal axis including corticotropin-releasing hormone (CRH) (Geracioti et al. 2008) and cortisol (Yehuda et al. 2002) have been demonstrated in both stressed and nonstressed studies, and may moderate the observed results. As an example, maltreated children with internalizing symptoms demonstrated a blunting of expected diurnal decrease in cortisol throughout the day, suggesting changes in the HPA-axis that may be related to changes in the ANS (Cicchetti et al. 2010). Because NE is involved in HPA-axis function and hemostasis, examination of normal and potential aberrations regarding interaction and negative as well as positive feedback between these two systems may be informative. Second, the sample is relatively small, and does not allow for extensive analysis of covariates that may help explain the observed findings. Third, saliva was collected in the subjects' homes and not in a standardized laboratory setting, although this approach was chosen to minimize secondary stress effects associated with travel to the clinical research setting, and both parents and participants were trained and were required to demonstrate competency in appropriate salivary collection, and all participants documented the timing and duration of saliva collection at home. Finally, although we focused on a relatively brief time period post-abuse, it is possible that dynamic changes in ANS function occur over that time period with relation to symptom severity, and we are underpowered to detect any time by symptom interaction effects.

Conclusions

Adolescent girls with a history of sexual abuse demonstrated a correlation between levels of sAA and symptoms of posttraumatic stress. Overall morning elevation of sAA in sexually abused girls appears to be associated with PTSD symptomatology as well as intrusive symptoms and hypervigilance, reflecting alterations in the ANS, and possibly increased sympathetic tone. In addition, dissociative symptoms may be related to afternoon levels of sAA, reflecting a potentially different biological profile within traumatized individuals who have dissociative symptomatology.

Clinical Significance

These data raise the possibility that alterations in the dynamic secretion of sAA are directly related to the pathophysiology of sexual abuse-related PTSD symptoms in adolescent girls, and that investigation into the utility of antiadrenergic agents for the treatment of pediatric PTSD is warranted.

Disclosures

Dr. Keeshin has received research support from the American Academy of Child and Adolescent Psychiatry and from the Doris Duke Foundation. Dr. Strawn has received research support from the American Academy of Child and Adolescent Psychiatry, Eli Lilly, Lundbeck, National Institute of Mental Health, Forest Research Laboratories, and Shire. Dr. Out was supported by a Rubicon award (446-10-026) from the Netherlands Organization for Scientific Research. Dr. Granger is the founder and Chief Scientific and Strategy Advisor at Salimetrics LLC and Salivabio LLC. Samples were assayed using commercially available Salimetrics assays. The project was not funded by Salimetrics nor did Salimetrics have any input into the process by which the data were gathered, the design of the study, the analysis of the data, or the interpretation of the findings. Salimetrics did not review the manuscript prior to submission and had no input into the content of the manuscript. These relationships of Dr. Granger's are managed by the policies of the conflict of interest committee at Arizona State University. Dr. Putnam reports no biomedical conflicts of interest. Although given the nature of this study, these relationships are not believed to represent conflicts of interest, they are provided in the spirit of full disclosure.

References

American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 4th ed., Text Revision. Washington, DC: American Psychiatric Association; 2000.

American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 5th ed. Washington, DC: American Psychiatric Association; 2013.

Armstrong JG, Putnam FW, Carlson EB, Libero DZ, Smith SR: Development and validation of a measure of adolescent dissociation: The Adolescent Dissociative Experiences Scale. J Nerv Ment Dis 185:491–497, 1997.

Bosch JA, Veerman ECI, de Geus EJ, Proctor GB: α-Amylase as a reliable and convenient measure of sympathetic activity: Don't start salivating just yet! Psychoneuroendocrinology 36:449–453, 2011.

Cicchetti D, Rogosch FA, Gunnar MR, Toth SL: The differential impacts of early physical and sexual abuse and internalizing problems on daytime cortisol rhythm in school-aged children. Child Dev 81:252–269, 2010.

Copeland WE, Keeler G, Angold A, Costello EJ: Traumatic events and posttraumatic stress in childhood. Arch Gen Psychiatry 64:577–584, 2007.

De Bellis MD, Baum AS, Birmaher B, Keshavan MS, Eccard CH, Boring AM, Jenkins FJ, Ryan ND: A.E. Bennett Research Award. Developmental traumatology. Part I: Biological stress systems. Biol Psychiatry 45:1259–1270, 1999.

De Bellis MD, Lefter L, Trickett PK, Putnam FW: Urinary catecholamine excretion in sexually abused girls. J Am Acad Child Adolesc Psychiatry 33:320–327, 1994.

Delahanty DL, Royer DK, Raimonde AJ, Spoonster E: Peritraumatic Dissociation is inversely related to catecholamine levels in initial urine samples of motor vehicle accident victims. J Trauma Dissociation 4:65–80, 2003.

Feldman R, Vengrober A, Eidelman-Rothman M, Zagoory-Sharon O: Stress reactivity in war-exposed young children with and without posttraumatic stress disorder: relations to maternal stress hormones, parenting, and child emotionality and regulation. Dev Psychopathol 25:943–955, 2013.

Geracioti TD, Baker DG, Ekhator NN, West SA, Hill KK, Bruce AB, Schmidt D, Rounds-Kugler B, Yehuda R, Keck PE, Kasckow JW: CSF norepinephrine concentrations in posttraumatic stress disorder. Am J Psychiatry 158:1227–1230, 2001.

Geracioti TD, Baker DG, Kasckow JW, Strawn JR, Jeffrey Mulchahey J, Dashevsky BA, Horn PS, Ekhator NN: Effects of trauma-related audiovisual stimulation on cerebrospinal fluid norepinephrine and corticotropin-releasing hormone concentrations in post-traumatic stress disorder. Psychoneuroendocrinology 33:416–424, 2008.

Gordis EB, Granger DA, Susman EJ, Trickett PK: Salivary alpha amylase-cortisol asymmetry in maltreated youth. Horm Behav 53: 96–103, 2008.

Jensen CF, Keller TW, Peskind ER, McFall ME, Veith RC, Martin D, Wilkinson CW, Raskind MA: Behavioral and neuroendocrine responses to sodium lactate infusion in subjects with posttraumatic stress disorder. Am J Psychiatry 154:266–268, 1997.

Keeshin BR, Strawn JR: Psychological and pharmacologic treatment of youth with posttraumatic stress disorder: an evidence-based review. Child Adolesc Psychiatr Clin N Am 23:399–411, 2014.

Keeshin BR, Strawn JR, Out D, Granger DA, Putnam FW: Cortisol awakening response in adolescents with acute sexual abuse related posttraumatic stress disorder. Depress Anxiety 31:107–114, 2014.

Kung S, Espinel Z, Lapid MI: Treatment of nightmares with prazosin: A systematic review. Mayo Clin Proc 87:890–900, 2012.

Lanius RA, Vermetten E, Loewenstein RJ, Brand B, Schmahl C, Bremner JD, Spiegel D: Emotion modulation in PTSD: Clinical and neurobiological evidence for a dissociative subtype. Am J Psychiatry 167:640–647, 2010.

McFall ME, Veith RC, Murburg MM: Basal sympathoadrenal function in posttraumatic distress disorder. Biol Psychiatry 31:1050–1056, 1992.

Mellman TA, Kumar A, Kulick-Bell R, Kumar M, Nolan B: Nocturnal/daytime urine noradrenergic measures and sleep in combat-related PTSD. Biol Psychiatry 38:174–179, 1995.

Nater UM, La Marca R, Florin L, Moses A, Langhans W, Koller MM, Ehlert U: Stress-induced changes in human salivary alpha-amylase activity – associations with adrenergic activity. Psychoneuroendocrinology 31:49–58, 2006.

Ohan JL, Myers K, Collett BR: Ten-year review of rating scales. IV: Scales assessing trauma and its effects. J Am Acad Child Adolesc Psychiatry 41:1401–1422, 2002.

Pearl E: Childhood Trust Events Survey: Child and Caregiver Versions. 2nd ed. Cincinnati: Trauma Treatment Training Center; 2000.

Pervanidou P, Chrousos GP: Post-traumatic stress disorder in children and adolescents: from Sigmund Freud's 'trauma' to psychopathology and the (Dys)metabolic syndrome. Horm Metab Res 39:413–419, 2007.

Simeon D, Guralnik O, Knutelska M, Yehuda R, Schmeidler J: Basal norepinephrine in depersonalization disorder. Psychiatry Res 121: 93–97, 2003.

Strawn JR, Geracioti TD: Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder. Depress Anxiety 25:260–271, 2008.

Strawn JR, Keeshin BR: Successful treatment of posttraumatic stress disorder with prazosin in a young child. Ann Pharmacother 45:1590–1591, 2011.

Tabachnick BG, Fidell LS: Computer-assisted research design and analysis. Salt Lake City, Utah: Pearson Education; 2000.

Thoma MV, Joksimovic L, Kirschbaum C, Wolf JM, Rohleder N: Altered salivary alpha-amylase awakening response in Bosnian War refugees with posttraumatic stress disorder. Psychoneuroendocrinology 37:810–817, 2012.

Trickett PK, Noll JG, Susman EJ, Shenk CE, Putnam FW: Attenuation of cortisol across development for victims of sexual abuse. Dev Psychopathol 22:165–175, 2010.

Yehuda R, Halligan SL, Bierer LM: Cortisol levels in adult offspring of Holocaust survivors: Relation to PTSD symptom severity in the parent and child. Psychoneuroendocrinology 27:171–180, 2002.

Yehuda R, Siever LJ, Teicher MH, Levengood RA, Gerber DK, Schmeidler J, Yang R-K: Plasma norepinephrine and 3-methoxy-4-hydroxyphenylglycol concentrations and severity of depression in combat posttraumatic stress disorder and major depressive disorder. Biol Psychiatry 44:56–63, 1998.

Address correspondence to:
Brooks R. Keeshin, MD
Center for Safe and Healthy Families
Eccles Primary Children's Outpatient Services Building
81 N. Mario Capecchi Drive
Salt Lake City, UT 84113

E-mail: Brooks.Keeshin@hsc.utah.edu