The Effect of Comorbid Depression and Sexual Abuse during Childhood on Glucocorticoid and Mineralocorticoid Receptor Sensitivity of Patients with Post-Traumatic Stress Disorder

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Introduction

Posttraumatic Stress Disorder (PTSD) is a highly prevalent disorder [1], and diagnosis requires the presence of a stressor event that threatened the person's emotional or physical integrity [2].

Studies of the Hypothalamic-Pituitary-Adrenal (HPA) axis and autonomic nervous system are critical because they establish a relationship between the body/mind and the environment. Consequently, the activity of these systems has been highly scrutinized in PTSD patients. Although acute stressors activate the HPA axis, studies of combat veterans, Holocaust survivors, refugees, and abused subjects, all of whom have been diagnosed with PTSD, reveal a paradoxical decrease in urine or blood cortisol levels compared to healthy controls and other diagnostic groups [3]. While this counterintuitive finding has been replicated, other findings have not been as consistent [4]. Differences in the type and timing of the psychological trauma, as well as symptom patterns, the presence of comorbid conditions, personality type, and genetic disposition (among other factors) may contribute to this inconsistency [4].

Hypocortisolism in PTSD occurs in the context of increased HPA-axis sensitivity to negative glucocorticoid (GC) feedback [3], despite a marked and sustained increase in the Corticotrophin Releasing Factor (CRF) concentration in the Cerebrospinal Fluid (CSF) [5]. The evidence of a blunted ACTH response to CRF stimulation in PTSD patients supports the hypothesis that its pathology includes elevated levels of hypothalamic CRF activity and the consequent down regulation of pituitary CRF receptors [3]. Together, this constellation of neuroendocrine findings in PTSD patients reflects the sensitization of the HPA as when these patients are exposed to stressors. This neuroendocrine pattern distinguishes PTSD from Major Depressive Disorder (MDD), a frequently comorbid (but distinct) disorder, which could partially be responsible for the inconsistency of HPA axis studies in PTSD.

Another factor that could be responsible for this inconsistency is a history of childhood maltreatment. There is extensive literature evaluating HPA axis function in individuals with a history of childhood maltreatment. Heim et al. used the Trier Social Stress Test (TSST) to evaluate neuroendocrine and autonomic responses in four groups of women who had been carefully categorized according to the presence or absence of early-life abuse and current major depression [6]. Women with a history of childhood abuse, with or without current major depression, exhibited increased ACTH responses to stress compared with controls (6-fold greater when abused depressed women were compared to controls). Abused women who were not currently

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depressed exhibited normal cortisol responses, despite their increased ACTH response, potentially indicating adrenal adaptation to central sensitization as a marker of resilience against depression after early stress.

Heim et al. who analyzed their data using multiple regression techniques, showed that childhood maltreatment was the strongest predictor of ACTH responsiveness, followed by a number of abuse events, adulthood trauma and depression [7]. Studies of early-life stress in laboratory animals had previously suggested that HPA axis and autonomic nervous system hyper-reactivity, presumably due to CRH hyper secretion, may be a long-lasting consequence of childhood abuse [8]. In contrast, Carpenter et al. found that men and women with a history of childhood maltreatment and no history of depression had decreased cortisol responses to the TSST [9].

Heim et al. used pharmacological challenge tests, CRH and ACTH stimulation tests, on depressed and non-depressed women with and without a history of childhood maltreatment [10,11]. They found that abused women who were not depressed exhibited increased ACTH responses to CRH, but both groups of depressed women (with and without childhood maltreatment) exhibited a blunted ACTH response to CRH, which is consistent with many previous studies of major depression [12,13].

Abused women without depression had a lower cortisol response than other groups after the ACTH stimulation test [10]. Laboratory studies on early-life stress using non-human primate models have demonstrated similar results [14,15]. Heim et al. hypothesized that their results could reflect both a sensitization of the pituitary and a counter-regulatory adaptation of the adrenal gland in abused women without current depression. Because cortisol has inhibitory effects on the central CRH and noradrenergic systems, a relatively decreased availability of cortisol, as a consequence of childhood trauma, might facilitate disinhibition of central stress responses. When subjected to even more stress, such women might then repeatedly hyper secrete CRH, eventually resulting in pituitary CRH receptor down regulation and symptoms of depression through CRH effects in extra-hypothalamic circuits [8].

However, caution is required when interpreting such findings, as underscored by Carpenter et al. who used multiple regression analysis in an effort to disentangle the effects of depression and early life stress on CSF CRH in adults [16]. In their study of depressed and healthy control adults, perceived early-life stress was significantly correlated with concentrations of CSF CRH, but the presence of depression was not. However, the relationship between CSF CRH and early-life stress was complex: CSF CRH concentrations were negatively correlated (i.e., lower) with adversity in perinatal and pre-teen years (ages 6-13 years), but were positively correlated (i.e., higher) with stress in the preschool years (ages 0-5 years). These findings were interpreted in light of considerable preclinical research in laboratory animals, demonstrating that the timing of exposure to stressors in early life is critically important in determining their long-term effects on neurobiology and behavior.

Heim et al. used the combined dexamethasone/CRH (Dex/CRH) test to study HPA axis function in men with and without major depression and childhood abuse [17]. Abused men demonstrated increased cortisol responses compared to non-abused men, regardless of the presence of major depression. Increased responses were associated with exposure to both sexual and physical abuse and were correlated with the severity of abuse. Their results suggested that childhood maltreatment is associated with impaired glucocorticoid-mediated feedback control of the HPA axis under stimulated conditions.

Once again, however, specific factors relating to the nature of the abuse can markedly affect the results of such studies. Carpenter et al. (2009) recently reported their findings using the Dex/CRH test in a large sample of 230 adults without major Axis I disorders. [18] Using a general linear model analysis, the authors tested for a large number of potentially confounding variables, including age, gender, education level, socioeconomic adversity in childhood, current depressive and anxiety symptoms, smoking, exogenous hormone use (for contraception or estrogen replacement) and types of maltreatment. They found that a history of self-reported childhood emotional abuse was independently and significantly associated with diminished cortisol response to the Dex/CRH test, whereas physical abuse, sexual abuse, emotional neglect and physical neglect had no significant independent effects.

The Dexamethasone Suppression Test (DST) is used to evaluate GC negative feedback, particularly at the pituitary level [19]. Although greater cortisol and ACTH suppression after the DST has been found in PTSD patients, this finding does not explain much about the contribution of higher brain areas to the activity of the HPA axis because dexamethasone does not easily penetrate the blood-brain barrier; therefore, it does not bind to the Mineralocorticoid Receptor (MR) like cortisol and has different pharmacokinetics from endogenous corticoids [20]. The Prednisolone Suppression Test (PST) avoids this problem because its pharmacological properties bear great similarity to endogenous cortisol, and its effect on the HPA axis is closer to normal physiological adaptations [21]. Prednisolone has been used to probe the HPA axis in psychiatric patients since 2006. Juruena et al. [22] specifically compared the effects of prednisolone with dexamethasone in the same depressed patients. Moreover, in this sample of depressive adults, 83% of which had a history of childhood trauma such as parental neglect or antipathy, physical abuse, or sexual abuse, the authors concluded that that the additional effects of prednisolone on the MR explain the different responses to these glucocorticoids in the same sample of depressed subjects.

The present study was carried out to test the hypothesis that comorbidity with MDD and previous history of childhood sexual abuse would interfere in the activity of GC negative feedback. To test this hypothesis, salivary cortisol levels were evaluated before and after PST in a group of subjects who had been exposed to highly traumatic events sometime within the last three years, who either did or did not develop posttraumatic stress disorder (PTSD+ and PTSD−, respectively). PTSD is a unique condition compared to other psychiatric disorders in that having a severe traumatic event is a necessary condition to consider in the diagnostic. In this way, PTSD is an organism's pathological reaction to this traumatic event, which requires that researchers use subjects that were exposed to traumatic events but did not develop PTSD as a control group in contrast to other psychiatric disorders such as MDD.

Materials and Methods

Subjects

Subjects were drawn from a randomly sampled epidemiological survey including 3,000 participants conducted in São Paulo, Brazil that initially identified the subjects enrolled in the present study [23].

The inclusion criteria for the epidemiological survey were the following: being the victim of urban violence consistent with criterion A of DSM-IV for a PTSD diagnosis, a diagnosis of PTSD and being between 18 and 60 years of age. Upon enrollment, each subject was assessed through the WHO-Composite International Diagnostic
Interview (CIDI). Exclusion criteria included a lifetime history of bipolar or any psychotic disorder, substance dependence or abuse (except nicotine and caffeine) in the previous 6 months and having a MDD diagnosis prior to the traumatic event.

From the epidemiological survey, the following two groups were formed: individuals who were exposed to a traumatic life experience resulting in a PTSD diagnosis (cases or PTSD+1) and those who were similarly exposed but did not meet the criteria for a PTSD diagnosis (controls or PTSD-). The epidemiological survey interviewer informed subjects about the case-control study procedures [24] and invited them to participate in the present study. Those who agreed were invited by phone or mail to come to the outpatient clinic. The subjects were informed about the procedures, which included psychometrics and neuropsychological, imaging, genetics, and HPA axis studies. Many subjects did not participate because they had to take prednisolone; control subjects had a higher refusal rate.

In addition to the CIDI, all patients were administered the Structured Clinical Interview for DSM-IV Axis I and Axis II (SCID-I and SCID-II, respectively) by a trained psychiatrist. Patients were eligible for inclusion in the study if they met DSM-IV criteria A for a diagnosis of PTSD [25,26]. Subjects who were considered PTSD+ by CIDI but not by SCID-I were assigned to the control group. Patients were excluded from the study altogether if they 1) met SCID criteria for diagnosis of borderline personality disorder, bipolar disorder, panic disorder, generalized anxiety disorder, obsessive-compulsive disorder, major depressive disorder just prior to the traumatic event, or showed psychoactive substance dependence in the last 6 months; 2) had an HPA-related disease (regardless of duration, e.g., Cushing's or Addison's disease); 3) were currently using any psychotropic medication, such as antidepressants, antipsychotics, tranquilizers, or mood stabilizers; 4) were not free of psychotropic for 2 weeks for most antidepressants and anxiolytics, or 6 weeks in the case of fluoxetine and 1 year for antipsychotics; 5) were using drugs that altered the HPA axis or 6) were not able to understand the consent form.

Subjects were evaluated using the Early Trauma Inventory (ETI) [27] to investigate any reported history of abuse or other traumatic events during their childhood or adolescence. The validity of the Portuguese version of the ETI has been established previously [28]. Additionally, patients completed the Clinician-Administered Posttraumatic Stress Scale (CAPS) [29] and the Beck Depression Inventory (BDI) [30]. These questionnaires have also been adapted to and validated for use in Brazilian Portuguese [31,32].

All subjects were physically healthy based on a complete medical history. They were not taking any hormonal medications (including oral contraceptives) and had no history of hypersensitivity to corticosteroids. Women were only tested during the follicular phase of the menstrual cycle.

Urine tests for illicit drugs and pregnancy were conducted before starting the PST protocol. For this protocol, all subjects were instructed to take a 5 mg prednisolone capsule at 10:00 PM and then abstain from alcohol, coffee, tea, and food for 30 minutes after awakening. Subjects were then assessed for cortisol levels using Salivettes at awakening, 30 minutes later, and then again at 12:00 PM, 4:00 PM and 6:00 PM.

The epidemiological survey identified 167 subjects with PTSD, 64 with subclinical PTSD and 90 controls. Of these, 144 subjects came to the facility and agreed to be interviewed, 43 of which had confirmed PTSD after SCID-I administration and 101 of which had no psychiatric diagnostics. Seventy-five subjects refused to join the HPA axis protocol, 18 did not show up for the next interview with saliva samples, and the samples of 3 subjects were considered technical losses (Figure 1 for details on inclusion/exclusion procedures).

The study protocol was approved by the Research Ethical Committee of the University Federal de São Paulo (UNIFESP-Brazil). The subjects were seen as part of the Program for Victims of Violence of the Department of Psychiatry of UNIFESP, and they gave voluntary written informed consent to participate in this study.

**Instruments**

- **Structured Clinical Interview for DSM-IV Axes I and II (SCID-I and SCID-II)** [25,26] are semi-structured interviews that determine whether a subject can be diagnosed with any Axis I or Axis II disorder, respectively, according to DSM-IV criteria [2].
- **Clinician-Administered PTSD Scale (CAPS)** [29] is a structured interview developed to diagnose PTSD and rate its severity. It contains 30 items designed to assess the frequency and severity of PTSD-related symptoms. Scores can range from 0 to 136 and are classified as follows: subclinical 0 to 19; mild 20 to 39; moderate 40 to 59; severe 60 to 79; and extreme 80 and above.
- **Beck Depression Inventory (BDI)** [30] is a 21-item self-report inventory designed to measure the severity of depression. Scores can range from 0 to 63 and are classified as follows: minimal 0 to 11; mild 12 to 19; moderate 20 to 35; and severe 36 to 63.
- **Early Trauma Inventory (ETI)** [27] is a 56-item semi-structured interview that measures traumatic experiences taking place during childhood and the teenage years. Experiences are divided into the following four clusters: physical (9 items), sexual (11 items), psychological/behavioral abuse (8 items), and general trauma (24 items). All ETI items are evaluated according to their frequency and duration, the stage of development in
which the event occurred, and the impact that the event had on the subject. A general score is calculated by multiplying the frequency of each positive item by its duration (measured in number of years). The scores of individual items within each cluster are then added up to calculate the total severity of early trauma for a given cluster. Clusters with more items are given greater weight in the total score because there are differences in the number of items in each cluster; we weighted the scores by dividing the sum of each positive answer within a cluster by the number of items in the cluster. Therefore, the total score was the sum of all positive items divided by 56 or the total number of items. In this way, both the cluster and total scores varied from 0 to 1, with 0 indicating no early trauma and 1 indicating extreme early trauma.

- **Peri-traumatic Dissociative Experiences Questionnaire, Self-Report Version (PDEQ-SRV)** [33] assesses dissociative experiences that occurred during and immediately following (defined as minutes and hours) a traumatic event. Participants rated the degree to which they experienced depersonalization, de-realization, amnesia, out-of-body experiences, altered time perception and body image on a 5-point Likert scale (1 = not at all true, 2 = slightly true, 3 = somewhat true, 4 = very true, 5 = extremely true). The PDEQ-10SRV was scored as the mean item response across all items, and it was translated and culturally adapted from American English to Brazilian Portuguese by Fizman et al. [34].

- **Prednisolone Suppression Test (PST).** The dexamethasone suppression test (DST) was originally developed to evaluate negative feedback of the HPA axis by measuring plasma cortisol levels after the intake of 1 mg of dexamethasone [35]. Recently, lower dexamethasone doses have been used to detect an increase in negative feedback [22,36-38]. However, dexamethasone has pharmacodynamics and pharmacokinetic characteristics that are distinct from endogenous GC. Specifically, dexamethasone binds only to the GR [39], does not bind to corticosterone-binding globulin (CBG) [40], and has a much longer half-life than cortisol [41]. Prednisolone, another synthetic corticosteroid used in suppression tests, is more similar to cortisol in its GR [42] and CBG [40] binding properties as well as its half-life [43], in addition to being able to bind mineralocorticoid receptors (MR).

Cortisol, both in vitro and in vivo, has a much higher affinity for the MR [39,44] than for the GR [39,44]. In contrast, dexamethasone binds with a very high affinity to the GR both in vitro and in vivo [39,44,45], whereas it does not bind to the MR in vivo [39,46], and in vitro the dexamethasone–MR complex is much less stable than the dexamethasone–GR complex [46]. In studies measuring steroid binding in living cells, prednisolone and cortisol have shown a similar affinity for the MR, whereas dexamethasone affinity is approximately 70% lower [47]. In studies measuring the relative potency of these steroids to activate GR function, prednisolone has the same [48] or slightly higher (two-fold) [49] activity than cortisol, whereas dexamethasone potency is four- to nine-fold that of cortisol [48,49].

Due to these characteristics, prednisolone represents new possibilities to evaluate the effect of both the MR and the GR on HPA functioning in psychiatric disorders [50,51]. For example, a reduction in both MR and GR function has been described in patients with major depression, and both MR and GR are influenced by antidepressant treatment [52-54]. On the other hand, a recent study suggests that the response to prednisolone does not change with symptomatic improvement. This is in contrast with findings using other measures of HPA axis function, such as basal cortisol levels or the response to dexamethasone, suggesting that the prednisolone suppression test may offer specific biological and clinical information related to its action on both the GR and the MR [54].

Previous studies have shown that 20 mg/day of prednisolone induces cortisol suppression [55]. Pariante et al. [21], who developed the PST in healthy controls, report that prednisolone suppresses salivary cortisol to a larger extent than plasma cortisol, and compared to dexamethasone, plasma–salivary correlations are more consistent in the PST. They propose that administration of 5 mg prednisolone, which suppresses approximately 30% of salivary cortisol secretion, is the ideal tool to investigate enhanced or impaired glucocorticoid-mediated negative feedback on the HPA axis in large samples of patients with psychiatric disorders [50,51].

The Salivettes® kits brought back by the subjects were prepared and sent to a laboratory to be stored for further analysis. After sample collection from all the subjects, cortisol levels were analyzed from the stored saliva using the ELISA method. All samples were assayed in triplicate using a cortisol kit from Salimetrics. The samples were analyzed at Genese Produtos Diagnósticos Ltd., and all data were kept in a data bank.

**Statistical analysis**

All sociodemographic (gender, age) and clinical variables (PTSD, depressive, and anxiety symptoms, severity of early life abuse, and peri-traumatic dissociative experience) were analyzed with the chi-square, student’s T-test or univariate analysis of variance, as appropriate, compared to groups of victims of violence with and without PTSD. The significance level used was 5%. According to SCID-I, subjects were separated into the following three groups: PTSD+, PTSD+MDD, and PTSD- (controls). To compare salivary cortisol concentrations across time, data were subjected to a non-parametric repeated measures ANOVA for ordinal data [56]. The following groups were compared: PTSD+ and PTSD-, history and absence of early trauma, and presence or absence of PTSD and/or MDD.

To evaluate the factors that influenced salivary cortisol concentration in PTSD patients, we used a general linear model (GLM) regression using the gamma distribution family. The response variable was cortisol levels after awakening, and the clarifying variables included in the initial model were the following: group, age, gender, CAPS, BDI, BAI, ETI 1 (general trauma), ETI 2 (physical trauma), ETI 3 (emotional trauma), ETI 4 (sexual abuse), PDEQ and comorbid MDD.

**Results**

We examined 48 subjects (35 females and 13 males), of which 34 were in the PTSD+ group and 14 were in the PTSD- group. The average age of the entire group was 37.8 (s.d. = 12.4) years. There was no difference between groups (PTSD+ vs. PTSD-) regarding age or gender (Table 1). The most frequent types of traumatic events were the following: robbery with a gun (12), homicide of a close relative (11), gender (Table 1). The most frequent types of traumatic events were the following: robbery with a gun (12), homicide of a close relative (11), kidnapping with imprisonment (6), sexual abuse (6), physical violence (5), severe accident (4), and domestic violence (4).

The PTSD+ and PTSD- groups differed in CAPS, BDI and BAI scores. As expected, victims of violence who developed PTSD had more PTSD, depressive and anxiety symptoms compared to those who did not develop an Axis I diagnosis. However, there were no significant differences between groups regarding history of early
traumas as measured by either ETI total or ETI cluster (general trauma, physical abuse, emotional abuse, and sexual abuse) scores. There were no differences between groups regarding peri-traumatic dissociative experiences when comparing their PDEQ scores (Table 1).

Nineteen (39.6%) patients with PTSD presented comorbid MDD, 15 (31.2%) patients with PTSD alone, and 14 (29.2%) were victims of violence who did not fulfill criteria for an Axis I diagnosis (i.e., the control group).
Comparison of salivary cortisol concentrations through non-parametric repeated measures ANOVA for ordinal data revealed that there is an interaction effect between group and time at the 5% significance level, revealing that there are differences among the groups (QA\text{between-subject} = 1.154, df = 1.862, p = .2828; QA\text{within-subject} = 16.129, df = 3.108, p = .0000; QA\text{interaction} = 2.482, df = 5.665, p = .00296). PTSD+ subjects had lower salivary cortisol concentrations than PTSD- ones (Table 2).

In analyzing cortisol curves, we identified differences at awakening and 30 minutes after between PTSD+ group compared to the PTSD+/MDD+ and control groups (Figure 2). The general linear model (GLM) regression showed a negative correlation between salivary cortisol levels after awakening and CAPS (-.0069; se = .0016; $p = .06616$) and BAI, (-.0094; $se = .0035; p = .01199$) and ETI (sexual abuse) (-.7222; $se = .3792; p = .06616$) scores; higher clinical scores were associated with lower cortisol concentrations after awakening.

**Discussion**

These results confirmed previous findings that PTSD patients have a blunted response to exogenous GC (in this case, prednisolone), which is likely due to increased GR and MR responsiveness. It is important to reiterate that control subjects were also exposed to traumatic events, which reinforces the fact that the HPA axis dysfunction is specific to PTSD development.

Separating the patients with PTSD alone from those with comorbid MDD showed that a blunted awakening response to exogenous GC was specific to PTSD and that the presence of MDD counterbalances PTSD-related dysfunction to “normalize” the curve of patients with both diagnoses. This was our a priori reason for excluding patients, who were either depressed previously or when victimized, and the data confirm our rationale; we avoided including individuals with a previously affected stress response system.

Another factor that showed a negative correlation with cortisol levels after exogenous GC was the presence of sexual abuse during childhood, so the more severe the sexual abuse and its impact on the individual was, the lower the salivary cortisol levels were after awakening in response to prednisolone. In our sample, only sexual abuse during childhood, but not other types of early abuse such as general, physical, and psychological trauma, was correlated with this cortisol dysfunction. This finding raises some important points for discussion. The literature shows that presence of sexual abuse during childhood is a potent risk factor for developing an Axis I diagnosis during adulthood [57]. Some authors have found a correlation between early abuse and lower cortisol concentrations after a psychosocial stressor test and pharmacological challenge, an effect that is independent of the presence of an Axis I diagnosis [16,58,59]. The question raised here is whether the presence of severe sexual abuse during childhood and this specific HPA axis response to stress could be the specific characteristics of a group of patients. Another aspect to consider is whether subjects with a particular type of HPA axis response are at a greater risk for PTSD development after a severe adulthood stressor: PTSD related to increased GR super sensitivity should be investigated further. Research should also address whether the presence of these specific characteristics (GR sensitivity plus sexual abuse during childhood) depends on the timing of the trauma (considering epigenetic phenomena). Interestingly, we did not find differences between controls and PTSD patients on any ETI scores, including the total and sexual abuse scores; therefore, sexual abuse was not exclusive to those who developed PTSD after a traumatic event. This shows the complexity involved in combining multiple factors to determine the neurobiology of psychopathological conditions. More studies are necessary to better understand this mechanism.

Our findings reinforce the idea that PTSD symptoms are related to hyper-responsive GC receptors. The PST allows us to include the MR as part of this dysfunction as it plays roles that are different from dexamethasone, which acts exclusively on GR [22]. The findings were only related to PTSD symptoms, with more severe PTSD symptoms related to greater receptor sensitivity to prednisolone.

Jerjes et al. (2007) found an enhancement of negative feedback control of the HPA axis in patients with Chronic Fatigue Syndrome (CFS), another low-HPA axis activity similar to PTSD. The authors found that salivary cortisol was lower in CFS subjects before prednisolone treatment than in controls. Urinary cortisol metabolites were lower in CFS subjects before prednisolone treatment, but were not significant. Both measures were significantly lower in CFS subjects after the prednisolone administration [60]. The mean percentage suppression of both salivary cortisol and urinary cortisol metabolites was significantly higher in CFS subjects compared to the controls after PST [60,61]. Despite the fact that 40% of our sample had PTSD+MDD, the presence of PTSD symptoms had a predominant effect on the HPA axis by lowering cortisol levels after awakening after exogenous GC administration as demonstrated by our comparison of PTSD+ (either with or without MDD) with controls.

Nonetheless, the present study has some drawbacks that must be addressed. These include the small number of patients, the mixed gender of the sample, and the lack of more rigid control over the cortisol sampling time (because the subjects were at home). We did not calculate area under the cortisol concentration curve because of this last drawback.

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