

Alterations in Stress Reactivity After Long-Term Treatment with Paroxetine in Women with Posttraumatic Stress Disorder

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ABSTRACT: Posttraumatic stress disorder (PTSD) is typically accompanied by both acute and chronic alterations in the stress response. These alterations have mostly been described in individuals under baseline conditions, but studies have also used a challenge model to assess the role of the hypothalamic-pituitary-adrenal (HPA) axis in the stress response. The purpose of this article was to assess the effect of long-term treatment with the selective reuptake inhibitor (SSRI), paroxetine, on stress reactivity in patients with PTSD. We assessed diurnal salivary cortisol and urinary cortisol as well as cortisol, heart rate, and behavioral responses to a standardized cognitive stress challenge, in 13 female patients with chronic PTSD before and after 12 months of paroxetine treatment. Treatment resulted in a significant decrease in PTSD symptoms. Twenty-four-hour urinary cortisol was lower compared to base line after successful treatment. Treatment resulted in a decrease of salivary cortisol levels on all time points on a diurnal curve. Despite similar stress perception,

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cortisol response to the cognitive stress challenge resulted in a 26.5% relative decrease in stress-induced salivary cortisol with treatment. These results suggest that successful treatment with SSRI in chronic PTSD is associated with a trend for a decrease in baseline diurnal cortisol and with reduced cortisol reactivity to stress.

KEYWORDS: cortisol; PTSD; stress; paroxetine; SSRI; challenge; HPA axis

INTRODUCTION

The last decade has seen a rapid expansion of studies on hypothalamic-pituitary-adrenal (HPA) axis reactivity in posttraumatic stress disorder (PTSD). These studies have shown an array of alterations related to HPA axis function, with varying specificity for the disorder. While, baseline studies of the HPA axis have yielded mostly mixed results, findings of increased corticotrophic-releasing hormone (CRH) in cerebrospinal fluid (CSF)^{1,2} and decreased plasma cortisol over a 24-h period^{3,4} have been more consistent. Mixed results have been reported in 24-h urine excretion⁵⁻⁷ and single time point plasma cortisol studies have been reported. For example, low cortisol in urine and plasma samples has been reported in some studies of adults with PTSD⁸⁻¹⁰ but not in others.¹¹⁻¹⁹ In a recent study, cortisol was assessed in CSF showing increased levels in PTSD patients.²⁰ Additionally, some studies demonstrated flattened free cortisol response after awakening.⁸⁻¹⁰

Several pharmacological challenge paradigms have been used to further assess HPA axis function.²¹ The outcome of CRH challenges,²²⁻²⁵ adrenocorticotrophic hormone (ACTH) challenges,^{22,24} and metyrapone challenges²⁶⁻²⁸ are so far inconclusive. In contrast, despite methodological differences, the low-dose (0.5 mg) dexamethasone suppression test in plasma has consistently found enhanced suppression of cortisol in subjects with PTSD,²⁹⁻³⁴ as well as enhanced suppression of ACTH.^{34,35} Only one study that measured salivary cortisol showed no group difference in cortisol suppression.³⁶

Among the factors that contribute to inconsistencies in outcome in base line and challenge designs are differences in study populations, such as early childhood abuse versus (adult) combat trauma as well as differential contributions of early-life trauma on later-life psychopathology in general.

In PTSD, the response of the HPA axis to nonpharmacological challenges has been less extensively studied. To date, in PTSD patients, two studies assessing HPA axis reactivity to a cognitive stress challenge have been performed. Bremner *et al.*, using a series of arithmetic tasks under time pressure showed significant differences between PTSD patients and healthy controls in cortisol levels during the anticipation phase as well as during and after challenge.²² Elzinga *et al.*, assessed cortisol responsivity to traumatic reminders using a personalized traumatic script in abused women with and without current PTSD,³⁷

and also showed increased cortisol levels in patients with PTSD during the challenge paradigm.

It has been hypothesized that the inability to dampen responses to cues that do not represent true threat, is related to stress-sensitized dysfunctional neural circuitry. These nonpharmacological challenge studies, when performed before and after a specific treatment intervention, can provide important information about the effects of that treatment on biological alterations and reactivity.

The purpose of the current study was to assess the salivary cortisol and autonomic response to a cognitive stress challenge paradigm following chronic paroxetine treatment. We hypothesized that PTSD patients who responded to treatment with paroxetine would show decreased salivary cortisol response after treatment.

While the efficacy of the selective reuptake inhibitor (SSRI), paroxetine, has been reported for clinical measures of PTSD,^{38,39} no studies have yet looked at the effects of SSRIs on HPA axis functioning in PTSD. An important question is whether the alterations found on HPA axis functioning in PTSD patients are reversible. Tucker *et al.* demonstrated that 10 weeks of open label administration of fluvoxamine dampened autonomic reactivity to trauma scripts in PTSD compared to controls, the reported efficacy of fluvoxamine.⁴⁰ Nikisch *et al.* looked at long-term (16 weeks) citalopram administration in patients with major depression and found reduced responsiveness of HPA axis.⁴¹ Their results showed a time-dependent reduction of ACTH and cortisol response during the combined dexamethasone/CRH test both in treatment responders and nonresponders. Rinne *et al.* found that 12 weeks of fluvoxamine treatment⁴² in women with Borderline Personality Disorder (BPD) resulted in a reduction of the hyperresponsiveness of the HPA axis on the dexamethasone/CRH test.

SUBJECTS AND METHODS

Subjects

Patients in this study were part of a larger open label study on the effects of paroxetine on memory and hippocampal volume. Participating patients in this study were male and female outpatients diagnosed with PTSD. They were recruited through advertisements in the local newspapers. Diagnosis was on the basis of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria for PTSD, as determined with the Structured Clinical Interview for DSM-IV (SCID⁴³), consensus diagnosis by three research psychiatrists (EV, MV, and JDB), and a score of greater than 50 on the Clinician Administered PTSD Scale⁴⁴ (CAPS, current symptoms version). In case of comorbid disorders, the PTSD symptom onset had to predate the onset of comorbid

psychopathology. History of childhood trauma was assessed with the early trauma inventory⁴⁵ (ETI).

Some patients ($n = 6$) in this study were part of a national double-blind trial of paroxetine versus placebo in PTSD. Following 12 weeks of participation in a double-blind study, subjects were offered participation in the current study of 9 months open trial treatment of 10–30 mg paroxetine per day. Assessments of these patients were performed before entering the double-blind phase; in all other patients this was performed before open label treatment. Patients with a serious medical or neurological illness were excluded, on the basis of history and physical examination, lab testing, and electrocardiogram. Patients with a history of psychotherapeutic treatment within 3 months of the study start, and a positive toxicology screening were also excluded. Female subjects were required to have a negative pregnancy test before the study. Patients with a history of alcohol or drug abuse 6 months prior to the study, as well as current alcohol abuse or drug use were excluded from participation. All subjects were medication free for at least 4 weeks before participation in the study. During the study no concomitant antidepressant or neuroleptic medication was allowed. After complete explanation of the study procedures and before entering the study, written informed consent was obtained. This study was approved by the Investigational Review Board (IRB) of Yale University and was prepared in accordance with the ethical standards laid down in the Declaration of Helsinki.

A total of 28 patients passed the inclusion criteria and started their participation in the open label study. Eleven patients (5 female, 6 male) of this group participated in the double-blind trial and received placebo or 20–50 mg of paroxetine for 12 weeks prior to open label treatment. Twenty-three completed the complete open label study protocol (9 male and 14 female). We were able to collect posttreatment data for this study in 5 male and 14 female patients. Two of the males, and one of the females had to be left out of the total subject sample, because either cortisol could not be assessed on account of too little saliva throughout the cognitive challenge, or noncompliance with the challenge protocol. Because including only three males would skew our sex distribution, we decided to leave the males out of further analysis. For the analysis we used data of 13 females, with an average age of 42.9 years ($SD = 10.7$). Four of the females were premenopausal, and nine were postmenopausal. Of these patients 12 were white, and 1 was Hispanic. The average score on the CAPS was 87.8 ($SD = 21.7$). Nine patients had a lifetime diagnosis of major depressive disorder (MDD), and six patients had a current depressive disorder. Three patients were also diagnosed with comorbid generalized anxiety (GAD) without agoraphobia, and two met criteria for social phobia. In four patients PTSD was the sole diagnosis. Onset of PTSD was typically in childhood, and in all cases preceded the other comorbid disorders, including affective disorders, by at least several years. In nine patients trauma occurred before the age of 18 years, in four patients primary trauma happened later in life. In all cases of

early childhood trauma this was reported as sexual abuse. In later life traumas the witnessing of murder, assault, torture, and a motor vehicle accident were reported as the primary trauma. Mean score on the ETI-self report scale in the early childhood trauma group was 80 (SD = 37.8). For the other patients this was 42.5 (SD = 43).

Diurnal Cortisol and 24-h Urine Collection

All participating patients collected saliva over a 24-h time period using the same salivettes as in the cognitive challenge procedure. This involved collection of saliva at five time points (morning awaking, 8:00 AM, 12:00 AM, 4:00 PM, and 8:00 PM) before and after treatment. They were also instructed to collect urine over a 24-h time period and repeated this after treatment. Collection of saliva and urine and the cognitive challenge were performed on separate days.

Challenge Procedure

The cognitive challenge tests were on the basis of a protocol previously used in studies of aging^{46,47} and included arithmetic (multiplication, division, addition, and subtraction), cognitive tasks (Stroop task, e.g., looking at the word red spelled in color green and naming the color green), problem solving, matching figures to numbers and memory for figure–number pairing, and unscrambling words.²² All subjects were studied in the time interval from 1:00 PM to 3:30 PM. Subjects were placed in a hospital bed with application of a Dynamap cuff for measurement of heart rate and blood pressure, and baseline ratings of distress and salivary cortisol. Distress was assessed on a 10-point scale, ranging from 0 to 10. Subjects rested in a hospital bed for 60 min while listening to a tape with relaxing music and sounds (with headphone). Measurement of heart rate, blood pressure, and salivary cortisol was performed at 60 min, 10 min, and 5 min before the initiation of the cognitive challenge. At the start of the challenge a physician wearing a white laboratory coat entered the room, took place behind a desk, and initiated a series of computational tasks under time pressure, for each test in similar order. This lasted for 20 min. Each individual task was scored by the rater and performed under time pressure. Negative feedback regarding the score and the time spent in the task, was consistently given, and the level of difficulty was increased until subjects were unable to successfully complete the tasks. At the end of the cognitive challenge the physician left the room. The patient was debriefed by the research assistant who explained the procedure in terms of an “unfriendly physician,” not in terms of the setup. Heart rate and blood pressure were measured at 2, 4, 6, 7, 10, 12, 14, 15, 16, 18, 20, 30, 35, 45, 60, 75, and 90 min after the initiation

of the challenge. Salivary cortisol was measured at 0, 10, 15, 20, 30, 35, 45, 60, 75, and 90 min after the initiation of the challenge. Saliva samples were collected using salivette collection devices, salivette tubes were centrifuged in 0 to 4°C to prepare the saliva and stored at -70°C. The test scores on the cognitive tasks were not included in the analysis. The physicians assessing the arithmetics in the pretreatment situation were different from those who assessed the arithmetics in the posttreatment situation.

Treatment Phase

In patients that participated in the double-blind clinical trial, paroxetine was started after a wash out of 2 weeks. In all other patients, paroxetine was prescribed in the first visit after the pretreatment assessments. All patients started open label with a dose of 10 mg daily and were titrated up to 20 mg in 4 days. If patients reported side effects or were concerned about any symptoms during treatment they could call a 24-h answering service that was linked to an on-call physician. If patients did not respond to 20 mg by week 8, the paroxetine dosage was increased up to a maximum of 30 mg/day. Follow-up visits were scheduled every other week from weeks 1 to 12 and every 4 weeks from weeks 12 to 36. No systematic psychotherapeutic procedure was administered in the study phase. Medication was dispensed every visit to enhance compliance and reduce chances of misuse. After successful treatment all assessments were performed again.

Data Analysis and Statistical Analysis

The saliva was analyzed for cortisol using an ^{125}I immunoradiometric assay kit available from Diagnostic Products Corporation (Los Angeles, CA). Samples and standards (200 μL) were determined in duplicate; standard concentration ranged from 67 pg/mL to 3.0 ng/mL. Day-to-day coefficients of variation for low (398 pg/mL) and high (4.12 ng/mL) concentration quality assessment (QC) were made. After assessment of urinary volume, cortisol was assessed using the same assay kit. In subjects with a single inadequate or missing sample, data were interpolated where appropriate. Subjects with three or more adjacent samples missing were excluded from the analysis. The bulk of the samples was analyzed in one single run, some samples were run in a second analysis, and some samples were rerun in case of errors.

Clinical effect was analyzed using a paired samples *t*-test. Two-tailed tests of significance were used to assess group differences at discrete time points. In addition, a repeated measures analysis of variance (ANOVA) with time as repeated measure was used to compare cortisol, heart rate, and blood pressure response to the cognitive challenge, and in relation with diurnal cortisol and

CAPS score, before and after treatment with paroxetine. The level of significance for all analyses was set at 0.05. We calculated the area under the curve (AUC) after normalizing both cortisol curves at the beginning of the mental arithmetic (T0), and compared the calculated AUC for the first 60 min after the start of the challenge (T0–T60).

RESULTS

Clinical Effect

Paroxetine treatment resulted in a mean 56% reduction (87.8 [SD = 21.7] to 38.8 [SD = 36.7]) in PTSD symptoms as measured with changes from base line on the CAPS total score ($t = 4.77$; $df = 24$; $P < 0.001$) among study completers. Improvement was significant for all symptom cluster scores (reexperiencing: 25.6 [SD = 8.3] to 9.0 [SD = 10.1] avoidance/numbing: 33.7 [SD = 10.0] to 13.5 [SD = 12.0], hyperarousal: 27.0 [SD = 6.4] to 10.8 [SD = 7.5]).

Baseline Effects: 24-h Urinary and Diurnal Salivary Cortisol Effects

After treatment the mean baseline cortisol concentration in 24-h urine was reduced by 11%, the cortisol concentration changed from 2.88 (SD = 0.85) to 2.35 (SD = 1.26) $\mu\text{g/dL}$ (NS) (FIG. 1). This reduction correlated significantly with the reduction in CAPS score ($P < 0.01$). Treatment also resulted in a decrease of salivary cortisol levels on all time points on the diurnal curve. Mean decrease on the five time points after treatment was 38%. Repeated measurements analysis showed a significant effect for treatment ($F = 8.6$; $df = 9$, $P = 0.009$). Regression analysis showed that the 4 PM time point best predicted the cortisol reduction in diurnal curve ($t = -2.6$; $P = 0.02$) (TABLE 1, FIG. 2).

Stressful Challenge Effects: Distress, Heart Rate, Blood Pressure, and Cortisol

Both cognitive challenges (pre- and posttreatment) were experienced as stressful. This was assessed by subjective units of distress (SUDS), ranging from 0 (not at all) to 100 (maximally stressed). In the pretreatment phase, the baseline SUDS increased from a mean of 21.5 (SD = 20.2) at –60 min and 13.5 (SD = 16.1) at –5 min to 32.8 (SD = 27.9) at 20 min after start of the challenge. Posttreatment the SUDS increased from 16.8 (SD = 15.9) at –60 min

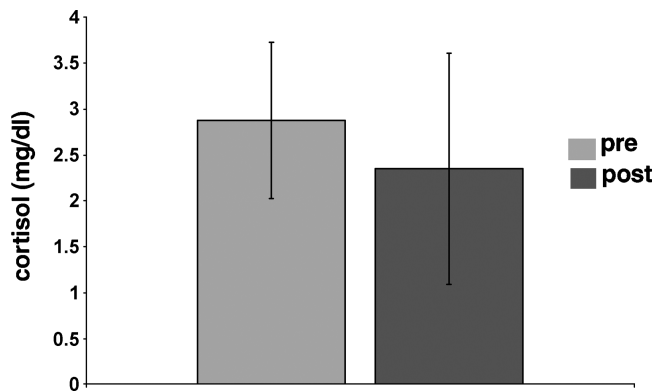


FIGURE 1. Twenty-four-hour cortisol in urine before and after treatment.

and 13.0 (SD = 16.1) at –5 min to 30.1 (SD = 28.8) at 20 min after start of the challenge. The individual difference between –60 and –5, and between –5 and 20 was significant in the pretreatment challenge ($P < 0.05$). In the posttreatment challenge only the –5- and 20-min comparison was significant ($P < 0.05$) (FIG. 3).

The pretreatment cognitive challenge resulted in a significant increase in heart rate when min “0” was compared with min 2, 6, 8, 10, 12, 14, 15, 16,18, and 20 ($P < 0.05$, FIG. 4). This was not observed in the posttreatment challenge. There was no significant effect of treatment on heart rate ($F = 0.85$; $P < 0.36$). There was also no significant time effect on systolic blood pressure for either pre- or posttreatment challenge (FIG. 5).

For diastolic blood pressure there was a positive time effect ($F = 3.191$; $df = 20$; $P < 0.001$). Time by treatment phase was not significant for either systolic or diastolic blood pressure, and there was no between-group effect for both measurements. There was no significant increase in systolic blood pressure after the start of the challenge in both treatment phases. Pretreatment cognitive

TABLE 1. Twenty-four hour urinary cortisol and diurnal cortisol before and after long-treatment with paroxetine in PTSD

	Pretreatment		Posttreatment		<i>p</i>
	avg (μg/dl)	stdev	avg (μg/dl)	stdev	
24 h urinary cortisol	2.880	0.850	2.350	1.260	ns
Diurnal cortisol					
wake up	0.388	0.139	0.259	0.172	ns
8 am	0.357	0.147	0.205	0.228	ns
12 pm	0.124	0.057	0.109	0.103	ns
4 pm	0.150	0.088	0.053	0.024	0.007
8 pm	0.108	0.198	0.042	0.042	ns

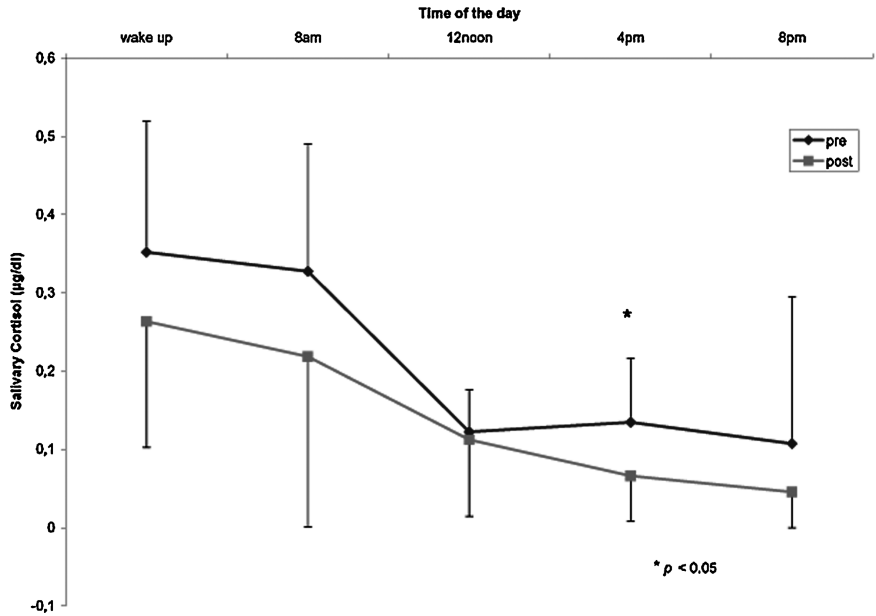


FIGURE 2. Diurnal curve for salivary cortisol before and after treatment.

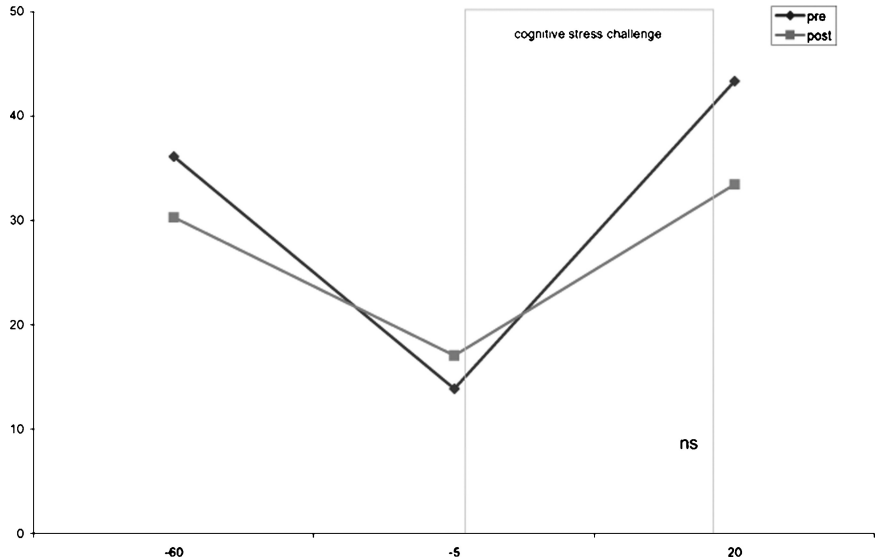


FIGURE 3. Subjective distress before and after cognitive stress challenge, before and after treatment.

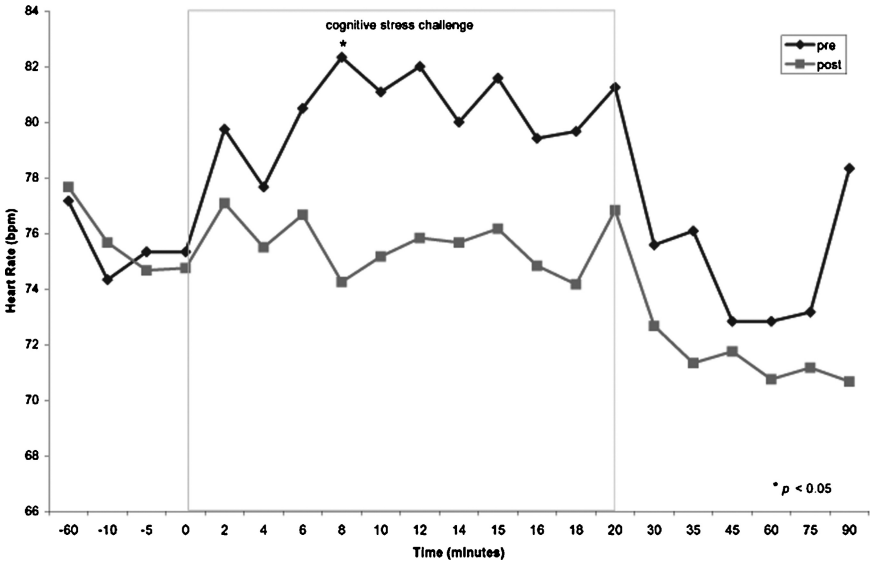


FIGURE 4. Heart rate during cognitive stress challenge before and after treatment.

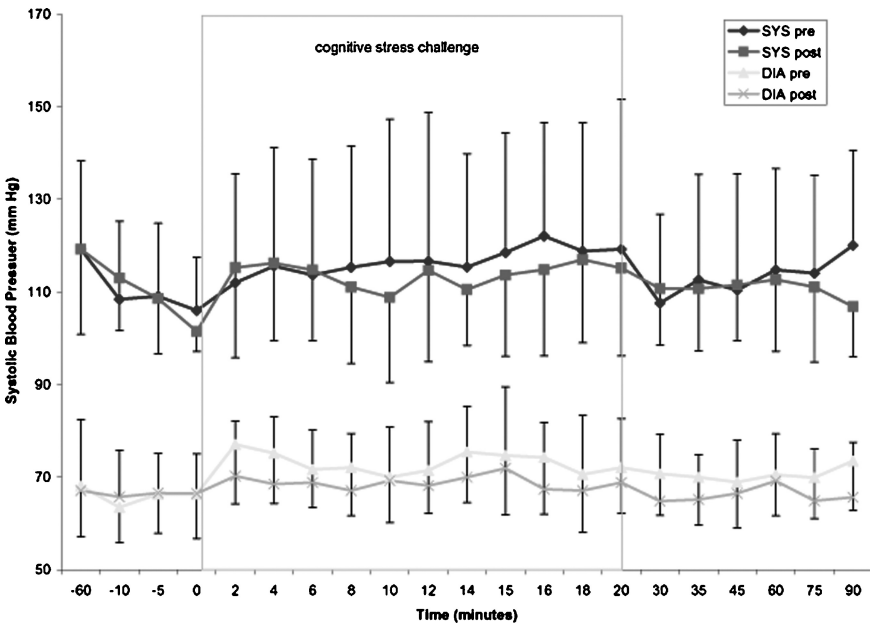


FIGURE 5. Blood pressure during cognitive stress challenge before and after treatment.

challenge resulted in a significant increase in diastolic blood pressure when min "0" min was compared with 2, 4, 6, 10, and 14 min ($P < 0.05$) and 2, 4, 6, 8, 14, and 16 min for posttreatment diastolic blood pressure (FIG. 5).

The pretreatment phase showed an increase of cortisol just after the start of the procedure. When the stress of mental arithmetic started, cortisol typically increased after 15 min, and peaked from 20 to 35 min after start of the challenge. Cortisol remained higher than base line after completion of the mental arithmetic. In the posttreatment phase, the cortisol elevation was almost completely absent. The peak concentration of cortisol occurred at the same time points as pretreatment, but at a significantly lower concentration. Repeated measures analysis showed a significant effect for treatment phase ($F = 52.7$; $P = 0.007$). Mean cortisol reduction across the challenge was 60%. At all time points except 15 and 90 min (respectively, $P = 0.6$ and $P = 0.7$) the difference between the pre- and posttreatment cortisol levels was significant (see FIG. 6). Cortisol dropped significantly from -60 to 15 min in the pretreatment ($t = 2.45$; $df = 10$; $P = 0.034$). When mean cortisol value at three time points T0, T10, and T15 was compared with mean of three subsequent time points T20, T30, and T35, there was a 5.4% differential increase when these time points were compared with the same ones in the posttreatment phase. There was no statistically significant difference between these time points in both the pre- and the posttreatment phase. When both salivary cortisol curves were normalized at the 0-min value, the calculated AUC for the first 60 min after the start of the challenge (0–60 min) showed a 26.5% relative decrease in stress-induced cortisol response between the pre- and posttreatment assessment (FIG. 6).

DISCUSSION

In this study, successful clinical treatment with paroxetine in individual patients with PTSD led to a reduction (trend) in 24-h urinary and diurnal salivary cortisol, and a significantly lower cortisol response to cognitive stress challenge. The neuroendocrine changes were positively correlated with improvements in PTSD symptoms as measured with the CAPS. Diurnal salivary and 24-h urinary cortisol results in this study are especially interesting since several other studies have reported decreased baseline cortisol in PTSD when compared with control groups without PTSD.^{4,48} As reported in the introduction, two studies have recently reported increased cortisol response to cognitive stress challenge²² and to traumatic reminders in abuse-related PTSD.³⁷ These studies demonstrate intact stress-induced cortisol reactivity in the presence of lower baseline cortisol among individuals with PTSD. Our data show that in the presence of a subjectively similar stressful situation, successfully treated PTSD patients demonstrate a significant reduction of cortisol as expressed by the AUC. We also looked at the relative increase from T20, typically the moment cortisol starts to increase after prolonged stress, we found a 5.4% larger relative increase in cortisol posttreatment compared to the pretreatment. This

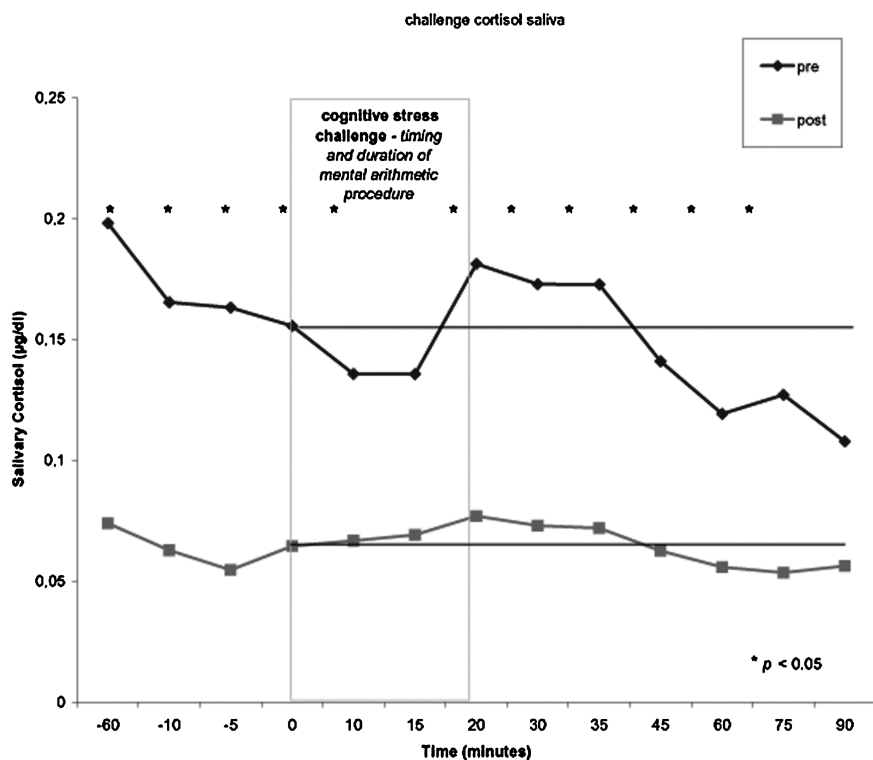


FIGURE 6. Salivary cortisol during cognitive stress challenge before and after treatment.

not only suggests an intact HPA axis, but also a system in which the adrenals are producing a lower absolute amount of cortisol in response to the stressful mental arithmetic, after treatment with paxil compared to before treatment (i.e., a 26% decrease in the AUC from T0 to T60 after treatment). After treatment we also found lower total cortisol concentration in 24-h urine and diurnal cortisol, however, these findings were not significant. This may be partly on account of small sample size, since effect size analyses for these differences revealed that values (24-h cortisol: $d = 0.66$) might be significant with greater sample size.

Several interrelated mechanisms may have contributed to these findings. The role of serotonin in the regulation of the HPA axis has long been recognized.^{49,50} PTSD has been associated with alterations in platelet serotonin receptor binding.⁵¹⁻⁶² Serotonin has been shown to enhance glucocorticoid signaling,⁶³ and increase the number and functional capacity of corticoid receptors in the hippocampus,^{64,65} which may influence glucocorticoid responsiveness.⁶⁶ Recently, increased glucocorticoid sensitivity was found to be associated with hypocortisolism, reflected by the absence of cortisol response

after awakening and lower daytime cortisol levels in Bosnian war refugees with PTSD.⁹ Low levels of cortisol in PTSD can be considered to disinhibit mediators that normally are downregulated by glucocorticoids. This is consistent with higher number of glucocorticoid receptor (GR) on peripheral lymphocytes in PTSD.^{48,67} Different study designs assessed lymphocyte GR function and number as a model for central GR functioning. *In vitro* measurements of lymphocyte sensitivity to dexamethasone showed enhanced GR sensitivity.^{9,67}

In an earlier study, Rinne *et al.* reported a reduction in the hyperresponsiveness of the HPA axis after treatment with fluvoxamine in women with BPD, with the greatest changes seen in women with BPD and early childhood abuse.⁴² In another study using a metachlorophenylpiperazine (mCPP) challenge to probe serotonergic function in this research, Rinne and colleagues also found the greatest behavioral responses in women with BPD and early abuse.⁶⁸ These studies suggest that it is not the neuropsychiatric disorder *per se*, but rather a history of traumatic childhood in combination with psychopathology that is an important determinant of this biological response pattern.⁶⁹ One explanation may be that early-life events compromise glucocorticoid signaling, and contribute to elevated sympathetic tone, CRH hypersecretion, and changes in immune system function.⁶⁶ These notions are consistent with the hypothesis that trauma spectrum disorders, including PTSD, depression, and BPD, all share a common biological basis that is linked to traumatic stress exposure.

The present study is the first to assess a long-term treatment effects on stress reactivity in PTSD. An earlier paper described positive effects of long-term paroxetine treatment on memory and hippocampal volume in patients with PTSD.⁷⁰ The long-term treatment design used in this study provided an opportunity to measure effects of the medication on neurobiology that could be missed in studies of a shorter duration.

There are several limitations to the current study that warrant cautious interpretation. First, all patients were on paroxetine during the time of second cognitive challenge. The alterations in stress responsivity could therefore be a direct effect of the medication. A better design would involve tapering and stopping of medication before the second stressful challenge. Second, we do not know whether baseline cortisol was decreased or increased compared to that in a healthy control group, which makes it more difficult to interpret the cortisol change. Further, we did not include a placebo control group in this study for three reasons. Firstly, it is not known if relatively short-term treatment (e.g., 12 weeks) would result in alterations in stress-regulating systems; and second, it was felt that it would be unethical to provide long-term (12 months) placebo treatment to subjects with PTSD. Of note, after having experienced the first challenge, the second challenge could have been anticipated in a different way. Including a healthy control group in this study design, where subjects were challenged at the same time points as the PTSD patients, could have provided more information on the learning effect. It is possible that the lower cortisol effect would be explained by prior experience of the procedure

rather than an effect of paroxetine. Our data on the SUDS, however, suggest that after treatment, the subjects perceived the procedure as equally distressing compared to the pretreatment challenge. It can be argued that subjects who had experienced the first challenge as unpleasant may have anticipated that the second challenge would be even more unpleasant. A recent study looking at habituation of cortisol responses to repeated psychosocial stress demonstrated that the general decline in cortisol response was only modest.⁷¹ In addition, to reduce habituation, and stimulate novelty, we chose to introduce another physician who performed the second challenge (posttreatment). A comparison of percentage increase in T0–T15 with T20–T35 showed an even larger cortisol increase in the posttreatment phase, however, this did not show in the AUC values that represent the effect of the total challenge procedure. As a final limitation cortisol was the only biological parameter assessed in this study. To study HPA axis regulation in more detail, the separate components of the stress axis should be measured, including corticotrophin-releasing factor (CRF), vasopressin, and ACTH. Moreover, the HPA axis and the locus coeruleus/noradrenergic system have mutual interconnections. PTSD may also be associated with dysfunctional connectivity between these systems. It would have been informative to assess these different stress systems within one study. Additionally, we did not collect a second control diurnal profile, to rule out day-to-day intraindividual variations.⁷² Finally, the sample size is small and therefore this study should be replicated with a larger number of patients. The age of onset of the traumatic experience in our sample varied, as did the trauma type; in nine patients trauma occurred before 18 years of age, in four the onset was in adult life. Our sample size was too small to calculate the magnitude of the effect on stress challenge in these two subgroups. It should also be mentioned that this was a completer analysis and all the males were left out of the analysis. Even though most women were postmenopausal, the data warrant caution in the absence of measurement of the phase of the menstrual cycle in the participating premenopausal women in the pre- and posttreatment time period. Gonadal steroids are known to exert influence on the reactivity of the HPA axis in humans.^{73,74}

Finally, we would like to stress that the design of this study has important strengths that should be further explored in future studies. Future studies identifying biological alterations in relation to stress reactivity and HPA axis function should help to develop a better understanding of the direction and magnitude of neurohormonal changes and their relation to the pharmacological treatment of PTSD.

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