Eur Arch Psychiatry Clin Neurosci (2002) 252 : 161–165 DOI 10.1007/s00406-002-0374-5

ORIGINAL PAPER

Murad Atmaca · Murat Kuloglu · Ertan Tezcan · Süleyman Onal · Bilal Ustundag

Neopterin levels and dexamethasone suppression test in posttraumatic stress disorder

Received: 13 February 2002 / Accepted: 13 June 2002

Abstract Neopterin has recently gained growing importance as an immunological marker in psychiatric disorders. In the present study, we aimed to evaluate whether the dexamethasone suppression test (DST) and neopterin were associated with posttraumatic stress disorder (PTSD). Fourteen patients with PTSD and 14 controls were enrolled in the study. A clinical evaluation and measurements of cortisol and neopterin levels before and after DST were performed. Additionally, all patients were assessed by Clinician Administered PTSD Scale (CAPS). There was a significantly higher DST nonsuppression in the patient group than control group. There were positive correlations between the duration of illness and CAPS, basal cortisol or postdexamethasone cortisol levels in the patient group. The mean neopterin levels for both before and after DST were significantly lower in the patient group than control group. In conclusion, our results suggest that not only the patients with PTSD have considerable DST nonsuppression but also PTSD may be associated with neopterin.

Key words PTSD · neopterin · DST · cortisol

Yrd. Doç. Dr. Murad Atmaca (✉) · M. Kuloglu · E. Tezcan
Firat (Euphrates) Üniversitesi
Firat Tip Merkezi
Psikiyatri Anabilim Dali
23119 Elazig, Turkey
Tel.: +90-424/233-3555
or: +90-424/2282-2300
Fax: +90-424/238-8096
E-Mail: matmaca_p@yahoo.com

S. Onal
Fırat University
School of Medicine, Department of Immunology
Elazig, Turkey

B. Ustundag
Fırat University
School of Medicine, Department of Clinical Biochemistry
Elazig, Turkey

Introduction

Recently, neopterin which is a biopterin precursor released by macrophages has gained a growing importance as an immunological marker in psychiatric disorders (O'Hanlon et al. 1996; Korte et al. 1998). It is produced by activation of cyclohydrolase, which is stimulated by interferon-γ (INF-γ). It has been accepted as an important indicator of the cellular immune system, since INF-γ is released by T-lymphocytes (Huber et al. 1984; Besedovsky et al. 1986). However, the cell types related to humoral immunity (e. g., B-lymphocytes) do not release measurable level of neopterin (Huber et al. 1984). In addition, neopterin is a bypass product in the synthesis of tetrahydrobiopterin which is a cofactor of tyrosine hydroxylase, an essential enzyme in catecholamine neurotransmitter synthesis (Wachter et al. 1989). Plasma neopterin is frequently increased in states of stimulated cellular immunity such as organ transplantation and autoimmune illness (Kobryn et al. 1989; Neale et al. 1990). Controversial results have been reported by studies evaluating neopterin in psychiatric patients, (Maes et al. 1994; O'Toole et al. 1998; Korte et al. 1998).

Posttraumatic stress disorder (PTSD), a stress reaction with a high rate of comorbidity, is classified under anxiety disorders in DSM-IV (Maes et al. 2000; Grabe et al. 2000). PTSD is one of the rare disorders which is defined with etiology in DSM-IV. However trauma alone is not enough to explain the occurrence of PTSD because many people experience a traumatic stressor but few develop PTSD. Probable posttraumatic biological factors seem to be effective on the development of PTSD. Stressor-related autonomic hyperactivity and changing the activity of the hypothalamo-pituitary-adrenal (HPA) axis have been established in PTSD. The HPA axis is obviously sensitive to stress-related disorders. It was reported that glucocorticoids (the last product of the HPA axis) could cause suppression of cell-mediated immunity and consequently could cause reduction in

neopterin levels. To the best of our knowledge, there has been no study in the literature concerning neopterin in PTSD. Therefore, we chose to specifically measure neopterin to see whether its eventual alterations might have an etiopathogenetic significance in the patients with PTSD. Thus, we planned to evaluate as follows: 1) whether the DST was associated with PTSD, and 2) whether neopterin levels were affected by PTSD.

Methods

Subjects

The study group consisted of 14 patients (8 females, 6 males) with a mean age of 32.48±5.34 years (range 18–41) who had applied to the Firat University Scool of Medicine Department of Psychiatry and were diagnosed with PTSD according to DSM-III-R, and met the admission criteria. Written consent to participate in the study was obtained from the subjects after they were thoroughly informed about the research details. The research protocol was approved by the Fırat University School of Medicine Ethics Committee. There were no significant differences in age and female/male ratio between the patient and control groups. The mean CAPS score of the patient group was 52.46±19.54. The mean duration of illness for the patient group was 3.62±2.55 years.

All subjects were free of all medications for at least the previous two weeks. Female subjects were tested in different menstrual phases. The vast majority of those were in the early follicular phase (n = 6), and the remaining in the late luteal phase. Each patient underwent diagnostic evaluation by one trained psychiatrist using the Structured Interview for DSM-III-R Outpatient Form (SCID-OP) (Spitzer et al. 1987). The patients with any kind of axis I comorbidity were excluded. Additionally, all subjects were evaluated by a semistructured questionnaire form which was arranged in accordance with clinical experience and available information sources and included gender, age, marital status, education, socieconomic status, duration of illness and the Clinician Administered PTSD Scale (CAPS) (Blake et al. 1990), adapted to Turkish patients with established validity and reliability (Aker et al. 1999). CAPS is a widely used instrument in the assessment of PTSD. It has 25 items. While a total of seventeen questions asks for PTSD symptoms of DSM-III-R, the remaining section is under the subscale of 'associated symptoms'. Each question is assessed by a clinician giving 1–4 points.

A total of 14 healthy control subjects (mean age = 29.88±6.04 years; range 21–44) in accordance with exclusion criteria, were chosen from the hospital staff. Controls were interviewed with the nonpatient version of the SCID (SCID-NP) to exclude any axis I disorder (Spitzer et al. 1990).

All patients underwent physical examination, total biochemistrical evaluation, chest X-ray, urinalysis and ECG. All participants were carefully assessed to rule out autoimmune, pulmonary, infectious diseases and neoplasms. Exclusion criteria were the history of any endocrinological condition, gestation, obesity, alcohol abuse and dependence, recent or present infection, the history of immunologic disease and oral contraceptive use.

DST procedure

In the procedure of the DST, the blood samples for the determination of cortisol were drawn on the first day from all subjects at 08.00 h after overnight fasting, no drinking of alcohol or coffee and not smoking for 12 hours. Blood samples were obtained after an hour rest period at a comfortable place by controlling tension. A cannula into a forearm vein was inserted and blood was drawn 45 minutes after inserting the cannula. Serum cortisol level was measured using a commercially available kit (Immulite 2000 cortisol, Diagnostic Products Corporation, Los Angeles, CA, USA) utilizing the chemiluminoassay method. On the same day at 23.00 h, 1 mg dexamethasone was administered. The cortisol levels were determined on the following day at 16.00 h, again by the same blood taking procedure. Procedures established by Carroll et al. (1981) were strictly followed, the cutoff point for nonsuppression being 5 µg/dl. Intra- and inter-assay coefficients of variation were < 6.1 % and 7.8 %, and sensitivity was 0.20 µg/dl.

Neopterin procedure

In the neopterin procedure, the blood drawing procedure was the same. Blood samples were taken from the subjects into dry tubes at 8.00 h on the first day and on the following day again at 16.00 h. The sera were collected by centrifuging the tubes at 250 x *g*. The sera were stored at –20 °C until assessment of neopterin levels. Serum neopterin levels of the subjects were measured by the ELISA (enzyme linked immunoassay) method (Neopterin; BRAHMS Diagnostica GmbH, 16761 Berlin, Germany).

Statistical analysis

Obtained data were evaluated by SPSS for windows 9.0 (SPSS, 1998). The comparisons within group (for both cortisol and neopterin levels before and after DST in each group) were performed by using Wilcoxon's rank sum test, whereas those between intergroups were carried out by using independent samples t test. The chi-square test was used to compare the proportion of non-suppression between groups. For correlation evaluations, the Spearman correlation (two-tailed) test was used. $p < 0.05$ was considered to be significant.

Results

The mean basal cortisol levels were 12.91±3.78 µg/dl in the patient group and 10.76±2.22 µg/dl in controls, respectively ($p > 0.05$). The mean postdexamethasone cortisol levels were 4.76±1.56 and 2.19±1.04 µg/dl for the patient group and controls, respectively ($p < 0.01$). After DST, changes in cortisol levels were statistically significant both in patients ($p < 0.01$) and controls ($p < 0.01$). While 11 (70.6 %) out of 14 patients had DST nonsuppression, none of the control group had DST nonsuppression ($p < 0.0001$).

There were positive correlations between the duration of illness and CAPS ($r = 0.29$, $p < 0.05$), basal cortisol ($r = 0.34$, $p < 0.05$) or postdexamethasone cortisol ($r = 0.44$, $p < 0.01$) in the patient group. In addition, there was a correlation between CAPS and basal ($r = 0.64$, $P < 0.01$) or postdexamethasone cortisol levels ($r = 0.68$, $p < 0.01$) in the patient group.

The baseline neopterin levels were significantly lower in the patients compared with healthy controls (4.34±1.04 vs. 8.03±2.13 nmol/ml, respectively). A significant difference between the patient and control groups was found ($P < 0.001$). After DST, the mean neopterin level in the patients (1.99±0.73 nmol/ml) was significantly ($p < 0.01$) decreased compared with that found at baseline, whereas no significant change in controls was found (7.33±1.98 nmol/ml, $p > 0.05$). There was a statistically significant difference in the decrease of neopterin levels in the patient group compared with that in control group ($p < 0.01$).

Statistical significant correlations between baseline

neopterin levels and baseline cortisol ($r = -0.37$, $p < 0.05$), postdexamethasone cortisol ($r = -0.64$, $p < 0.01$), the duration of illness ($r = -0.37$, $p < 0.05$), or CAPS scores ($r = -0.40$, $p < 0.05$) were found in the patient group. On the other hand, there were significant correlations between neopterin levels after DST and postdexamethasone cortisol ($r = -0.26$, $P < 0.05$), the duration of illness ($r = -0.37$, $P < 0.05$) or CAPS scores ($r = -0.43$, $P < 0.05$). In the control group, a significant correlation was only found between baseline neopterin and baseline cortisol levels ($r = -0.32$, $P < 0.05$).

The distribution of baseline and postdexamethasone cortisol and neopterin levels for groups are presented in Figs. 1 and 2.

Discussion

As far as we know, this is the first study regarding neopterin in the patients with PTSD. The main findings of our study are as follows: 1) The patients with PTSD have statistically significant higher baseline and after DST cortisol levels and statistically significant lower neopterin levels compared to controls, 2) there were positive correlations between the duration of illness and CAPS, baseline cortisol or postdexamethasone cortisol levels in the patient group. In addition, there was a correlation between CAPS and baseline or postdexamethasone cortisol levels in the patient group, 3) statistically significant correlations between baseline neopterin levels and baseline cortisol, postdexamethasone cortisol, the duration of illness, or CAPS scores were found in the patient group, and there were significant correlations between neopterin levels after DST and postdexamethasone cortisol, the duration of illness or CAPS scores.

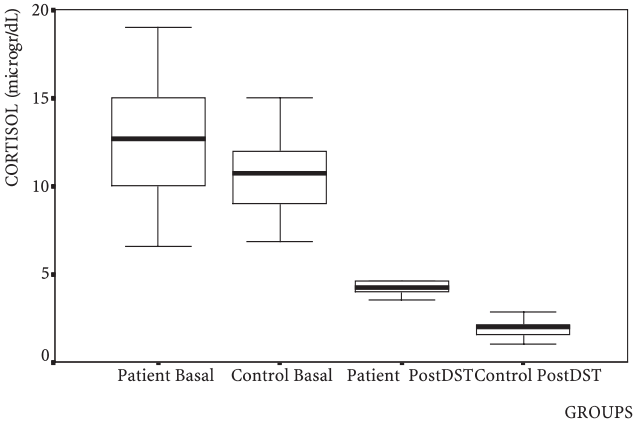

Fig. 1 The distribution of basal and postdexamethasone cortisol levels in the groups.

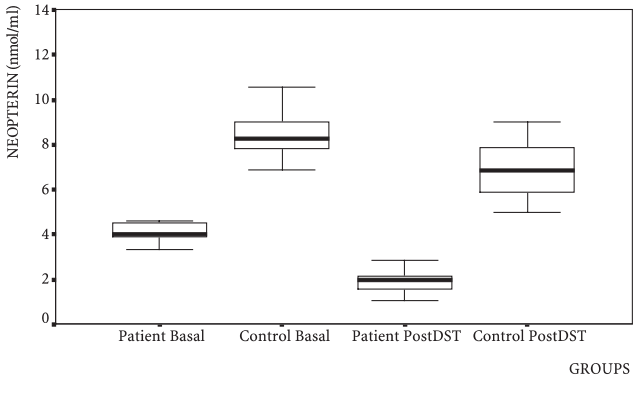

Fig. 2 The distribution of basal and postdexamethasone neopterin levels in the groups.

In PTSD, the studies focusing on the HPA axis have proposed that the classical profile of increased adrenocortical activity and resultant dysregulation of this system described in studies of stress and major depression; the data support the hypothesis of an enhanced negative feedback sensitivity of the HPA axis in PTSD (Yehuda et al. 1990; Yehuda et al. 1995a). An enhanced sensitization of the HPA axis as reflected by a stronger negative feedback inhibition is compatible with some preclinical models of stress. Although stress is generally associated with an overactivation of the HPA axis and a resultant resistance of the HPA axis to glucocorticoid feedback signals (Natelson et al. 1988; Young et al. 1990), the HPA axis may become sensitized under certain conditions of stress. Glucocorticoid receptors in hippocampus which is dense with those that play a prominent role in stress-induced feedback inhibition of the HPA axis and, thus, may be an important aspect of HPA dysregulation in PTSD (Yehuda et al. 1991; Yehuda et al. 1995a; Seed et al. 2000). In contrast to the findings mentioned above, some have suggested that the HPA axis may be overactivated in PTSD similar to that seen in depressive disorders. Pitman and Orr (1990) demonstrated a greater urinary cortisol excretion than normal. In addition, in another study, blunted ACTH response to corticotropin-releasing factor has been shown (Smith et al. 1989). Data from DST studies about PTSD have remained inconclusive with regard to abnormal cortisol metabolism in PTSD. Of the studies in PTSD patients meeting concurrent criteria for major depression, two studies (Kudler et al. 1987; Olivera et al. 1990) have demonstrated a nonsuppressive response to dexamethasone in some patients. In our study, not only were baseline and post-DST cortisol levels for the patients higher compared with controls, but also statistically significant higher DST nonsuppression in the patients were found. These results are in conflict with the research which has demonstrated lower baseline plasma cortisol levels, lower mean 24-hour urinary cortisol excretion and a series of alterations that are distinct from those reported in major depressive disorder. It has been concluded that cortisol nonsuppression is rare in posttraumatic stress disorder unless there is concomitant major depression (Kudler et al. 1987; Yehuda et al. 1990; Yehuda et al. 1994; Yehuda et al. 1995b).

In the studies concerning neopterin in psychiatric patients, controversial results have been reported. O'-Toole et al. (1998) reported a slight suppression of

neopterin levels in depressed patients compared to matched controls. They suggested that neopterin might not be a useful indicator for the severity and the duration of a depressive episode. Korte et al. (1998) evaluated the neopterin levels of 29 inpatients with acute schizophrenia and reported that the patients, especially those with clinical improvement, had significantly higher levels of neopterin compared to controls. In our study, both before and after DST neopterin levels in the patients with PTSD were decreased compared with the control group and there was also a significant difference between the mean neopterin level before and after DST in the patient group but not in the control group. Several mechanisms can be proposed to explain these findings. First, PTSD seems to be characterized by increased basal and postdexamethasone cortisol levels. This situation may be leading to suppression of T-cells since glucocorticoids (the last product of the HPA axis) could cause suppression of cell-mediated immunity and consequently could result in decreased neopterin levels. The presence of statistically significant negative correlations between baseline neopterin levels and baseline cortisol or postdexamethasone cortisol, and between neopterin levels after DST and postdexamethasone cortisol supports this. Meanwhile with respect to the post-DST statistically significant lower neopterin levels in the patient group, the suppressive effects of dexamethasone itself, a synthetic glucocorticoid with a long half-life, should be taken into consideration. Dexamethasone also suppresses other pituitary hormones including prolactin and thyroid-stimulating hormone (TSH) (Olazabal et al. 2000; Coiro et al. 2001). Second, it was reported that norepinephrine, a neurotransmitter frequently reported to be increased in PTSD, and the HPA axis might lead to the inhibition of cytokine secretion. Thus, since neopterin has been accepted as an important indicator of the cellular immune system (Huber et al. 1984; Besedovsky et al. 1986), decreased neopterin levels in PTSD may be expected. The suppression of cellular immunity in PTSD has been supported by means of determining a lower number of lymphocytes, T cells, and decreased natural killer cell activity (Inoue-Sakurai et al. 2000; Kawamura et al. 2001). Moreover, neopterin is the first intermediate in the synthesis of tetrahydrobiopterin, a cofactor for the hydroxylation of phenylalanine and tryptophan in a rate-limiting step in the biosynthesis of dopamine, noradrenaline and 5-hydroxytryptamine (5-HT) (Fuchs et al. 1988). It has been reported that the pathophysiology of PTSD may involve dysfunction of several brain structures, especially locus coerulous, amygdala and hippocampus, as well as noradrenergic, dopamine and corticotropin-releasing factor neurochemical systems. Severe psychologic traumas lead to the parallel activation of the systems mentioned above, producing an array of adaptive behavioral and physiologic responses necessary for survival; however, these acute responses appear to reveal the maladaptive neurobiologic sequelae (Charney et al. 1993). These changes may be associated with PTSD symptoms becoming chronic and the poor response to trauma. In summary, there are close relations between PTSD and neopterin. However, it is controversial that lower neopterin is a cause or result in PTSD. Although neopterin is a reliable, easy-to-measure and well-established soluble marker of immune activation, the evaluation of only one immune parameter was too small to allow for a conclusive interpretation of immunological changes in PTSD.

In conclusion, the present study suggests that PTSD may be associated with neopterin but our sample is too small to allow us to conclude that this alteration may be an important biological indicator for this disorder. Our finding is only a suggestion and more comprehensive and detailed studies, in which in addition to neopterin, more immunological parameters are used, are needed to decipher the exact roles of immunological changes in PTSD.

References

1. Aker AT, Ozeren M, Basoglu M, Kaptanoglu C, Erol A, Buran B (1999) The validity and the reliability study of Clinician Administered Post-Traumatic Stress Disorder Scale. Türk Psikiyatri Dergisi 10: 286–293 (in Turkish)
2. Besedovsky H, del Rey A, Sorkin E, Dinarello C (1986) Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. Science 233: 652–654
3. Blake DD, Weathers FW, Nagy LM, Kaloupek DG, Gusman FD, Charney DS, Keane TM (1995) The development of a Clinician-Administered PTSD Scale. J Trauma Stress 8: 75–90
4. Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Haskett RF, James NM, Kronfol Z, Lohr N, Steiner M, de Vigne JP, Young E (1981) A specific laboratory test for the diagnosis of melancholia. Arch Gen Psychiatry 38: 15–22
5. Charney DS, Deutch AY, Krystal JH, Soutwick SM, Davis M (1993) Psychobiologic mechanisms of posttraumatic stress disorder. Arch Gen Psychiatry 50: 294–305
6. Coiro V, Volpi R, Capretti L, Speroni G, Pilla S, Cataldo S, Rianconcini M, Balzani E, Chiodera P (2001) Effect of dexamethasone on TSH secretion induced by TRH in human obesity. J Investig Med 49: 330–334
7. Fuchs D, Hausen A, Reibnegger G, Werner ER, Dieric MP, Wachter H (1988) Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. Immunol Today 9: 150–155
8. Grabe HJ, Meyer C, Hapke U, Rumpf HJ, Freyberger HJ, Dilling H, John U (2000) Lifetime-comorbidity of obsessive-compulsive disorder and subclinical obsessive-compulsive disorder in Northern Germany. Eur Arch Psychiatry Clin Neurosci 251: 130–135
9. Huber C, Batchelor JR, Fuchs D, Hausen A, Lang A, Niederwieser D, Reibnegger G, Swetly P, Troppmair J, Wachter H (1984) Immune response-associated production of neopterin: release from macrophages primarily under control of interferon-gamma. J Exp Med 160: 310–316
10. Inoue-Sakurai C, Maruyama S, Morimoto K (2000) Posttraumatic stress and lifestyles are associated with natural killer activity in victims of the Hanshin-Awaji earthquake in Japan. Prev Med 31: 467–473
11. Kawamura N, Kim Y, Asukai N (2001) Suppression of cellular immunity in men with a past history of posttraumatic stress disorder. Am J psychiatry 158: 484–486
12. Kobryn A, Pacsa A, White AG, Kumar MS, Abouna GM (1989) Are peripheral blood lymphocytes the source of elevated b2 microglobulin in renal transplant patients? Transplant Proc 21: 302–303

13. Korte S, Arolt V, Peters M, Weitzsch C, Rothermundt M, Kirchner H (1998) Increased serum neopterin levels in acutely ill and recovered schizophrenic patients. Schizophrenia Res 32: 63–67
14. Kudler H, Davidson J, Meador K, Lipper S, Ely T (1987) The DST and posttraumatic stress disorder. Am J Psychiatry 144: 1068–1071
15. Maes M, Scharpe S, Meltzer HY, Okayli G, Bosmans E, D'Hondt P, Vanden Bossche BV, Cosyns P (1994) Increased neopterin and interferon-gamma secretion and lower availability of L-tryptophan in major depression:further evidence for an immune response. Psychiatry Res 54: 143–160
16. Maes M, Mylle J, Delmeire L, Altamura C (2000) Psychiatric morbidity and comorbidity following accidental man-made traumatic events: incidence and risk factors. Eur Arch Psychiatry Clin Neurosci 250: 156–162
17. Natelson BH, Ottenweller JW, Cook J, Pirm SL, McCarty R, Tapp WNL (1988) Effect of stressor intensity on habitutation of the adrenocortical stress response. Physiol Behav 43: 41–46
18. Neale TJ, Hill JH, Cooke R, Dunbar PR (1990) Neopterin quantitation indicates participation of cell mediated immunity (CMI) in human glomerulonephritis (GN). Kidney Int 37: 442
19. O'Hanlon M, Salter S, Scull D, Labib M (1996) Neopterin levels in alcohol-dependent patients. Ann Clin Biochem 33: 536–539
20. Olazabal I, Muroz J, Ogueta S, Obregon E, Garcia-Ruiz JP (2000) Prolactin (PRL)-PRL receptor system increases cell proliferation involving JNK (c-Jun amino terminal kinase) and AP-1 activation: inhibition by glucocorticoids. Mol Endocrinol 14: 564–575
21. O'Toole SM, Chiappelli F, Rubin RT (1998) Plasma neopterin in major depression: relationship to basal and stimulated pituitary-adrenal cortical axis function. Psychiatry Res 79: 21–29
22. Olivera AA, Fero D (1990) Affective disorders, DST, and treatment in PTSD patients: clinical observations. J Traumatic Stress 3: 407–414
23. Pitman R, Orr S (1990) Twenty-four hour urinary cortisol and catecholamine excretion in combat-related pottraumatic stress disorder. Biol Psychiatry 27: 245–247
24. Seed JA, Dixon RA, McCluskey SE, Young AH (2000) Basal activity of the hypothalamic-pituitary-adrenal axis and cognitive function in anorexia nervosa. Eur Arch Psychiatry Clin Neurosci 250: 11–15
25. Smith MA, Davidson J, Ritchie JC, Kudler H, Lipper S, Chappell P, Nemeroff CB (1989) The corticotropin-releasing hormone test in patients with posttraumatic stress disorder. Biol Psychiatry 26: 349–355
26. Spitzer RL, Williams JBW, Gibbon M, First M (1987) Structured Interview for DSM-III-R (SCID). New York State Psychiatric Institute, New York, Biometrics Research
27. Spitzer RL, Williams JBW, Gibbon M, First M (1990) Structured Interview for DSM-III-R, Nonpatient Version (SCID-NP, Version 1.0). American Psychiatric Press, Washington, DC
28. Wachter H, Fuchs D, Hausen A, Reibnegger G, Werner E (1989) Neopterin as marker for activation of cellular immunity: immunologic basis and clinical application. Adv Clin Chem 27: 81–141
29. Yehuda R, Boisoneau D,Lowy MT, Giller EL (1995a) Dose response changes in plasma cortisol and lymphocyte glucocorticoid receptors following dexamethasone administration in combat veterans with and without posttraumatic stress disorder. Arch Gen Psychiatry 52: 583–593
30. Yehuda R, Kahana B, Binder-Brynes K, Southwick SM, Mason JW, Giller EL (1995b) Low urinary cortisol excretion in Holocaust survivors with posttraumatic stress disorder. Am J Psychiatry 152: 982–986
31. Yehuda R, Lowy MT, Southwick SM, Shaffer D, Giller EL (1991) Lymphocyte glucocorticoid receptor number in posttraumatic stress disorder. Am J Psychiatry 148: 499–504
32. Yehuda R, Soutwick SM, Nussbaum G, Wahby V, Mason JW, Giller EL (1990) Low urinary cortisol excretion in patients with PTSD. J Nerv Ment Dis 178: 366–369
33. Yehuda R, Teicher M, Trestman RL, Levengood RA, Siever LJ (1994) Cortisol regulation in post-traumatic stress disorder: a chronobiological analysis. Ann N Y Acad Sci 746: 378–380
34. Young EA, Akana S, Dallman MF (1990) Decreased sensitivity to glucocorticoid fast feedback in chronically stressed rats. Neuroendocrinology 51: 536–542