Advances in Understanding Neuroendocrine Alterations in PTSD and Their Therapeutic Implications

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ABSTRACT: The findings from investigations of the neuroendocrinology of posttraumatic stress disorder (PTSD) have highlighted alterations that have not historically been associated with pathologic processes, and have, accordingly, raised several questions about the nature of the findings and their relationship to PTSD. The most infamous of these observations-–low cortisol levels-–has been the subject of much discussion and scrutiny because the finding has been both counterintuitive, and not uniformly reproducible. This fact notwithstanding, novel therapeutic approaches to the treatment of PTSD are in large part predicated on the assumption that glucocorticoid levels may be lower in PTSD. This article summarizes important neuroendocrine observations in cortisol and provides strategies for understanding what has emerged over the past two decades, to be a complex and sometimes contradictory literature.

KEYWORDS: posttraumatic stress disorder; cortisol; neuroendocrine alterations; novel therapeutic strategies; hypothalamic-pituitary-adrenal axis

INTRODUCTION

An observable biologic change—even one that is directly associated with severity of symptoms or presence of a disorder—does not always constitute a core pathophysiologic process. Biologic alterations may be present in disorders because they are correlates of, or proxies for, other pathophysiologic processes, or because they represent compensatory mechanisms of adaptation. Some biologic alterations may predate the development of disorder. In the case of posttraumatic stress disorder (PTSD), there may be specific biologic markers present prior to a person's exposure to a traumatic event that in turn may

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Ann. N.Y. Acad. Sci. 1071: 137–166 (2006).
doi: 10.1196/annals.1364.012

affect the response to trauma by increasing or decreasing risk for the development of, and/or recovery from, PTSD. Studies using prospective, longitudinal designs have tended to support the possibility that cortisol levels may constitute measures associated with risk for PTSD (see articles by Marmar, Bryant, and Delahanty, this volume), and have also helped place other disparate observations regarding cortisol levels in PTSD into proper perspective. Although there is still a great deal of confusion about the role of cortisol and related neuroendocrine alterations in PTSD, novel therapeutic approaches to the treatment of PTSD are in large part predicated on the assumption that glucocorticoid levels may be lower in PTSD, or in the early aftermath of trauma exposure, resulting in a lack of containment of sympathetic nervous system (SNS) or other stress-related activity that may be restored with appropriate pharmacologic intervention.[1–4] Accordingly, it is important to understand what lies at the root of these seemingly discrepant findings, and this is the subject of the current article.

Certainly the initial observation of low cortisol in a disorder precipitated by extreme stress[5] directly contradicted the popular "glucocorticoid cascade hypothesis"[6] that was emerging as a cogent rationale for anti-glucocorticoid treatments in depression, and other psychiatric disorders thought to be driven by hypercortisolism. In discussing their results, Mason *et al.* commented that the observation of lower 24-h mean urinary cortisol excretion in combat Vietnam veterans with PTSD was indeed surprising since "certain clinical features such as depression and anxiety [in PTSD] might have been expected to be associated with increased activity of the pituitary-adrenal cortical system."[7] The majority of reports published since the initial observation of low urinary cortisol excretion in PTSD support the idea that cortisol alterations in PTSD are different from those observed in acute and many types of chronic stress, as well as those associated with major depressive disorder (e.g., for review see Yehuda[7]). More importantly, however, the seemingly paradoxical nature of the initial finding has led to follow-up studies demonstrating important differences in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis in PTSD, which appear to reflect adaptations to fundamental alterations in glucocorticoid responsiveness and signaling. The cumulative observations have provided a context for interpreting the more diverse range of baseline cortisol levels in PTSD and delineating areas for future investigations. What remains uncertain at this time concerns the etiology of observed changes in glucocorticoid responsiveness, the question of whether this alteration is confined to a specific subgroup of trauma survivors with PTSD, the relationship between glucocorticoid responsiveness and other HPA and neurochemical alterations documented in PTSD, and most importantly, the extent to which symptom improvement can be achieved by manipulating HPA axis regulation in trauma survivors.

There are now several hundred peer-reviewed journal articles that report on various aspects of HPA axis functioning in PTSD. This article is not intended

to summarize each and every published observation, but rather to discuss approaches to the interpretation of neuroendocrine results in consideration of discrepant observations. There is a tendency to try to reach consensus regarding contrasting views in the literature by designating majority findings as more true than findings occurring less frequently, often with very little attempt at evaluating the meaning of disparate observations, or attempting to determine whether specific methodologic considerations compromise the findings or their interpretation, or merit that data from well-constructed studies be more heavily weighted. This article takes the position that it is necessary to learn not only from the independent observations that comprise the literature—the sum of the parts—but also from the conflicting literature as a unit.

CORTISOL LEVELS IN PTSD: STRATEGIES FOR EVALUATING A COMPLEX LITERATURE

When compared to levels in normal controls, ambient cortisol concentrations in PTSD over a 24-h period have been reported as significantly lower,[8–14] significantly higher,[15–17] and not significantly different.[18,19] This collection of findings raises the question of whether cortisol levels are important at all to the pathophysiology of this disorder. If cortisol levels are important to PTSD pathophysiology, and all findings merit consideration as scientifically valid conclusions, then it is necessary to provide a conceptual framework to facilitate an understanding of these seemingly disparate reports. This can be achieved by more clearly defining the meaning of cortisol levels in the overall assessment of neuroendocrine function and the methodologic limitations of obtaining interpretable cortisol data. In this context, the following issues should be considered in the interpretation of the literature on cortisol alterations in PTSD.

Cortisol Levels Associated with PTSD are Within the Normal Endocrinologic Range

With very few exceptions, cortisol levels reported in the literature for persons with PTSD are within the normal endocrinologic range.[5] Even studies demonstrating statistically significant differences between subjects with and without PTSD are not suggestive of endocrine pathology. The clinical significance of cortisol alterations within the normal endocrinologic range has not been fully established and will require follow-up studies examining adrenal production of cortisol and region-specific degradation of glucocorticoids. That cortisol alterations are at best, subtle, and not easily differentiated from normal values, is important to note since this may have implications for why it has been difficult to observe group differences in cortisol levels in small studies.

Group Differences in Cortisol Levels in PTSD may be Obscured by Other Individual Differences Related to Cortisol

The problem of establishing group differences relating to ambient cortisol levels (whether measured as an integrated measure over 24 h or at circumscribed times in the diurnal cycle) are further magnified in consideration of individual variability associated with cortisol levels in the normal population, including the known impact of factors, such as age, gender, body weight, height, metabolism, medical illness, mood, substance use, including alcohol and nicotine, and environmental stress. Studies that report on cortisol levels in subjects that are homogenous with respect to the above variables, or in which these variables have been controlled for in statistical analyses, have generally been more likely to observe significant differences in cortisol related to PTSD than those that have not (reviewed in Yehuda[7]). Furthermore, studies in which cortisol levels have been measured more carefully and under controlled conditions, such as every hour or half-hour via indwelling catheter in a clinical research center, have demonstrated overall reductions in cortisol levels across the diurnal cycle, but more importantly have shown that for large portions of the day and night, cortisol levels between persons with and without PTSD are not distinguishable.[11,14] Because of the marked fluctuations in cortisol levels over a normal 24-h period and the marked variability in individual sleep cycles and activity levels, detecting significant differences within the normal endocrinologic range using single point plasma or salivary estimates or cortisol is likely to require extremely large and homogenous samples. Particularly noteworthy in this context is the finding of low 8:00 AM cortisol levels in Vietnam veterans with PTSD compared to those without PTSD.[20] Significant differences were detected possibly because of subject homogeneity and the large sample size (of approximately 2400 subjects). Even so, the authors noted that the actual difference in cortisol levels in the PTSD group was only 4%. In contrast, evaluation of a large, heterogenous epidemiologic sample failed to detect differences in cortisol related to PTSD.[21,22]

Cortisol Levels are not Useful Diagnostic Marker for PTSD Just as They are not Useful as Diagnostic Makers for any Other Psychiatric Disorder

The failure to observe reliable group differences in cortisol levels simply on the basis of the diagnosis (as it is currently formulated) and the lack of an established range that would be reflective of PTSD, make cortisol levels poor diagnostic markers for PTSD. However, cortisol levels are not currently used as diagnostic markers for any psychiatric disorder in which there have been reports of significantly altered levels of this hormone compared to normals, and in which HPA alterations are considered a central component of pathophysiology. In studies of major depressive disorder, increased cortisol concentrations have

been noted, but have been demonstrated to occur in only a proportion of those studied (reviewed below).

Thus, though not useful as diagnostic indicators, studies using cortisol levels as dependent variables can be informative in the context of longitudinal studies because they will provide data concerning the stability of this measure over time[23–29]; in the context of multidisciplinary studies in which the influence of cortisol levels on other neurochemical, immune, neuroanatomic, medical, or cognitive measures have been evaluated,[30–37] and in examining the hormonal responses to environmental, neurochemical provocation or in response to treatment.[38–44] In this regard, one of the most interesting observations in PTSD has concerned the relative contributions of the sympathetic adrenal medullary and the HPA axis to the regulation of the response to stress in PTSD, both with respect to the "focal" trauma from which PTSD may have arisen, and the response to provocations in those already exposed. Although in the former case, cortisol levels have been reported to be lower in trauma-exposed persons at risk for the development of PTSD, levels in those with PTSD following exposure to environmental provocation are elevated.[41,42] The assessment of cortisol levels in PTSD should be done in the context of clearly formulated experimental questions that transcend simplistic queries regarding whether absolute levels of basal cortisol differ in persons with PTSD compared to persons without PTSD.

That Cortisol Levels are not Elevated in PTSD is an Important (i.e., "Positive") Finding

Initial observations of low and even normal cortisol levels in PTSD in the context of expecting significantly high levels of this stress hormone have presented a challenge to the field of biological psychiatry in that it was forced to reevaluate the well-established paradigm that the magnitude of stress responses can be evaluated as a function of cortisol elevations. It has only been more recently—and may in part be a result of studies of the neuroendocrinology of PTSD—that investigators have reconsidered the pathophysiologic significance of insufficient glucocorticoid signaling in stress-related neuropsychiatric disorders.[45] In any event, the necessity for clarification of basal hormone levels has served as the catalyst for more informative neuroendocrine studies that have provided more relevant information about the regulation of the HPA axis and its role in PTSD pathophysiology.

The Variability Across Published Studies Regarding Cortisol Levels is Informative

In cases where separate studies examine cortisol alterations in diverse populations, the lack of variability in cortisol alterations implies that alterations associated with low cortisol may only present in specific subtypes of PTSD

or trauma-exposed persons. There has been accruing evidence that cortisol-related alterations in PTSD reflect (one of possibly several) preexisting vulnerability factors that might increase the probability of developing PTSD following trauma exposure. In several prospective, longitudinal studies lower cortisol levels in the acute aftermath of trauma were associated with either the subsequent development of PTSD, or with the well-established risk factor of prior trauma exposure.[46–50] Accordingly it has been hypothesized that in some persons, reduced cortisol levels at the time of a trauma may result in a failure to inhibit stress-induced biologic responses (e.g., during and immediately following a traumatic event), leading to a perpetuation of physiological/emotional distress and, ultimately, to PTSD.[51,52] Regardless of whether pre- or peritraumatic stress hormone levels directly facilitate the development of PTSD, the proposition that cortisol levels are associated with some aspect of pre-exposure vulnerability to PTSD provides a plausible explanation for the variability in observations of cortisol levels in PTSD[53] in that lower cortisol levels may only be present in trauma survivors showing specific risk factors.

There are Often Correlations Within the PTSD Group with Indices of PTSD Symptom Severity

Baker *et al.*[18] failed to find group differences between Vietnam veterans with PTSD compared to nonexposed controls, but did report a negative correlation between 24-h urinary cortisol and PTSD symptoms in combat veterans. A negative correlation between baseline plasma cortisol levels and PTSD symptoms, particularly avoidance and hyperarousal symptoms, were observed in adolescents with PTSD.[54] Rasmusson *et al.*[19] failed to observe a significant difference in urinary cortisol between premenopausal women with PTSD and healthy women, but noted an inverse correlation between duration since the trauma and cortisol levels, implying that low cortisol is associated with early traumatization, a finding consistent with the observation of an inverse relationship between childhood emotional abuse and cortisol levels in adult children of Holocaust survivors.[55] Greater PTSD and depression severity was also associated with lower awakening cortisol in active duty police officers,[56] and in women with breast cancer.[57] Low cortisol levels were also recently found to be inversely related to PTSD symptom severity in a subgroup of combat veterans with PTSD displaying the BC11 GC genotype, a polymorphism of the glucocorticoid receptors (GRs) associated with increased glucocorticoid sensitivity.[58]

Cortisol levels have also been correlated with findings from brain imaging studies in PTSD. In one report, there was a positive relationship between cortisol levels and hippocampal *N*-acetylaspartate (NAA), a marker of cell atrophy presumed to reflect changes in neuronal density or metabolism, in subjects with PTSD, suggesting that rather than having neurotoxic effects, cortisol levels in

PTSD may have a trophic effect on the hippocampus.[59] Similarly, cortisol levels in PTSD were negatively correlated with medial temporal lob perfusion, while anterior cingulate perfusion and cortisol levels were positively correlated in PTSD, but negatively correlated in trauma survivors without PTSD, possibly reflecting an augmented negative hippocampal effect secondary to increased sensitivity of brain GR, which would account for the inverse correlation in PTSD despite equal cortisol levels in both the PTSD and non-PTSD groups.[60] The strong correlations between cortisol levels and other aspects of PTSD pathology suggest that optimally, understanding the relevance of cortisol to PTSD may require comprehensive investigations in persons with PTSD who differ with respect to symptom severity or other characteristics.

Methodologic Details Regarding How Cortisol was Determined are Relevant to Understanding Discrepancies in the Literature and Should not be Ignored

Methodologic details regarding the collection and processing of biologic samples, as well as details regarding how cortisol levels were ultimately determined, are given surprisingly little attention in the PTSD literature, perhaps because few investigators who have published studies of cortisol levels from participants they have recruited and evaluated, have been in a position to take responsibility for establishing and testing appropriate collection procedures and/or biologic assays. Accordingly, there has been a focus on methodologic issues relating to individual differences in such factors as age, gender, height, weight, menstrual status, and nicotine use in subjects (and checking for correlations in individual data sets between these variables and hormone concentrations for the purpose of identifying covariates is critical), but what has often been ignored are issues related to collection or assay of the biologic specimen. Certainly the fewest methodologic problems are likely to result from cortisol assayed from blood samples, since once collected, blood samples usually require centrifugation at room temperature (to extract either plasma or serum), and can be frozen (at –30'C), thawed and refrozen, without notably affecting cortisol concentrations. Of course, cortisol levels in plasma can be artificially elevated under conditions where routine venipuncture does not go smoothly (e.g., yet that this occurred may not be known to relevant parties, and even if it were, there is no way to statistically account for this). For this reason, studies in which blood samples are withdrawn via an indwelling catheter offer a significant advantage to those in which they are not. Samples collection from saliva and urine must first undergo extraction procedures prior to assay, and error can be introduced from many sources, including from preservatives in the collection tubes (required for analysis of catecholamines). Salivary samples can be contaminated with food particles. With urine samples there is the additional problem of insuring completeness of collections if subjects are asked to collect samples

at home. Home sampling is also problematic because it is impossible to ensure that subjects have adhered to the protocol with respect to collection times (for saliva), completeness of collection (for urine), as well as dietary or exercise restrictions.

Typical procedures for the assessment of cortisol include radioimmunoassay (RIA) and gas chromatography-mass spectrometry (GC-MS). However, the levels of "free" cortisol measured by RIA and GC-MS are not necessarily similar, since the former method will not distinguish free cortisol from its inactive metabolites and the latter will. This can be particularly problematic in the evaluation of cortisol from urinary samples. Furthermore, there can be differences in the sensitivity for the detection of cortisol levels in the low, medium, or high ranges even when commercially available RIA kits are used, depending on the number and concentration of internal standards, which should be optimized on the basis of whether expected cortisol levels are in the low, medium, or high ranges. These methodologic considerations are important because they contribute sources of variability that may obscure group differences that are expected to be subtle to begin with. The astute reader not only considers group differences, but evaluates absolute levels of hormones, and considers statistical outliers not only to evaluate the impact the number will have on the group mean and standard deviation, but also from the perspective of whether the value is plausible.

UNANSWERED QUESTIONS ABOUT BASAL CORTISOL LEVELS THAT REQUIRE RESOLUTION

Although it is not clear what more can be resolved from studies comparing 24-h integrated mean levels of cortisol in PTSD with another group, the assessment of cortisol at different time points throughout the day may be informative with respect to circadian rhythmicity of cortisol. One of the initial rationales for performing a comprehensive circadian rhythm analysis was to corroborate and extend findings from the 24-h urine excretion studies and those using single-point estimates and to ensure that findings from 24-h urine studies did not result from group differences in adherence to the collection protocol or individual differences in sleep/wake cycles, activity, diet, and other daily patterns. Thus, an initial study of circadian parameters in PTSD was conducted by obtaining 49 consecutive blood samples from three groups of subjects—Vietnam combat veterans with PTSD, subjects (largely veterans) with major depression, and nonpsychiatric comparison subjects—every 30 min over a 24-h period under carefully controlled laboratory conditions. This study demonstrated lower mean basal cortisol levels, primarily in the late evening and early morning hours in PTSD compared to the other groups. The major difference between PTSD and non-PTSD groups was that cortisol levels were lower in the late night and very early AM, and remained lower for a longer

period of time in PTSD during hours when subjects are normally sleeping. By the time of awakening, the peak cortisol release, however, was comparable in PTSD subjects and age-matched subjects. In a second study of women who had been sexually assaulted in childhood, cortisol levels were obtained every 15 min over a 24-h period. Here too, significantly low cortisol levels were observed in PTSD, this time in the afternoon and evening, but not morning, hours.

In Yehuda *et al.*, the raw cortisol data were subjected to single and multioscillator cosinor analyses to determine circadian rhythm parameters.[11] PTSD subjects displayed a greater dynamic range of cortisol that was reflected in an increased amplitude-to-mesor ratio occurring because relative to other groups, subjects with PTSD showed lower cortisol levels at the nadir of the cycle for longer periods of time, but did not show differences in peak cortisol levels. In contrast, depressed patients showed an increased mean cortisol release over the 24-h cycle, with a decreased amplitude-to-mesor ratio, and an elevated trough. The findings imply a potential for a greater reactivity of the system in PTSD, since reactivity, in part, is a function of the range of cortisol released over the diurnal cycle, which in PTSD, was greater.

It has been extremely difficult to evaluate from other studies, whether true differences in circadian rhythmicity exist. In one study, the above pattern showing increased/no increased cortisol in the morning, but lower cortisol at the evening of the cycle was also demonstrated.[61] However, another study showed that the greater AM to P.M. decline in PTSD occurred because subjects with PTSD went from a higher morning cortisol to a comparable evening cortisol levels in controls.[62] Yet a third pattern can be found in reports of low morning cortisol levels in PTSD, which results in a flattened rhythm. Given the recent emphasis on the importance of cortisol slope and range of cortisol, it is of interest to resolve issues related to circadian rhythm in PTSD. One suggestion is that such changes are related to longitudinal course, chronicity, or age of subjects.[63] Interestingly, the variance associated with findings of morning cortisol levels, in particular, may result from subtle protocol differences. Those that base morning samples on a certain time of day may yield dramatically different information than those that standardize to individual wake time. A recent report showed that although there were no absolute differences in cortisol levels associated with awakening samples, subjects with PTSD did not show the expected increase in cortisol at 30-min post-awakening, as did the other groups,[64] an effect that persisted, ultimately resulting in an overall decrease in cortisol secretion in PTSD, and a correlation between AUC of cortisol and overall PTSD symptomology. The authors suggest that failure to differentiate between salivary samples at immediate awakening and 30-min post-awakening may result in an inability to differentiate between subjects with and without PTSD (e.g., Ref. 65).

Even though in studies where fewer points are considered, it becomes difficult to do the kind of mathematical analyses that are needed to confirm

changes in circadian rhythm, it is nonetheless useful to determine whether there are differences even between peak and trough in disparate groups of PTSD subjects, in consideration of individual differences related to age, gender, type of trauma, age at exposure, and other risk or developmental factors in the context of well-designed studies.

RESULTS OF NEUROENDOCRINE CHALLENGES IN PTSD

What has been perplexing about the neuroendocrinology of PTSD is not simply that cortisol levels are low or even normal, but rather, that they are so despite evidence that corticotropin-releasing factor (CRF) levels appear to be increased.[66–68] PTSD is also associated with increased cortisol suppression in response to dexamethasone (DEX) administration[69–84] in most, but not all[85–87] studies. More recently, the adrenocorticotrophin hormone (ACTH) response to cortisol injection has also been found to be reduced.[88] Both these effects likely result from increased responsiveness of central or peripheral GR.[89–93] The profile of alterations in PTSD is clearly different from that observed in studies of acute and chronic stress and major depressive disorder, which have been associated with increased CRF, increased cortisol levels, and reduced cortisol suppression in response to DEX.[94–96] The presence of these alterations has made it difficult to discount cortisol observations in the normal or low range. On the other hand, while the results of neuroendocrine challenge studies have provided support for the fact that there are alterations in the HPA axis in PTSD, when seen in the aggregate, they have not always presented a uniform understanding of PTSD. As with the findings of ambient hormone levels in PTSD, it is important to be able to critically evaluate the literature so as to know which conclusions can be made with more certainty than others. This involves the ability to evaluate whether or not a particular challenge test has been constructed and interpreted appropriately.

Results of studies using the low-dose dexamethasone suppression test (DST) perhaps provide the most consistent findings in PTSD, in part because the DST is a relatively straightforward test that has been applied in a consistent manner, and in part because this test provides unambiguous information regarding negative feedback inhibition. In contrast, results using corticotrophin-releasing factor (CRF) challenge testing have been conflicting, in part because these findings must be interpreted in the context of other HPA axis alterations. Studies using probes, such as metyrapone have been inconclusive because of the disparate methodologies used. However, particularly problematic are cases in which interpretation is challenging because of the contradictory nature of the findings in any single given report. An analysis of findings from these different challenge tests is presented below to illustrate some of the interpretative issues that have arisen in trying to integrate the disparate observations.

Findings of DST Support the Idea of Increased Glucocorticoid Responsiveness

The DST provides a direct test of the effects of GR activation in the pituitary on ACTH secretion. Cortisol levels following DEX administration therefore provide an estimate of the strength of negative feedback inhibition, provided that the adrenal response to ACTH is not altered. There are several hundred published studies reporting on the use of the DST in depression, all reporting that approximately 40–60% of patients with major depression demonstrate a failure to suppress cortisol levels below 5.0 ug/100 dL in response to 1.0-mg of DEX.[95] Nonsuppression of cortisol results from a reduced ability of DEX to exert negative feedback inhibition on the release of CRF and ACTH,[96] thus, it is plausible that more extreme reductions in cortisol in response to DEX imply an enhanced negative feedback inhibition.

The initial DST studies in PTSD using the 1.0-mg dose of DEX did not consider the possibility of a hypersuppression to DEX, but rather tested the hypothesis that patients with PTSD might show a nonsuppression of cortisol similar to patients with major depressive disorder. A large proportion of the PTSD subjects studied also met criteria for major depression. Four[97–100] out of five[101] of the earlier studies noted that PTSD did not appear to be associated with cortisol nonsuppression, using the established criterion of 5 ug/100 mL at 4:00 P.M. A more recent study did not use the established criterion to determine nonsuppression, but nonetheless reported a greater mean cortisol in PTSD compared to normal subjects at 8:00 AM[61] In this study, Thaller *et al.*[61] reported that DEX resulted in a 67% suppression in PTSD ($n = 34$) compared to an 85% suppression in comparison ($n = 17$) subjects. Similarly, Atmaca *et al.*, showed a significantly higher DST nonsuppression in the PTSD group (63.12%) compared to healthy controls (79.6%) using the 1.0-mg DST.[86]

Although the 1.0-mg DST studies primarily focused on evaluating failure of normal negative feedback inhibition, Halbreich *et al.* noted that post-DEX cortisol levels in the PTSD group were particularly lower than subjects with depression and even comparison subjects,[98] leading Yehuda *et al.* to hypothesize that PTSD patients would show an enhanced, rather than reduced cortisol suppression to DEX and administered lower doses of DEX—0.50 mg and 0.25 mg—to examine this possibility.[69,70] A hyperresponsiveness to low doses of DEX, as reflected by significantly lower post-DEX cortisol levels, was observed in combat Vietnam veterans compared to nonexposed subjects. The finding of an exaggerated suppression of cortisol in response to DEX was also observed in adult survivors of childhood sexual abuse,[72] children who survived the Armenian earthquake,[71] Gulf War soldiers who were still in active duty,[73] Holocaust survivors,[76] older combat veterans, [79] depressed women with PTSD resulting from early childhood abuse,[82] and in a mixed group of trauma survivors with PTSD.[79] Only two studies, one[85] using a mixed civilian group, and one examining adolescents exposed to multiple traumatic events[87]

failed to find cortisol differences in response to DEX. However, the main difference between these studies and others is in the use of saliva samples obtained at home, rather than plasma samples obtained at confirmed standard intervals. Since the amount of free (active) cortisol is assessed preferentially in saliva, it may be that different findings relate to the higher fraction of bound cortisol in PTSD, a possibility further supported by the findings of increased corticosteroid-binding globolin (CBG) in PTSD.[102]

Attempts to Examine Negative Feedback Inhibition, With Metyrapone Have Produced Conflicting Results

Metyrapone is a drug that prevents adrenal steroidogenesis by blocking the conversion of 11-β-deoxycortisol to cortisol, thereby unmasking the pituitary gland from the influences of negative feedback inhibition. A sufficiently high dose of metyrapone results in an almost complete suppression of cortisol for several hours, effectively removing negative feedback inhibition of cortisol on the pituitary and hypothalamus. Eliminating cortisol negative feedback allows a direct examination of pituitary release of ACTH. If negative feedback inhibition of cortisol were exaggerated, as implied by the findings of enhanced cortisol suppression following DEX, ACTH levels in response to metyrapone would be expected to be elevated. In this initial study, metyrapone was administered in the morning, when HPA axis activity is relatively high and maximal pituitary activity can be achieved, so that group differences in pituitary capability could be examined. A very high dose of metyrapone—2.5-mg metyrapone—was used so as to result in an almost complete reduction in cortisol levels in both PTSD and normal subjects (i.e, the removal of negative feedback inhibition), but in a higher increase in ACTH and 11-β-deoxycortisol in combat Vietnam veterans with PTSD, compared to nonexposed subjects.[103] In the context of low cortisol levels and increased cerebrospinal fluid (CSF) and CRF levels, the findings supported the hypothesis of a stronger negative feedback inhibition in PTSD, but more importantly, helped establish that pituitary and adrenal insufficiency were not the likely culprits for low cortisol levels in PTSD (since the former would be associated with an attenuated ACTH response and the latter would not necessarily affect the ACTH response).

To date, there have been three other investigations in which effects of metyrapone have been investigated. None of the studies reported a similar finding of increased ACTH levels in response to metyrapone. The study most comparable to Yehuda *et al.* was that published by Kellner *et al.,* which examined the effects of metyrapone (at doses proportional to body weight) at 12 PM compared to the administration of placebo at that time of the day before in a group of 10 mixed civilians with PTSD and 10 comparison subjects.[104] Though metyrapone significantly increased ACTH, no group differences were observed. Kanter *et al.* failed to find evidence for an exaggerated negative

feedback inhibition using a different type of metyrapone stimulation paradigm.[102] In this study, a lower dose of metyrapone was administered over a 3-h period (750 mg at 7:00 am and 10:00 AM), but rather than simply examining the ACTH response to this manipulation, cortisol levels were reintroduced by means of an infusion, allowing the effects of negative feedback inhibition to be evaluated more systematically. Under conditions of enhanced negative feedback inhibition, the introduction of cortisol following metyrapone administration should result in a greater suppression of ACTH in PTSD. However, no significant differences in the ACTH response to cortisol infusion between PTSD and comparison subjects (but a nonsignificant trend, $P = 0.10$, for such a reduction) were observed. There was, however, a reduced response of 11-β-deoxycortisol. The authors concluded that their findings provided evidence of subclinical adrenocortical insufficiency.

Interpreting this study is complicated by the fact that metyrapone did not accomplish a complete suppression of cortisol in this study in either the PTSD or comparison group, thus, negative feedback inhibition was diminished, but not fully suppressed. In fact, metyrapone administration produced a greater suppression of cortisol in comparison subjects without differentially affecting ACTH, prior to cortisol infusion. Thus, it is entirely plausible that a statistically more robust decrease in ACTH (e.g., there was a nonsignificant trend for this) following cortisol infusion was not observed in the PTSD group because the endogenous cortisol present may have already been high enough to suppress ACTH secretion in the PTSD group. Interestingly, however, although metyrapone did not result in as great a decline in cortisol in PTSD, it did result in the same level of cortisol inhibition implying differences in the activity of the enzyme 11-β-hydroxylase. In the absence of an attenuated ACTH response, such a finding would support the idea of a reduced adrenal output. However, the trend for an ACTH response suggests that part of the failure to achieve statistical significance may have also occurred because of limited power, particularly given the lack of evidence for increased ambient ACTH levels in PTSD relative to normal controls. Dose-response studies using the higher versus lower dose of metyrapone should certainly be conducted to further address this critical issue.

Finally, metyrapone administration was used to evaluate CRF effects on sleep. In this study, 750 mg of metyrapone was administered at 8:00 AM every 4 h for 16 h, and cortisol, 11-β-deoxycortisol and ACTH levels were measured at 8:00 AM, the following morning. Cortisol, 11-β-deoxycortisol, and ACTH levels were increased in the PTSD group relative to the controls, suggesting that the same dose of metyrapone did not produce the same degree of adrenal suppression of cortisol synthesis.[105] Under these conditions, it is difficult to evaluate the true effect on ACTH and 11-β-deoxycortisol, which depends on achieving complete cortisol suppression, or at least the same degree of cortisol suppression, in the two groups. The endocrine response to metyrapone in this study does not support the model of reduced adrenal capacity since this would

have been expected to yield a large ratio of ACTH to cortisol release, yet the mean ACTH/cortisol ratio prior to metyrapone was no different in PTSD versus controls. On the other hand, the mean ACTH/cortisol ratio post metyrapone was lower, though nonsignificantly, suggesting, though rather inconclusively, the possibility of an exaggerated negative feedback rather than reduced adrenal capacity.

In conclusion, in contrast to studies using DEX, studies using metyrapone as a neuroendocrine probe has produced mixed findings. However, unlike the DST literature in which similar methodologies were used, these studies were all different with respect to time of day of administration (affecting whether examination of pituitary functioning is at its maximum or relatively quiescent), dose of metyrapone (affecting whether negative feedback is actually removed), and time elapsed from metyrapone administration to assessment of neuroendocrine response. Thus, although this powerful endocrine tool is potentially informative about negative feedback inhibition and other aspects of HPA functioning, more studies are needed.

Discrepancies Between Reported Findings and Their Interpretation

Studies in which two neuroendocrine challenges are performed in the same patients are potentially very informative, but sometimes, a contradiction between two findings in the same subjects merely points to design flaws in the study rather than the "complexity" of the HPA axis in PTSD. Rasmusson *et al.*[19] reported an augmented ACTH response to CRF in 12 women with PTSD compared to 11 healthy controls, which contradicted a prior report by Smith *et al.* showing a decrease in ACTH in response to CRF in combat veterans with PTSD,[106] and a more recent study by Kellner *et al.*[107] showing no differences in either ACTH or cortisol levels in response to CRF. The authors also performed a neuroendocrine challenge with 250 ug of cosyntropin (ACTH) in 10 of 12 PTSD and 7 of 11 controls to determine the response of the pituitary gland to this maximally stimulating dose. In response to this challenge, subjects with PTSD showed an increased cortisol response in ACTH, leading the authors to conclude on the basis of findings from both challenge tests, that PTSD is characterized by an increased reactivity of both the pituitary and adrenal which likely results from CRF hypersecretion (though this was not measured in the current study, nor manifest in basal assessments of ACTH or cortisol levels). A blunted ACTH response to CRF is classically considered to reflect CRF hypersecretion since such hypersecretion would result in a downregulation of pituitary ACTH receptors.[108–110]

The authors did not emphasize, what is perhaps the most intriguing and informative observation in the study, and that is that the magnitude of the ACTH response appeared to be much higher than the cortisol response in the PTSD subjects. The ACTH response was 87% greater in the subjects with PTSD, but the cortisol response was only 35% higher, suggesting that the

increased ACTH level did not result in a comparable stimulation of cortisol. Thus, the results of the CRF test clearly suggested a reduced adrenal activity for cortisol production or release. Thus, the results of the CRF test clearly suggested a reduced adrenal activity, since otherwise, cortisol levels would have been imparably increased following CRF in relation to the ACTH levels. In contrast, the demonstration of an increased cortisol response to cosyntropic suggests an increased, rather than reduced capacity for the adrenal to respond to ACTH. On the other hand, the demonstration of an increased cortisol response to cosyntropin suggests the opposite: an increased capacity for the adrenal to respond to ACTH. In part, the discrepancy between findings of the two endocrine challenge tests could be resolved by controlling for time of day of administration of ACTH (the methods section noted that this ranged from 8:15 AM to 4:15 PM), or by evaluating the relationship between the subset of persons who received both tests. Indeed, to conclude a greater reactivity of the pituitary gland in the context of a more reduced cortisol response would be plausible, but obviously not compatible with CRF hypersecretion, nor with the lack of group difference in ambient cortisol levels.

The above example illustrates the importance of understanding the rationale behind employing particular endocrine challenge tests, and anticipating possible outcomes and explanations. The CRF challenge test is potentially informative about the status of the pituitary gland. A blunted ACTH response would be expected in response to CRF administration, if under unstimulated conditions, there is increased hypothalamic CRF release. A blunted ACTH response to CRF could also occur under conditions of more exaggerated glucocorticoid feedback inhibition of the pituitary. However, an augmented ACTH response in one group versus another would generally be suggestive of decreased ambient hypothalamic CRF release, but could be further confirmed by administering metyrapone. The purpose of measuring both ACTH and cortisol in response to CRF is to further determine discrepancies between pituitary and adrenal responses to CRF that might identify alterations in the adrenal gland or negative feedback inhibition. Thus, if there were a relatively lower cortisol than ACTH response to CRF, this is indicative of endocrine perturbations that are not simply consequences of hypothalamic CRF release at all. The cosyntropin test would be an appropriate follow-up to the observation of higher ACTH than cortisol responses to CRF, but would only confirm the CRF findings in their demonstration of decreased cortisol, not increased cortisol responses. To the extent that pituitary and adrenal glands would be demonstrated to be "hyperresponsive" to challenge in the face of normal ambient hormone levels, this constellation might implicate changes in feedback sensitivity or glucocorticoid responsiveness.

GLUCOCORTICOID RESPONSIVENESS IN PTSD

Type II GR are expressed in ACTH and CRF producing neurons of the pituitary, hypothalamus, and hippocampus, and mediate most systemic

glucocorticoid effects, particularly those related to stress responsiveness.[111] The initial investigation of GR in PTSD was on the basis of the knowledge that low circulating levels of a hormone or neurotransmitter could result in increased numbers of available receptors[112] that improve response capacity and facilitates homeostasis. Reciprocally, alterations in the number and sensitivity of both type I (mineralocorticoid) and type II GR can significantly influence HPA axis activity, and in particular, can regulate hormone levels by mediating the strength of negative feedback. Either way, on the basis of initial observations of low cortisol levels, it seemed important to focus on responsiveness of glucocorticoids by examining alterations in GR.

The problem with this line of investigation has historically been a methodologic one, in that it has been difficult to directly measure GR in relevant tissue. Furthermore, estimates of cytosolic (rather than nuclear or total) GR number only provide partial information about GR number and responsiveness. Yet, in view of the finding that lymphocyte and brain GR were found to share similar regulatory and binding characteristics,[113] an initial study was undertaken to examine peripheral cytosolic GR number in the lymphocyte. A greater number of 8:00 AM, but not 4:00 PM, type II GR was reported in Vietnam veterans with PTSD compared to a normal comparison group.[90] Subsequently, an inverse relationship was observed between 24-h urinary cortisol excretion and lymphocyte GR number in PTSD and depression (i.e., low cortisol and increased receptor levels were observed in PTSD whereas in major depressive disorder, elevated cortisol and reduced receptor numbers were observed).[9]

Following the administration of 0.25-mg dose of DEX, it was possible to observe that the cortisol response was accompanied by a concurrent decline in the number of cytosolic lymphocyte receptors.[68] This finding contrasted the observation of a reduced decline in the number of cytosolic lymphocyte receptors in major depression, implying that the reduced cortisol levels following DEX administration may reflect an enhanced negative feedback inhibition in PTSD.[114]

Observations regarding the cellular immune response in PTSD are also consistent with enhanced GR responsiveness in the periphery. In one study, beclomethasone-induced vasoconstriction was increased in women PTSD subjects compared to healthy, nontrauma exposed comparison subjects.[90] Similarly, an enhanced delayed-type hypersensitivity of skin test responses was observed in women who survived childhood sexual abuse versus those who did not.[33] Because immune responses, like endocrine ones, can be multiply regulated, these studies provide only indirect evidence of GR responsiveness. However, when considered in the context of the observation that PTSD patients showed increased expression of the receptors in all lymphocyte subpopulations, despite a relatively lower quantity of intracellular GR as determined by flow cytometry, and in the face of lower ambient cortisol levels,[89] the findings more convincingly support an enhanced sensitivity of the GR to glucocorti-

coids. Furthermore, Kellner *et al.* reported an absence of alterations of the minerlocorticoid receptor in PTSD as investigated by examining the cortisol and ACTH response to spironolactone following CRH stimulation.[115]

The demonstration of increased glucocorticoid responsiveness in the live lymphocyte, as evidenced by the greater effects of DEX on lysozyme activity is also noteworthy. Mononuclear leukocytes isolated from the blood of 26 men with PTSD and 18 men without PTSD were incubated with a series of concentrations of DEX to determine the rate of inhibition of lysozyme activity; a portion of cells was frozen for the determination of GR. Subjects with PTSD showed evidence of a greater sensitivity to glucocorticoids as reflected by a significantly lower mean lysozyme IC_{50-DEX} [nM]. The lysozyme IC_{50-DEX} was significantly correlated with age at exposure to the first traumatic event in subjects with PTSD.[88] Since the GR gene uses different promotors in different target tissues,[116] there is appropriate concern about whether a change observed with respect to GR sensitivity in lymphocytes mirrors what occurs in the brain. However, that there are detectable differences in a gene product related to the activity of GR (i.e., in this case, lysozyme activity) in any target tissue (i.e., even the lymphocyte) is evidence of altered GR sensitivity, which might reflect either naturally occurring variants in GR polymorphisms that result in increased GR sensitivity or epigenetic modifications, such as cytosine methylation of GR promotors, either of which may promote increased cortisol signaling. For example, under certain environmental conditions, such as in response to a traumatic event, increased cortisol signaling would alter or interact with psychological and biologic risk factors to result in the PTSD clinical phenotype. This would be an analogous situation to that illustrated in a recent pharmacogenetic study, in which patients who carried a specific variation in the *FKBP5* gene, which encodes a co-chaperone of heat shock protein 90, responded much faster to antidepressants than a group that did not carry this mutation.[117]

The relationship between lysozyme IC_{50} and early trauma suggests that enhanced glucocorticoid responsiveness as reflected by this model is related to the PTSD risk factor of early trauma exposure. Yet it is impossible to know from the above results whether the IC_{50} is related to a cause or a consequence of this risk factor (early environmental exposure). Furthermore, this association does not explain the finding of lower IC_{50} in PTSD in the absence of early trauma, suggesting that this correlate is multiply determined.

There are several other unanswered questions regarding glucocorticoid responsiveness in PTSD that require further investigation. For example, while the lysozyme IC_{50-DEX} in lymphocytes appears to be associated with early traumatization, the cortisol response to DEX, which is also a measure of glucocorticoid responsivenss, is not correlated with early traumatization,[79] though one study did report a relationship between lysozyme IC_{50} and DST suppression in normal volunteers.[118] Thus, the link between glucocorticoid responsiveness and enhanced negative feedback inhibition should be further

investigated. This is particularly important insofar as studies in depression have shown a normalization of the DST in remitted depressives,[119–122] which argues that this measure of feedback inhibition may be related to transient phenomenon in PTSD, such as symptom severity. On the other hand, cortisol hypersuppression has recently been demonstrated in association with the risk factor of parental PTSD in adult children of Holocaust survivors and may therefore, constitute a stable marker in PTSD.[123] Consistent with this is a recent report that negative feedback alterations were premorbidly not different in high-risk probands with a positive family history for depression who developed later an affective disorder compared to controls.[124] Rather, reduced negative feedback inhibition developed only when high-risk subjects became affected. However, in this high-risk group, DST nonsuppression persisted upon successful treatment.[124] Thus, whether any of the neuroendocrine alterations observed in PTSD are transient or stable may be dependent on specific risk factors and their interactions with environmental events.

PUTATIVE MODELS OF HPA AXIS ALTERATIONS IN PTSD

Cortisol levels are often found to be lower than normal in PTSD, but can also be similar to or greater than those in comparison subjects. Findings of changes in circadian rhythm suggest that there may be regulatory influences that result in a greater dynamic range of cortisol release over the diurnal cycle in PTSD. Studies using the DST have consistently demonstrated that there is enhanced negative feedback inhibition of cortisol, at least at the level of the pituitary. An enhanced negative feedback inhibition certainly explains why ambient cortisol levels may be normal or even lower in the face of hypothalamic CRF hypersecretion, but more importantly, is compatible with the idea that there may be transient elevations in cortisol. It would be expected that the regulatory influences responsible for enhancing negative feedback inhibition (e.g., glucocorticoid responsiveness) would result in shorter-lived increases in cortisol on account of a more efficient containment of ACTH release. In contrast to other models of endocrinopathy, which identify specific and usually singular primary alterations in endocrine organs and/or regulation, the explanation that enhanced negative feedback inhibition explains other HPA alterations in PTSD is in large part descriptive and offers little explanation for why some individuals show such alterations of the HPA axis following exposure to traumatic experiences while others do not.

Another explanation for low cortisol levels in PTSD, and at least some of the other alterations observed, involves reduced adrenal output. This model certainly provides a reasonable explanation for why ambient cortisol levels would be lower than normal, and even for the relatively smaller magnitude of

differences in ACTH relative to cortisol, but does not account for why basal ACTH levels are not significantly higher in PTSD than in comparison subjects, particularly in light of evidence of CRF hypersecretion. One of the challenges in elucidating a neuroendocrinology of PTSD is in being able to resolve the apparent paradox that cortisol levels are low when CRF levels appear to be elevated, as well as to accommodate a dynamic process that accounts for observed diurnal fluctuations and potential responsivity to environmental cues. Heim *et al.* have again argued that in response to early trauma, CRF hypersecretion may result in a downregulation of pituitary CRF receptors leading to a decreased ACTH response.[40] However, it is not quite clear according to this why in such cases CRF hypersecretion would lead to pituitary desensitization and low cortisol as opposed to the more classic model of HPA dysfunction articulated for major depressive disorder in which the effect of hypothalamic CRF release on the pituitary would ultimately result in hypercortisolism. Furthermore, though findings of increased CRF levels in PTSD are important to the theory of enhanced negative feedback inhibition in PTSD, they are not necessarily relevant to theories of adrenal insufficiency. That is, to the extent that there are increases in CRF, these would not necessarily occur as a direct response to reduced adrenal output, but might have a different origin. Under conditions of reduced adrenal output, it is possible that compensatory changes in hypothalamic CRF might occur to the extent that there is a weaker negative feedback inhibition as a result of decreased cortisol output. But if this were occurring, it would be difficult to find an explanation for why the ACTH response to CRF and psychological stressors were augmented in relation to early traumatization.

Results of studies examining the cortisol response to DEX are compatible with both the enhanced negative feedback inhibition model and adrenal insufficiency. However, in the latter case, one would not expect that a reduced cortisol level to result from, or even be accompanied by change in the GR, but rather, would reflect reduced adrenal output rather than an enhanced containment of ACTH. Findings of a blunted ACTH response to CRF are compatible with the enhanced negative feedback model, but not the adrenal insufficiency hypothesis. Adrenal insufficiency would not be expected to result in a blunted ACTH response to CRF. On the contrary, primary adrenal insufficiency is characterized by an increased ACTH at baseline, and in response to CRF. Findings demonstrating an augmented ACTH to metyrapone are also consistent with enhanced negative feedback inhibition, but not adrenal insufficiency. Adrenal insufficiency is also not compatible with findings showing a greater activation of cortisol in the context of reduced ACTH responses to pituitary challenges.

There are other possible explanations that have not yet been carefully considered. Differences in glucocorticoid production in the adrenals, and glucocorticoid degradation in a variety of target tissues are also likely to be relevant to the neuroendocrine alterations in PTSD. These have not yet been

carefully investigated. Furthermore, heterologous influences on the HPA axis, such as regulation of the pituitary ACTH or adrenal cortisol by other neuropeptides and factors must also be considered and studied. Regardless of which model proves to be more explanatory, it appears clear that the majority of findings support the idea of an increased cortisol signaling capacity so that lower levels of cortisol efficiently suppress HPA function. Such an alteration could conceivably lead to a situation in which afferent pathways in at least some tissues are exposed to reduced levels of cortisol, which might, in turn, result in increased sympathetic activation, also noted in PTSD.[125–129]

FUTURE DIRECTIONS: THE POTENTIAL RELEVANCE OF EPIGENETICS

Findings suggesting that cortisol levels might have been low prior to exposure to a focal trauma as well as those indicating that the most severe abnormalities of adult glucocorticoid metabolism are seen with earliest age of trauma exposure, suggest that alterations are present as a result of developmental factors that then serve as regulators of long-term HPA axis dynamics. The recent observation that some cortisol-related alterations are observed in the infant[130] and adult[131] offspring of parents with PTSD also implies the possibility of transgeneral transmission relating to early glucocorticoid programming.[132] Glucocorticoid programming has been shown to occur both pre- and postnatally, and implicates epigenetic changes in association with HPA alterations in PTSD.[133]

Epigenetics refers to a transgenerationally transmissible functional change in the genome that can be altered by environmental events and does not involve an alteration of DNA sequence.[134,135] Several mechanisms of stable epigenetic gene regulation have been described in different organisms; of which the best characterized in the mammalian genome is DNA methylation at the cytosine site in CpG dinucleotides.[136] Methylation of polymerase II promotors is an efficient way of gene silencing, and accordingly, provides a concrete molecular mechanism through which genetic–environmental interactions occur.[137] Interest in epigenetics is sparked both by the general need to develop molecular markers that reflect the impact of the environment on gene activity, and by the realization that such mechanisms are certainly likely to be important with respect to PTSD. There is good evidence that DNA methylation is a mechanism operative in programming the activity of genes regulating HPA activity by early life events (i.e., differences in maternal care),[138,139] paralleling observations that early life events are associated both with the development of PTSD[131–142] and the HPA axis alterations[143,144] described in this condition. Such changes in the rat pups result in permanent changes in hippocampal GR expression and HPA function[145] and provide a clear molecular link between early environment

and gene expression and function. Interestingly, the alterations observed are in the same direction as those described in PTSD (i.e., increased GR sensitivity, enhanced cortisol response to DEX, lower cortisol levels), offering proof of principle that environmental exposures can result in such changes.

Although it is not immediately obvious what the similarities between effects of "positive" maternal behaviors (i.e., increased licking and grooming) and PTSD risk might be (i.e., *prima facie,* the latter would be expected to be associated with deprivation), in all other aspects, the possibility of defining the pathways by which environmental risk factors might directly alter GR expression, thus forming a basis for individual differences in endocrine function and, perhaps, vulnerability, appears to constitute an extremely relevant finding to PTSD. The parallels are indeed striking. In theory, different cells and tissues are particularly sensitive to changes in methylation at different times during development, though in some instances, such as the development of cancer,[145] DNA methylation appears to be central for the process throughout life. This is entirely congruent with findings in PTSD that point to a greater prevalence of PTSD following events occurring at specific developmental stages, though this disorder can develop throughout one's lifetime.[146]

The GR may be a particularly relevant target of epigenetic regulation on the basis of the observations that this receptor is subject to programming by early life events that result in permanent changes in physiology throughout life in rats.[138,139] A similar process in humans may likely be relevant for the development of PTSD. Furthermore, such changes are capable of being transmitted intergenerationally,[147,148] which also fits with recent observations of HPA axis alterations in both infants and adult offspring of parents with PTSD. For obvious reasons, genetic analyses will simply not detect environment–gene activity connections, and though endocrine studies can in principle detect them, endocrine activities often are determined by more recent life events that may obfuscate the impact of earlier events.[149] One of the major limitations of studying endocrine aspects of PTSD has been the inability to know for certain whether what is being measured constitutes a change associated with PTSD pathophysiology, the trauma that produced it, or the earlier risk factor. Though we have suggested above that neuroendocrine aspects of PTSD may be somewhat stable, it is inarguable that the pattern of DNA methylation is far more stable and is more likely to reflect earlier life events, rather than the cumulative effects of stress.

CONCLUSIONS

The HPA axis alterations in PTSD support the idea that different HPA axis alterations may be associated with different aspects of PTSD including risk for the development of this disorder. Clearly there is evidence that some features of the HPA axis may be altered prior to the exposure to a focal trauma.

Understanding that components of the HPA axis are not uniformly regulated (e.g., circadian rhythm patterns, tonic cortisol secretion, negative feedback inhibition, and the cortisol response to stress are differentially mediated), is critical in the integration of disparate findings, as is an appreciation for the fact that the HPA axis is a fundamentally dynamic system that may show transient increases or hyperresponsivity under certain environmental conditions.

The disparate observations observed in the neuroendocrinology of PTSD underscore the important observation of Mason *et al.*, that HPA response patterns in PTSD are fundamentally in the normal range and do not reflect endocrinopathy. In endocrinologic disorders, where there is usually a lesion in one or more target tissues or biosynthetic pathways, endocrine methods can usually isolate the problem with the appropriate tests, and then obtain rather consistent results. In psychiatric disorders, neuroendocrine alterations may be subtle, and therefore, when using standard endocrine tools to examine these alterations, there is a high probability of failing to observe all the alterations consistent with a neuroendocrine explanation of the pathology in tandem. The next generation studies will hopefully be able to apply more rigorous tests of neuroendocrinology of PTSD on the basis of the appropriate developmental issues and in consideration of the longitudinal course of the disorder, and the individual differences that affect these processes. No doubt such studies will require a closer examination of a wide range of biologic responses including the genetic, cellular, and molecular mechanisms involved in adaptation to stress.

ACKNOWLEDGMENTS

This work was supported by NIMH R01-2- 49555 and NIMH R01-0-64675 and VA MERIT grant.

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