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Interferon-alpha-Induced Inflammation is Associated with Reduced Glucocorticoid Negative Feedback Sensitivity and Depression in Patients with Hepatitis C Virus

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Abstract

Major medical illnesses are associated with increased risk for depression and alterations in hypothalamic-pituitary-adrenal (HPA) axis function. Pathophysiological processes such as inflammation that occur as a part of medical illnesses and their treatments have been shown to cause depressive symptoms, and may also affect the HPA axis. We previously reported that patients with hepatitis C virus chronically administered interferon (IFN)-alpha develop increased evening plasma cortisol concentrations and a flattened diurnal cortisol slope, which correlated with increased tumor necrosis factor (TNF) and its soluble receptor 2 (sTNFR2). Increased TNF and sTNFR2 were further correlated with depression and fatigue scores. The current study examined whether flattened cortisol slope might be secondary to reduced glucocorticoid receptor (GR) sensitivity, by measuring glucocorticoid negative feedback to dexamethasone (DEX) administration followed by corticotropin releasing hormone (CRH) challenge. In an exploratory analysis, 28 male and female patients with hepatitis C virus were studied at baseline (Visit 1) and after 12 weeks (Visit 2) of either IFN-alpha plus ribavirin (n=17) or no treatment (n=11). Patients underwent dexamethasone DEX-CRH challenge, neuropsychiatric assessments, and measurement of plasma TNF and sTNFR2 during each visit. IFN-alpha did not affect neuroendocrine responses following CRH but did increase post-DEX cortisol, which was correlated with flattening of the diurnal cortisol slope ($r=0.57$, $p=0.002$) and with increased depression scores ($r=0.38$, $p=0.047$).

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Conflict of Interest Statement

All authors declare that there are no conflicts of interest, and all financial disclosures are listed for each author: In the previous 12 months *Charles L. Raison* has served as an advisor for Otsuka-Lundbeck and PamLab, on the speakers bureau for Sunovion, PamLab and Merck and delivered non-branded promotional lectures for Otsuka; *Jennifer C. Felger*, *Ebrahim Haroon*, *Bobbi J. Woolwine*, and *Andrew H. Miller* have nothing to declare.

Furthermore, the change in post-DEX cortisol was associated with IFN-alpha-induced increase in sTNFR2 ($r=0.51$, $p=0.006$), which was in turn correlated with depression ($r=0.63$, $p<0.001$) and fatigue ($r=0.51$, $p=0.005$) scores. Whereas the relationship between sTNFR2 and depression scores were independent of the change in post-DEX cortisol, the correlation between post-DEX cortisol and depression scores was not significant when controlling for sTNFR2. These findings suggest that inflammation induced in patients with hepatitis C virus during IFN-alpha therapy precipitates decreased GR sensitivity to lead to a flattened diurnal cortisol slope. Decreased GR sensitivity may in turn further increase inflammation and its ultimate effects on behavior. Treatments that target inflammation and/or GR sensitivity may reduce depressive symptoms associated with medical illnesses.

Keywords

HPA-axis; inflammation; hepatitis C virus; dexamethasone; depression; fatigue

1. Introduction

Medical illnesses such as chronic viral infections, cancer and cardiovascular disease are associated with increased risk for depression as well as with changes in hypothalamic-pituitary-adrenal (HPA) axis function, including reduced glucocorticoid receptor (GR) sensitivity (Sephton et al., 2000, Abercrombie et al., 2004, Evans et al., 2005, Nikkheslat et al., 2015). One pathophysiologic process that may account for relationships among medical illness, depression and HPA dysregulation is inflammation (Haroon et al., 2012, Slavich, 2015). Increased inflammation has been observed in both medically ill and medically healthy patients with depression, and administration of inflammatory stimuli and cytokines to humans and laboratory animals causes depressive behaviors (Musselman et al., 2001b, Raison et al., 2006, Howren et al., 2009, Dowlati et al., 2010, Zunszain et al., 2013). Indeed, administration of the inflammatory cytokine interferon (IFN)-alpha for malignant melanoma or hepatitis C virus (HCV) leads to a range of depressive symptom including anhedonia, anxiety, sleep disturbances and fatigue (Raison et al., 2005, Felger and Lotrich, 2013), all of which have been found to correlate with induction of other cytokines and inflammatory signaling pathways (Prather et al., 2009, Raison et al., 2010c, Felger et al., 2011, Felger et al., 2012). Similar relationships between inflammatory markers and depressive symptoms have been observed in other medical illnesses, particularly in cancer patients undergoing treatment with chemotherapy and radiation that, like IFN-alpha, activate endogenous production of inflammatory cytokines and gene expression pathways (Bower et al., 2002, Bower et al., 2011, Torres et al., 2013).

Medical illnesses such as cancer and the administration of IFN-alpha have also been associated with changes in HPA axis function (Ticher et al., 1996, Rosmond and Bjorntorp, 2000, Sephton et al., 2000, Abercrombie et al., 2004, Matthews et al., 2006, Raison et al., 2010a). For example, women with breast cancer have been found to exhibit higher blood cortisol concentrations following oral dexamethasone administration, which correlated with a more flattened cortisol slope (Spiegel et al., 2006). Moreover, administration of IFN-alpha caused flattened cortisol slope and increased evening cortisol concentrations in patients with

hepatitis C virus, both of which correlated with depression and fatigue scores. Depression and fatigue scores were in turn positively correlated with induction of the inflammatory cytokine tumor necrosis factor (TNF) and its soluble receptor 2 (sTNFR2) (Raison et al., 2010a).

A number of studies have indicated that a flattened cortisol slope is associated with increased morbidity and mortality in the context of a variety of illnesses including diabetes, cardiovascular disease and cancer (Ticher et al., 1996, Rosmond and Bjorntorp, 2000, Abercrombie et al., 2004, Matthews et al., 2006, Schrepf et al., 2015). The mechanism of this altered diurnal cortisol rhythm in inflammation-related illness is unknown, although data suggests that changes in GR sensitivity may be involved (Pace et al., 2007). In the current study, we examined whether the flattened cortisol slope in patients with hepatitis C virus administered IFN-alpha may occur secondary to alterations in glucocorticoid negative feedback sensitivity as measured by the dexamethasone (DEX) suppression test followed by administration of corticotropin releasing hormone (CRH) (the DEX-CRH challenge) (Heim et al., 2008, Heim et al., 2009), and whether changes in sensitivity to DEX were associated with previously described IFN-alpha-induced increases in plasma TNF and sTNFR2 and depressive behaviors (Raison et al., 2010a).

2. Methods and Materials

2.1 Participants

Twenty-eight HCV-positive subjects (14 males, 14 females) were enrolled. Exclusion criteria included decompensated liver disease; liver disease from any cause other than HCV; unstable cardiovascular, endocrinologic, hematologic, renal or neurologic disease (as determined by physical exam and laboratory testing); infection with HIV (as reported by the subjects' treating physician); and history of schizophrenia or bipolar disorder and/or a diagnosis of major depression or substance abuse/dependence within 6 months of study entry, as determined by Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders- Fourth Edition (First et al., 1997). Patients were required to be off all antidepressant, antipsychotic, or mood stabilizer medications for at least 4 weeks prior to study entry (8 weeks for fluoxetine). Subjects were also required to discontinue other agents that might affect study results (i.e., narcotic analgesics, benzodiazepines, and anti-inflammatory agents) at least 2 weeks prior to sample collection. The subjects reported on herein represent a subsample of subjects included in previous studies on the effects of IFN-alpha on depressive behaviors, cognitive performance, neuroendocrine function, gene expression, and inflammatory responses (Raison et al., 2009, Raison et al., 2010a, Raison et al., 2010b, Felger et al., 2011, Felger et al., 2013, Felger et al., In press). All subjects provided written informed consent, and study procedures were approved by the Emory University Institutional Review Board.

2.2 Study Design

Study participants were enrolled in a longitudinal study examining immune, neuroendocrine, and neuropsychiatric variables at baseline and after either no treatment or chronic treatment with IFN-alpha/ribavirin (Raison et al., 2008, 2010a, Raison et al., 2010b, Felger et al.,

2011, Felger et al., 2012, Felger et al., 2013). For purposes of this study, DEX-CRH challenge involved administration of DEX at 2300 hours followed by collection of 6 post-DEX cortisol and adrenocorticotrophic hormone (ACTH) samples from 1230 to 1300 hours the following day (DEX suppression test) prior to administration of CRH and collection of 6 subsequent samples over 2 hours (CRH challenge) as previously described (Heim et al., 2008, Heim et al., 2009). The DEX-CRH challenge was performed at baseline (Visit 1) and at 12 weeks (Visit 2) from a subset of HCV+ patients treated with IFN-alpha plus ribavirin (n=17) or untreated HCV+ patients awaiting IFN-alpha/ribavirin therapy (control subjects, n=11). All subjects who underwent IFN-alpha treatment received either pegylated IFN-alfa-2b (Pegintron, Schering Plough, Kenilworth, NJ; 1.5µg/kg)(n=9) or pegylated IFN-alfa-2a (Pegasys, Roche-Genentech, San Francisco, CA; 180mg)(n=8) weekly administered subcutaneously plus oral ribavirin (800–1400 mg) daily. Participation in the treatment versus control group was determined by the patients and their physicians based on scheduling constraints and personal preferences and was not based on standardized criteria or controlled by study protocol.

For DEX-CRH challenge at Visits 1 and 2, subjects were admitted to the Emory University Clinical Research Network (CRN) for evaluation. To allow for accommodation to the CRN environment and to control for the effects of sleep and waking times on diurnal cortisol/ACTH and cytokine rhythms, subjects were admitted to the CRN at least 12 h prior to commencing blood draws for evaluation of hormones, TNF and sTNFR2. Lights out occurred at 2200 hours, and all subjects were awakened at 0715 hours. The following morning, subjects were awakened and served breakfast. An intravenous catheter was inserted at 0800 hours and blood was collected every hour from 0900 to 2100 hours to measure diurnal cortisol slope, and mean diurnal TNF and sTNFR2 concentrations (due to lack of significant diurnal change in this cytokine and its receptor) prior to conducting the DEX-CRH test during Visit 1 compared to Visit 2 as described (Raison et al., 2010a). During this time, neuropsychiatric assessments were also conducted. For the DEX-CRH challenge, a dose of 1.5 mg dexamethasone (Roxane Laboratories, Columbus, OH) was administered orally at 2300 hours following collection of the 2100 hour diurnal sample. On the following day, an intravenous catheter was inserted at 1200 hours and 6 blood samples were collected every 30 minutes starting at 1230 hours to determine mean post-DEX cortisol and ACTH concentrations as a measure of DEX suppression, immediately followed by post-CRH challenge samples. Immediately after the 1500 hour sample, a bolus intravenous injection of 1 µg/kg ovine CRH (Ferring, Suffern, NY, USA) was administered. Blood samples were collected at 5, 15, 30, 60, 90, and 120 min after CRH injection to determine maximal post-CRH hormone responses and the area under the curve (AUC). Blood was collected into chilled EDTA-coated tubes and immediately centrifuged at $1000 \times g$ for 10 min at 4°C, and plasma was removed and frozen at –80°C until assay. Subjects were asked to rest quietly during blood sampling. Urine drug screens were conducted at both study visits to rule out substance abuse. Control subjects participated in all study procedures in parallel with IFN-alpha/ribavirin-treated patients. The timeline of diurnal plasma sampling, behavioral assessments, DEX-CRH challenge administration and respective sampling intervals are detailed in Figure 1.

2.3 Neuropsychiatric Assessments

Neuropsychiatric assessments were conducted at baseline and 12 weeks of study participation. Depression was evaluated using the Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979). The MADRS is a 10-item, clinician-administered scale that assesses the severity of depressive symptoms. Fatigue was assessed using the self-report, 20-item Multidimensional Fatigue Inventory (MFI) (Smets et al., 1995).

2.4 Sample Analysis

Commercially available immunoradiometric assay and radioimmunoassay kits were used for the assessment of plasma ACTH (ALPCO Diagnostics, Salem, NH, USA and Nichols Institute Diagnostics, San Juan Capistrano, CA, USA when available) and cortisol (DiaSorin Stillwater, MN, USA) respectively (Raison et al., 2010a). Intra and inter-assay coefficients of variation respectively were 2.8 and 5.7% (ALPCO) or 4.5 and 6.3% (Nichols) for ACTH and 8.5 and 12.7% for cortisol. Due to our previous findings that TNF and sTNFR2 were the only plasma cytokines to increase during IFN-alpha administration, our studies focused on plasma concentrations of TNF and sTNFR2, which were measured by high-sensitivity and Quantikine enzyme-linked immunosorbent assay (ELISA) kits, respectively (R&D Systems, Minneapolis, MN, USA). Assays were performed according to the manufacturer's specifications, and were run in duplicate. Inter- and intra-assay variability were reliably <12% for TNF and <10% for sTNFR2. All biological samples were analyzed by research staff blinded to the clinical status of study participants.

2.5 Statistical Analysis

Differences between groups were assessed using *t* tests, Chi-squared Tests, or Fisher's Exact Test for categorical variables. To determine the effect of treatment on hormone responses to the DEX-CRH challenge, the change in mean post-DEX plasma cortisol and ACTH, and change in maximum and AUC post-CRH plasma cortisol and ACTH from Visit 1 to Visit 2 were calculated as post minus pre (i.e. the *delta*; Visit 2 - Visit 1). Multivariate general linear models were used to examine the effects of treatment (IFN-alpha plus ribavirin versus control) on *delta* cortisol and ACTH measures following DEX and CRH while adjusting for age, sex, race, body mass index (BMI), and history of major depression (MD). Post-CRH ACTH data for one IFN-alpha-treated subject was excluded from multivariate analysis due to a significant Grub's test for statistical outliers ($p < 0.05$). To determine the relationship between DEX sensitivity and cortisol slope, we calculated the diurnal cortisol slope by natural log-transforming cortisol values and using the β value of the regression of 13 samples collected every hour from 0900 to 2100 hours on the day prior to DEX administration, as described previously (Sephton et al., 2000, Abercrombie et al., 2004, Giese-Davis et al., 2004, Bower et al., 2005, Raison et al., 2010a). The *delta* diurnal cortisol values (change in diurnal slope from Visit 1 to Visit 2) were used for statistical comparison with *delta* post-DEX cortisol. Because larger beta values (i.e. values closer to zero) reflect a flatter slope (Abercrombie et al., 2004), positive *delta* slope values reflected flattening of the diurnal slope over time. To determine relationships between inflammation and DEX sensitivity, Pearson correlation coefficients were calculated to evaluate the association

between the *delta* post-DEX mean cortisol and the *delta* for TNF and sTNFR2 (change in mean diurnal concentrations from Visit 1 to Visit 2) in all subjects. To further evaluate associations between inflammation and DEX suppression in patients administered IFN- α , *delta* post-DEX cortisol was compared between patients who exhibited “high” versus “low” *delta* sTNFR2 (versus $<$ the median of *delta* sTNFR2) the by T test. To determine relationships between inflammation or DEX sensitivity with behavior, Pearson correlation coefficients were calculated to evaluate the association between the *delta* post-DEX mean cortisol and the *delta* for sTNFR2 with depression and fatigue (as measured by MADRS and MFI scores, respectively) in all subjects. All significant linear relationships were assessed in linear models using backward (*significance level stay* = 0.05) and forward (*significance level entry* = 0.05) selection while controlling for age, sex, race, body mass index (BMI), and history of major depression (past MD). Partial correlation coefficients were calculated to establish independent relationships between TNF and sTNFR2 and change in hormone responses while controlling for change in behavior, and vice versa, using linear regression models that included covariates. Tests of significance were two-tailed with $\alpha < 0.05$, and all statistics were conducted using SPSS software (Chicago, IL, USA).

3. Results

3.1 Subject Characteristics

As shown in Table 1, no significant differences between IFN- α /ribavirin-treated subjects and controls were observed for relevant clinical characteristics including age, race, sex, BMI and past history of substance abuse. However, there was a trend toward a significant difference between IFN- α -treated subjects and controls for history of MD ($p=0.082$). Therefore, history of MD was included as a covariate in all linear regression and general linear models, along with age, sex, race and BMI. It should also be noted that there were no differences between IFN- α -treated and control subjects in baseline depression (MADRS) and fatigue (MFI) scores. However, IFN- α -treated subjects displayed significant increases in depression (MADRS score: $t=-3.58$, $df=26$, $p=0.001$) and fatigue (MFI score: $t=-4.25$, $df=26$, $p<0.001$) after 12 weeks of treatment compared to controls. Eight out of 17 patients administered IFN- α plus ribavirin (47%) exhibited MADRS scores ≥ 15 , consistent with clinically significant depressive symptoms (Potter et al., 2004, Felger et al., 2011, Felger et al., 2012).

3.2 DEX-induced suppression of cortisol was significantly decreased in patients administered IFN- α compared to controls, and correlated with the diurnal cortisol slope

To evaluate the effect of IFN- α administration on plasma hormone responses to DEX-CRH challenge, the change in mean post-DEX concentrations of cortisol and ACTH from Visit 1 (baseline) to Visit 2 (week 12) of the study (*deltas*), and the *delta* maximal and AUC cortisol and ACTH responses to CRH were compared in IFN- α -treated subjects ($n=17$) and controls ($n=11$) while controlling for age, sex, race, BMI and history of MD. The mean *delta* post-DEX cortisol, but not ACTH, was significantly higher in IFN- α -treated patients compared to controls ($F[1,27]=7.85$, $p=0.011$) (Table 2), indicating that post-DEX cortisol suppression was significantly reduced by 12 weeks of IFN- α /ribavirin

administration compared to control. Data in Table 2 are presented as the mean ($\pm$ SD) for Visit 1 and Visit 2 that were used to calculate the *deltas*, along with the *deltas* ($\pm$ SD), which were statistically compared. No other significant effects of IFN-alpha/ribavirin administration on post-DEX suppression or post-CRH stimulation were observed (all $p>0.05$).

Consistent with our previous findings (Raison et al., 2010a), IFN-alpha-treated patients compared to controls exhibited significantly increased *delta* diurnal cortisol slope (more positive change in the β values) prior to DEX administration when controlling for age, sex, race, BMI and history of MD ($F[1,26]=17.93$, $p<0.001$) (Table 2), indicating a flattened cortisol slope following IFN-alpha exposure (mean *delta* slope IFN-alpha: 0.02 ± 0.04 , mean *delta* slope control: -0.01 ± 0.03). Interestingly, *delta* post-DEX cortisol was significantly positively correlated with *delta* diurnal cortisol slope ($r=0.57$, $df=26$, $p=0.002$), and *delta* post-DEX cortisol remained the most significant predictor of *delta* cortisol slope in both backward and forward linear regression models including age, sex, race, BMI and history of MD ($p<0.01$).

3.3 Increased plasma sTNFR2 was associated with decreased suppression of cortisol in IFN-alpha-treated patients and the sample as a whole

Relationships between post-DEX cortisol suppression and induction of TNF and sTNFR2 were examined by correlating *delta* post-DEX cortisol with *delta* TNF (control mean Visit 1 = 1.59 ± 1.05 , mean Visit 2 = 1.66 ± 1.11 , $\Delta=0.07\pm0.33$; IFN-alpha mean Visit 1 = 2.03 ± 0.95 , mean Visit 2 = 2.59 ± 0.97 , $\Delta=0.56\pm0.52$) and sTNFR2 (control mean Visit 1 = 2.06 ± 0.69 , mean Visit 2 = 2.20 ± 0.93 , $\Delta=0.13\pm0.36$; IFN-alpha mean Visit 1 = 2.78 ± 0.82 , mean Visit 2 = 3.87 ± 1.12 , $\Delta=1.11\pm0.59$). This exploratory analysis in all patients revealed a significant positive association between *delta* post-DEX cortisol and *delta* sTNFR2 ($r=0.51$, $df=26$, $p=0.006$) (Figure 2a) but not TNF, and *delta* sTNFR2 remained the most significant predictor of *delta* post-DEX cortisol in both backward and forward linear regression models including age, sex, race, BMI and past MD ($p<0.01$).

To examine whether the association between glucocorticoid sensitivity and inflammation was mediated by IFN-alpha-induced increases in post-DEX cortisol and mean sTNFR2, the relationship between *delta* post-DEX cortisol and *delta* sTNFR2 was examined by correlation in IFN-alpha-treated subjects only ($n=17$). Although the study was not powered to detect a statistically significant relationship between *delta* post-DEX cortisol and *delta* sTNFR2 in the IFN-alpha-treated subjects alone ($r=0.43$, $df=15$, $p=0.089$), *delta* sTNFR2 was the most significant predictor of *delta* post-DEX cortisol in both backward and forward linear regression models including age, sex, race, BMI and past MD when entry and stay criteria were relaxed to $p<0.10$. Additionally, when the IFN-alpha treated patients were stratified by those exhibiting “high” versus “low” (versus $<$ the median) *delta* sTNFR2 (0.69 ng/ml plasma), those patients with high IFN-alpha-induced increases in sTNFR2 had significantly higher IFN-alpha-induced increases in post-DEX cortisol ($t=-2.3$, $df=9$, $p<0.05$) (Figure 2b).

3.4 Increased plasma sTNFR2 was associated with increased depression and fatigue, independent of decreases in glucocorticoid sensitivity

Delta post-DEX cortisol and *delta* sTNFR2 were correlated with *delta* depression and fatigue scores to examine associations between decreased post-DEX cortisol suppression and increased inflammation with behavioral change. Interestingly, *delta* sTNFR2 was significantly associated with *delta* MADRS ($r=0.63$, $df=26$, $p<0.001$) (Figure 3a) and MFI scores ($r=0.51$, $df=26$, $p=0.005$) (Figure 3b) in all patients, and *delta* sTNFR2 remained the most significant predictor of *delta* MADRS and *delta* MFI in both backward and forward linear regression models including age, sex, race, BMI and past MD (all $p<0.001$). *Delta* post-DEX cortisol was also significantly associated with *delta* MADRS ($r=0.38$, $df=26$, $p=0.047$), which remained significant in backward and forward linear regression models controlling for covariates ($p<0.05$). *Delta* post-DEX cortisol was not significantly correlated with *delta* MFI ($r=0.34$, $df=26$, $p=0.082$).

To determine whether the relationship between *delta* sTNFR2 and *delta* MADRS scores was independent of *delta* post-DEX cortisol, or vice versa, partial correlation coefficients were calculated by including both predictors in a linear regression model with the covariates and *delta* MADRS as the dependent variable. Interestingly, *delta* sTNFR2 remained a highly significant predictor of *delta* MADRS score ($r=0.55$, $df=26$, $p=0.003$), whereas *delta* post-DEX cortisol and *delta* MADRS score were no longer associated when controlling for sTNFR2 ($r=0.09$, $df=26$, $p=0.668$). Similarly, a positive relationship between *delta* sTNFR2 and *delta* MFI score was maintained when controlling for *delta* post-DEX cortisol ($r=0.38$, $p=0.057$). Furthermore, partial correlation coefficients were also calculated which revealed that the above mentioned relationship between *delta* sTNFR2 and *delta* post-DEX cortisol was significant when controlling for the covariates and the *delta* MADRS scores ($r=0.46$, $df=26$, $p=0.033$), indicating that the association between increase in inflammation and decrease in glucocorticoid sensitivity was independent of an increase in depression.

4. Discussion

IFN- α administration to HCV+ patients led to significantly increased cortisol concentrations following DEX administration, suggesting that IFN- α exposure produced a decrease in GR sensitivity. Consistent with our previous findings (Raison et al., 2010a), IFN- α administration was also associated with flattening of the diurnal cortisol slope, which correlated with increased post-DEX cortisol. Increases in post-DEX cortisol concentrations were further correlated with IFN- α -induced increases in the TNF soluble receptor, sTNFR2. Although both the increase in post-DEX cortisol and the induction of sTNFR2 correlated with increased depression scores, the relationship between post-DEX cortisol and increased depression was not significant when controlling for the increase in sTNFR2. Conversely, the relationship between increased sTNFR2 and increased depression occurred independently of effects on post-DEX cortisol, and vice versa. These findings support the idea that flattening of the diurnal cortisol slope in patients administered IFN- α may be due to inflammatory cytokine effects on GR sensitivity. Moreover, these findings are consistent with the idea that inflammation and the production of cytokines

during medical illness may independently contribute both to depressive symptoms and HPA axis dysregulation.

Although IFN- α -treated patients did meet clinical criteria for DEX suppression of cortisol (post-DEX cortisol <5 $\mu\text{g/dl}$, equivalent 50 ng/ml or $\mu\text{g/L}$) (Hubain et al., 1998), the mean post-DEX cortisol (Vegiopoulos and Herzig, 2007) increased by an average of 23.8%, with the patients that exhibited the most change showing a $\sim 60\%$ increase in post-DEX cortisol. Our findings of decreased post-DEX suppression of cortisol along with flattened cortisol slope in patients with hepatitis C virus administered IFN- α is similar to that which has been observed in patients with metastatic breast cancer (Spiegel et al., 2006), and in patients with major depression (Pariante and Miller, 2001, Jarcho et al., 2013). Decreased sensitivity to DEX administration and flattened slope are related manifestations of HPA axis dysfunction, which has been shown to contribute to metabolic disease, including obesity and insulin resistance (Dallman et al., 2007, Vegiopoulos and Herzig, 2007, Champaneri et al., 2013). Therefore, understanding mechanisms that contribute to decreased DEX sensitivity in patients with psychiatric and medical illness may be relevant to preventing further stress-related metabolic complications and co-morbidities.

One of the most interesting findings of the present study was that although a moderate correlation between IFN- α -induced changes in DEX sensitivity and depression existed, this relationship was no longer significant when controlling for induction of sTNFR2. However, relationships were observed between increase in sTNFR2 with both depressive behavior and change in DEX sensitivity, which were both statistically independent of each other. Therefore, it is likely that inflammatory processes such as TNF signaling that have been shown to contribute to the potent effects of IFN- α on behavior may also affect the HPA axis. Indeed, we have previously found that increased TNF and sTNFR2 (which increases in response to increased TNF activity and is stable in the blood) were associated with IFN- α -induced depression (Raison et al., 2010a).

In terms of the mechanisms by which IFN- α may have led to decreases in DEX sensitivity, *in vitro* studies have demonstrated that IFN- α can decrease GR function through activation of signal transducer and activator of transcription 5 (STAT5), which was in turn found to bind to the GR in the nucleus and thereby prevent GR-mediated gene transcription (Hu et al., 2009, Pace et al., 2011). Other IFN- α -induced cytokines (e.g. TNF) can also activate inflammatory signaling pathways such as nuclear factor kappa B (NF- κB) and mitogen activated protein kinases (MAPKs), which can additionally inhibit GR signaling and GR translocation from the cytoplasm to the nucleus (Pariante et al., 1999, Wang et al., 2004, Engler et al., 2008). Indeed NF- κB , a lynchpin molecule through which cytokines activate inflammation, is well known to inhibit GR function via nuclear protein-protein interactions (Smoak and Cidlowski, 2004). Moreover, there appears to be a reciprocal relationship between signaling through NF- κB versus GR with repeated demonstration of increased activation of NF- κB -associated genes in conjunction with reduced activation of GR-regulated genes in cancer patients and their caregivers, as well as other populations experiencing significant psychosocial stress (Cole et al., 2007, Miller et al., 2008, Bower et al., 2011). Activation of inflammatory signaling pathways including p38 MAPK by interleukin (IL)-1 has also been shown to disrupt GR signaling through blocking

GR translocation from the cytoplasm to the nucleus (Pariante et al., 1999, Wang et al., 2004, Engler et al., 2008). This decrease in GR function can then further contribute to increased inflammation due to the inability of the GR to reduce inflammatory transcriptional pathways (Pariante et al., 1999, Smoak and Cidlowski, 2004, Nikkheslat et al., 2015). Pharmacological strategies that block inflammatory signaling pathways, such as STATs, NF- κ B, and MAPKs, or inflammatory cytokines, such as TNF, may have therapeutic potential in reversing depressive symptoms and may also increase GR sensitivity in patients with high inflammation (Monk et al., 2006, Tying et al., 2006, Raison et al., 2013, Lopresti et al., 2014).

Interestingly, we did not observe an effect of IFN-alpha administration on ACTH following DEX suppression, or on the response in either ACTH or cortisol following CRH challenge. These results may indicate that the effects of IFN-alpha on GR sensitivity are occurring primarily in the periphery. This is consistent with the finding that increase in peripheral sTNFR2 was associated with depressive behavior, which likely involves cytokine effects on brain neurotransmitters and neurocircuitry (Felger and Lotrich, 2013, Miller et al., 2013), independently of the change in post-DEX cortisol, which may reflect IFN-alpha effects on GR sensitivity in the periphery. However, these hypotheses are largely speculative and cannot be determined conclusively from the results obtained from this exploratory study. Additionally, IFN-alpha administration and the associated increase in cytokines may exert a direct stimulatory effect on cortisol production (Silverman et al., 2005). Indeed, although the precise mechanisms are unknown, circulating and intra-adrenal cytokines have been shown to directly increase glucocorticoid release by both potentiating the effects of ACTH and by stimulating steroidogenesis (Judd and MacLeod, 1995, Tkachenko et al., 2011).

Although this study provides interesting data suggesting that inflammation in medical illness may independently influence the HPA axis function and behavior, the sample size was small. Nevertheless, this exploratory analysis revealed moderate effect sizes ($r=0.5-0.6$) for relationships between induction of sTNFR2 and change in DEX sensitivity and behavior in the sample as a whole ($n=28$). However, we were underpowered to detect significant relationships specifically within the IFN-alpha-treated patients ($n=17$). It is worth noting that hepatitis C virus infection may be associated with a low-grade inflammation that is not as potent as the effects of IFN-alpha administration, but that may have contributed to relationships between change in sTNFR2, DEX sensitivity and behavior over time, even in control subjects not receiving IFN-alpha. Another limitation of this study includes the fact that all of the IFN-alpha-treated patients were concomitantly treated with ribavirin, which may contribute to the depressive burden of IFN-based hepatitis treatment (Asnis et al., 2004). However, IFN-alpha monotherapy for malignant melanoma has been associated with profound induction of depression and fatigue (Musselman et al., 2001a, Raison et al., 2005, Capuron et al., 2007), indicating that treatment effects on behavior in this study were likely attributable to specific effects of IFN-alpha. Additionally, the present study was not a randomized experiment in which patients were experimentally allocated to treatment and control groups. Therefore, a trend for the treatment group to have a higher rate of past MD was observed. To limit the potential effect of history of MD or other demographic and clinical variables, appropriate covariates were controlled for in statistical models.

5. Conclusions

Overall, this study provided evidence that chronic IFN- α administration may decrease glucocorticoid negative feedback sensitivity through direct effects on the GR, or through GR effects via activation of TNF-related inflammatory signaling pathways, which in turn lead to flattening of the diurnal cortisol slope. Furthermore, peripheral inflammatory activity was found to be related to increased depressive symptoms in these patients, independent of the change in DEX sensitivity. These results support the hypothesis that increased inflammation in medical illness may contribute to decreased glucocorticoid sensitivity which in turn may further increase inflammation and its ultimate effects on behavior and HPA axis function.

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Highlights

- Interferon (IFN)-alpha decreased glucocorticoid sensitivity in association with flattened diurnal cortisol slope in patients with hepatitis C virus
- Decreased glucocorticoid sensitivity was correlated with increased IFN-alpha-induced inflammation, as measured by induction of soluble tumor necrosis factor receptor 2 (sTNFR2)
- Increased sTNFR2 predicted increased depression and fatigue scores, independent of the change in glucocorticoid sensitivity
- Inflammation in medical illness can decrease glucocorticoid sensitivity, which may in turn further increase inflammation and its ultimate effects on behavior

Visit 1 (Baseline) and Visit 2 (12 weeks IFN-alpha or control)

Fig. 1. Timeline of procedures during study Visit 1 (baseline) and study Visit 2 (12 weeks)
Patients with hepatitis C virus administered IFN-alpha plus ribavirin therapy or controls patients were admitted to the inpatient research clinic for all procedures. Timeline denotes time of day for hourly diurnal blood sampling, behavioral assessments, DEX and CRH administration and respective post-DEX-CRH sampling intervals.

Fig. 2. Increased *delta* post-dexamethasone (DEX) cortisol (the change from Visit 1 to Visit 2) in IFN-alpha-treated and control hepatitis C patients was associated with increased *delta* soluble tumor necrosis factor receptor 2 (sTNF-R2)

The *delta* post-DEX cortisol concentrations (mean of 6 samples collected every 30 minutes between 1230 to 1500 hours following administration of DEX at 2100 hours the evening before) were positively correlated with the *delta* mean diurnal sTNF-R2 in hepatitis C patients administered interferon (IFN)-alpha therapy and control patients assessed at baseline and 12 weeks (A). *Delta* post-DEX cortisol was significantly higher in IFN-alpha-treated subjects who exhibited “high” versus “low” (or < the median) *delta* sTNF-R2 (B). Data are summarized as mean $\pm$ SE, * $p<0.05$

Fig. 3. Increased *delta* post-dexamethasone (DEX) cortisol (the change from Visit 1 to Visit 2) in IFN-alpha-treated and control hepatitis C patients was associated with increased *delta* depression and fatigue scores

The *delta* post-DEX cortisol concentrations (mean of 6 samples collected every 30 minutes between 1230 to 1500 hours following administration of DEX at 2100 hours the evening before) were positively correlated with the *delta* Montgomery-Asberg Depression Rating Scale (MADRS) (A) and Multidimensional Fatigue Inventory (MFI) (B) scores in hepatitis

C patients administered interferon (IFN)-alpha therapy and control patients assessed at baseline and 12 weeks.

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Table 1

Characteristics of Study Participants

Characteristic	Control (n=11)	IFN-alpha (n=17)	p-value*
Age (mean, SD)	45.5 (7.3)	47.4 (6.1)	0.450
Sex (n, %) Males	5 (45.5)	9 (52.9)	0.700
Race (n, %)			0.334
Caucasian	6 (54.5)	8 (47.1)	
Black	5 (45.5)	6 (35.3)	
Hispanic	0 (0)	2 (11.8)	
Asian American	0 (0)	1 (5.9)	
History MD (n, %)	0 (0)	4 (23.5)	0.082
History Substance Abuse (n, %)	8 (72.7)	18 (69.2)	0.800
BMI (mean, SD)	28.5 (5.7)	31.4 (4.4)	0.142
Baseline MADRS (mean, SD)	3.5 (5.1)	3.1 (4.0)	0.807
Baseline MFI (mean, SD)	39.7 (12.8)	40.4 (15.2)	0.903
12 week MADRS (mean, SD)	3.3 (3.9)	14.8 (10.1)	0.001
12 week MFI (mean, SD)	35.5 (13.3)	65.5 (20.8)	<0.001

BMI, body mass index; MADRS, Montgomery-Asberg Depression Rating Scale; MD, major depression; MFI, Multidimensional Fatigue Inventory.

* Significant relationships are indicated in bold.

Table 2

Change in Cortisol and ACTH responses to DEX-CRH challenge and diurnal cortisol slope from Visit 1 to Visit 2 (Delta) in IFN-alpha-treated and control subjects.

	Control (n=11)			IFN-alpha (n=17)		
	Visit 1	Visit 2	Delta	Visit 1	Visit 2	Delta
<i>Post-DEX (mean, SD)</i>						
Mean cortisol (µg/dl)	1.27 (0.38)	1.32 (0.29)	0.05 (0.34)	1.22 (0.26)	1.50 (0.37)	0.28 (0.31) *
Mean ACTH (pg/ml)	6.47 (2.40)	6.60 (3.03)	0.13 (1.25)	5.77 (2.48)	4.96 (2.37)	-0.81 (1.74)
<i>Post-CRH (mean, SD)</i>						
Max cortisol (µg/dl)	7.77 (5.39)	6.47 (4.56)	-1.31 (3.05)	4.79 (3.65)	4.17 (3.17)	-0.90 (2.47)
Max ACTH (pg/ml)	27.77 (11.04)	25.45 (9.88)	-2.32 (8.16)	22.07 (14.03)	23.54 (18.90)	-1.58 (8.65)
AUC cortisol	14.98 (15.63)	12.21 (11.82)	-2.77 (11.65)	6.98 (9.10)	4.75 (7.10)	-2.91 (6.06)
AUC ACTH	57.92 (38.78)	48.76 (30.98)	-9.16 (23.78)	38.91 (35.96)	48.20 (57.49)	0.56 (15.04)
<i>Diurnal (mean, SD)</i>						
Slope cortisol	-0.08 (0.03)	-0.09 (0.03)	-0.01 (0.03) ***	-0.10 (0.03)	-0.08 (0.03)	0.023 (0.04)

Post-dexamethasone (DEX) cortisol and adrenocorticotrophic hormone (ACTH) concentrations were the mean of 6 samples collected every 30 minutes between 1230 to 1500 hours following administration of DEX at 2100 hours the evening before. Post-corticotropin releasing hormone (CRH) cortisol and ACTH were collected at 5, 15, 30, 60, 90 and 120 minutes after i.v. CRH administered immediately following the 1500 hour blood draw. Maximum (Max) values reflect the highest hormone values post-CRH, and post-CRH area under the curve (AUC) was calculated from the 1500 hour sample collected immediately prior to CRH administration. Slope was calculated as the β value of the regression of natural log transformed plasma cortisol concentrations measured from 0900 to 2100 hours prior to DEX administration (larger β -values—closer to 0—reflect a flatter slope).

*
p<0.05

p<0.001 significantly different from control when controlling for age, race, sex, body mass index and history of major depression.