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Cortisol Levels Are Positively Correlated with Hippocampal *N*-Acetylaspartate

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Abstract

Background—This study examined the relationship of hypothalamic–pituitary–adrenal measures and hippocampal *N*-acetylaspartate (NAA) in posttraumatic stress disorder (PTSD) patients and control subjects.

Methods—Eleven patients with combat-related PTSD and 11 control subjects were evaluated with magnetic resonance spectroscopy as well as by morning salivary cortisol samples before and after administration of low-dose dexamethasone (.5 mg).

Results—Left hippocampal NAA was strongly associated with both pre-dexamethasone cortisol levels ($n = 22$, $r = .53$, $p = .013$) and post-dexamethasone cortisol levels ($n = 22$, $r = .63$, $p = .002$). After accounting for clinical symptom severity and hippocampal volume, cortisol levels accounted for 21.9% of the variance ($F = 5.6$, $p = .004$) in left hippocampal NAA and 12.6% of the variance ($F = 3.2$, $p = .035$) in right hippocampal NAA.

Conclusions—This study shows a positive relationship between cortisol levels and hippocampal NAA in subjects without hypercortisolemia. Within the range of values seen in our subjects, cortisol may have a trophic effect on the hippocampus.

Keywords

Posttraumatic stress disorder; hippocampus; cortisol; magnetic resonance spectroscopy; *N*-acetylaspartate; magnetic resonance imaging

Introduction

Hippocampal volume reduction and lower cortisol levels have been found in subjects with posttraumatic stress disorder (PTSD) (Yehuda 1997), but no study has examined the relationship of these two findings. Some have suggested that elevations in glucocorticoids may damage hippocampal neurons (Sapolsky et al 1990; Woolley et al 1990). This hypothesis is supported by studies showing an inverse relationship between cortisol and hippocampal volume in subjects with hypercortisolemia (Lupien et al 1998; Starkman et al 1999).

We have recently reported that *N*-acetylaspartate (NAA) is reduced in the hippocampus of PTSD patients in the absence of hippocampal volume loss (Schuff et al 2001). *N*-acetylaspartate occurs in high concentrations in neurons and is virtually undetectable in other tissue types (Birken and Oldendorf 1989). Levels of NAA are presumed to reflect changes of neuronal density or metabolism, in contrast to magnetic resonance imaging (MRI), which reflects nonspecific changes of both neurons and glia. Given the evidence for hippocampal abnormalities in PTSD, as indexed in our study by reduced NAA, we examined the relationship between clinical symptom levels, morning cortisol levels before and after dexamethasone administration, and hippocampal NAA in the sub-sample of PTSD and control subjects from the Schuff et al (2001) study who had complete cortisol and spectroscopy data.

Methods and Materials

Medically healthy combat veterans (subjects with PTSD: $n = 11$, mean age 51.1 ± 2.5 years; control subjects: $n = 11$, mean age 52.0 ± 2.4 years) were recruited from the San Francisco Veterans Affairs Medical Center and from the community. All subjects were given details of the study and were asked to sign a written, informed consent form if they wished to participate. The study protocol and consent form were approved by the Committee on Human Research at the University of California, San Francisco. The Structured Clinical Interview for DSM-IV Diagnosis (SCID; Spitzer et al 1992) was used to exclude current psychiatric disorders other than PTSD, including major depression, and a history of alcohol or substance abuse during the previous 5 years. Diagnosis and severity of PTSD was assessed with the Clinician-Administered PTSD Scale (CAPS; Blake et al 1995). Subjects with history of head trauma, neurologic disorder, or systemic illness affecting brain function were excluded.

Magnetic resonance imaging and ^1H MR spectroscopic imaging (MRSI) acquisition and processing were described in detail elsewhere (Schuff et al 2001). In brief, the subjects were scanned on a 1.5-T VISION MR system (Siemens AG, Munich, Germany) using a double spin echo sequence with repetition time (TR)/echo time (TE)₁/TE₂ = 2500/20/80 msec, 3-mm slice resolution, and a volumetric magnetization-prepared rapid gradient echo sequence with TR/TE/inversion time (TI) = 10/4/300 msec, 15° flip angle, $1.0 \times 1.0 \text{ mm}^2$ in-plane resolution, and 1.4-mm-thick coronal partitions for structural MRI. A point-resolved spectroscopy ^1H MRSI (Bottomley 1987) sequence with TR/TE = 1800/135 msec and 1.1-cm^3 MRSI voxels was used to acquire water-suppressed ^1H MR spectra simultaneously from both hippocampi.

Volumes of the left and right hippocampus and entorhinal cortex (ERC) were measured by manually drawing the boundaries of these structures on the coronal T1-weighted, magnetization-prepared rapid acquisition gradient echo images, following previously published protocols (Insausti et al 1998; Watson et al 1992). To account for variations in head size, hippocampal and ERC volumes were normalized to the total intracranial volume, which was determined based on tissue-segmented MRI data using software developed in-house (Tanabe et al 1997). The masks from manual tracing of the left and right hippocampus were also incorporated into the segmentation data as separate tissue classes. Reliability of manual measurements of hippocampal volumes was .99 (determined as intraclass correlation between

two subsequent MRI evaluations on nine randomly selected subjects). The ^1H MRSI data were mildly filtered in the spectral and spatial domain to improve signal-to-noise ratio before Fourier reconstruction, resulting in approximately 1.6-cm^3 MRSI voxels. Peak areas of NAA, as well as creatine- and choline-containing compounds were estimated using fully automated spectral fitting software developed in-house (Soher et al 1998). To transform peak areas into concentrations of the metabolites, the amounts of gray matter, white matter, cerebro-spinal fluid (CSF), and left and right hippocampal tissue in each MRSI voxel were estimated using information from the tissue-segmented MRI data that were coregistered with the ^1H MRSI data. Magnetic resonance spectroscopic imaging voxels that best covered the right or left hippocampus in each subject were selected based on the estimated amount of hippocampal tissue in the voxel. Finally, the metabolite values were corrected for the amount of CSF in the MRSI voxels, to obtain concentrations (in arbitrary units), and also normalized to the median ventricular CSF intensity from each subject, as measured with proton density MRI, to compensate for instrumental variations. Similar MRSI studies from this laboratory measuring hippocampal NAA measurements found intraclass correlations to be between .77 and .91 (Wiedermann et al 2001).

Separate collections of saliva in a Salivette tube (Sarstedt, NC) at 8:00 AM were obtained before and following the ingestion of dexamethasone (.5 mg) at 11 PM. Salivary cortisol was assayed by radioimmunoassay kit (Abbott Laboratories, Abbott Park, IL). The second sample was assayed for dexamethasone to ensure compliance. Cortisol levels from subjects with undetectable dexamethasone levels were excluded.

Relationships between the outcome and predictor variables were conducted with two-tailed t tests. Relationships of cortisol levels with PTSD severity, hippocampal volume, and NAA were tested using Pearson correlation coefficients. Hierarchical linear regression models were developed to explain left and right hippocampal NAA in the combined sample.

Results

Similar to our earlier report (Schuff et al 2001), left and right hippocampal NAA (mean [SD]) was significantly reduced in our subsample of PTSD compared with control subjects: (left hippocampus: PTSD NAA = 2.72 [.67], control NAA = 3.61 [.96], $t = -2.5$, $p = .019$; right hippocampus: PTSD NAA = 2.61 [1.36], control NAA = 3.85 [.57], $t = -2.8$, $p = .012$). There were no differences between PTSD and control subjects in hippocampal or entorhinal cortex volumes. Predexamethasone cortisol (mean [SD]) levels (PTSD = 866 [312] ng/dL; control = 987 [428] ng/dL, $t = -.76$, $p = .46$) and postdexamethasone cortisol levels (PTSD = 324 [126] ng/dL; control = 433 [315] ng/dL, $t = -1.07$, $p = .30$) were all within a normal range. Repeated-measures analysis of covariance using log-transformed cortisol values, covarying for day 2 dexamethasone levels, showed no effect of PTSD status on cortisol levels ($F = 1.1$, $p = .31$).

We tested bivariate associations between the predictors and both left and right hippocampal NAA. Clinician-Administered PTSD Scale total score was negatively associated with left NAA ($r = -.44$, $p < .05$) and right NAA ($r = -.45$, $p < .05$) in the combined sample, but not separately within the two groups. Left hippocampal NAA was positively correlated with left ERC volume ($r = .47$, $p < .05$), and right NAA was correlated with right ERC volume at a trend level ($r = .38$, $p = .07$). As expected, NAA and hippocampal volumes were not correlated because the ^1H MR spectra from hippocampus were corrected for tissue volume. Left hippocampal NAA was positively associated with both predexamethasone cortisol levels ($r = .53$, $p = .013$) and postdexamethasone cortisol levels ($n = 22$, $r = .63$, $p = .002$) (see Figure 1). The correlation between left NAA and postdexamethasone cortisol levels appeared stronger in control subjects ($r = .70$, $p = .02$) compared with PTSD subjects ($r = .37$, $p = .27$), though this is not a significant difference (Fisher $z = .96$, $p = .34$). There was no association between right hippocampal NAA

and the hypothalamic–pituitary–adrenal (HPA) measures. There were no significant correlations between percent suppression of cortisol by dexamethasone and left or right hippocampal NAA.

A hierarchical linear regression model was developed to explain both left and right hippocampal NAA in the combined sample. In step 1, CAPS total score accounted for 14.9% of the variance in left hippocampal NAA [$F(1,22) = 4.7, p = .043$]. In step 2, left hippocampal volume and ERC volume added 15.3% of incremental variance [$F(3,22) = 4.0, p = .023$]. In step 3, entering pre- and postdexamethasone cortisol levels accounted for an additional 21.9% of the variance [$F(5,22) = 5.6, p = .004$]. For right NAA, in step 1, CAPS total score accounted for 15.8% of the variance [$F(1,22) = 4.9, p = .038$]. In step 2, right hippocampal volume and ERC volume added 5.8% of incremental variance [$F(3,22) = 2.9, p = .062$]. In step 3, entering pre- and postdexamethasone cortisol levels accounted for an additional 12.6% of the variance [$F(5,22) = 3.2, p = .035$].

Discussion

The major finding is that cortisol levels were positively correlated with hippocampal NAA. This suggests that within the range of normal (as opposed to hypercortisolemic) levels, cortisol has a trophic effect on hippocampal neurons. This is consistent with studies in rodents, which show that lowering cortisol levels results in a loss of hippocampal granule cells in the dentate gyrus (Sloviter et al 1989). Hence the relationship between cortisol and the hippocampus may vary depending on the levels of glucocorticoids exhibited by particular groups. In addition, given that the direct relationship between cortisol and NAA was stronger in control subjects, other factors associated with group status, separate from cortisol levels, may have a contributing role. Finally, given that other brain areas were not measured in this study, we cannot be certain that the relationship of cortisol and NAA is specific to the hippocampus.

Alternatively, reduced hippocampal NAA may be associated with altered hippocampal input to the hypothalamus, consistent with studies showing that the hippocampus regulates the output of the HPA axis (Jacobson and Sapolsky 1991). It is also possible that the positive association is caused by an unmeasured third variable that regulates both hippocampal neurogenesis and cortisol output. In any case, the association between cortisol and the hippocampus appears to be complex, and additional work is needed to clarify mediating and moderating variables that influence this relationship.

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Figure 1. Scatter plot of post-dexamethasone salivary cortisol levels and hippocampal N-acetylaspartate (NAA). NAA, N-acetylaspartate; PTSD, posttraumatic stress disorder.