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Associations between CSF cortisol and CSF norepinephrine in cognitively normal controls and patients with amnesic MCI and AD dementia

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Abstract

Objective—This study evaluated the effects of Alzheimer’s disease (AD) on the relationship between the brain noradrenergic system and hypothalamic pituitary adrenocortical axis (HPA). Specifically, relationships between cerebrospinal fluid (CSF) norepinephrine (NE) and CSF cortisol were examined in cognitively normal participants and participants with AD dementia and amnesic mild cognitive impairment (aMCI). We hypothesized there would a positive association between these two measures in cognitively normal controls, and that this association would be altered in AD.

Methods—421 CSF samples were assayed for NE and cortisol in controls (n=305), participants with aMCI (n=22) and AD dementia (n=94). Linear regression was used to examine the association between CSF cortisol and NE, adjusting for age, sex, education, and body mass index.

Results—Contrary to our hypothesis, CSF cortisol and NE levels were not significantly associated in controls. However, higher cortisol levels were associated with higher NE levels in AD and aMCI participants. Regression coefficients $\pm$ standard errors for the change in cortisol per 100 pg/ml increase in NE are as follows: controls 0.0 ± 0.2 , $p=1.0$; MCI, 1.4 ± 0.7 , $p=0.14$; and AD 1.1 ± 0.4 , $p=0.032$. Analysis with MCI and AD participants combined strengthened statistical significance (1.2 ± 0.3 , $p=0.007$).

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Conclusions—Enhanced responsiveness of the HPA axis to noradrenergic stimulatory regulation in AD and disruption of the blood brain barrier may contribute to these findings. Because brainstem noradrenergic stimulatory regulation of the HPA axis is substantially increased by both acute and chronic stress, these findings are also consistent with AD participants experiencing higher levels of acute and chronic stress.

Keywords

cortisol; norepinephrine; Alzheimer's disease; Mild Cognitive Impairment; cerebrospinal fluid; stress

INTRODUCTION

The relationships between the central nervous system (CNS) noradrenergic system and the hypothalamic pituitary adrenocortical (HPA) axis are complex, and the levels of at which these systems interact are multiple. Noradrenergic and adrenergic input to HPA axis activation can involve direct projections and indirect paths between brain structures and signaling through peripheral sympathetic input ^{1,2}. One significant pathway includes brainstem projections thought to provide the substantial proportion of regulatory input to corticotropin releasing factor (CRF) producing neurons in the paraventricular nucleus (PVN) of the hypothalamus. These include noradrenergic projections, the larger proportion from the nucleus of the solitary tract (NTS) but also from the locus coeruleus (LC) ¹, likely mediated through alpha-1 adrenoreceptors in the PVN ³.

Previous cerebrospinal fluid (CSF) studies suggest dysregulation of the HPA axis and the CNS noradrenergic system in Alzheimer's disease (AD). Cross-sectional studies examining CSF cortisol have shown higher levels in older compared to younger participants, with even higher levels in older participants with AD ⁴⁻⁶. Neurodegeneration of the LC with substantial neuronal loss and neurofibrillary tangle formation occurs early in the course of AD ^{7,8}. Compensatory upregulation of norepinephrine (NE) biosynthetic capacity in surviving LC neurons and upregulation of noradrenergic postsynaptic receptors in AD ^{9,10} may contribute to the pathophysiology of agitation and aggression in AD ^{11,12}.

Given the role of CNS noradrenergic regulation of the HPA axis, we hypothesized that in normal controls there would be a positive relationship between CSF cortisol and CSF NE. We also hypothesized that this relationship would be altered in AD dementia and aMCI. Therefore, we examined the cross-sectional association between CSF cortisol and NE from samples in a large CSF repository. Our aims were to examine this relationship in cognitively normal controls and whether CSF cortisol and NE associations differed by presence of aMCI and AD dementia. We also added a sensitivity analysis combining AD dementia and aMCI in one group, based on the conceptualization that aMCI is the earliest clinical manifestation of AD.

METHODS

Study participants

We analyzed CSF samples and data from an existing CSF repository from an academic multicenter study. Study procedures pertaining to this repository and multicenter study have been previously described^{13,14}. The sites involved were the University of Washington, University of California, San Diego, Oregon Health and Science University, University of Pennsylvania, Indiana University, and University of California, Davis. The repository includes samples from participants aged 20 to 100 years with normal cognition, aMCI, and AD. (This repository also contains samples from participations with other neurodegenerative conditions including Dementia with Lewy bodies, Parkinson's Disease, and Frontotemporal dementia, and traumatic brain injury).

For this analysis, we extracted data from the repository for participants who had normal cognition, aMCI, or AD dementia. We excluded participants taking medications that are known to affect CSF cortisol or NE concentrations, including corticosteroids, stimulants, serotonin-norepinephrine reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors, and Parkinson disease medications (including carbidopa/levodopa and dopamine agonists).

Diagnoses for normal cognition, aMCI, and AD dementia, were based on the following criteria. Criteria for normal cognition included a Mini-Mental State Examination (MMSE)¹⁵ score of 26 to 30 and Clinical Dementia Rating (CDR) Scale¹⁶ score of 0, Logical Memory¹⁷ score of 6 or greater (a test of delayed verbal recall) and no evidence or history of cognitive decline. Criteria for patients with aMCI included a CDR of 0.5 and a Logical Memory score of less than 6. Participants with AD dementia met NINCDS-ADRDA criteria for probable AD and had a Clinical Dementia Rating Scale of 1 or more.

The repository protocol required all participants be medically stable and have stable medications for 4 weeks prior to the lumbar puncture. Exclusionary criteria included current substance use disorders, a current major depressive episode, and chronic psychiatric conditions such as schizophrenia, bipolar disorder and PTSD.

All procedures were approved by the institutional review boards for participating institutions, and all participants provided written informed consent before study enrollment.

CSF collection

Sample collections occurred between 0900 and 1100 hours with subjects fasting. CSF was divided into sequential 0.5 ml aliquots into polypropylene tubes, frozen immediately on dry ice at the bedside, and then stored at -70 C° until assayed. Tubes assigned to the norepinephrine assay also contained the additive glutathione.

Norepinephrine and cortisol assays

For measurement of CSF NE concentration, CSF was extracted using the alumina extraction method optimized by Holmes et al¹⁸. Extracted samples were separated by high-pressure liquid chromatography (HPLC) using a reverse phase C-18 column and measured by

electrochemical detection (Coulechem II, ESA, Inc., Chelmsford, MA) with 3,4-dihydroxybenzylamine (DHBA) as an internal standard ¹⁴. The intra-assay coefficient of variation (CV) is 7.1% (based on measurements of the internal reference standard DHBA) and the inter-assay CV is 10.8% (based on measurement of pooled CSF samples repeated in each assay).

CSF cortisol was measured with ImmuChem Cortisol Coated Tube RIA Kits (ICN/MP Biomedical, Santa Ana, CA) following the commercial protocol modified for their use with CSF. Because of the low protein content of CSF cortisol relative to plasma, steroid-free Stripped Human Serum (ICN/MP Biomedical, Santa Ana, CA) was added to samples to bring their protein content roughly equivalent to the protein in the kit standards that are in a “human serum matrix.”

Statistical analysis

Linear regression was used to determine the association between CSF NE or CSF cortisol (the dependent variables) and age, and the association between the CSF variables and sex, education and body mass index (BMI) adjusting for age (i.e. with linear age as a model covariate).

To determine if mean CSF cortisol or CSF NE levels differed by AD/aMCI vs. cognitively normal participants, a three diagnostic category variable (controls (i.e., cognitively normal), aMCI or AD) was added as an independent covariate to the above model (using 2 dummy variables). Pair-wise comparisons of AD, aMCI and controls were tested for significance using simultaneous inference which adjust for multiple comparisons within a model ¹⁹.

Linear regression was used to assess relationships between CSF cortisol (the dependent variable) and CSF NE, adjusting for age, sex, education, and BMI. Interaction terms for NE by diagnostic category or age were added to the regression models to assess whether associations between NE and cortisol differ by age or diagnostic category. Slopes of change in CSF cortisol by NE for each diagnostic category and predicted mean cortisol for each group at selected values of NE were estimated from this model. Hypothesis testing to determine if each slope was different from zero and the computation of 95% confidence intervals (CI) for predicted mean cortisol values across selected NE values and group were carried out using simultaneous inference as above.

Three sets of sensitivity analyses were also carried out: 1) regressions where age, as an independent covariate, was modeled as nonlinear using a restricted cubic spline; 2) regressions of cortisol or NE either excluding potential outliers or transforming the data to address possible violations of linear regression assumptions, and 3) regressions using two category variable for diagnostic category, combining AD and aMCI.

Type 1 error was set at $p=0.05$. Summary statistics in the text are reported as means $\pm$ standard errors (SE), [95% confidence intervals (CI)], unless where otherwise indicated. Analyses were carried out using R 3.2.3 ²⁰, with regressions carried out using the rms package ²¹, simultaneous inference statistics estimated using the multcomp package ¹⁹

RESULTS

Study participant characteristics

Study participant characteristics are summarized in Table 1. The study sample included 305 individuals with normal cognition, 22 individuals with MCI, and 94 individuals with AD. Study groups had generally similar characteristics with regards to sex, BMI and education.

To characterize this sample, we examined these participant characteristics in relation to CSF cortisol and CSF NE. Linear regression analysis showed both CSF cortisol and CSF NE increased significantly with subject age ($p < 0.0001$) by a mean $\pm$ SE of 0.7 ± 0.1 ng/ml (95% CI [0.5, 0.9] and 8.4 ± 2.1 pg/ml (95% CI [4.3, 12.4]) per 10 years of participant age increase, respectively. Adjusting for age, mean CSF cortisol was slightly higher for men than women by 0.8 ± 0.3 ng/ml (95% CI [0.2, 1.3], $p = 0.012$) but mean CSF NE did not differ significantly by sex ($p = 0.28$). Age adjusted mean cortisol was not associated with education, but age adjusted mean NE decreased by 2.8 ± 1.3 pg/ml per increase in one year of education ([0.2, 5.4], $p = 0.033$). (This association between CSF NE and education was no longer significant when diagnostic category was added to the regression model as a covariate, $p = 0.22$.) No association for either CSF cortisol or NE was found with BMI ($p > 0.11$).

CSF Cortisol and NE concentrations by diagnostic category

Figure 1 presents scatterplots of CSF cortisol (A) and CSF NE (B) by age with separate symbols and slopes by diagnostic category. There were significant differences in mean cortisol concentrations among controls, aMCI and AD dementia groups adjusting for age, sex, education and BMI. Compared to controls, CSF cortisol was higher by 1.6 ± 0.4 ng/ml (95% CI [0.7, 2.5], $p < 0.001$) in the AD dementia group. Mean CSF cortisol for the aMCI group was higher than controls by 0.8 ± 0.7 ng/ml (95% CI [-0.8, 2.5]) and lower than the AD dementia group by the same amount (0.8 ± 0.7 ng/ml, 95% CI [-0.9, 2.5]), but neither difference was significant ($p > 0.44$). There was no significant difference in mean CSF NE among the control, aMCI and AD dementia groups ($p > 0.60$).

Association between CSF cortisol and NE

Linear regression, adjusting for age, sex, education, BMI and diagnostic category showed no significant association between cortisol (the dependent variable) and NE overall (regression coefficient $\pm$ SE per 100 pg/ml increase in NE [95% CI], 0.3 ± 0.2 [-0.1, 0.7], $p = 0.11$). There was no evidence that the association between cortisol and NE differed by age (linear age by NE interaction, $p = 0.45$).

However, when testing for the effect of diagnostic category on the cortisol versus NE association, a significant interaction was found (interaction term: NE by category; $p = 0.021$). In cognitively normal controls (Figure 2), no association between CSF cortisol and NE was found (regression coefficient $\pm$ SE per increase in 100 pg/ml [95% CI]: 0.0 ± 0.2 , 95% CI [-0.6, 0.5], $p = 1.0$). In aMCI and AD dementia participants, higher cortisol levels were associated with higher NE levels (regression coefficient [95% CI]: aMCI, 1.4 ± 0.7 [-0.3, 3.2], $p = 0.14$; and AD 1.1 ± 0.4 [0.1, 2.2], $p = 0.032$).

The linear regression model above was used to calculate mean cortisol levels per increased NE levels (Table 2). In AD dementia participants, an increase in CSF NE from 100 to 300 pg/ml was associated with an increase in cortisol from 13.7 to 16.0 ng/ml. In aMCI participants, the same increase in CSF NE was associated with an increase in cortisol from 12.5 to 15.4 ng/ml. In contrast, cognitively normal participants' mean cortisol level remained constant with increasing NE concentrations.

Sensitivity Analyses

Sensitivity analysis using age as a non-linear variable demonstrated similar results as the primary findings above, further confirming age does not explain the relationship between CSF cortisol and NE found in AD dementia patients.

Another sensitivity analysis using regression models using log(NE) instead of NE as the dependent variable to address right skewness in the NE variable also yielded similar findings. When a participant with cortisol > 30 ng/ml was excluded, differences in mean cortisol between the controls and aMCI and AD groups remained significant. However, the exclusion of this participant attenuated the difference in the cortisol vs. NE slopes by diagnostic category, (NE by diagnostic category interaction $p=0.079$).

When diagnostic category was modeled with two categories (AD dementia and aMCI combined vs. controls), diagnostic category differences in mean cortisol and in the cortisol vs. NE slopes were strengthened. Mean CSF cortisol for the AD/aMCI group was higher than controls by 1.3 ± 0.4 ng/ml ($p<0.001$). Mean CSF cortisol increased by 1.2 ± 0.3 ng/ml per increase in CSF NE of 100 pg/ml, ($p=0.002$, NE by category interaction; $p = 0.007$). These associations remained significant with the exclusion of the high cortisol outlier.

DISCUSSION

This analysis is the first, to our knowledge, to examine the association between CSF cortisol and CSF NE in cognitively normal controls, participants with aMCI, and participants with AD dementia. We show that contrary to our hypothesis, there was not a significant positive association between CSF cortisol and CSF NE in control participants. In contrast, we did find a positive association between CSF cortisol and CSF NE in AD dementia participants, a finding strengthened when the aMCI and AD dementia participants were combined in the analysis.

Although stimulatory regulation of the HPA axis by central noradrenergic system input is well established, this mechanism is one among multiple neural and endocrine regulators of the HPA axis. These range from direct projections and indirect paths between brain structures, signaling through peripheral sympathetic input, and glucocorticoid negative feedback^{2,22}. Given the complexity of the relationships between these two stress systems, it is not surprising that we did not find the association between CSF cortisol and CSF NE in controls we had anticipated. These multiple regulatory mechanisms likely obscure finding a significant relationship between CSF NE and CSF cortisol, particularly as the latter hormone is derived from the periphery and must cross the blood brain barrier.

There are several phenomena in AD that could increase the likelihood of detecting a significant relationship between NE and cortisol, one of which is the blood brain barrier. Normally, access of cortisol to the central nervous system is restricted by tight junctions and by efflux transporters, the primary of which is P-glycoprotein ²³. In AD, P-glycoprotein has been shown to be expressed at lower levels and have decreased function; tight junctions are loosened, and focal necrosis also increase permeability of the blood brain barrier ^{24–26}. Together, these changes provide greater access to the brain and CSF but decrease the efficiency of cortisol efflux, likely resulting to higher CSF cortisol in those with AD. This process in AD would favor detection of a significant association between NE and cortisol in the CSF compartment.

Another possibility is raised by demonstration in preclinical studies that chronic stress enhances PVN excitability, through multiple mechanisms including reduction of feedback inhibition and increased input from afferent connections ²². One such mechanism involves the noradrenergic system, as chronic stress enhances noradrenergic innervation ¹. NE has been suggested to play a key role in chronic stress enhancement of HPA axis excitability ²⁷. It is likely that the impaired memory, executive function, and reasoning ability of AD patients make everyday challenges more stressful. Such elevations in chronic stress could give the noradrenergic component of HPA axis stimulatory regulation a more dominant role, and thus make a relationship between CSF NE and CSF cortisol more detectable in this study.

Finally, there are known alterations in the noradrenergic system in AD ²⁸. Marked degeneration of the LC is one of the earliest changes that occur in the course of AD ^{7,29–31}, and more recently, accumulation of tau in the LC has been found to occur early in AD as well ⁸. Post-mortem studies indicate increased biosynthetic capacity in remaining LC neurons ⁹ and upregulation of numbers of post-synaptic alpha-1 adrenoreceptors present in AD patients compared to controls in the hippocampus and frontal cortex ^{10,32}. We have previously proposed that this apparent compensatory upregulation contributes to symptoms such as agitation and aggression in AD ^{12,33}. How these changes in AD may influence HPA axis activation is less clear. In animal studies, LC projections to the PVN are present, although the substantial proportion noradrenergic input comes from the NTS ²². There also may not be detectable differences in NE measured in the hypothalami of AD subjects compared to controls ³⁴. However, given the early and substantial changes in the noradrenergic system in AD, as well as our previous work suggesting compensatory upregulation of this system, an alteration in the relationship between CSF NE and cortisol compared to controls is a consistent finding, notwithstanding uncertainty over the exact mechanism by which this association occurs.

One separate area of significance of this study is that it replicates several other previous CSF studies. We show that higher cortisol concentrations occur in older versus younger adults and in AD patients versus controls ^{4,5,35,36}. We had previously demonstrated in this sample that CSF NE increases with age; the analysis described in this report also replicates smaller studies revealing similar CSF NE concentrations in controls and AD participants ^{33,37}.

There are several limitations to this study. It is a cross-sectional examination of stress systems known to fluctuate depending on the time of day and environment. To minimize this variability, the study design standardized lumbar puncture procedures including time of day, and included a large number of participants in relation to most CSF studies, which better accommodates variability. This study also may not have taken into account potential medication effects, although we excluded participants taking medications known to affect CSF NE or cortisol levels (e.g., certain antidepressants, Parkinson's disease medications). Another limitation is that this analysis presents associations only and does not elucidate mechanisms. However, associations seen in this study provide rationale for future work investigating underlying pathophysiology.

In summary, this study reports a positive association between CSF cortisol and CSF NE levels in AD patients but not in cognitively normal controls. This association likely reflects alterations in the HPA axis and noradrenergic systems in the CNS of AD patients. Given the complexity of the relationships between these two systems, we propose several possible mechanisms ranging from how cortisol is cleared from the CSF to neurobiological changes in chronic stress and AD. The underlying mechanism of this association may not be clear from this study alone, but findings support future studies examining the relationships between these two stress systems in AD.

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A. CSF cortisol increases with age and is higher in AD/aMCI than in normal controls ($p < 0.001$).

B. CSF NE increases with age but is not affected by AD/aMCI ($p>0.70$).

Figure 1. Scatterplot of CSF cortisol (A) and norepinephrine (B) concentrations by age and diagnostic category. Slopes are estimated from linear regression of cortisol on age and diagnostic category.

Figure 2.

Scatterplot of CSF cortisol by CSF norepinephrine and diagnostic category. Slopes are estimated from linear regression of CSF cortisol on CSF NE, diagnostic category, age, sex, education and BMI, and averaged across age, sex, education and BMI.

There is a significant positive correlation between CSF cortisol and CSF NE in AD/aMCI.

Table 1
Subject characteristics by diagnostic category: mean $\pm$ SD (range) or frequency (%)

	Controls			MCI (n=22)	AD (n=94)
	All (n=305)	Age < 50 (n=97)	Age 50 (n=208)		
Age	56.1 $\pm$ 18.3 (20–100)	33.6 $\pm$ 9.4 (20–49)	66.7 $\pm$ 10.0 (50–100)	72.3 $\pm$ 8.2 (56–84)	71.2 $\pm$ 9.3 (52–88)
Male	139 (46%)	53 (55%)	86 (41%)	13 (59%)	49 (52%)
Education	16.1 $\pm$ 2.7 (10–27)	16.4 $\pm$ 2.7 (11–27)	16.0 $\pm$ 2.7 (10–25)	16.5 $\pm$ 2.7 (12–21)	15.8 $\pm$ 3.1 (6–25) *
Body Mass Index	26.0 $\pm$ 4.0 (18–41)	25.7 $\pm$ 4.3 (19–41)	26.1 $\pm$ 3.8 (18–40)	24.7 $\pm$ 3.4 (18–33) *	25.9 $\pm$ 3.4 (18–36) †

* unavailable for 1 participant

† unavailable for 3 participants

Table 2

Mean CSF cortisol level in ng/ml (95% CI) per NE concentration, predicted from regression analysis of CSF cortisol on CSF NE by diagnostic category adjusting for age, sex, education and BMI *

NE (pg/ml)	Cortisol (ng/ml)		
	Controls Age 50 (n=305)	aMCI (n=22)	AD (n=94)
100	12.9 (12.1, 13.7)	12.5 (10.0, 15.1)	13.7 (12.3, 15.0)
200	12.9 (12.2, 13.6)	14.0 (12.1, 15.9)	14.8 (13.8, 15.9)
300	12.9 (11.9, 13.9)	15.4 (12.5, 18.3)	16.0 (14.2, 17.8)

* From linear regression of cortisol concentration (the dependent variable) on NE concentration by category interaction with co-variates age, sex, education and BMI.