BRIEF REPORT

Smaller Left Anterior Cingulate Cortex Volumes Are Associated with Impaired Hypothalamic-Pituitary-Adrenal Axis Regulation in Healthy Elderly Men

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Context: Studies in animals suggest that the limbic prefrontal cortex, including the anterior cingulate cortex, is involved in regulation of the hypothalamic-pituitary-adrenal (HPA) axis, but human data are lacking.

Objective: This study tested the hypothesis that smaller anterior cingulate cortex volumes are associated with HPA axis dysregulation in healthy older men.

Design and Participants: Comparison was made of volumes of bilateral anterior cingulate cortex, hippocampus, and superior frontal gyrus (control region) volumes in two groups of 10 healthy men, aged 65–70 yr, who showed nonsuppression or suppression of cortisol levels in response to low dose (250 μg) dexamethasone. Analysis of brain volumes was performed blind to the cortisol levels.

Setting: This study was performed at a tertiary care clinical research center.

Results: Nonsuppressors had significantly smaller left anterior cingulate cortex volumes than suppressors (5757 vs. 7817 mm³; P = 0.01). Right anterior cingulate cortex, bilateral hippocampus, and bilateral superior frontal gyrus volumes were not significantly different between nonsuppressors and suppressors.

Conclusions: Smaller left anterior cingulate cortex volumes may be associated with HPA axis dysregulation in humans. These results substantiate evidence from animal studies indicating an important role for the anterior cingulate cortex in suprahypothalamic feedback regulation of the HPA axis. The results also have implications for disorders in which HPA axis dysregulation and abnormalities of the anterior cingulate cortex are frequently observed, such as depression and Alzheimer’s disease. (J Clin Endocrinol Metab 91: 1591–1594, 2006)

CORTISOL HYPERSECRETION IS frequently observed in major brain disorders, including depression and Alzheimer’s disease, and is more common with increasing age (1). High cortisol levels (e.g. in Cushing’s disease) also cause brain disorders, such as depression and cognitive impairment (2); therefore, understanding the central mechanisms of plasma cortisol control is important. Cortisol levels are regulated by the hypothalamic-pituitary-adrenal (HPA) axis, which is subject to suprahypothalamic control. The hippocampus is established to be an important cortical site of (largely inhibitory) feedback regulation of the HPA axis (3). In animals, lesions of the hippocampus cause glucocorticoid hypersecretion (4, 5); in humans, hippocampal atrophy is associated with HPA axis dysregulation in Alzheimer’s disease and depression (6, 7). We have previously reported that hippocampal volumes were not associated with cortisol levels or their regulation in healthy, young-elderly men (8), but others have reported a correlation between rising cortisol levels and hippocampal atrophy in older healthy subjects (9).

However, multiple recent studies in the rat suggest that in addition to the hippocampus, the limbic medial prefrontal (including anterior cingulate) cortex plays an important role in glucocorticoid regulation (10–14). For example, lesions of this region result in higher peak glucocorticoid levels and slower return to basal glucocorticoid levels after a stressor. The human medial prefrontal cortex differs markedly from the structure in rodents, yet finding extrahippocampal regions of HPA axis control in the human cortex is of substantial interest.

Recently, human functional neuroimaging and opportunistic lesion studies have shown that the anterior cingulate cortex (ACC), part of the medial prefrontal cortex, plays an important integrative role in the cortical response to diverse stressors (15). Furthermore, structural and functional abnormalities of the ACC are present in those same disorders in
which glucocorticoid dysregulation is frequently observed, i.e. depression (16, 17) and Alzheimer’s disease (18).

Two published studies provide evidence of an association between abnormalities of the prefrontal cortex and HPA dysregulation in humans. First, in a combined sample of young (n = 9; age range, 19–30 yr) and old (n = 11; age range, 59–76 yr) subjects, Wolf et al. (19) found significant negative correlations between basal (but not posthydrocortisone) ACTH levels, and ACC and hippocampus volumes. Correlations with orbitofrontal and parahippocampal regions were not significant. Second, Gold et al. (20) examined the brain volumes and results of the dexamethasone/CRH test in a group of 54 normal and hypertensive subjects and found that posttest cortisol output was associated with worsened global and prefrontal cortex atrophy.

However, human studies exploring direct links between volumes of ACC and feedback regulation of glucocorticoid levels are lacking. Therefore, we measured ACC volumes in two groups of healthy, unmedicated, elderly men who differed in showing either resistance (nonsuppression) or sensitivity (suppression) of cortisol levels after very low-dose dexamethasone. We hypothesized that nonsuppression would be associated with smaller volumes of the ACC. We also measured volumes of the hippocampus, which is believed to be involved in HPA axis control, and, as a control, the superior frontal gyrus (SFG), which is not thought to participate in HPA axis regulation.

Subjects and Methods

Subjects and dexamethasone suppression testing

We examined healthy, nondepressed, nondemented, unmedicated, community-dwelling, right-handed, male, unpaid volunteers, aged 65–70 yr, who were participants in a larger study (n = 97) (8). The protocol was approved by the Lothian health ethics committee.

Subjects provided written consent after discussion of the experimental procedures. Each participant had undergone a very low dose (250 μg) dexamethasone suppression test, with the dexamethasone tablet taken at 2300 h and blood drawn at 0900 h the following morning, as previously described (8). From these, we selected two groups of 10 subjects who were at the extremes of these postdexamethasone cortisol levels. We thus compared two groups: nonsuppressors and suppressors. Data from basal 0900 h plasma cortisol assays were also available. Cortisol concentrations were measured with an in-house RIA (8). The mean intra-assay coefficient of variation was 6.2%. The interassay coefficient of variation was calculated through quality control samples included in each assay. The calculated coefficient of variation was 6.2%. The interassay coefficient of variation was calculated through quality control samples included in each assay. The calculated coefficient of variation was 6.2%.

Data analysis

General linear modeling analysis of covariance was used to test differences in ACC and SFG volumes between suppressors and nonsuppressors. Intracranial area was used as a covariate to control for differences in estimated intracranial capacity.

Results

Suppressors had a mean postdexamethasone cortisol level of 71.5 nmol/liter (sd, 24.4; range, 40–110), with a mean basal (0900 h) cortisol level of 362.8 nmol/liter (sd, 125.0; range, 183–556). Nonsuppressors had a mean postdexamethasone cortisol level of 526.9 nmol/liter (sd, 89.6; range, 373–632), with a mean basal (0900 h) cortisol level of 643.4 nmol/liter (sd, 166.5; range, 386–898). Postdexamethasone and basal cortisol levels were significantly different between the groups (P < 0.001).

Suppressors and nonsuppressors did not differ in age, body mass index, or previously estimated intelligence as determined by the National Adult Reading Test.

Nonsuppressors had significantly lower left adjusted ACC volumes (nonsuppressors, 5757 mm³; suppressors, 7817 followed. Volumes were adjusted for intracranial area, a validated estimate of intracranial volume (8). The intraclass correlations were 0.96 (ACC) and 0.97 (SFG), indicating high intrarater reliability. Representative coronal sections are shown in Fig. 1. Bilateral hippocampal volumes were also measured, as previously described (8).

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Neuroimaging

Imaging was performed in an Elscint (now GE Medical Systems, Haifa, Israel) Prestige MR scanner (1.9 T). Structural image acquisition followed a three-view localizer and consisted of a coronal, T1-weighted, three-dimensional gradient-echo sequence covering the entire brain and skull [echo time, 9.254 msec; repetition time, 28.5 sec; tip angle, 25°; slice thickness, 1.5 mm (no interslice gap) × 1.18-cm field of view; matrix, 180 × 190].

Image analysis was performed using Analyze software (Mayo Clinic, Rochester, MN). All analyses were performed blind to all other details of the participants. An intensity threshold separating the brain from the meninges was imposed for semiautomated analysis of both regions. The landmarks for delineating the regions of interest were adapted from published methods (16, 21). Both ACC and SFG were measured bilaterally from the first slice in which they appeared to the slice containing the mamillary bodies, with continuous reference to other orthogonal planes for guidance. In subjects in whom a double-parallel cingulate gyrus was present, the method described by Yamashue et al. (21) was followed. Volumes were adjusted for intracranial area, a validated estimate of intracranial volume (8). The intraclass correlations were 0.96 (ACC) and 0.97 (SFG), indicating high intrarater reliability. Representative coronal sections are shown in Fig. 1. Bilateral hippocampal volumes were also measured, as previously described (8).

Fig. 1. A and B, Representative images of ACC. Coronal view showing SFG and ACC. Nonsuppressor, with left ACC smaller than right ACC (A). Suppressor, with left and right ACC of similar dimensions (B).
mm$^3$, df = 2; $F = 8.29; P = 0.01)$. Right ACC and bilateral SFG volumes were not different between the groups (Table 1).

Adjusted hippocampal volumes were not significantly different between the groups, although the volumes were lower in nonsuppressors on both sides (Table 1). Covarying for hippocampal volumes in the analyses with ACC volumes did not significantly alter the results. For the difference between left adjusted ACC volumes Cohen’s, the df was $-1.37$, indicating a large effect size (by $t$ test using adjusted ACC volumes).

**Discussion**

The novel finding in this study is that smaller left ACC volumes are associated with dysregulation of the HPA axis in humans. This suggests that like the hippocampus, the ACC may be a cortical site of HPA axis regulation in humans. This is of interest because dysregulation of the HPA axis and abnormalities of the ACC are frequently observed in depression, Alzheimer’s disease, and ageing. The present findings extend multiple recent animal studies demonstrating a role for the medial prefrontal cortex in regulation of the HPA axis. Two implications of these results are that 1) abnormalities of the left ACC may contribute to HPA dysregulation; and/or 2) chronic higher glucocorticoid exposure may be associated with acquired atrophy of the ACC.

The time scale of any link between left ACC volumes and HPA dysregulation is uncertain. ACC volumes were adjusted for estimated intracranial area, but, nevertheless, in this cross-sectional study it is not possible to know whether variations in ACC volume are life long or due to ageing-related or disease processes. However, in adult rats, damage to the medial prefrontal cortex causes HPA axis dysregulation (10, 11, 14). Thus, acquired pathological changes in ACC might, in principle, partly explain the HPA axis dysregulation frequently observed in depression, Alzheimer’s disease, and ageing (22). Moreover, HPA axis dysregulation itself might play a causal role; chronic stress and high doses of exogenous glucocorticoids adversely alter dendritic morphology in the medial prefrontal cortex in rats (23, 24), and high dose glucocorticoids cause atrophy of the medial prefrontal cortex in rats (25).

The differences between results for right and left ACC volumes were unexpected. However, some recent studies suggest a lateralization of cortical control of glucocorticoid levels, with the left side showing a predominantly inhibitory influence (12). Functional neuroimaging and electroencephalogram studies in depression, in which there is cortisol hypersecretion and dysregulation of the HPA axis, have also frequently shown asymmetry of function of prefrontal and other brain regions (26–29). Additionally, two recent studies have suggested relatively greater vulnerability of left-sided anterior cingulate cortex with high dose glucocorticoid treatment (25) and in affective disorders (17), but clearly these findings require additional investigation.

We previously reported that adjusted hippocampal volumes were not associated with postdexamethasone cortisol levels in the cohort ($n = 97$) from which the subjects in the present study were drawn (8). Reanalysis of hippocampal volumes in nonsuppressors vs. suppressors (to mirror the ACC analyses in the present paper) did not yield significant differences, although the results were in the expected direction. This may indicate that compared with the hippocampus, 1) the left ACC is more sensitive to chronic high glucocorticoids; or 2) long-term variations in this structure are more closely associated with long-term variations in HPA axis regulation. However, the relatively small sample size and good health of this cohort may have masked the association between hippocampal atrophy and HPA axis dysregulation reported in other studies that have examined more diseased and older groups (6, 9).

Some methodological limitations must be noted. The sample was all male and was selected for good health, so the findings may not apply to other populations. The study was cross-sectional. Also we cannot exclude reverse causation, that insensitivity to glucocorticoid suppression of HPA activity drives down left ACC volume, although this mechanism is perhaps less likely in the light of findings from animal studies.

The present study provides novel evidence directly linking smaller left ACC volumes with HPA axis dysregulation in humans. Future research will explore links between ACC and glucocorticoid dysregulation in depression, Alzheimer’s disease, and ageing.

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**TABLE 1. Adjusted volumes (mm$^3$) of anterior cingulate cortex, hippocampus, and superior frontal gyrus in suppressors and nonsuppressors**

<table>
<thead>
<tr>
<th></th>
<th>Nonsuppressors</th>
<th>Suppressors</th>
<th>$P$ value</th>
</tr>
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<tbody>
<tr>
<td>Left ACC</td>
<td>5,757 (493)</td>
<td>7,817 (493)</td>
<td>0.01</td>
</tr>
<tr>
<td>Right ACC</td>
<td>8,156 (522)</td>
<td>7,833 (522)</td>
<td>0.49</td>
</tr>
<tr>
<td>Left hippocampus</td>
<td>3,407 (123)</td>
<td>3,615 (123)</td>
<td>0.45</td>
</tr>
<tr>
<td>Right hippocampus</td>
<td>3,442 (132)</td>
<td>3,763 (132)</td>
<td>0.11</td>
</tr>
<tr>
<td>Left SFG</td>
<td>46,106 (2,556)</td>
<td>43,943 (2,556)</td>
<td>0.81</td>
</tr>
<tr>
<td>Right SFG</td>
<td>44,749 (2,170)</td>
<td>46,356 (2,170)</td>
<td>0.76</td>
</tr>
</tbody>
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ANCOVA with intracranial area as covariate. Degrees of freedom = 2. Volumes are estimated marginal means. SE values are in parentheses.
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