Hypothalamic Blood Flow Correlates Positively With Stress-Induced Cortisol Levels in Subjects With Social Anxiety Disorder

Fredrik Åhs, MS, Tomas Furmark, PhD, Åsa Michelgård, MS, Bengt Långström, PhD, Lieuwe Appel, PhD, Oliver T. Wolf, PhD, Clemens Kirschbaum, PhD, and Mats Fredrikson, PhD

Objective: The adrenal excretion of cortisol in animals is dependent on the production of corticotropin-releasing factor in the paraventricular nucleus of the hypothalamus. The a priori hypothesis of this study was that hypothalamic regional cerebral blood flow (rCBF) would correlate positively with salivary cortisol levels in patients with social anxiety disorder (SAD) during anxiety provocation. Another objective was to evaluate whether salivary cortisol levels correlated with rCBF in other brain areas. **Method:** Regional CBF was measured with oxygen-15-labeled water and positron emission tomography during a public speaking task before and after placebo treatment in 12 subjects with Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition-defined SAD. Cortisol concentrations in saliva were measured 15 minutes after the task. The a priori hypothesis of a salivary cortisol-dependent activation of the hypothalamus was studied with region-of-interest analysis. In addition, the covariation between rCBF and salivary cortisol was studied in the whole brain using the general linear model. **Results:** The region-of-interest analysis revealed a positive correlation between salivary cortisol and hypothalamic rCBF. In the whole brain analysis, a positive covariation between rCBF and salivary cortisol levels was found in a midbrain cluster encompassing the hypothalamus with its statistical maximum in the mamillary bodies. Negative covariations were observed in the medial prefrontal cortex as well as in the motor and premotor cortices. **Conclusion:** Like in animals, stress-induced cortisol excretion in humans may be inhibited by activity in the medial prefrontal cortex and enhanced by activity in the hypothalamus. **Key words:** rCBF, PET, cortisol, symptom provocation, hypothalamus, medial prefrontal cortex.

ACC = anterior cingulate cortex; **ACTH** = adrenocorticotropic hormone; **BA** = Brodmann area; **CRF** = corticotropin-releasing factor; **fMRI** = functional magnetic resonance imaging; **MNI** = Montreal Neurological Institute; **MTL** = medial temporal lobe; **PET** = positron emission tomography; **PTSD** = posttraumatic stress disorder; **PVN** = paraventricular nucleus of the hypothalamus; **rCBF** = regional cerebral blood flow; **ROI** = region of interest; **SAD** = social anxiety disorder.

INTRODUCTION

Cortisol is extensively used as a marker of stress (1) and negative affect (2) often in studies of psychiatric disorders like depression (3), posttraumatic stress disorder (PTSD) (4), and social anxiety disorder (SAD) (5). However, brain mechanisms underlying cortisol excretion are incompletely understood, particularly in humans.

Animal studies support that activity in the paraventricular nucleus of the hypothalamus (PVN) induces release of the corticotropin-releasing factor (CRF) to the pituitary gland in turn regulating the adrenocorticotropic hormone (ACTH) (6). The release of ACTH is under inhibitory control from the hippocampus (7) and regions in the medial prefrontal cortex (8,9), areas with dense distributions of glucocorticoid and mineralocorticoid receptors (10,11). Only a few brain imaging studies have related measures of central neural activity to cortisol excretion in humans, all during resting state conditions. In patients with schizophrenia, studied before and after neuroleptic treatment, differences in glucose metabolism in a hypothalamic region of interest (ROI) after treatment were negatively correlated to differences in plasma cortisol (12). In depressed patients, plasma cortisol levels were positively correlated with glucose metabolism in the left amygdala (13). In traumatized volunteers with and without PTSD, a negative correlation between resting plasma cortisol levels and measures of regional cerebral blood flow (rCBF) was observed in the anterior cingulate cortex (ACC) (14). Negative correlations between rCBF and cortisol were obtained in the medial temporal lobe in patients with combat related PTSD (14). In healthy volunteers, Neylan et al. (15) reported a positive correlation between hippocampal N-acetylaspartate activity, a marker of neuronal integrity and synaptic abundance, and salivary cortisol levels. However, to the best of our knowledge, no study in humans has related stress-induced cortisol to the corresponding brain activation patterns indexed by rCBF.

Hence, the present study aimed at correlating measures of cortisol excretion during stress to rCBF. Stress was induced through a public speaking task in 12 patients with SAD. Subjects participated in a larger study on the effects of drug treatment on brain activity (16). We relate salivary cortisol levels to rCBF measured during public speaking before and after a double-blind 6-week placebo treatment period. Based on animal research (6), we predicted a positive linear relationship between hypothalamic rCBF and cortisol levels, but with no specific hypothesis regarding other brain areas.

METHODS

The positron emission tomography (PET) protocol and design have been described in detail elsewhere (16). Briefly, 12 adults (seven women, mean age = 32.4 years, standard deviation [SD] = 6.5), who met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for SAD as defined by the Structured Clinical Interview for DSM-IV (SCID) were included. The study was approved by the local ethics and radiation safety committees and subjects signed informed consent. Subjects participated in the study during the spring and fall of 2002.

Regional CBF was measured using a Siemens Ecat+ PET scanner with an axial field of view of 155 mm operated in the three-dimensional mode. PET

From the Departments of Psychology (F.Å., T.F., M.F.) and Neuroscience (Å.M.), Uppsala University, Uppsala, Sweden; Uppsala Imanet AB (B.L., L.A.), Uppsala, Sweden; the Department of Psychology (O.T.W.), University of Bielefeld, Bielefeld, Germany; and Biopsychology (C.K.), Technical University of Dresden, Dresden, Germany.

Address correspondence and reprint requests to Fredrik Åhs, MS, Department of Psychology, Uppsala University, Box 1225, SE-751 42 Uppsala, Sweden. E-mail: Fredrik.Ahs@psyk.uu.se

Received for publication January 31, 2006. Revision received June 23, 2006.

This study was supported by the Swedish Research Council, the Bank of Sweden Tercentenary Foundation, GlaxoSmithKline, and the Swedish Brain Foundation.

DOI: 10.1097/01.psy.0000242120.91030.d8

0033-3174/06/6806-0859

data were collected in three frames of 30 seconds and corrected for attenuation and scatter. Subjects were positioned in the PET scanner and asked to prepare a short speech about a travel experience. After ^{15}O-water tracer injection, subjects spoke for 180 seconds in the presence of a silently observing audience of six to eight persons while being video recorded. The PET scanner started automatically at 50,000 counts/second when the tracer bolus reached the brain. A second PET scan using the same procedure was made after 6 weeks of placebo treatment consisting of a daily, orally administered pill taken together with 100 mL of orange juice. Subjects completed the Spielberger's state anxiety inventory, STAI-S (range = 20–80) (17) 2 hours before (baseline) and right after the two speeches to determine anxiety evoked by the speaking task.

Salivary cortisol samples were collected 2 hours before (baseline) and 15 minutes after both speeches using Salivette devices (Sarstedt, Nümbrecht, Germany) and stored at −20°C until biochemical analysis. Saliva was thawed and spun at 3000 rpm for 10 minutes and cortisol concentrations were determined by a time-resolved immunoassay with fluorometric detection as described elsewhere (18). The time of day for the two assessments varied between individuals but were as far as possible tried to be held constant within subjects (mean absolute difference = 50 minutes, standard error = 13).

Whole brain statistical analyses were performed using the SPM99 software (Wellcome Department of Cognitive Neurology, London, U.K.). Images were realigned, normalized, and smoothed using a 12-mm Gaussian kernel. Statistical parametric maps using the general linear model (19) were computed with cortisol measures after speech from both PET assessments as a covariate of interest and sex as a nuisance variable. Sex was used as a nuisance variable because a previous study reported larger salivary cortisol concentrations for men than for women in response to a public speaking task (20). Subject specific regressors were used to model the within subject error variance (19). Cortisol measures after speech were analyzed because they reflect the cortisol excretion during the speech in the PET scanner. The resulting t-maps were thresholded at a t-value of 1.96 in accordance with a previous correlative study on autonomic nervous activity and rCBF (21). Locations of activation patterns are expressed as x, y, z coordinates in Talairach (22) space. Anatomic labeling was done according to the detailed brain atlas of Mai (23).

To test the a priori hypothesis of a positive correlation between rCBF in the hypothalamus and salivary cortisol, mean rCBF values from the hypothalamus were extracted using MarsBaR (http://marsbar.sourceforge.net) from a ROI defined in Montreal Neurological Institute (MNI) space in WFU PickAtlas (24). Because the measurements from the two assessments pre- and postplacebo treatment could not be considered statistically independent, hypothalamic rCBF values and cortisol values after speech were collapsed over the two speeches to form mean values. The mean values were then used to compute the Pearson product moment correlation coefficient in SPSS (version 12; SPSS, Inc., Chicago, IL). Also, the mean values of the time of day for the two cortisol assessments after speech were correlated to the mean cortisol levels to control for the possible confound imposed by diurnal fluctuations of cortisol excretion.

RESULTS

State anxiety ratings increased from baseline to speech both before (mean ± SD, baseline 40.50 ± 10.54, speech 59.75 ± 10.38; $t(11) = 5.56$; $p = .0002$) and after placebo treatment (mean ± SD, baseline 41.83 ± 11.56, speech 55.50 ± 10.89; $t(11) = 3.47$; $p = .005$). Ratings were not different over time (baseline $t(11) = 0.43$; $p = .67$; speech $t(11) = 1.53$; $p = .15$). Cortisol levels did not increase significantly from baseline to speech neither before (mean ± SD, baseline 12.40 ± 7.20, speech 14.70 ± 11.44; $t(11) = 0.80$; $p = .44$) nor after placebo treatment (mean ± SD, baseline 8.02 ± 4.08, speech 11.42 ± 8.60; $t(11) = 1.45$; $p = .18$). Cortisol levels were lower during baseline ($t(11) = 3.07$; $p = .01$) and tended to be lower after speech ($t(11) = 2.07$; $p = .06$) after placebo treatment. Regional CBF changed with placebo treatment in the cerebellum only (16).

Positive Covariation Between Regional Cerebral Blood Flow and Cortisol

Computation of the product moment correlation coefficient between rCBF in the hypothalamus and salivary cortisol supported the a priori hypothesis of a positive relationship ($r = 0.68$; $p = .014$, Fig. 1). Because two of the 12 subjects showed exceedingly high cortisol levels (>1.9 standard deviations above the mean), the correlation coefficient was recomputed using log-transformed data. The correlation remained ($r = 0.60$, $p = .039$), showing that it could not be explained by the possible nonnormal distribution of the data. Another concern was that the cortisol levels could reflect diurnal fluctuations rather than stress that was induced from the speaking task. However, there was no correlation between time of the day and cortisol levels ($r = -0.227$, $p = .478$). Also, the two subjects with the highest cortisol levels were not tested at an earlier time of the day than the rest of the subjects.

In the exploratory SPM99 analysis, measures of rCBF and salivary cortisol levels covaried positively in a midbrain cluster of 2624 voxels with its statistical maximum in the mamillary bodies (Talairach coordinates 0, −12, −13; z = 3.97; $p = .019$ corrected), a part of the hypothalamus. The cluster extended ventrally into the brain stem/pons (Fig. 2A).

Negative Covariation Between Regional Cerebral Blood Flow and Cortisol

Cortisol and rCBF covaried negatively in a cluster encompassing the medial prefrontal cortex and another located in the premotor/motor cortices with the maxima in Brodmann areas (BA) 32 (Talairach coordinates 4, 38, 18; z = 3.56; $p = .021$ corrected) and 6 (Talairach coordinates 4, −28, 70; z = 4.99;

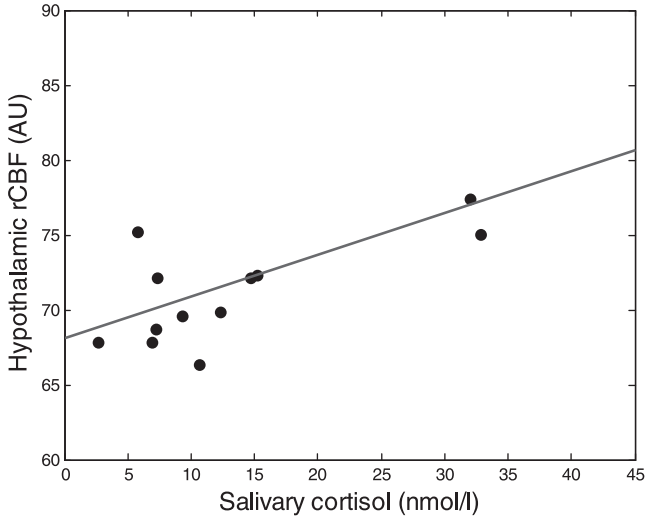

Figure 1. A scatter plot of mean salivary cortisol levels from after the two speeches and mean hypothalamic regional cerebral blood flow (rCBF) during the two speeches showing a positive correlation ($r = 0.68$, $p = .014$) consistent with an excitatory influence of hypothalamic activity on cortisol excretion.

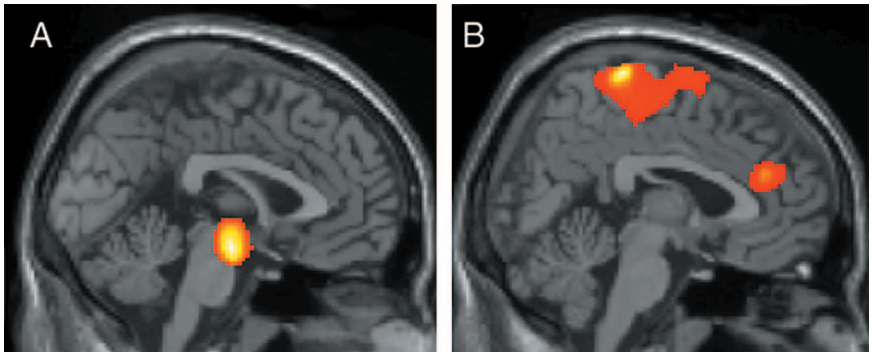

Figure 2. Sagittal images from the exploratory analysis illustrating a positive covariation (**A**) between stress induced salivary cortisol levels and regional cerebral blood flow in a midbrain cluster ranging from the thalamus to the hypothalamus and extending into the brain stem pons. A negative covariation (**B**) was observed in the medial prefrontal cortex (Brodmann areas 9, 10, and 32) and in the motor and premotor cortices (Brodmann areas 4 and 6).

$p = .00025$ corrected), respectively. The medial prefrontal cluster extended into BA 9 and 10, and the premotor/motor cluster encompassed BA 4 and 6 bilaterally (Fig. 2B).

DISCUSSION

We observed a positive covariation between rCBF in the hypothalamus and salivary cortisol excretion in patients with SAD during a stressful public speaking task. Negative covariations were observed in the medial prefrontal cortex and motor/premotor areas. The positive covariation is consistent with reports in animals in which hypothalamic activity originating in the PVN enhances the release of CRF.

Similar to Bonne et al. (14), we observed a negative covariation between cortisol levels and neural activity in the medial prefrontal cortex with the focus in the ACC. Increased ACTH levels have also been related to reduced volumes of the ACC in humans (25). In animals, lesions to the ACC result in increased cortisol in response to restraint stress (8) and an increased expression of cFOS in the PVN (9), suggesting an inhibitory influence. However, a recent perfusion functional MRI (fMRI) study implicated a possible excitatory role for the anteromedial prefrontal cortex in the control of cortisol during mild to moderate cognitive stress (26).

Cortisol levels covaried negatively with activity in the premotor and motor areas (BA 4 and 6). Elevated cortisol levels have previously been associated with behavioral inhibition (27). Systemic injections of cortisol have also shown dampening effects on locomotion in rats (28). It is unlikely that activity in the motor cortex should have an inhibitory influence on cortisol excretion. Rather, we speculate that the reduction of neural activity in the presence of higher cortisol levels could be mediated by the release of CRF from afferent nerve terminals, because CRFs seem to have a depressant-like action on neural activity in the sensorimotor cortex (29).

This study has several limitations. First, the low spatial resolution of PET precludes precise localization of specific hypothalamic subnuclei such as the PVN. In addition, because measurements of other hormones, for example vasopressin, were lacking in this study, it could not be excluded that these hormones did not correlate with hypothalamic activity as well. Therefore, we cannot study the segregation of the covariation between cortisol and rCBF on one hand and other hormones linked to cortisol on the other hand. Last, no baseline PET scans were acquired that prevented an analysis of changes in cortisol levels and changes in rCBF between a resting and a stress state.

Although previous reports on brain activity and cortisol excretion exist (12–15), general conclusions may not be feasible because designs and data processing differ. Bonne et al. (14) studied plasma cortisol excretion and rCBF in the resting state in newly traumatized individuals. Consistent with animal data, suggesting hippocampal inhibition of cortisol excretion (6), they reported a negative correlation between rCBF in the medial temporal lobe (MTL) and cortisol in patients with PTSD. We did not observe such a negative correlation. Because our study was performed during symptom provocation, the relation between rCBF in the MTL and cortisol may be altered. Also, we did not observe any relation between amygdala activity and cortisol excretion, whereas Drevets and coworkers (13) reported a positive association in patients with major depressive disorder.

Most reports on the relation between brain activity and cortisol excretion may not reflect general regulatory processes but mechanisms specific for disorders like SAD, schizophrenia (12), depression (13), or traumatized individuals (14). Hence, a study in normal healthy individuals performing a stressful task is warranted to explore brain mechanisms generally involved in cortisol control. At present, consistent with previous animal literature, data tentatively suggest that activity in the medial prefrontal and frontal cortex, as well as the hypothalamus, relate to the control of cortisol excretion in humans.

REFERENCES

1. Herman JP, Cullinana WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary–adrenocortical axis. Trends Neurosci 1997;20:78–84.
2. Fredrikson M, Sundin Ö, Frankenheuser M. Cortisol excretion during the defensive reaction in humans. Psychosom Med 1985;47:312–9.
3. Burke HM, Davis MC, Otte C, Mohs DC. Depression and cortisol responses to psychological stress: a meta-analysis. Psychoneuroendocrinology 2005;30:846–56.
4. Yehuda R. Risk and resilience in posttraumatic stress disorder. J Clin Psychiatry 2004;65(suppl 1):29–36.

5. Furlan PM, DeMartinis N, Scheizer E, Rickels K, Lucki I. Abnormal salivary cortisol levels in social phobic patients in response to acute psychological but not physical stress. Biol Psychiatry 2001;50:254–9.
6. Brück K. Functions of the endocrine system. In: Schmidt RF, Thews G, eds. Human Physiology. Berlin-Heidelberg: Springer Verlag, 1983: 658–87.
7. Jacobson L, Sapolsky R. The role of the hippocampus in feedback regulation of the hypothalamic–pituitary–adrenocortical axis. Endocr Rev 1991;12:118–34.
8. Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic–pituitary–adrenal responses to stress. J Neurosci 1993;13:3839–47.
9. Figueiredo HF, Bruestle A, Bodie B, Dolgas CM, Herman JP. The medial prefrontal cortex differentially regulates stress-induced c-fos expression in the forebrain depending on type of stressor. Eur J Neurosci 2003;18: 2357–64.
10. Sanchez MM, Young LJ, Plotsky PM, Insel TR. Distribution of corticosteroid receptors in the rhesus brain: relative absence of glucocorticoid receptors in the hippocampal formation. J Neurosci 2000;20:4657–68.
11. Seckl JR, Dickson KL, Yates C, Fink G. Distribution of glucocorticoid and mineralocorticoid receptor messenger RNA expression in human postmortem hippocampus. Brain Res 1991;591:332–7.
12. Wik G. Energy metabolism in the hypothalamus and plasma cortisol levels in patients with schizophrenia. Horm Metab Res 1996;28:205–6.
13. Drevets WC, Price JL, Bardgett ME, Reich T, Todd RD, Raichle ME. Glucose metabolism in the amygdala in depression: relationship to diagnostic subtype and plasma cortisol level. Pharm Biochem Behav 2002; 71:431–47.
14. Bonne O, Gilboa A, Louzoun Y, Brandes D, Yona I, Lester H, Barkai G, Freedman N, Chisin R, Shalev AY. Resting regional cerebral perfusion in recent posttraumatic stress disorder. Biol Psychiatry 2003;54:1077–86.
15. Neylan TC, Schuff N, Lenoci M, Yehuda R, Weiner MW, Marmar CR. Cortisol levels are positively correlated with hippocampal N-acetylaspartate. Biol Psychiatry 2003;54:1118–21.
16. Furmark T, Appel L, Michelgård Å, Wahlstedt K, Åhs F, Zancan S, Jacobsson E, Flyckt K, Grohp M, Bergström M, Pich EM, Nilsson LG, Bani M, Långström B, Fredrikson M. Cerebral blood flow changes after treatment of social phobia with the neurokinin-1 antagonist GR205171, citalopram, or placebo. Biol Psychiatry 2005;58:132–42.
17. Spielberger CD, Gorsuch RL, Lushene RE. Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press, 1970.
18. Dressendorfer RA, Kirschbaum C, Rohde W, Stahl F, Strasburger CJ. Synthesis of a cortisol–biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. Steroid Biochem Mol Biol 1992;43:683–92.
19. Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: a general linear approach. Hum Brain Mapp 1995;2:189–210.
20. al'Absi M, Wittmers LE. Enhanced adrenocortical responses to stress in hypertension-prone men and women. Ann Behav Med 2003;25:25–33.
21. Fredrikson M, Furmark T, Tillfors Olsson M, Fischer H, Andersson J, Långström B. Functional neuroanatomical correlates of electrodermal activity: a positron emission tomographic study. Psychophysiology 1998; 35:179–85.
22. Talairach J, Tournoux P. Co-planar Stereotaxic Atlas of the Human Brain. Stuttgart: Georg Thieme Verlag, 1988.
23. Mai JK, Assheuer J, Paxinos G. Atlas of the Human Brain. San Diego: Elsevier Academic Press, 2004.
24. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19:1233–9.
25. Wolf OT, Convit A, de Leon MJ, Caraos C, Quadri SF. Basal hypothalamo-pituitary–adrenal axis activity and corticotropin feedback in young and older men: relationship to magnetic resonance imaging derived hippocampus and cingulate gyrus volumes. Neuroendocrinology 2002;75:241–9.
26. Wang J, Rao H, Wetmore GS, Furlan PM, Korczykowski M, Dinges DF, Detre JA. Perfusion functional MRI reveals cerebral blood flow pattern under psychological stress. Proc Natl Acad Sci USA 2005;49:17804–9.
27. Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioural inhibition in children. Child Dev 1987;58:1459–73.
28. Isobe Y, Torii T, Kawaguchi T, Nishino H. Dexamethasone induces different wheel running activity than corticosterone through vasopressin release from the suprachiasmatic nucleus. Brain Res 2004;1028:219–24.
29. Froc DJ, Christie BR. Corticotrophin-releasing hormone decreases synaptic transmission in rat sensorimotor cortex *in vivo*. Neuropharmacology 2005;134:965–73.