CEREBROSPINAL FLUID CONCENTRATIONS OF CORTICOTROPIN-RELEASING HORMONE, VASOPRESSIN, AND SOMATOSTATIN IN DEPRESSED PATIENTS AND HEALTHY CONTROLS: RESPONSE TO AMITRIPTYLINE TREATMENT

I. Heuser,[1]* G. Bissette,[2] M. Dettling,[1] U. Schweiger,[1] U. Gotthardt,[1] J. Schmider,[1] C.-H. Lammers,[1] C.B. Nemeroff,[3] and F. Holsboer[1]

The effect of amitriptyline upon hypothalamic-pituitary-adrenal [HPA]-system-regulating neuropeptides (corticotropin-releasing hormone [CRH], vasopressin, somatostatin) was studied in a group of depressed elderly patients and controls. A first lumbar puncture was performed in 37 depressed in-patients. This was followed by a 6-week medication phase with amitriptyline. Upon its completion a second cerebrospinal fluid (CSF) sample was obtained in 18 of these 37 patients. In 25 healthy controls a first lumbar puncture was done; eleven of these individuals agreed to take 75 mg/d amitriptyline for 6 weeks and to participate in the follow-up CSF study. Within the group of depressed patients amitriptyline led to a significant decrease of CSF CRH in treatment responders only ($F_{1,16} = 5.2$; $P < 0.02$). Also, in normal controls CSF CRH concentration tended to decrease with amitriptyline treatment (t-test; $P < 0.09$). No effects of amitriptyline upon vasopressin or somatostatin were observed. In normal controls ($r = 0.4$; $P < 0.02$) and in patients ($r = 0.4$; $P < 0.03$) age correlated positively with baseline CSF somatostatin. A trend for CSF CRH to increase with aging was found only in controls ($r = 0.3$; $P < 0.09$); patients did not show a significant association here. Finally, CSF neuropeptide concentration at baseline did not differ between the group of depressed patients and healthy controls. Our study corroborates the evolving concept that antidepressants effect various components of the HPA system with the net result of a reduction in its activity. In addition, we found CSF CRH and CSF somatostatin concentrations to be better reflections of age than of depression and, finally, that during aging and during depression the HPA system changes in similar directions. Depression and Anxiety 8:71–79, 1998.

Key words: *amitriptyline; corticotropin-releasing hormone; vasopressin; somatostatin*

INTRODUCTION

Patients with depression frequently have symptom clusters (sleep disturbances, altered eating behavior, decreased libido, cardiovascular changes, cognitive deficits and changes in hormone secretion) which strongly suggest the pathological involvement of the hypothalamic-pituitary-adrenal (HPA) axis in affective disorders; it is equivocally established that dysregulation of the HPA system is very common in patients with major depression [1]. In depressed patients the findings of sustained hypercortisolemia, cortisol escape from dexamethasone suppression (DST), a blunted ACTH response to CRH and exaggerated ACTH and

[1]Max Planck Institute of Psychiatry, Clinical Institute, Munich, Germany
[2]Medical Center, University of Mississippi, Jackson, Mississippi
[3]Dept. of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia

Contract grant sponsor: NIMH; Contract grant number: MH-42088.

*Correspondence to: Isabella Heuser, M.D., Central Institute of Mental Health, J 5, 68159 Mannheim, Germany. E-mail: isabella @as200.zi-mannheim.de

Received for publication 11 September 1997; Accepted 4 March 1998

cortisol responses after dexamethasone pretreatment *and* CRH stimulation (DEX/CRH-test) are all indications of an overactive and/or dysregulated HPA system [2–5].

The hypothesis has been advanced that hypothalamic hypersecretion of corticotropin-releasing-hormone (CRH) contributes to the hyperactivity of the HPA system in patients with major depression. Indeed, several studies have demonstrated significant elevation of CRH in cerebrospinal fluid (CSF) in drug-free patients with major depression [6,7]. Also, cisternal CRH concentrations were found to be higher in depressed suicide victims in comparison to "sudden death" controls [8]. However, others found no differences in CSF CRH concentrations between depressed patients and controls [9,10]. Finally, elevated CSF CRH concentrations in depressed patients decrease significantly after successful treatment with antidepressants or ECT, and HPA axis function (DST, DEX/CRH test) normalizes with resolution of depressive psychopathology [11–15,5].

However, CRH is not the sole HPA system regulator. Studies in both laboratory animals and humans have provided evidence that arginine-vasopressin (AVP) is able to potentiate the ACTH-stimulating effects of CRH at the pituitary [16–19]. Hence, one might posit altered CSF AVP concentrations in depression. One CSF study reported significantly decreased AVP concentrations in depressed patients in comparison to healthy controls [20], whereas another found no differences [21].

Somatostatin (SOM) is another hypothalamic neuropeptide involved in the regulation of the HPA system. SOM has mainly inhibitory effects upon release of other pituitary hormones, a fact which led McCann [22] to propose changing the name of SOM to "panhibin." In clinical studies, CSF SOM was mostly found to be decreased in depressed patients, especially in those who were DST-nonsuppressors [23–28]. A post-mortem study found no differences in cortical SOM-immunoreactivity, affinity or binding capacity of SOM-receptors between depressed patients and controls [29]. However, in contrast to antidepressive treatment effects upon CSF CRH concentrations, no significant changes were observable in CSF SOM after ECT [13] and at least one study found CSF SOM not to differ among various psychiatric patients [30].

Neuroendocrine research about changes of the HPA axis across the life-cycle has advanced our knowledge about the major role this system plays in controlling certain aging processes and in modulating development of pathology which becomes more likely with increasing age. For example, in aging rats basal glucocorticoid plasma concentrations increase and adrenocortical recovery from stress is delayed [31]. In humans, aging is associated with a higher likelihood of nonsuppression in the DST, enhanced cortisol responses to mental stress and increased release of ACTH and cortisol in the DEX/CRH test [32–34]. To date however, it still remains to be elucidated which component of the HPA system contributes to age-related HPA changes: experimental studies in rats report an increased activity of central AVP neurons with aging, possibly due to an age-associated desensitization of renal AVP receptors [35]. Post-mortem studies in humans strongly support the hypothesis that AVP neurons in the PVN are hyperactivated in aging [36–38] and in depression.

Finally, animal studies demonstrated reductions of SOM prohormone mRNA expression in cortical brain areas—but not in the hypothalamus—of aged rats, an increased in vitro release of SOM from aged hypothalami, a marked loss of SOM receptors in various cortical and subcortical brain areas and an age-associated increased pituitary sensitivity to SOM [39–42]. In humans, most CSF studies found no effect of age upon SOM concentrations [27,43–45].

In the current study, we sought to delineate the effects of depression, age and antidepressant medication upon the HPA-system-regulating neuropeptides by treating depressed patients and elderly, non-depressed probands for 6 weeks with an antidepressant. Before and after the medication interval a lumbar puncture for determination of CSF concentrations of CRH, AVP and SOM was performed.

SUBJECTS AND METHODS

SUBJECTS

A total of 37 in-patients (26 female; 11 male) who fulfilled DSM-III-R criteria [46] for a major depressive episode participated in the study after providing informed consent. Patients with mood-incongruent psychotic features were not included. The mean age of the patients was 46.8 ± 17.0 years (mean ± SD)(range 22–82 years) and the mean body-mass index (BMI) was 23.6 ± 4.1. The Hamilton Depression Rating Scale score (HDRS)[47] before treatment averaged 26.3 ± 4.1 (range 18–40); after treatment the mean HDRS was 11.6 ± 7.5 (range 1–26). Age of onset of depressive illness (= first major depressive episode ever to occur) was 40 ± 17 years (range 17–82 years). Over all, duration of the affective disorder varied from <1 to 39 years, with a mean of 8.1 ± 11 years. Number of depressive episodes varied from one to 14, with a mean of 3 ± 3. Duration of the index episode averaged 15.2 ± 17.2 weeks.

Eighteen of the 37 patients agreed to be restudied after completion of a 6-week treatment trial with amitriptyline (75 mg/d); the mean age of this patient subgroup was 48 ± 15 years. Nine of these patients were considered responders to treatment and nine were nonresponders (response was defined as a drop of at least 50% in the HDRS score after treatment). Mean HDRS of responders before treatment was 25.2 ± 6.4, that of nonresponders, 27.9 ± 5.3; after treatment responders had an HDRS score of 5.6 ± 4.3; nonresponders scored 18.4 ± 3.2.

HEALTHY CONTROLS

Twenty-five healthy controls (eight females, 17 males) with a mean age of 65.5 ± 15.4 years, ranging from 23 to 85 years, and a mean BMI of 24.2 ± 3.7 were studied at baseline. Eleven of these 25 individuals agreed to take 75 mg/d amitriptyline for 6 weeks and to undergo follow-up study. The mean age of this subgroup of probands was 68 ± 10 years (seven males, four females). All individuals were in good medical condition, free of any psychoactive drugs and without a personal or family history of psychiatric or neurodegenerative disorders. Two of the elderly volunteers (one male, one female) received antihypertensive medication and one other female received digitalis; none of the elderly females received estrogen replacement therapy.

STUDY DESIGN

In patients, any previous psychopharmacological medication was discontinued upon admission to the hospital, and thereafter patients were kept medication-free for at least 5 d to achieve drug-free conditions. None of the patients had been pretreated with fluoxetine; thereafter the first lumbar puncture (PRE) was performed. Then the 6-week medication phase with amitriptyline (75 mg/d) was started and upon its completion the second CSF sample was obtained.

Normal volunteers were hospitalized at approximately 1300 the day prior to the CSF study; after the lumbar puncture probands remained in the hospital for an observation period of at least 6 h. Those 11 who received amitriptyline (75 mg/d) at 2200 every day for 6 weeks were readmitted to the hospital for a second lumbar puncture.

LUMBAR PUNCTURES

After 12 h of fasting and an overnight controlled bedrest, starting at 2200 the previous night, the procedure was performed at 0800 with the subject in the lateral decubital position. An atraumatic needle was advanced between the fourth and fifth lumbar intervertebral space and four aliquots of 2.5 ml spinal fluid were obtained. Aliquots were immediately placed on dry ice and after completion of the lumbar puncture stored at –80°C. The neuropeptides were measured only in the third and fourth aliquots to prevent the confound of a possible rostral-caudal gradient.

ASSAYS

The frozen spinal fluid samples were transported by air in a styrofoam box containing dry ice to the United States (Dr. Garth Bissette, Department of Psychiatry, Duke University Medical Center, Durham, NC) for peptide analyses. All samples were coded before transportation and the code was made available to the laboratory only after all the samples had been analyzed. CRH, AVP and SOM were measured using sensitive and specific radioimmunoassay procedures described earlier [6,26]. Each neuropeptide assay measured the pretreatment and posttreatment CSF samples in a single batch using freshly prepared radioactive tracers.

STATISTICS

Differences in group means (patients vs. controls) of the CSF neuropeptide data were compared separately for PRE- and POST-treatment time points using ANCOVA, factorial design with age as covariate to account for the fact that on average patients were younger than controls. Differences between responders and nonresponders before and after treatment were analyzed by ANOVA. Statistical analysis of treatment effects within the control group and within each of the response groups was carried out separately for each neuropeptide using paired *t*-tests. Correlations between neuropeptide concentrations and background variables (e.g., HAMD scores, duration of illness) and relationships between the three neuropeptides were explored by Pearson's product-moment coefficient. All hormone data were log-transformed prior to statistical analysis to correct for heteroscadacity. Statistical significance was accepted at $P < 0.05$. Results are reported as means ± standard deviation of the non-log transformed data in the text and in the tables.

RESULTS

ANCOVA analysis for the CSF-PRE concentrations revealed that diagnosis (patients vs. controls) did not affect CSF-PRE concentrations of any neuropeptide studied (Table 1). However, a significant age effect upon CRH ($F_{1,58} = 6.8$; $P < 0.01$) and a pronounced impact of the covariate on SOM ($F_{1,58} = 9.7$; $P < 0.003$), but not upon AVP ($F_{1,58} = 2.9$; $P < 0.1$), emerged: correlational analysis indicated that within the group of normal controls age correlated positively with SOM PRE (r = 0.4; $P < 0.02$), the same was true for the group of patients (r = 0.4; $P < 0.03$). A trend

TABLE 1. CSF neuropeptide concentrations (pg/ml) in depressed patients and controls before (pre) and after (post) amitriptyline

	Depressed patients (pre, n = 37; post, n = 18)	Normal controls (pre n = 25; post n = 11)
Age** (years)	46.8 ± 17.2	65.5 ± 15.4
CRH pre	49.6 ± 18.4	49.6 ± 13.3
CRH post	45.2 ± 12.4	41.5 ± 11.4
AVP pre	3.7 ± 0.8	3.7 ± 0.7
AVP post	3.8 ± 0.5	4.1 ± 0.5
SOM pre	31.4 ± 9.9	38.6 ± 8.8
SOM post	33.9 ± 9.8	34.9 ± 8.6

***Normal controls were significantly older than patients (*t*–test;* P < *0.001). CRH, corticotropin–releasing hormone; AVP, arginine–vasopressin; SOM, somatostatin.*

for CSF CRH to increase with age was found only in controls (r = 0.3; $P < 0.09$); patients did not show a significant association (r = 0.2; $P < 0.2$). However, after pooling patients and controls, the correlation between CSF CRH-PRE and age remained significant (r = 0.3; $P < 0.02$). Finally, in neither group did AVP correlate significantly with age. Also, no interaction effect between age and diagnosis was found.

Comparing group means (patients vs. controls) for CRH, AVP and SOM at baseline (PRE) between strictly age-matched individuals confirmed that patients and probands did not differ in these parameters (Table 2).

Nine of the 18 depressed patients who agreed to have a second spinal tap after completion of antidepressant treatment were considered treatment responders (RES) and nine did not respond sufficiently to 6 weeks of amitriptyline (Non-RES). Treatment responders were on an average 10 years older (53.9 ± 22.9 years) than treatment nonresponders (44.5 ± 10.8 years), but this difference did not attain statistical significance. There was no difference between responders and nonresponders in age of onset (responders, 41.1 ± 21.6 years; nonresponders, 36.4 ± 13.8 years), or number of previous episodes (responders, 2.1 ± 2; nonresponders, 4.2 ± 4.6). There was a trend for CSF CRH-PRE to be higher in non-RES in comparison to RES but the difference was not significant (Table 3). After treatment, however, there was a statistically significant ($F_{1,16} = 5.2$; $P < 0.02$) lower CSF CRH concentration in RES as opposed to non-RES. No difference was found in either AVP or SOM CSF concentrations before or after amitriptyline treatment between the two response groups. Moreover, in patients there was no correlation between severity of depression as measured with the HDRS and any of the CSF neuropeptide concentrations at baseline.

CSF CRH concentrations tended to decrease (paired *t*-test; $P < 0.09$) also in normal controls after amitriptyline, whereas the other neuropeptides did not change significantly.

With regard to the relationship between the different peptides no significant associations were found.

TABLE 2. CSF-neuropeptide concentrations in elderly depressed patients and strictly age-matched controls before amitriptyline

	Depressed patients[a] (n = 11)	Normal controls[b] (n = 22)
Age (years)	68 ± 10	69 ± 6
CRH (pg/ml)	48 ± 13	51 ± 14
AVP (pg/ml)	4.1 ± 0.8	3.8 ± 0.7
SOM (pg/ml)	35 ± 10	40 ± 7

[a]*Seven females, four males.*
[b]*Eight females, 14 males.*
CRH, corticotropin–releasing hormone; AVP, arginine–vasopressin; SOM, somatostatin.

TABLE 3. CSF-neuropeptide concentrations (pg/ml) in treatment responders (RES) and nonresponders (Non-RES) before (pre) and after (post) amitriptyline

	RES (n = 9)	Non–RES (n = 9)
CRH pre	45.1 ± 12.6	50.1 ± 11.1
CRH post	38.9 ± 7.9	51.6 ± 13.3
AVP pre	4.1 ± 0.5	3.8 ± 1.1
AVP post	3.9 ± 0.5	3.8 ± 0.4
SOM pre	32.8 ± 7.7	31.6 ± 11.4
SOM post	34.4 ± 10.0	33.4 ± 10.2

CRH, corticotropin–releasing hormone; AVP, arginine–vasopressin; SOM, somatostatin.

However, plotting correlational matrices for PRE concentrations of CRH and SOM of all patients gave the impression that, in those patients with CSF CRH-PRE levels ranging at or above 50 pg/ml, a positive association between these two neuropeptides might exist, which was absent in the "lower" (<50 pg/ml) CSF CRH group (Table 4). Indeed, in the former group, there was a statistically significant positive correlation (r = 0.8; $P < 0.02$) between PRE levels of CSF CRH and SOM which was not found for the latter group (r = 0.2; $P < 0.3$). However, within the group of the 18 restudied patients POST-CRH and -SOM were significantly associated after treatment (r = 0.5; $P < 0.02$). Likewise, in normal controls PRE-CSF SOM and -CRH were significantly correlated (r = 0.6; $P < 0.002$). In contrast, no relevant correlations were seen between either CSF CRH and AVP, the HAMD scores and any neuropeptide levels or among any background variable and CSF CRH, AVP and SOM concentrations in the patient group.

DISCUSSION

The major findings of this study were as follows. 1) CSF neuropeptide concentrations did not differ between depressed patients and healthy controls. 2) With aging, CSF concentrations of somatostatin, but not of vasopressin, increased, whereas only in healthy

TABLE 4. Depressed patients grouped according to "high" (≥50 pg/ml) and "low" (≤50 pg/ml) pre CSF CRH

	CSF CRH "high"	CSF CRH "low"
N	10	27
Age (years)	46 ± 15	47 ± 18
HDRS	25 ± 5	26 ± 7
CRH	61 ± 9	41 ± 5
SOM	34 ± 11	30 ± 9
AVP	4 ± 1	4 ± 1

HDRS, Hamilton Depression Rating Scale; CRH, corticotropin–releasing hormone; AVP, arginine–vasopressin; SOM, somatostatin.

individuals, but not in depressed patients, a trend for an age-associated increase in CSF CRH was found. 3) CSF concentrations of CRH and SOM were tightly correlated across all subjects with the exception of the subgroup of untreated depressed patients with lower CRH levels. AVP and CRH or AVP and SOM showed no correlations. 4) Amitriptyline led to a significant decrease of CSF CRH in responders to antidepressant treatment. Similarly, in normal controls CSF CRH concentrations tended to decrease with amitriptyline treatment; the two other neuropeptides studied were not affected by medication.

On the basis of findings related to the sites of origin of CSF levels of CRH, these CSF concentrations are probably derived from brain regions outside the median eminence, such as hippocampus, cerebral cortex, amygdala, bed nucleus of the stria terminalis, and cortical areas [48,49].

Our finding of comparable CSF concentrations of CRH in depressed patients and normal probands is in contrast to a series of earlier studies using single-point CSF samples, in which significant elevation of CSF CRH in drug-free depressed patients as opposed to controls has repeatedly been demonstrated [50]. However, our findings are consistent with those of Roy et al. [9], who also found no differences in mean CSF CRH between a group of unipolar and bipolar depressed patients and healthy controls.

Another study also reported similar CSF concentrations between 62 healthy volunteers and 34 depressed patients, whose age range was similar to that of the patients we studied [10]. One possible explanation to reconcile these discrepancies with regard to CSF CRH in depression might be: CSF CRH-PRE was almost identical in our younger patients and elderly controls; a trend for CRH to be positively associated with aging was found only in the control probands. Thus one might expect CSF CRH concentrations in our younger patients to be at least somewhat lower than in the old controls. Because this was not the case, one might speculate that in depressed patients, a possibly existing state-dependent CRH overactivity was masked or overridden by the greater impact age might have on its concentration. Disagreeing with such an explanation are, of course, the results of the ANCOVA, where we controlled for age effects with regard to CSF concentrations, and of our separate analysis, where we compared CSF concentrations between strictly age-matched individuals and found them to be similar in depressed patients and controls. A somewhat indirect support for our negative findings concerning CSF CRH levels is given by two studies reporting (in contrast to the findings by Nemeroff et al. [51]) no significant alterations in either CRH immunoreactivity or receptor binding in the frontal cortex of patients with major depression when compared with normal controls [52,53].

Our study is in agreement with others reporting significant reductions in posttreatment relative to pretreatment samples of CSF CRH concentrations after antidepressants or ECT in depressed patients, and with another study demonstrating a significant dose-dependent reduction in CRH CSF concentration following 2 d of desimipramine administration to healthy volunteers [13,14,45,54,55]. In addition, a recent study found no differences in CSF CRH between antidepressant-treated depressed patients and healthy controls [53]. Those patients of our study that were considered responders to 6 weeks of amitriptyline exhibited a significant reduction in CSF CRH concentrations, and there was a trend for amitriptyline to lower CSF CRH concentrations in the normal volunteers. Recently the concept has evolved that antidepressants affect various components of the HPA system with the net result of a reduction of its activity [56–58]. These observations are corroborated by studies in rats demonstrating mRNA expression of CRH and of tyrosine hydroxylase in the locus coeruleus to be decreased after desipramine, while mRNA expression of hippocampal mineralocorticoid receptors (MR), which are crucially involved in feedback regulation of the HPA system, is increased [59]. In another study this group also showed that other antidepressants, such as fluoxetine, idazoxan and phenelzine, reduce CRH mRNA expression [60]. However, to date it is not clear whether these reductions in CRH mRNA expression are, first, of functional relevance and, second, not counterregulated by CRH-receptor adaptations.

Numerous studies have suggested that with aging the HPA system becomes more active in rats and humans [61–63,33,34]. Although it still remains uncertain which component of the HPA system undergoes which changes, it has been reported that in male rats a progressive hypothalamic CRH deficiency with aging seems to be linked to elevated production of vasopressin in the hypothalamus, resulting in an age-associated hyperactivity of the HPA system [64]. Others, however, support the notion that an age-related decrease in pituitary CRH receptors is in response to an increased secretion of hypothalamic CRH [64–67]. With regard to humans, post-mortem studies suggested a significant age-dependent increase in the total number of CRH neurons in the nucleus paraventricularis (PVN) in non-depressed humans [68]. However, it is of relevance in the context of our study that in depressed patients the increase that was observed was not age dependent. Obviously, as also seen in our study, the positive correlation between age and CRH may be masked if the individual is also depressed.

It has been established that AVP is a cosecretagogue of CRH at the corticotroph [17,69] and that under conditions of chronic stress in rats the number of CRH cells and AVP colocalization in CRH-containing vesicles increase [70–72]. Also, post-mortem studies in severely depressed humans found a threefold increase in CRH neurons coexpressing AVP together with an increase in the total number of AVP-immunoreactive neurons in the PVN [68,73]. Similar results have been

obtained in studies measuring CRH and AVP mRnA expression [38].Recently a strong positive correlation between CSF CRH and CSF AVP in medicated, depressed patients has been shown [55] and earlier, in a small sample of depressed patients, slight, albeit statistically significant decreases in both CSF CRH and AVP following a minimum of a 6-week treatment with 20 mg fluoxetine were observed [14]. These findings are in contrast to our data where AVP appeared to be remarkably inert across the two groups and was not changed after treatment. We were also unable to detect any age- or depression-dependent variations in CSF AVP, which disagrees with post-mortem studies, indicating tightly coregulated activation of CRH- and AVP-containing neurons [74]. These authors investigated brains of 13 subjects between the ages of 23 and 91 years. Colocalization of CRH and AVP in the PVN was only found in individuals older than 40 years and, with two exceptions, from 52 years onwards in all subjects this colocalization was detectable. Given these post-mortem findings, it might very well be that in both our groups of younger, but depressed patients and older, but healthy controls, AVP systems were activated to a similar degree, albeit for different reasons; thus our failure to detect differences in CSF levels. However, if such a scenario accounted for our results, one would expect 1) strong interaction effects between depression and aging to emerge and 2) a substantial positive correlation between CRH and AVP to be detectable; neither was the case. Therefore, we draw the conclusion that the role of AVP in humans is of minor importance in depression and aging. However, it is important to point out that CSF measurements remain an indirect measure of extracellular fluid concentrations of neuropeptides and are no substitute for post-mortem brain tissue studies.

In contrast to the majority of previous studies we found similar CSF SOM levels in patients and controls and a positive correlation between age and SOM. Also, a complicated relationship between CRH and SOM was apparent: while in healthy individuals CRH and SOM were clearly coregulated, this was seen only in unmedicated patients with relatively high (>50 pg/ml) CSF CRH. Surprisingly, after antidepressant treatment, despite a mean CRH level of 45.2 pg/ml, POST-CSF SOM and CRH from depressed patients were correlated similar to normal controls. Decreasing growth hormone secretory activity is one of the endocrine hallmarks of aging. The activity of the somatotropic system is mediated by two hypothalamic hormones: growth hormone-releasing hormone (GHRH) and somatostatin (growth hormone-inhibiting hormone). Although the precise underlying mechanism which leads to a diminished GH activity in aging still remains unclear, several studies in humans suggest that this might be due at least in part to an increase in basal SOM secretion [75]. However, one has to consider that the SOM measured in these two aforementioned studies may have originated mainly in the gut rendering inferences to hypothalamic and/or cortical SOM difficult. As mentioned in the Introduction, the majority of CSF studies in depressed or healthy humans were unable to detect an effect of age upon CSF SOM. However, evidence from animal experiments suggests that with aging, central components of the SOM system change in ways that might lead to the net result of increased secretion of this neuropeptide [76–78]. Thus, our findings of an age-associated increase in CSF SOM corroborate these data from the preclinical area.

Numerous studies found CSF SOM to be significantly decreased in depressed patients as opposed to normal controls [50]. We were unable to replicate these findings and interpret our results as follows. Because age was strongly and positively correlated with CSF SOM, if CSF SOM were indeed decreased in depression, one would expect our considerably younger depressed patients to have much lower SOM concentrations than the elderly controls. In fact, the depressed patients did have lower CSF SOM levels than the healthy probands, but this was not statistically significant after controlling for age. Also, there was no difference in strictly age-matched depressed patients and healthy controls, although SOM was insignificantly higher in controls, again supporting the notion that age, but not depression, is a major modulator of CSF SOM.

Interestingly, in our sample SOM and CRH were tightly coregulated. Somatostatin appears to play a major role in the regulation of the HPA-system activity, although its precise underlying mechanisms remain unclear. It was shown that intracerebroventricular administration of cysteamine, which depletes central SOM stores, leads to an inhibition of dexamethasone-induced corticosterone suppression [79]. In another study, all but one of the healthy volunteers who received 80 mg of prednisone for 5 d showed a reduction in CSF SOM supporting previous animal and in vitro experiments [80]. Also, a significant negative correlation was reported between post-dexamethasone plasma cortisol concentration and CSF SOM in depressed patients [25], supporting the notion that either SOM might counterregulate HPA-system activity or that glucocorticoids suppress SOM. Paradoxically, in our study depressed patients with higher CSF CRH and thus, presumably, elevated plasma cortisol showed a linear and positive relation between the two neuropeptides before amitriptyline, whereas after treatment this relationship was also seen in the, by then, "lower level" CRH patients. Taken together with a study by Kling et al. [81], who found an inverted U-shape relationship between post-dexamethasone cortisol and CSF-SOM concentrations in depressed patients, this suggests the following hypothesis: in depression only an as yet unidentified factor delineates or modulates a threshold above which SOM activity increases in concert with heightened HPA-system activity and eventually exerts inhibitory effects upon the HPA system as reflected by decreasing concentrations of CRH while SOM is still high.

In conclusion, we found CSF CRH and SOM concentrations to be better reflections of age than of depression. This is strongly supported by the fact that neither of these two, tightly coregulated neuropeptides varied with the severity of depression. The fact that amitriptyline led to a reduction in CSF CRH only in treatment responders and the finding that before initiation of treatment prospective nonresponders had higher, albeit statistically not significant, levels of CRH, support the notion that CRH is causally involved in the etiology of depression. This is corroborated by our findings that the elderly healthy controls, who had CSF CRH levels similar to the depressed patients before amitriptyline and had concentrations after treatment comparable to the depressed treatment responders, also exhibited markedly lower CRH after amitriptyline than the depressed nonresponders. Finally, the finding that in elderly controls CRH and SOM were coregulated in a similar fashion as in depressed patients after treatment and, second, that amitriptyline lowered CRH also in the elderly healthy individuals strengthens the notion that during aging and during depression the HPA system undergoes changes in a similar direction.

REFERENCES

1. Holsboer F, Spengler D, Heuser I (1992) The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. Prog Brain Res 93:385–417.
2. Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Haskett RF, McJames N, Kronfol Z, Lohr N, Steiner M, de Vigne JP, Young E (1981) A specific laboratory test for the diagnosis of melancholia. Arch Gen Psychiatry 38:15–22.
3. Gold PW, Chrousos G, Kellner C, Post R, Roy A, Augerios P, Schulte H, Oldfield E, Loriaux DL (1984) Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. Am J Psychiatry 141:619–627.
4. Holsboer F, von Bardeleben U, Gerken A, Stalla GK, Müller OA (1984) Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor (h-CRF) in depression. N Engl J Med 311:1127.
5. Holsboer-Trachsler E, Stohler R, Hatzinger M (1991) Repeated administration of the combined dexamethasone/hCRH stimulation test during treatment of depression. Psychiatry Res 38:163–171.
6. Nemeroff CB, Widerlöv E, Bissette G, Walléus H, Karlsson I, Eklund K, Kilts CD, Loosen PT, Vale W (1984) Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science 226:1342–1344.
7. Banki CM, Bissette G, Arato M, O'Connor L, Nemeroff MS, Nemeroff CB (1987) CSF corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. Am J Psychiatry 144:7.
8. Arato M, Banki CM, Bissette G, Nemeroff CB (1989) Elevated CSF-CRF in suicide victims. Biol Psychiatry 25:355–359.
9. Roy A, Pickar D, Paul S, Doran A, Chrousos GP, Gold PW (1987) CSF corticotropin-releasing hormone in depressed patients and normal control subjects. Am J Psychiatry 144:641–645.
10. Kling MA, Roy A, Doran AR, Calabrese JR, Rubinow DR, Whitfield Jr HJ, May C, Post RM, Chrousos GP, Gold PW (1991) Cerebrospinal fluid immunoreactive corticotropin-releasing hormone and adrenocorticotropin secretion in Cushing's disease and major depression: potential clinical implications. J Clin Endocrinol Metab 72:260–271.
11. Holsboer F, Liebl R, Hofschuster E (1982) Repeated dexamethasone suppression test during depressive illness. Normalization of test result compared with clinical improvement. J Affective Disord 4:93–101.
12. Greden JF, Gardner R, King D, Grunhaus L, Carroll BJ, Kronfol Z (1983) Dexamethasone suppression test in antidepressant treatment of melancholia—the process of normalization and test-retest reproductibility. Arch Gen Psychiatry 40:493–500.
13. Nemeroff CB, Bissette G, Akil H, Fink M (1991) Neuropeptide concentrations in the cerebrospinal fluid of depressed patients treated with electroconvulsive therapy. Corticotropin-releasing factor, -endorphin and somatostatin. Br J Psychiatry 158:59–63.
14. De Bellis MD, Gold PW, Geracioti Jr TD, Listwak SJ, Kling MA (1993) Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. Am J Psychiatry 150:656–657.
15. Heuser IJE, Schweiger U, Gotthardt U, Schmider J, Lammers C-H, Dettling M, Holsboer F (1996) Pituitary-adrenal-system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and in normal controls. Am J Psychiatry 153:93–99.
16. Gillies GE, Linton EA, Lowry PJ (1982) Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. Nature 299:355–357.
17. von Bardeleben U, Holsboer F, Stalla GK, Müller OA (1985) Combined administration of human corticotropin-releasing factor and lysine vasopressin induces cortisol escape from dexamethasone suppression in healthy subjects. Life Sci 37:1613–1618.
18. Whitnall MH, Mezey E, Gainer H (1985) Colocalization of corticotropin-releasing factor and vasopressin in median eminence neurosecretory vesicles. Nature 317:248–250.
19. Lightman SL, Young WS III (1988) Vasopressin, oxytocin, dynorphin, enkephalin and corticotropin-releasing factor mRNA stimulation in the rat. J Physiol (Camb) 403:511–523.
20. Gjerris A, Hammer M, Vendsborg P, Christensen NJ, Rafaelsen OJ (1985) Cerebrospinal fluid vasopressin: changes in depression. Br J Psychiatry 147:696–701.
21. Sørensen PS, Gjerris A, Hammer M (1985) Cerebrospinal fluid vasopressin in neurological and psychiatric disorders. J Neurol Psychiatry 48:50–57.
22. McCann SM (1982) Physiology and pharmacology of LHRH and somatostatin. Ann Rev Pharmacol Toxicol 22:491–515.
23. Gerner RH, Yamada T (1982) Altered neuropeptide concentrations in cerebrospinal fluid of psychiatric patients. Brain Res 238:298–302.
24. Rubinow DR (1986) Cerebrospinal fluid somatostatin and psychiatric illness. Biol Psychiatry 21:341–365.
25. Doran AR, Rubinow DR, Roy A, Pickar D (1986) CSF somatostatin and abnormal response to dexamethasone administration in schizophrenic and depressed patients. Arch Gen Psychiatry 43:365–369.
26. Bissette G, Widerlov E, Walleus H, Karlsson I, Eklundt K, Forsman A, Nemeroff CB (1986) Alterations in cerebrospinal fluid concentrations of somatostatin-like immunoreactivity (SRIF-LI) in neuropsychiatric disorders. Arch Gen Psychiatry 43:1148–1154.
27. Sunderland T, Rubinow DR, Tariot PN, Cohen RM, Newhouse PA, Mellow AM, Mueller EA, Murphy DL (1987) CSF somatostation in patients with Alzheimer's disease, older de-

pressed patients, and age-matched control subjects. Am J Psychiatry 144:1313–1316.
28. Molchan SE, Lawlor BA, Hill JL, Martinez RA, Davis CL, Mellow AM, Rubinow DR, Sunderland T (1991) CSF monoamine metabolites and somatostatin in Alzheimer's disease and major depression. Biol Psychiatry 29:1110–1118.
29. Charlton BG, Leake A, Wright C, Fairbairn AF, McKeith IG, Candy JM, Ferrier IN (1988a) Somatostatin content and receptors in the cerebral cortex of depressed and control subjects. J Neurol Neurosurg Psychiatry 51:719–721.
30. Banki CM, Karmacsi L, Bissette G, Nemeroff CB (1992a) Cerebrospinal fluid neuropeptides in mood disorder and dementia. J Affective Disord 25:39–46.
31. Seeman TE, Robbins RJ (1994) Aging and hypothalamic-pituitary-adrenal response to challenge in humans. Endocr Rev 15:233–260.
32. Ferrier IN, Pascual J, Charlton BG, Wright C, Leake A, Griffiths HW, Fairbairn AF, Edwardson JA (1988) Cortisol, ACTH, and dexamethasone concentrations in a psychogeriatric population. Biol Psychiatry 3:252–260.
33. Heuser IJ, Gotthardt U, Schweiger U, Schmider J, Lammers C-H, Dettling M, Holsboer F (1994) Age-associated changes of pituitary-adrenocortical hormone regulation in humans: importance of gender. Neurobiol Aging 15:227–231.
34. Gotthardt U, Schweiger U, Fahrenberg J, Lauer Ch, Holsboer F, Heuser I (1995) Cortisol, ACTH, and cardiovascular response to a cognitive challenge paradigm in aging and depression. Am J Physiol 268:R865–R873.
35. Ravid R, Fliers F, Swaab DF, Zurcher C (1987) Changes in vasopressin and testosterone in the senescent Brown-Norway (BN/BiRij) rat. Gerontology 53:87–98.
36. Goudsmit E, Hofman MA, Fliers E, Swaab DF (1990) The supraoptic and paraventricular nuclei of the human hypothalamus in relation to sex, age and Alzheimer's disease. Neurobiol Aging 11:529–536.
37. Lucassen PJ, Salehi A, Pool CW, Gonatas NK, Swaab DF (1994) Activation of vasopressin neurons in aging and Alzheimer's disease. J Neuroendocrinol 6:673–679.
38. Raadsheer FC, van Heerikhuize JJ, Lucassen PJ, Hoogendijk WJG, Tilders FJH, Swaab DF (1995) Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression. Am J Psychiatry 152:1372–1376.
39. Florio T, Ventra C, Postiglione A, Schettini G (1991) Age-related alterations of somatostatin gene expression in different rat brain areas. Brain Res 557:64–68.
40. Sonntag WE, Gottschall PE, Meites J (1986) Increased secretion of somatostatin-28 from hypothalamic neurons of aged rats in vitro. Brain Res 380:229–234.
41. Sato H, Ota Z, Ogawa N (1991) Somatostatin receptors in the senescent rat brain: a quantitative autoradiographic study. Regul Pept 33:81–92.
42. Spik K, Sonntag WE (1989) Increased pituitary response to somatostatin in aging male rats: relationship to somatostatin receptor number and affinity. Neuroendocrinology 50:489–494.
43. Raskind MA, Peskind ER, Lampe TH, Risse SC, Taborsky GJ, Dorsa D (1986) Cerebrospinal fluid vasopressin, oxytocin, somatostatin, and -endorphin in Alzheimer's disease. Arch Gen Psychiatry 43:382–388.
44. Atack JR, Beal MF, May C, Kaye JA, Mazurek MF, Kay AD, Rapoport SI (1988) Cerebrospinal fluid somatostatin and neuropeptide Y concentration in aging and in dementia of the Alzheimer type with and without extrapyramidal signs. Arch Neurol 45:269–274.
45. Banki CM, Karmacsi L, Bissette G, Nemeroff CB (1992b) CSF corticotropin-releasing hormone and somatostatin in major depression: response to antidepressant treatment and relapse. Eur Neuropsychopharmacol 2:107–113.
46. American Psychiatric Association (1987) Diagnostic and Statistical Manual of Mental Disorders, 3rd ed. rev., DSM-III-R. Washington, DC: American Psychiatric Association.
47. Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56–62.
48. Post RM, Gold P, Rubinow DR, Ballenger JC, Bunney WE, Goodwin FK (1982) Peptides in cerebrospinal fluid of neuropsychiatric patients: an approach to central nervous system peptide function. Life Sci 31:1–15.
49. Owens MJ, Nemeroff CB (1988) The neurobiology of CRF: implications for affective disorders. In Schatzberg AF, Nemeroff CB (eds): The Hypothalamic-Pituitary-Adrenal Axis: Physiology, Pathophysiology, and Psychiatric Implications. New York: Raven Press, pp 1–36.
50. Plotsky PM, Owens MJ, Nemeroff CB (1995) Neuropeptide alterations in mood disorders. In Bloom FE, Kupfer DJ (eds): Psychopharmacology: The Fourth Generation of Progress. New York: Raven Press, pp 971–981.
51. Nemeroff CB, Owens MJ, Bissette G, Andorn AC, Stanley M (1988) Reduced corticotropin-releasing factor binding sites in the frontal cortex of suicide victims. Arch Gen Psychiatry 45:577–579.
52. Charlton BG, Cheetham SC, Horton RW, Katona CLE, Crompton MR, Ferrier IN (1988b) Corticotropin-releasing factor immunoreactivity in post-mortem brains from depressed suicides. J Psychopharmacol 2:13–18.
53. Leake A, Perry EK, Perry RH, Fairbairn AF, Ferrier IN (1990) Cortical concentrations of corticotropin-releasing hormone and its receptor in Alzheimer type dementia and major depression. Biol Psychiatry 28:603–608.
54. Veith RC, Lewis N, Langohr JI, Murburg MM, Ashleigh EA, Castillo S, Peskind ER, Pascualy M, Bissette G, Nemeroff CB, Raskind MA (1993) Effect of desipramine on cerebrospinal fluid concentrations of corticotropin-releasing factor in human subjects. Psychiatry Res 46:1–8.
55. Pitts AF, Samuelson SD, Meller WH, Bissette G, Nemeroff CB, Kathol RG (1995) Cerebrospinal fluid corticotropin-releasing hormone, vasopressin, and oxytocin concentrations in treated patients with major depression and controls. Biol Psychiatry 38:330–335.
56. Reul JMHM, Stec I, Söder M, Holsboer F (1993) Chronic treatment of rats with the antidepressant amitriptyline attenuates the activity of the hypothalamus-pituitary-adrenocortical system. Endocrinology 133:312–320.
57. Reul JMHM, Labeur MS, Grigoriadis DE, De Souza EB, Holsboer F (1994) Hypothalamic-pituitary-adrenocortical axis changes in the rat after long-term treatment with the reversible monoamine oxidase-A inhibitor moclobemide. Neuroendocrinology 60:509–519.
58. Barden N, Reul JMHM, Holsboer F (1995) Do antidepressants stabilize mood through actions on the hypothalamic-pituitary-adrenocortical system? TINS 28:6–11.
59. Brady LS, Whitfield HJ Jr, Fox RJ, Gold PW, Herkenham M (1991) Long-term antidepressant administration alters corticotropin-releasing hormone, tyrosine hydroxylase, and mineralocorticoid receptor gene expression in the rat brain. J Clin Invest 87:831–837.
60. Brady LS, Gold PW, Herkenham M, Lynn AB, Whitfield HJ Jr (1992) The antidepressants fluoxetine, idazoxan and phenelzine alter corticotropin-releasing hormone and tyrosine hydroxylase mRNA levels in rat brain: therapeutic implications. Brain Res 572:117–125.

61. Sapolsky RM (1991) Do glucocorticoid concentrations rise with age in the rat? Neurobiol Aging 13:171–174.
62. Van Eekelen JAM, Rots NY, Sutanto W, de Kloet, ER (1991) The effect of aging on stress responsiveness and central corticosteroid receptors in the Brown Norway rat. Neurobiol Aging 13:159–170.
63. Lupien S, Lecours AR, Lussier I, Schwartz G, Nair NPV, Meaney MJ (1994) Basal cortisol levels and cognitive deficits in human aging. J Neurosci 14:2893–2903.
64. Cizza G, Calogero AE, Brady LS, Bagdy G, Bergamini E, Blackman MR, Chrousos GP, Gold PW (1994) Male Fischer 344/N rats show a progressive central impairment of the hypothalamic-pituitary-adrenal axis with advancing age. Endocrinology 134:1611–1620.
65. Heroux JA, Grigoriadis DE, De Souza EB (1991) Age-related decreases in corticotropin-releasing factor (CRF) receptors in rat brain and anterior pituitary gland. Brain Res 542:155–158.
66. Hauger RL, Thrivikraman KV, Plotsky PM (1994) Age-related alterations of hypothalamic-pituitary-adrenal axis function in male Fischer 344 rats. Endocrinology 134:1528–1536.
67. Tizabi Y, Aguilera G, Gilad GM (1992) Age-related reduction in pituitary corticotropin-releasing hormone receptors in two rat strains. Neurobiol Aging 13:227–230.
68. Raadsheer FC, Hoogendijk WJG, Stam FC, Tilders FJH, Swaab DF (1994) Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. Neuroendocrinology 60:436–444.
69. Levin N, Roberts IL (1991) Positive regulation of proopiomelanocortin gene expression in corticotropes and melanotropes. Front Neuroendocrinol 12:1–22.
70. Whitnall MH, Smyth D, Gainer H (1987) Vasopressin coexists in half of the corticotropin-releasing factor axons present in the external zone of the median eminence in normal rats. Neuroendocrinology 45:420–424.
71. De Goeij DCE, Kvetnansky R, Whitnall MH, Jezova D, Berkenbosch F, Tilders FJH (1991) Repeated stress-induced activation of corticotropin-releasing factor neurons enhances vasopressin stores and colocalization with corticotropin-releasing factor in the median eminence of rats. Neuroendocrinology 53:150–159.
72. Bartanusz V, Jezova D, Bertini LT, Tilders FJH, Aubry JM, Kiss JZ (1993) Stress-induced increase in vasopressin and corticotropin-releasing factor expression in hypophysiotrophic paraventricular neurons. Endocrinology 132:895–902.
73. Purba JS, Hoogendijk WJG, Hofman MA, Swaab DF (1996) Increased number of vasopressin and oxytocin expressing neurons in the paraventricular nucleus of the human hypothalamus in depression. Arch Gen Psychiatry 53:137–143.
74. Raadsheer FC, Sluiter AA, Ravid R, Tilders FJH, Swaab DF (1993) Localization of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the human hypothalamus; age-dependent colocalization with vasopressin. Brain Res 615:50–62.
75. Rolandi E, Franceschini R, Messina V, Cataldi A, Salvemini M, Barreca T (1987) Somatostatin in the elderly: diurnal plasma profile and secretory response to meal stimulation. Gerontology 33:296–301.
76. Sonntag WE, Xu X, Ingram RL, D'Costa A (1995) Moderate caloric restriction alters the subcellular distribution of somatostatin mRNA and increases growth hormone pulse amplitude in aged animals. Neuroendocrinology 61:601–608.
77. Cuttler L, Welsh JB, Szabo M (1986) The effect of age on somatostatin suppression of basal, growth hormone (GH)-releasing factor-stimulated, and dibutyryl adenosine 3 ,5 -monophosposphate-stimulated GH release from rat pituitary cells in monolayer culture. Endocrinology 119:152–158.
78. Kowalski C, Micheau J, Corder R, Gaillard R, Conte-Devolx B (1992) Age-related changes in corticoreleasing factor, somatostatin, neuropeptide Y, methionine enkephalin and -endorphin in specific rat brain areas. Brain Res 582:38–46.
79. Ferrara C, Cocchi D, Müller EE (1991) Somatostatin in the hippocampus mediates dexamethasone-induced suppression of corticosterone secretion in the rat. Neuroendocrinology 53:428–431.
80. Wolkowitz OM, Rubinow DR, Breier A, Doran AR, Davis C, Pickar D (1987) Prednisone decreases CSF somatostatin in healthy humans: implications for neuropsychiatric illness. Life Sci 41:1929–1933.
81. Kling MA, Rubinow DR, Doran AR, Roy A, Davis CL, Calabrese JR, Nieman LK, Post RM, Chrousos GP, Gold PW (1993) Cerebrospinal fluid immunoreactive somatostatin concentrations in patients with Cushing's disease and major depression: relationship to indices of corticotropin-releasing hormone and cortisol secretion. Neuroendocrinology 57:79–88.