Corticotropin-Releasing Hormone mRNA Levels in the Paraventricular Nucleus of Patients With Alzheimer's Disease and Depression

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Objective: *Greater activity of the hypothalamic-pituitary-adrenal (HPA) axis is associated with specific neurological and psychiatric disorders, including Alzheimer's disease and depression. Hyperactivation of paraventricular corticotropin-releasing hormone (CRH) neurons may form the basis of this increased activity of the HPA axis.* *Method:* *Activation of the CRH neurons was determined through measurement of the amount of CRH-mRNA in the paraventricular nucleus by using quantitative, in situ hybridization histochemistry with systematically sampled frontal sections through the hypothalamus of routinely formalin-fixed and paraffin-embedded autopsy brain material of 10 comparison subjects, 10 patients with Alzheimer's disease, and seven depressed patients.* *Results:* *CRH-mRNA levels in the paraventricular nucleus of Alzheimer's patients were markedly higher than those of comparison subjects, whereas CRH-mRNA levels in the paraventricular nucleus of depressed patients were even higher than the levels of Alzheimer's patients.* *Conclusions:* *Paraventricular CRH neurons in Alzheimer's disease and depression are hyperactivated, and this hyperactivation may contribute to the etiology of these disorders.*

(Am J Psychiatry 1995; 152:1372–1376)

Various neurological and psychopathological conditions, e.g., depression, Alzheimer's disease, anorexia nervosa, and chronic active alcoholism, are accompanied by hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, while other psychopathologies are associated with less activity of this stress system (1, 2). For patients with Alzheimer's disease and depressed patients, plasma cortisol concentrations and daily cortisol excretion in urine are higher than normal (3–5). With these patients, provocation and suppression tests revealed marked changes in the activity of various components of the HPA axis. In general, the adrenal glands of these patients show hypersecretory responses to ACTH challenges compared to those of control subjects, whereas corticotropin-releasing hormone (CRH) challenge tests show suppressed ACTH responses but normal cortisol responses (6–8). In addition, the HPA system of patients suffering from Alzheimer's disease or depression is considerably less sensitive to the feedback actions of the synthetic glucocorticoid dexamethasone (9–11). It is generally hypothesized that the greater activity of CRH neurons in the hypothalamus drives the functional changes in these disorders (1, 2, 12). In addition, CRH has other behavioral and physiological effects (13, 14).

In rats, chronic or repeated activation of the HPA axis caused an increase in CRH-mRNA; in the number of CRH-expressing neurons in the paraventricular nucleus; and in the fraction of CRH neurons that coproduce, costore, and cosecrete vasopressin (15–19). Since similar changes were found after adrenalectomy, we recently hypothesized that these measures can be used as indices of the ante-mortem history of the activity state of these CRH neurons (20). In human autopsy material, recent immunocytochemical and morpho-

Received June 10, 1994; revisions received Oct. 24, 1994, and Feb. 10, 1995; accepted March 23, 1995. From the Graduate School Neurosciences Amsterdam, Netherlands Institute for Brain Research, and the Research Institute Neurosciences Vrije Universiteit, Medical Faculty, Department of Pharmacology, Amsterdam. Address reprint requests to Dr. Swaab, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, the Netherlands.

Supported by the Graduate School Neurosciences Amsterdam (project 89-23), the Netherlands Organization for Scientific Research (Department of Medical Sciences grant 900-552-083), and Mrs. E.J.M. Stevens.

Oligonucleotide was provided by Dr. C. Bonnefond of Sandoz Research Laboratories, Basel, Switzerland.

The authors thank Mr. B. Fisser for technical assistance; Mr. H. Stoffels for preparing the figure; Dr. M.A. Hofman for advice on the statistics; Drs. C.W. Pool and S.E.F. Guldenaar for technical suggestions; Mrs. W.T.P. Verweij for correcting the English; and the Netherlands Brain Bank (Dr. R. Ravid, coordinator) and Drs. J.L.G. Blauwgeers, E.J. Colon, P. Kölling, M. Kross, M.E.I. Schipper, E.N.H. Jansen Steur, and R.A.I de Vos for the brain material.

metrical studies revealed an age-dependent increase in the number of CRH-expressing neurons and in the fraction of these neurons that coexpress vasopressin (21–23). This finding is in agreement with results from functional neuroendocrine studies that demonstrated hyperactivation of the HPA axis during aging (24, 25). Compared to age-matched subjects, depressed patients showed a fourfold greater total number of CRH-expressing neurons and a threefold greater number of vasopressin-producing CRH neurons (26). These results were interpreted as evidence of hyperactivation of hypothalamic CRH neurons in depression (26). A surprising finding was that for patients with Alzheimer's disease, the number of CRH-expressing and vasopressin-coexpressing neurons did not differ from those of age-matched comparison subjects (22, 23). These immunocytochemical measures thus did not support a chronic hyperactivity of CRH neurons in Alzheimer's disease, as postulated by others (27, 28). In today's neuroscience, most information on activity changes in peptidergic neurons is derived from studies on mRNA of the neuropeptide precursor. In view of the previously mentioned discrepancies, in the present study we determined the amount of CRH-mRNA in the paraventricular nucleus by using quantitative, in situ hybridization histochemistry.

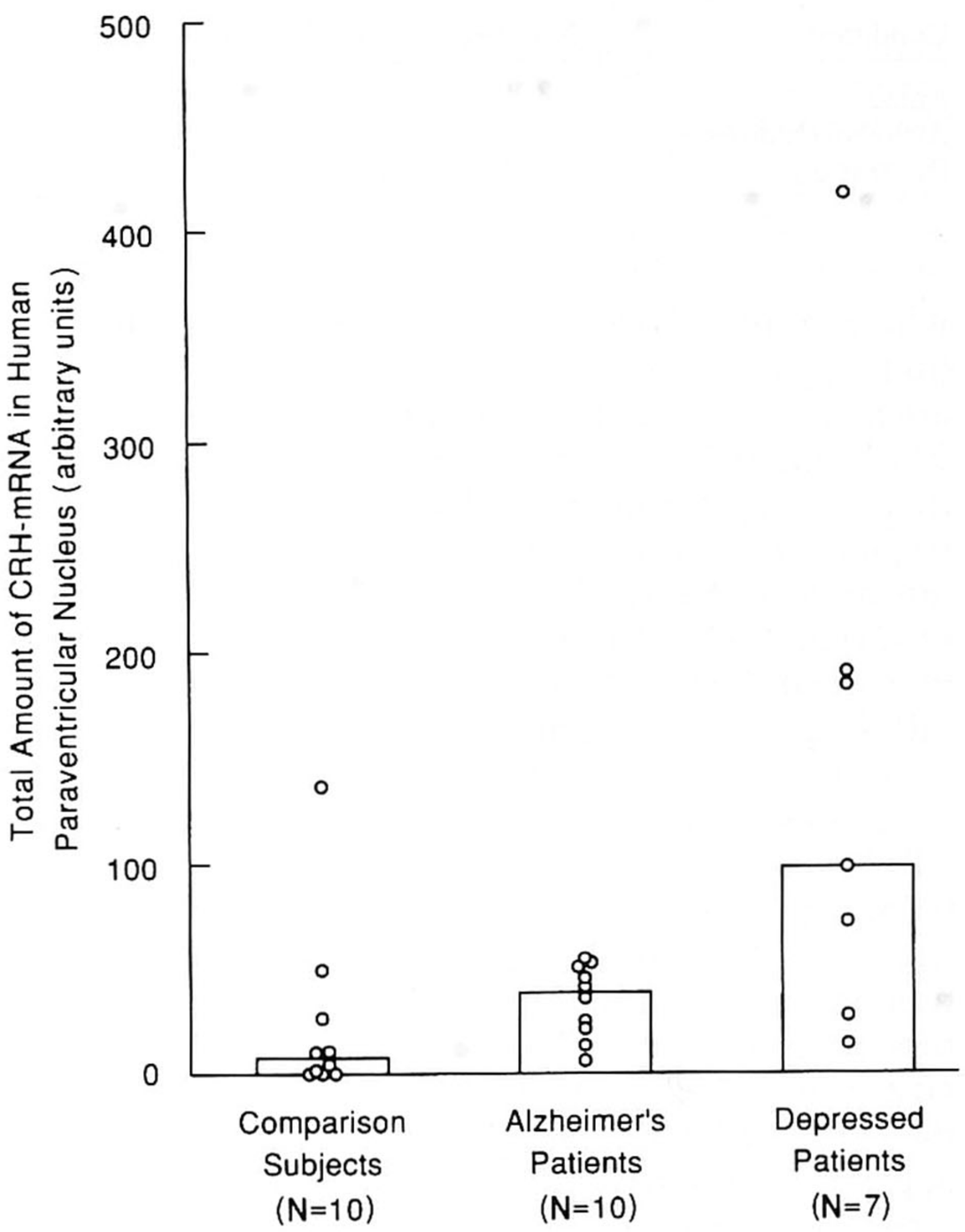
METHOD

Hypothalami from 10 comparison subjects (36 to 91 years of age; mean=65.7), 10 Alzheimer's patients (with a similar mean age), and seven depressed patients were obtained at autopsy according to the dissection protocol of the Netherlands Brain Bank. These samples were identical to those reported in a previous study (26). Comparison subjects had not suffered from a primary neurological or psychiatric disease. Alzheimer's disease was neuropathologically confirmed on the basis of number and distribution of senile plaques and neurofibrillary tangles (29). The clinical diagnosis of depression was based on DSM-III-R criteria and obtained from medical records.

A modification of the method of Lucassen et al. (30) was used for the in situ hybridization histochemistry. In short, 6-µm sections were taken at a distance of 300 µm throughout the total paraventricular nucleus and mounted on amino-alkyl-silane coated object slides. We used an oligonucleotide that was complementary to the nucleotide sequences 555-582 of the human CRH-mRNA sequence (31) and highly specific. The oligonucleotide was labeled with ^{35}S-ATP, purified on a Nensorb-20 column, and yielded 4.5 molecules of ATP per molecule of CRH-probe. Postfixation and HCl incubation were absent during the pretreatment of the tissue sections, and a 30-minute (10-µg/ml) proteinase-K incubation was used. Hybridization conditions were the same as those described by Lucassen et al. (30), except for the deionized formamide and final probe concentration (40% and 4,000 counts per minute/µl, respectively). The β-max X-ray film was exposed at room temperature for 20 days. The radioactive CRH-probe standards ranged from 62.5 to 4,000 counts per minute/µl. For the quantitative analysis of the hybridization signals on the β-max films by an image analysis system, we used a 40-mm focus ring on the camera. For each subject, the total hybridization signal was estimated as a measure for the total amount of CRH-mRNA, per left paraventricular nucleus (except for five patients for whom only the right paraventricular nucleus was present). The total hybridization signal was achieved by integrating the weighted mean densities corrected for background, multiplied by the labeled area of the paraventricular nucleus per section and by the number of sections in which the hybridization signal was present (arbitrary units). The substitution of the right paraventricular nucleus for the absent left paraventricular nucleus was valid, since no left-right differences in amount of CRH-mRNA were present for patients with both paraventricular nucleus sides intact (N=19) (Mann-Whitney U=175.0, Wilcoxon rank sum W=376.0, z=–0.16, p=0.88).

Data analysis required nonparametric statistics, since the data were discrete and unlikely to be normally distributed. Differences in total amounts of CRH-mRNA between patient groups were analyzed through use of the Mann-Whitney U-Wilcoxon rank sum W test by using SPSS-X followed by a two-tailed probability of z. Relations between the amount of CRH message and factors such as age, brain weight, post-mortem delay, fixation, and storage time of the tissue were determined by using Spearman's rho based on a two-tailed test (SPSS-X analyses). The results were considered statistically significant when $p \leq 0.05$.

FIGURE 1. Total Hybridization Signal for Human CRH-mRNA (Arbitrary Units) in the Paraventricular Nucleus for Comparison Subjects and Alzheimer's and Depressed Patients[a]



[a]Bars indicate median values. There was a significant difference between patients with Alzheimer's disease and comparison subjects (Mann-Whitney U=23.0, Wilcoxon rank sum W=0.78, z=–2.0, p=0.04). The amount of radioactivity in depressed patients was significantly higher than that in comparison subjects (Mann-Whitney U=7.0, Wilcoxon rank sum W=91.0, z=–2.7, p=0.006) and patients with Alzheimer's disease (Mann-Whitney U=15.0, Wilcoxon rank sum W=83.0, z=–2.0, p=0.05).

RESULTS

In the hypothalami of the comparison subjects, Alzheimer's patients, and depressed patients, CRH message was found only in the paraventricular nucleus,

TABLE 1. Activation Strategies of CRH Neurons in Aging, Alzheimer's Disease, and Depression

Condition	Activity			
	Number of CRH Neurons	Vasopressin Colocalization	CRH-mRNA in Paraventricular Nucleus	CRH-mRNA per Neuron
Aging	Increased	Increased	Unchanged	Unchanged
Alzheimer's disease	Unchanged	Unchanged	Increased	Increased
Depression	Greatly increased	Greatly increased	Greatly increased	Unchanged

which is in accordance with immunocytochemical findings demonstrating that CRH-expressing neurons are present only in the paraventricular nucleus (21–23, 26). Quantification of the autoradiograms showed that the total amounts of CRH-mRNA in the paraventricular nucleus of the three subject groups were significantly different (Kruskal-Wallis one-way analysis of variance: $H=10.1$, $df=2$, $0.01<p<0.001$). For Alzheimer's patients (N=10) the total amounts of CRH-mRNA were significantly higher than for comparison subjects (N=10) ($p=0.04$). The paraventricular nucleus of depressed patients (N=7) contained even more CRH-mRNA (depressed versus Alzheimer's patients: $p=0.05$, depressed versus comparison subjects: $p=0.006$) (figure 1). None of the three groups showed a correlation between the amount of CRH message and the factors of age ($0.06<p<0.29$), brain weight ($0.20<p<0.33$), post-mortem delay ($0.38<p<0.43$), or fixation time of the tissue ($0.20<p<0.38$), and there was no difference in gender ($0.08<p<0.47$). For the comparison subjects, a negative correlation was found between the storage time of the paraffin-embedded tissue and the total hybridization signal ($r_s=-0.77$, $p=0.005$). However, for the groups of Alzheimer's and depressed patients, this correlation was absent ($r_s=-0.04$, $p=0.46$, and $r_s=0.18$, $p=0.35$, respectively). After correction (32) for the bivariate relationship between the storage time of the tissue and the amount of CRH-mRNA in the comparison group ($r=-0.72$, $p=0.02$), the differences between the groups remained significant (Alzheimer's and depressed patients versus comparison group: $p=0.004$ and $p=0.03$, respectively).

DISCUSSION

The present results lead us to conclude that paraventricular CRH neurons are hyperactivated in Alzheimer's disease and depression. Hyperactivation of these CRH neurons might contribute to the etiology of these disorders, since hyperactivation of the HPA axis, resulting from hyperactive CRH neurons in the paraventricular nucleus, has been shown to correlate with the severity of the hippocampal atrophy and cognitive impairment in Alzheimer's patients (27, 33). In addition to these presumably cortisol-mediated effects (1, 2), direct effects of CRH on brain structures may also play a role in certain symptoms of these diseases (12–14). This view is based on observations showing that when CRH is injected directly into the brain of rats, it induces various behavioral effects including decreased food intake, decreased sexual activity, disturbed sleep and motor behavior, impaired learning, and increased anxiety (34–39).

The greater activity of the paraventricular CRH neurons in depressed patients is unlikely to be the result of the chronic administration of antidepressants, since these kinds of drugs have been found to attenuate rather than to enhance activity of CRH neurons. In rats, stress-evoked stimulation of CRH neurons is reduced by tianeptine (40), and chronic treatment with amitriptyline attenuates the activity of the HPA system (41). Long-term treatment of rats with imipramine, fluoxetine, idazoxan, and phenelzine has been shown to decrease CRH-mRNA levels in the paraventricular nucleus (42, 43). In healthy volunteers, the antidepressant desipramine has been shown to reduce CRH concentrations in CSF (44). Interference of antidepressants with our measurements would thus lead to an underestimation of the difference that we observed between comparison and depressed subjects in the state of activity of CRH-expressing neurons in the paraventricular nucleus.

By comparing the CRH-mRNA data (figure 1) with the number of CRH-expressing neurons in the paraventricular nucleus of the same subjects (21, 26), we found that the amount of CRH-mRNA per CRH neuron (i.e., the quotient of these measures) was greater for Alzheimer's disease patients ($p=0.02$) than for comparison subjects but remained unchanged in depression ($p=0.20$) and during aging ($p=0.36$). These data on CRH-mRNA and numbers of CRH neurons, together with our earlier observations on the numbers of CRH neurons coexpressing vasopressin in the same patients (23), lead us to postulate that CRH neurons show physiology-specific or pathology-specific changes (table 1). In Alzheimer's disease, unaltered numbers of CRH neurons are stimulated to produce more mRNA of CRH and may, therefore, show greater CRH turnover, whereas in depression, more neurons are recruited to produce CRH and vasopressin. Increased vasopressin production in CRH neurons increases the power of the HPA system, since vasopressin strongly potentiates the ACTH-releasing activity of CRH (45). Although discordant results have been reported, the shift in the composition of the signal produced by CRH neurons toward a more vasopressin-dominated production (20) is in accordance with a lower sensitivity of depressed patients to dexamethasone, since vasopressin-induced ACTH secretion is considerably less sensitive to the in-

hibitory effect of glucocorticoids than CRH-induced ACTH secretion (46).

In the present study we show that the combination of results obtained by in situ hybridization histochemistry and immunocytochemistry in post-mortem human brain material creates fascinating new possibilities for the study of those mechanisms that affect CRH neurons during pathological disorders. This generates new opportunities to study functional changes in central peptidergic systems in neuropathological, psychopathological, and other disorders.

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