Increased Cortisol Levels in Aging and Alzheimer's Disease in Postmortem Cerebrospinal Fluid

D. F. Swaab*, F. C. Raadsheer*,§, E. Endert†, M. A. Hofman*, W. Kamphorst‡ and R. Ravid*,¶

Graduate School Neurosciences, Amsterdam

*Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

†Laboratory for Endocrinology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

‡Department of Pathology, Free University Hospital, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

§Research Institute Free University, Medical Faculty, Department of Pharmacology, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

¶Netherlands Brain Bank, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

Key words: cerebrospinal fluid cortisol, Alzheimer's disease, aging, hypothalamo-pituitary-adrenal axis, cortisol neurotoxicity.

Abstract

The hypothalamo-pituitary-adrenal (HPA) axis is activated during aging and even more so in dementia. Increased levels of corticosteroids may be neurotoxic. Therefore we have investigated cortisol levels in cerebrospinal fluid (CSF) of Alzheimer patients and controls. Ventricular postmortem CSF was collected from clinically and neuropathologically well-defined Alzheimer patients ($n=26$) and control subjects ($n=21$). In the group of Alzheimer patients the mean CSF total cortisol level was 83% higher than that in the controls. In presenile Alzheimer patients (<65 years of age; $n=13$) the CSF-cortisol level was 5 times higher than that of presenile controls ($n=7$). In contrast, senile Alzheimer patients ($n=13$) and controls of over 65 years of age ($n=14$) did not show a significant difference in CSF-cortisol levels. The presence or absence of a difference in the cortisol-CSF levels in, respectively, presenile or senile Alzheimer patients as compared to controls was due to the 3.5-fold rise of CSF-cortisol in control subjects over 65 years of age as compared with controls under 65 years of age. The CSF-cortisol levels in presenile and senile Alzheimer patients were similar. No significant correlation was observed in the Alzheimer patients between age of onset of the dementia and CSF cortisol levels or duration of Alzheimer's disease and CSF cortisol levels.

The finding that in senile Alzheimer patients cortisol levels were similar to those of unaffected age-matched controls does not seem to support the cortisol neurotoxicity hypothesis. On the other hand, it should be noted that postmortem ventricular CSF cortisol levels were found to be 13–16 times higher than lumbar puncture CSF cortisol levels of ambulatory patients. This means that the ventricular CSF levels probably reflect the reaction of the HPA-axis to the process of dying rather than the basal levels of this system. The exact consequences of elevated HPA-axis activity for the human brain should be studied in more detail.

Various studies indicate that the hypothalamo-pituitary-adrenal (HPA) axis is activated during aging 1–6 and even more so in dementia (1, 7–9). Although activation of the HPA-axis is not always reflected in increased basal plasma cortisol levels (10), it is apparent in approximately 50% of the patients with dementia from the non-suppression of plasma cortisol following dexamethasone administration (1, 9, 11–13). Interestingly, the degree of hyperactivity of the HPA-axis in dementia was found to be correlated with the severity of cognitive impairment and hippocampal atrophy (9, 14). Animal experiments suggested that these correlations might even be meaningful since, on the one hand, hippocampal lesions lead to hyperactivity of corticotropin-releasing hormone (CRH) neurons (15) and, on the other hand, increased levels of corticosteroids may be neurotoxic and contribute to irreversible hippocampal damage (16, 17).

The present study was meant to investigate whether the hyperac-

tivity of the HPA-axis in aging and Alzheimer's disease was reflected in ventricular postmortem CSF-cortisol levels of neuropathologically confirmed Alzheimer patients and controls.

Evidence is presented for a hyperexcitability of the HPA-axis in Alzheimer patients, especially in presenile Alzheimer patients, and for an increasing excitability of the HPA-axis in the course of aging in controls.

Results

Alzheimer patients

The difference in ventricular CSF-cortisol levels between all Alzheimer patients ($n=26$) and controls ($n=21$), being $0.55 \mu\text{M}/1 \pm 0.07$ (SEM) and $0.29 \mu\text{M}/1 \pm 0.06$, respectively, was

statistically highly significant ($P=0.002$) (Fig. 1). A number of the studied variables appeared to influence these data.

Age, age at onset and duration of Alzheimer's disease

In controls a significant increase in ventricular CSF-cortisol was found with age (Spearman's $\rho=0.74$, $P<0.001$). Controls under 65 years of age ($n=7$) had levels of $0.11\pm0.04\ \mu\text{M/l}$ (Fig. 1). This level increased 3.5-fold in controls over 65 years of age ($n=14$) that had $0.38\pm0.08\ \mu\text{M/l}$ cortisol in CSF (Fig. 1), and was significantly different from that of young subjects ($P=0.005$).

Presenile Alzheimer patients (<65 years of age, $n=13$) had increased CSF-cortisol levels ($0.54\pm0.08\ \mu\text{M/l}$) which were 5 times higher than in young controls ($P=0.001$). The cortisol levels of presenile Alzheimer patients did not differ from those of senile Alzheimer patients ($0.56\pm0.12\ \mu\text{M/l}$) ($P=0.82$). The difference between senile Alzheimer patients ($n=13$) and old controls ($n=14$) was not significant ($P=0.25$).

No significant correlation was found between age at onset of the dementia and CSF cortisol levels in Alzheimer patients (presenile: $r=-0.259$, $P>0.75$; senile: $r=0.079$, $P>0.9$; presenile and senile: $r=-0.051$, $P>0.9$). In addition, no correlation was observed between duration of Alzheimer's disease and CSF cortisol (presenile: $r=-0.092$, $P>0.9$; senile: $r=0.200$, $P>0.75$; presenile and senile: $r=0.098$, $P>0.9$). The duration of

Alzheimer's disease was similar in presenile (8.0 ± 1.04 years) and senile (8.9 ± 1.17 years) patients (Table 2).

Sex, postmortem interval, brain weight, CSF-pH, season

Other variables did not affect the outcome. Cortisol levels in males were not significantly different from those in females ($P=0.20$). No relationship was found between ventricular CSF-cortisol levels in controls or Alzheimer patients on the one hand, and brain weight ($P=0.37$ and $P=0.25$ respectively) or post mortem interval ($P=0.51$ and $P=0.20$ respectively) on the other hand. There was a trend towards a negative correlation of CSF-cortisol and CSF-pH only for controls ($P=0.08$) but not for Alzheimer patients ($P=0.39$). When the group of controls was divided in a young (<65 years of age) and an old part, the trend disappeared ($P=0.72$ and $P=0.40$ respectively), indicating that the trend was not based on a causal relationship but that the old controls, who had higher ventricular CSF-cortisol levels than the young ones, died after a longer terminal phase.

There is, consequently, no reason to presume that the longer postmortem interval of the controls (6 h 56 min $\pm$ 47 min vs 3 h 53 min $\pm$ 11 min in Alzheimer patients) influenced our conclusions. The same goes for the larger brain weight in the controls as compared to the Alzheimer patients (1313 $\pm$ 26 g vs 1128 $\pm$ 36 g).

FIG. 1. Concentration of total cortisol in the ventricular cerebrospinal fluid (CSF) in $\mu\text{mol/l}$. Bars represent the mean level, the vertical lines denote the SEM, and n is the number of samples per group. CONTR. = control subjects, AD = Alzheimer patients. A. Comparison of all controls with all AD patients. B. Comparison of controls with AD patients, and young with old subjects after subdivision of the groups into subjects younger and older than 65 years of age. Note the increased CSF-cortisol levels in AD patients (A), the 5-fold increased CSF-levels in presenile AD patients (B), the increase in CSF-cortisol levels during aging in controls (B) and the lack of significance between senile controls and AD patients (B).

Lumbar puncture—CSF cortisol

A pilot study revealed cortisol levels that were 13–16 times lower in lumbar puncture (LP) CSF of ambulatory patients (mean levels of 0.018 ± 0.0014 and $0.042 \pm 0.014 \mu\text{M/l}$ respectively, for controls and Alzheimer patients) than in postmortem ventricular CSF. Although possible and probable Alzheimer patients had a mean lumbar CSF-cortisol level that was 2.3 times higher than in controls, this difference was not significant ($P=0.3$). The high cortisol levels observed in some of the demented patients (i.e. 0.080, 0.108 and $0.214 \mu\text{M/l}$), but not in controls, certainly warrant a further study of LP-CSF. Such a study has, however, to include a follow-up of such patients in order to obtain neuropathological confirmation of the diagnosis.

Discussion

The most remarkable finding of the present study is the clear hypercortisolism as measured in ventricular CSF of presenile Alzheimer patients. Cortisol levels were 5-fold higher in presenile Alzheimer patients than in age-matched controls. In senile Alzheimer patients no significant difference in CSF-cortisol levels was found as compared to controls, due to the increased CSF-cortisol levels in controls during aging. The activation of the HPA-axis in Alzheimer's disease, as judged by ventricular CSF-cortisol, agrees with measurements in plasma and urine (7–9, 18). The absence of a difference in ventricular CSF-cortisol levels between senile Alzheimer patients and old controls tallies with the similar plasma levels found by Goudsmit *et al.* (19) and Dodt *et al.* (1) in these 2 groups of subjects.

It has been proposed that the activation of the HPA-axis might contribute to the pathogenesis of the Alzheimer process (16, 17). These authors proposed that hypercortisolism could lower the threshold to whatever is the primary neurotoxic lesion in AD, e.g., in the hippocampus. Such lesions would, in turn, impair the inhibiting influence of the hippocampus on the HPA-axis, thus initiating a vicious circle. The finding that in senile Alzheimer patients cortisol levels were similar to those of age-matched controls who did not reveal the neuropathological Alzheimer changes in the brain was surprising and does not seem to support the cortisol neurotoxicity hypothesis. Moreover, if cortisol neurotoxicity would play a central role in the pathogenesis of Alzheimer's disease, it is difficult to explain at present why some presenile and senile Alzheimer patients, who are also clearly affected neuropathologically, did not have increased ventricular CSF-cortisol levels (Table 2). On the other hand it should be noted that postmortem ventricular CSF cortisol levels were found to be 13–16 times higher than lumbar puncture CSF cortisol levels of ambulatory patients. This means that the ventricular CSF levels probably reflect the response of the HPA-axis to the process of dying rather than the basal levels of this system. In that sense, ventricular CSF cortisol levels may be compared to the dexamethason suppression test. There are, however, also other observations that, at present, do not seem to fit into the cortisol neurotoxicity hypothesis. It is, e.g. difficult to explain why other chronic conditions that are said to lead at least to similar degrees of hypercortisolism, e.g. MID and depression (7, 22–24), do not lead to hippocampal damage or Alzheimer changes in the brain. One might, of course, put forward that more knowledge is

required about daily fluctuations of CSF-cortisol levels and about corticosteroid receptors in the possibly affected tissues in the human brain, such as the hippocampus (25, 26), in relation to the possible neurotoxic effect of increased cortisol levels in the human brain. On the other hand, in Cushing's syndrome, that is characterized by chronically elevated cortisol levels, reduced hippocampal formation volume and memory dysfunction (27) no Alzheimer changes or neuropathological hippocampal damage were reported (28). Moreover, in depressed patients with an activated HPA-axis (22–24) whose CRH neurons were found to be much more strongly activated than those found in patients with Alzheimer's disease, we found neither hippocampal cell loss nor abnormal numbers and/or distribution patterns of senile plaques, neurofibrillary tangles, dystrophic neurites, or Alz-50 immunoreactivity, although the oldest patient in the group we investigated was 80 years of age (29). In addition, we found a similar degree of activation of CRH neurons, but no neuropathological signs of Alzheimer's disease or hippocampal damage, in Multiple Sclerosis (MS) patients (30). The HPA-axis of MS patients also has an increased activity (31). The lack of Alzheimer changes or hippocampal damage in these 4 clinical conditions that are characterized by a chronically hyperactivated HPA-axis does not seem to support the glucocorticoid neurotoxicity hypothesis as developed in animal experiments. More neuroendocrine studies in combination with systematic neuropathological investigation are necessary in human disorders with HPA-axis hyperactivity in order to establish the exact consequences of chronically enhanced corticosteroid levels on the human brain.

The increase of ventricular CSF-cortisol levels with aging in controls supports earlier reports suggesting an activation of the HPA-axis (1, 5, 32). Also, the observation that during the process of aging an increasing number of CRH neurons in the paraventricular nucleus starts to express CRH (6) and to co-express vasopressin (3, 4), indicates a step-by-step activation of the HPA-axis in aging. The activation of the HPA-axis in controls during aging is controversial, however, since other studies indicated no change (cf. 10), or even a decline of the activity of this axis with age (33). These discrepancies might be partly due to the CSF-cortisol measurements being more likely to detect HPA-axis activity changes than the basal plasma cortisol levels in which the activation of the HPA-axis was often not reflected (10), possibly because the amount of corticosteroids varies less in CSF than in blood (34). Both 24-h urinary cortisol determinations (7) and ventricular CSF-cortisol levels (the present study) appear to be more valuable parameters for the measurement of HPA-axis hyperactivity than basal plasma levels. Moreover, changes in the responsiveness of the pituitary and adrenal in the course of aging (2, 24, 25) may also contribute to the ultimate cortisol levels.

In the present study total cortisol levels in postmortem ventricular CSF were found to be similar to the levels in plasma in ambulatory patients (19). This was rather unexpected, since previous studies reported that total cortisol levels in CSF are less than 10% of those in plasma (34, 35, 36). The CSF-cortisol levels reported in literature are thus close to free plasma cortisol levels (34, 36–38). In accordance with the literature the pilot study yielded some 13–16 times lower levels in lumbar puncture CSF of ambulant patients than the postmortem ventricular CSF-cortisol levels. Since, moreover, in living patients, ventricular CSF cortisol levels are not different from lumbar levels (34), our

high ventricular CSF-cortisol levels will be due to the fact that our CSF samples were obtained postmortem and the differences obtained in the present study indicate thus probable differences in the reaction of the HPA-axis to the process of dying. Whether the relatively high ventricular CSF-cortisol levels go together with higher cortisol plasma levels and lumbar puncture liquor cortisol around the moment of death remains to be studied. A theoretical explanation for the high ventricular cortisol-CSF levels was that, during the postmortem period, the blood-brain barrier might be damaged, resulting in a surge of transcortin, the carrier protein of cortisol (39), from the blood towards the CSF. This surge would then be larger in presenile Alzheimer patients than in controls. Transcortin measurements (CBG-RIA-100, Medgenix Diagnostics S.A. Fleurus, Belgium) in postmortem CSF of presenile Alzheimer patients and age-matched controls showed, however, that this is not the case. Rather the opposite was found. The transcortin levels in ventricular CSF of young controls were 0.44 ± 0.14 mg/l and in young Alzheimer patients 0.19 ± 0.10 mg/l and there was no significant difference between these two groups ($P=0.1$) (0.33 mg/l).

A number of additional factors that might theoretically also interfere with the results of our study, i.e. increased cortisol-CSF levels in aging and Alzheimer's disease, have been checked. Postmortem interval, sex differences and agonal state, as measured by pH (40, 41, Table 1, 2), appeared not to interfere with the outcome. In the course of aging and in Alzheimer's disease the magnitude of diurnal plasma fluctuations diminishes (32, 42). These changes are probably due to a degeneration of the hypothalamic clock, the suprachiasmatic nucleus, in these conditions (43, 44). We did not study diurnal changes in ventricular CSF-cortisol (cf. 34), since the data in the control group were not evenly

distributed over day and night. However, when the data were matched for clock hours of death, the increased cortisol levels we observed in aging and Alzheimer's remained unchanged. Therefore, diurnal changes have not confounded our results. We could not check for the possible effects of changes in CSF production rate or CSF-cortisol turnover in relation to the increase of CSF-cortisol levels (45). However, since the ventricles are enlarged in aging and Alzheimer's disease, the magnitude of the observed increased levels of cortisol during these conditions might have been larger rather than smaller. Concluding, we feel quite confident that the increased levels of CSF-cortisol in aging and Alzheimer's disease show a real neurobiological phenomenon reflecting the hyperactivity of the HPA-axis in those two conditions around the moment of death.

The pilot study indicated that, in a number of living patients as well, high cortisol levels may be found in lumbar puncture-CSF. The fact that this pilot study did not show statistical significance between the lumbar puncture-CSF-cortisol levels of Alzheimer patients and controls may be influenced by the fact that a number of the 'possible' and 'probable' Alzheimer cases will ultimately appear not to be Alzheimer patients after all (46). The fact that lumbar puncture-CSF-cortisol levels in the Alzheimer group had a mean that was twice as high as in controls, as well as the three very high cortisol levels that were not observed in the controls, warrants a further study of cortisol levels in lumbar puncture-CSF cortisol levels in Alzheimer patients who, eventually, become available for autopsy for confirmation of the diagnosis. Although CSF-cortisol levels may be a good research tool for postmortem estimation of the responsiveness of the HPA-axis, it is unlikely that increased CSF-cortisol levels might help in the diagnosis of Alzheimer's disease, since hypercortisolism

TABLE 1. Clinical and Pathological Data of Control Subjects Studied.

Case	Patient number	Sex	Age (y)	Pmd (h)	pH	BrW (g)	Time of death	Cort μ M/l	Diagnosis, cause of death
1	90-90.1	M	30	4 h 55	6.50	1325	18.00 h	0.05	Cardiac failure
2	89-228.5	M	51	5 h 15	6.67	1540	06.45 h	0.07	Myocardial infarction
3	90-160	M	57	4 h 25	8.05	1345	11.30 h	0.07	Myocardial infarction
4	91-186.2	M	59	7 h 00	8.77	1355	10.00 h	0.06	Acute cardiac arrest
5	90-234.3	M	60	3 h 55	7.23	1409	23.15 h	0.35	Myocardial infarction
6	90-48	M	61	12 h 05	6.77	1460	03.00 h	0.10	ND
7	91-230	F	61	5 h 40	6.35	1380	07.20 h	0.10	ND
8	90-60	M	68	7 h 17	6.35	1366	15.30 h	0.29	Myocardial infarction
9	90-204.5	M	69	4 h 55	7.45	1240	16.15 h	0.19	Myocardial infarction
10	89-270.6	M	69	7 h 00	6.57	1340	06.00 h	0.08	Dislocation C5-C6
11	90-134.0	F	70	7 h 10	6.32	1160	09.30 h	0.22	Cardiac failure, hypovolemic shock
12	90-47	M	71	19 h 35	6.77	1454	16.30 h	1.11	Metastatic urothelium carcinoma, pneumonia, sepsis
13	90-202	M	73	4 h 25	6.51	1330	11.30 h	0.13	Cardiac failure, lung emboli
14	91-120	M	74	6 h 15	6.22	1310	06.15 h	0.17	Postoperative myocardial infarction
15	90-98	M	74	3 h 55	6.39	1283	12.00 h	0.36	Myocardial infarction
16	90-90	M	76	6 h 00	6.27	1145	07.45 h	0.25	Bronchus carcinoma
17	89-294	F	80	8 h 00	6.07	1190	04.30 h	0.54	Mamma carcinoma
18	90-81	F	83	8 h 00	6.37	1195	10.30 h	0.29	Cardiac failure
19	90-142	F	84	4 h 25	6.23	1408	12.50 h	0.64	Pancreatic carcinoma, liver metastases, lung emphysema, diffuse cardiac ischaemia
20	90-65	M	88	11 h 00	5.98	1300	03.00 h	0.70	Bronchus carcinoma
21	90-206	F	90	4 h 30	6.70	1040	10.30 h	0.31	Lung emboli

Abbreviations used: Y: years; Pmd: postmortem delay; h: hours; BrW: brain weight; g: grams; Cort: cerebrospinal fluid-cortisol; μ M/l: μ mol per liter; F: female; M: male; ND: not determined.

TABLE 2. Clinical and Pathological Data of Alzheimer Patients Studied.

Case	Patient number	Sex	Age (y)	Age at onset (y)	Duration of disorder (y)	Pmd (h)	pH	BrW (g)	Time of death	Cort $\mu\text{M/l}$	Diagnosis, cause of death
22	89-166	M	40	35	5	2 h 50	6.46	1370	04.30 h	0.93	AD, cachexia
23	90-45	M	48	40	8	5 h 40	6.07	1435	18.00 h	0.47	AD, uremia, pneumonia
24	90-262	M	49	43	6	4 h 25	6.17	1426	09.10 h	0.48	AD
25	91-92	F	54	49	5	3 h 15	6.32	1055	02.45 h	0.85	AD, cachexia
26	91-214	M	58	51	7	4 h 45	6.67	1265	11.15 h	0.35	AD, cachexia, pulmonic insufficiency
27	89-128	F	58	53	5	3 h 05	6.11	1050	09.10 h	0.38	AD, cachexia
28	89-154	F	59	54	5	5 h 05	6.59	780	06.00 h	0.08	AD, cardiac failure
29	91-271	M	61	44	17	4 h 15	nd	846	10.15 h	0.44	AD, dehydration
30	90-41	M	63	49	14	5 h 00	6.32	1350	19.30 h	0.69	AD, pneumonia
31	90-66	M	64	55	9	5 h 55	6.22	1155	07.00 h	0.58	AD
32	89-112	M	64	57	7	5 h 30	6.12	1210	22.30 h	0.34	AD, cachexia
33	89-178	M	64	68	6	3 h 15	6.02	1355	14.45 h	1.06	AD, gastric perforation
34	88-325	F	64	54	10	2 h 30	6.44	800	15.45 h	0.37	AD, cachexia, dehydration
35	90-189	M	66	58	8	3 h 45	6.91	1350	09.00 h	0.11	AD, respiratory insufficiency
36	89-183	M	67	52	15	4 h 10	6.04	990	03.20 h	0.46	AD, cachexia, dehydration
37	90-68	F	72	57	15	1 h 10	6.51	1130	00.10 h	1.07	AD, pneumonia
38	91-71	M	73	58	15	4 h 30	6.49	1230	21.30 h	0.14	AD, emphysema
39	89-310	M	75	72	3	2 h 25	6.67	1200	02.05 h	0.38	AD
40	90-77	F	79	72	7	2 h 50	7.15	984	12.00 h	0.47	AD, cardiac failure
41	89-142	F	81	75	6	3 h 55	6.07	1030	18.15 h	0.83	AD, cachexia, decubitus
42	90-325	M	82	76	6	4 h 15	6.51	1121	06.51 h	1.60	AD, dehydration
43	90-64	F	83	70	13	3 h 15	6.89	990	18.00 h	0.47	AD, cardiac failure
44	90-78	F	83	74	9	3 h 20	6.57	1060	14.15 h	0.79	AD, dehydration
45	90-129	F	85	80	5	3 h 30	7.20	1015	09.30 h	0.09	AD, cachexia
46	91-158	M	87	82	5	3 h 25	nd	1006	00.05 h	0.19	AD, cachexia
47	89-347	F	95	86	9	3 h 35	6.80	970	13.55 h	0.72	AD

Abbreviations used: AD: Alzheimer's disease, others as Table 1.

has also been reported in other conditions, e.g. in depressed patients (22-24) also in CSF (36) in the Cushing syndrome (27), MS (31), and in multi-infarct dementia patients (7).

Materials and methods

Autopsies were performed within the framework of the Netherlands Brain Bank. Ventricular CSF was obtained at autopsy, generally between 1 to 12 h after death, from 26 Alzheimer patients and 21 controls without a neurologic or psychiatric disease. Before the brain was removed, ventricular CSF was collected and pH was determined immediately as a measure of agonal state. Brains of individuals who die after a long terminal phase accumulate lactic acid and have, therefore, lower pH (40) independent of the postmortem time (41). CSF was immediately centrifuged at 700 g. The supernatant was subsequently subdivided into 250-1000 μl aliquots that were kept at -80°C until assayed.

The following variables were included in the present study for both Alzheimer patients and controls; postmortem interval, CSF-pH, brain weight, sex, and age (Table 1, 2).

Neuropathology

All Alzheimer patients had a history of gradual intellectual deterioration, and the diagnosis 'probable Alzheimer's disease' was made according to the NINCDS-ADRDA criteria (cf. 46) excluding other specific causes of dementia by history, physical examination and laboratory tests. The demented patients had a global deterioration scale of 6-7 for severity of dementia (47).

The brains of both Alzheimer patients and controls were investigated in a systematic way by neuropathologists (Professor F. C. Stam, Dr W. Kamphorst, Free University, Amsterdam, The Netherlands or Dr D. Troost, Academic Medical Center, University of Amsterdam, The Netherlands), following the guidelines of the US National Institute on Aging for the diagnosis of Alzheimer's disease (48). The neuropathological diagnosis 'Alzheimer's disease' was made on the basis of the occurrence of many senile plaques, neurofibrillary tangles and a disorganized fiber pattern (dystrophic neurites) in Bodian and Congo stains of the hippocampus and 5 cortical areas (for details see 49) in formalin-fixed tissue. In

order to exclude Parkinson's changes, the substantia nigra was also examined. The controls were not demented and had no primary neurological or psychiatric diseases (Table 1, 2).

Cortisol assay

Total cortisol was measured by fluorescence polarization immunoassay on a TDx analyzer (Abbott Laboratories, North Chicago, IL, USA). This assay, initially developed for plasma cortisol assay, is also suitable for CSF-cortisol assay. Addition of cortisol (range 0.2-1.0 $\mu\text{M/l}$) yielded 99% recovery and dilution of CSF with high endogenous cortisol concentration showed a linear response. Interassay variation was 8.3%, 5% and 3% at concentrations of 0.12, 0.40 and 1.03 $\mu\text{M/l}$.

A pilot study was performed on lumbar puncture(LP)-CSF-cortisol levels of 15 ambulant possible or probable Alzheimer patients (46) between 55 and 89 years of age, and 15 non-demented neurological patients between 48 and 83 years of age as controls. For this study a more sensitive assay (Orion Diagnostica, Espoo, Finland) was used because of the much lower level of cortisol in these samples. Lumbar puncture CSF had been obtained earlier for clinical reasons, and was stored at -80°C . The samples were kindly provided by Dr H. P. H. Kremer, Department of Neurology, University of Leiden and Drs J. P. Haesen and W. van Pelt, Overvecht Hospital, Utrecht.

Statistics

Cortisol levels were analyzed by a two-tailed Mann-Whitney *U*-test using SPSS-X (SPSS Inc., Chicago, USA). Correlations of age, postmortem interval, brain weight and pH of CSF versus cortisol levels were analyzed by Spearman correlation coefficients (determined separately for controls and AD patients) using SPSS-X followed by a two-tailed test of significance. Significance was defined at the 0.05 level and values are expressed as mean $\pm$ SEM.

Acknowledgements

We are grateful to the Netherlands Brain Bank (coordinator R. Ravid) for the CSF samples, to W. T. P. Verweij and A. A. M. Janssen for their secretarial assistance and to H. Stoffels for the graphics.

Part of this study was supported by the Amstel project (Stimuleringsprogramma Gezondheidsonderzoek (SGO) of the Ministry of Science and Education), Mrs E. J. M. Stevens and the Amsterdam Neuroscience Network.

Accepted August 4 1994

References

1. Dodt C, Dittman J, Hruby J, Späth-Schwalbe E, Born J, Schüttler R, Fehm HL. (1991) Different regulation of adrenocorticotropin and cortisol secretion in young, mentally healthy elderly and patients with senile dementia of Alzheimer's type. *J Clin Endocrinol Metab.* 72: 272–276.
2. Friedman M, Green MF, Sharland DE. (1969) Assessment of hypothalamic-pituitary-adrenal function in the geriatric age group. *J Gerontol.* 24: 292–297.
3. 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.
4. Raadsheer FC, Tilders FJH, Swaab DF. (1994). Similar age related increase of vasopressin colocalization in paraventricular corticotropin-releasing hormone neurons in controls and Alzheimer patients. *J Neuroendocrinology.* 6: 131–133.
5. Weiner MF, Davis BM, Mohs RC, Davis KL. (1987). Influence of age and relative weight on cortisol suppression in normal subjects. *Am J Psychiatry.* 144: 646–649.
6. Raadsheer FC, Oorschot D, Verwer RWH, Tilders FJH, Swaab DF. (1994). Age-related increase in the total number of corticotropin-releasing hormone neurons in the human paraventricular nucleus in controls and Alzheimer's disease: comparison of the disector with an unfolding method. *J Comp Neurol.* 339: 447–457.
7. Maeda K, Tanimoto K, Terada T, Shintani T, Kakigi T. (1991). Elevated urinary free cortisol in patients with dementia. *Neurobiol Aging.* 12: 161–163.
8. Martignoni E, Petraglia F, Costa A, Bono G, Genazzani AR, Nappi G. (1990). Dementia of the Alzheimer type and hypothalamus-pituitary-adrenocortical axis: changes in cerebrospinal fluid corticotropin releasing factor and plasma cortisol levels. *Acta Neurol Scand.* 81: 452–456.
9. Gurevich D, Siegel B, Dumlaio M, Perl E, Chaitin P, Bagne C, Oxenkrug G. (1990). HPA-axis responsivity to dexamethasone and cognitive impairment in dementia. *Prog Neuro-Psychopharmacol & Biol Psychiat.* 14: 297–308.
10. Franceschi M, Airaghi L, Gramigna C, Truci G, Manfredi MG, Canal N, Catania A. (1991). ACTH and cortisol secretion in patients with Alzheimer's disease. *J Neurol Neurosurg Psychiat.* 54: 836–837.
11. Davis KL, Davis BM, Greenwald BS, Mohs RC, Mathé AA, Johns CA, Horvath T. (1986). B. Cortisol and Alzheimer's disease, I: Basal studies. *Am J Psychiatry.* 143: 300–305.
12. Greenwald BS, Mathé AA, Mohs RC, Levy MI, Johns CA, Davis KL. (1986). Cortisol and Alzheimer's disease, II: Dexamethasone suppression, dementia severity, and affective symptoms. *Am J Psychiatry.* 143: 442–446.
13. Spar JE, Gerner R. (1982). Does the dexamethasone suppression test distinguish dementia from depression? *Am J Psychiatry.* 139: 238–240.
14. De Leon MJ, McRae T, Tsai JR, George AE, Marcus DL, Freedman M, Wolf AP, McEwen B. (1988). Abnormal cortisol response in Alzheimer's disease linked to hippocampal atrophy. *Lancet.* 13: 391–392.
15. Herman JP, Schäfer MK-H, Young EA, Thompson R, Douglass J, Akil H, Watson SJ. (1989). Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis. *J Neurosci.* 9: 3072–3082.
16. Landfield PW, Waymire JC, Lynch G. (1978). Hippocampal aging and adrenocorticoids: quantitative correlations. *Science.* 202: 1098–1102.
17. Sapolsky RM, McEwen BS. (1986). Stress, glucocorticoids, and their role in degenerative changes in the aging process. In: Crook T, Bartus RT, Ferris S, Gershon S. eds. *Treatment Development Strategies for Alzheimer's Disease.* 151–171. Mark Powley Associates, Inc., Madison, CT, USA.
18. Martignoni E, Costa A, Sinforiani E, Liuzzi A, Chiodini P, Mauri M, Bono G, Nappi G. (1992). The brain as a target for adrenocortical steroids: cognitive implications. *Psychoneuroendocrinology.* 17: 343–354.
19. Goudsmit E, Scheltens P, Endert E, Fliers E, Swaab DF. (1994). Plasma levels of steroid hormones in ambulatory Alzheimer patients. *Psychoneuroendocrinology.* (in press).
20. Bondareff W, Mountjoy CQ, Roth M. (1981). Selective loss of neurones of origin of adrenergic projection to cerebral cortex (nucleus locus coeruleus) in senile dementia. *Lancet.* i: 783–784.
21. Rossor MN, Iversen LL, Reynolds GP, Mountjoy CQ, Roth M. (1984). Neurochemical characteristics of early and late onset types of Alzheimer's disease. *Br Med J.* 288: 961–964.
22. Coppen A, Abou-Saleh M, Milln P, Metcalfe M, Harwood J, Bailey J. (1983). Dexamethasone suppression test in depression and other psychiatric illnesses. *Brit J Psychiat.* 142: 98–504.
23. Dahl RE, Ryan ND, Puig-Antich J, Nguyen NA, All-Shabbout M, Meyer VA, Perel J. (1991). 24-hour cortisol measures in adolescents with major depression: a controlled study. *Biol Psychiat.* 30: 25–36.
24. 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. In: Swaab DF, Hofman MA, Mirmiran M, Ravid R, Van Leeuwen FW. eds. *The human hypothalamus in health and disease. Progress in Brain Research,* vol. 93, 385–417. Elsevier, Amsterdam.
25. De Kloet ER. (1991). Brain corticosteroid receptor balance and homeostatic control. *Frontiers in Neuroendocrinology.* 12: 95–164.
26. Kerr DS, Campbell LW, Thibault O, Landfield PW. (1992). Hippocampal glucocorticoid receptor activation enhances voltage-dependent Ca^{2+} conductances: relevance to brain aging. *Proc Natl Acad Sci USA.* 89: 8527–8531.
27. Starkman MN, Gebarski SS, Berent S, Scheingart DE. (1992). Hippocampal formation volume, memory dysfunction and cortisol levels in patients with Cushing's syndrome. *Biol Psychiatry.* 32: 756–765.
28. Trethowan WH, Cobb S. (1952). Neuropsychiatric aspects of Cushing's syndrome. *AMA Arch Neurol Psych.* 67: 283–309.
29. 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.* (in press).
30. Purba JS. (1994). The paraventricular nucleus of the human hypothalamus during aging and in several pathologies. Thesis, University of Amsterdam, The Netherlands.
31. Reder AT, Mackowiec RL, Lowy MT. (1994). Adrenal size is increased in multiple sclerosis. *Arch Neurol.* 51: 151–154.
32. Touitou Y, Sulon J, Bogdan A, Reinberg A, Sodeyoz JC, Demey-Ponsart E. (1983). Adrenocortical hormones, ageing and mental condition: seasonal and circadian rhythms of plasma 18-hydroxy-11-deoxy-corticosterone, total and free cortisol and urinary corticosteroids. *J Endocrinol.* 96: 53–64.
33. Sharma M, Palacios-Bois J, Schwartz G, Iskandar H, Thakur M, Quirion R, Nair NPV. (1989). Circadian rhythms of melatonin and cortisol in aging. *Biol Psychiatry.* 25: 305–319.
34. Uete T, Nishimura S, Ohya H, Shimomura T, Tatebayashi Y. (1970). Corticosteroid levels in blood and cerebrospinal fluid in various disease. *J Clin Endocr.* 30: 208–214.
35. Banki CM, Arato M, Papp Z, Kurcz M. (1984). Biochemical markers in suicidal patients. Investigations with cerebrospinal fluid amine metabolites and neuroendocrine tests. *Journal of Affective Disorders.* 6: 341–350.
36. Carroll BJ, Curtis GC, Mendels J. (1976). Cerebrospinal fluid and plasma free cortisol concentrations in depression. *Psychol Med.* 6: 235–244.
37. Marynick SP, Wood JH, Loriaux DL. (1980). Cerebrospinal fluid steroid hormones. In: Wood JH. ed. *Neurobiology of Cerebrospinal Fluid.* 605–611. Plenum Press, New York.
38. Wood JH. (1982). Neuroendocrinology of cerebrospinal fluids: peptides, steroids, and other hormones. *Neurosurgery.* 11: 293–305.
39. Schneider SL, Slaunwhite Jr WR. (1971). Transcortin. A comparison of the cortisol-binding globulin from human and cavian plasma. *Biochemistry.* 10(11): 2086–2093.
40. Hardy JA, Wester P, Winblad B, Gezelius C, Bring G, Eriksson A. (1985). The patients dying after long terminal phase have acidotic

- brains; implications for biochemical measurements on autopsy tissue. **J Neural Trans.** 61: 253–264.
41. Ravid R, Van Zwieten EJ, Swaab DF. (1992). Brain banking and the human hypothalamus—factors to match for, pitfalls and potentials. In: Swaab DF, Hofman MA, Mirmiran M, Ravid R, Van Leeuwen FW. eds. **The human hypothalamus in health and disease. Progress in Brain Research** vol. 93, 83–95. Elsevier, Amsterdam.
 42. Raskind M, Peskind E, Rivard M-F, Veith R, Barnes R. (1982). Dexamethasone suppression test and cortisol circadian rhythm in primary degenerative dementia. **Am J Psychiatry.** 139: 1468–1471.
 43. Swaab DF, Fliers E, Partiman TS. (1985). The suprachiasmatic nucleus of the human brain in relation to sex, age and dementia. **Brain Res.** 342: 37–44.
 44. Swaab DF, Roozendaal B, Ravid R, Velis DN, Gooren L, Williams R. (1987). Suprachiasmatic nucleus in aging, Alzheimer's disease, transsexuals and Prader-Willi syndrome. In: De Kloet ER, Wiegant VM, De Wied D. eds. **Neuropeptides and Brain Function. Progress in Brain Research**, vol. 72, 301–311, Elsevier, Amsterdam.
 45. Nilsson C, Lindvall-Axelsson M, Owman C. (1992). Neuroendocrine regulatory mechanisms in the choroid plexus-cerebrospinal fluid system. **Brain Res Rev.** 17: 109–138.
 46. McKahn G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADR work group under the auspices of the department of health and human services task force on Alzheimer's disease. **Neurology.** 34: 939–944.
 47. Reisberg B, Ferris SH, DeLeon MJ. (1982). The global deterioration scale for assessment of primary degenerative dementia. **Am J Psychiatry.** 139: 1136–1139.
 48. Khachaturian Z. (1985). Diagnosis of Alzheimer's disease. **Arch Neurol.** 42: 1097–1105.
 49. Van de Nes JAP, Kamphorst W, Ravid R, Swaab DF. (1993). The distribution of Alz-50 immunoreactivity in the hypothalamus and adjoining areas of Alzheimer's disease patients. **Brain.** 116: 103–115.