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Alzheimer's Disease

Pathophysiological Implications of Measurement of Plasma Cortisol, Plasma Dehydroepiandrosterone Sulfate, and Lymphocytic Corticosteroid Receptors

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Alzheimer's disease is often characterized by an increase in plasma cortisol without clinical evidence of hypercorticism. Twenty-three consecutive patients with Alzheimer's disease and 23 age- and sex-matched healthy controls were studied by measuring plasma cortisol and dehydroepiandrosterone sulfate (DHEAS) (by enzyme immunoassay), the number of type I and type II corticosteroid receptors in mononuclear leukocytes (by radioreceptorassay), and the lymphocyte subpopulations (by cytofluorimetry). Results are expressed in terms of median and range. In Alzheimer's disease, plasma cortisol was higher than in controls (median 0.74, range 0.47–1.21 vs 0.47, 0.36–0.77 mmol/L; $p < 0.001$). Plasma DHEAS, the DHEAS/cortisol ratio, and the number of type II corticosteroid receptors were significantly lower in AD than in controls (DHEAS: median 1.81, range 0.21–3.69 vs 3.51, 1.35–9.07 μmol/L; DHEAS/cortisol: 2.04, range 0.3–5.8 vs 6.8, range 2.7–24 and type II receptors: 1219, 1000–2700 vs 1950, 1035–2750 receptors per cell; $p < 0.001$). No correlation was found between the hormonal parameters, age, and mini-mental test score. These data support the hypothesis of a dysregulation of the adrenal pituitary axis in Alzheimer's disease, which is probably the consequence of damage to target tissues by corticosteroids.

Key Words: Alzheimer's disease; cortisol; dehydroepiandrosterone sulfate; corticosteroid type 1 receptors; corticosteroid type 2 receptors; lymphocyte subpopulations.

Introduction

It is known that aging can be associated with an altered regulation of the hypothalamic–pituitary–adrenal axis, an increase in cortisol, decreased adaptation to stress and reduced response to dexamethasone suppression test *(1–4)*. These abnormalities are more frequent and evident in Alzheimer's disease (AD), but the exact mechanism for the increase in plasma cortisol is not completely understood *(3–6)*. Elevated glucocorticoid levels produce hippocampal dysfunction, and the degree of hippocampal atrophy correlates strongly with the degree of cortisol elevation over time *(7)*. Plasma cortisol is also slightly increased in psychiatric diseases, such as depression or anorexia nervosa *(8)*. The activity of cortisol is mediated by the binding to corticosteroid receptors. These receptors are of two types: type I (mineralocorticoid) receptors, which are involved in the regulation of the circadian variations of cortisol at the suprahypothalamic and hypothalamic level, and type II (glucocorticoid) receptors, which are activated early in the morning or during stress *(9,10)*. The baseline secretion of corticotropin-releasing factor and the regulation of corticosteroid receptors at the brain level seem to be under the control of various neurotransmitters (cholinergic, adrenergic, etc.) *(11)*. Degeneration of the locus ceruleus and reduced norepinephrine in the locus ceruleus projection areas are found in AD and could promote the inflammatory responses to beta amyloid *(12)*. The finding that markers of cholinergic function are lost in AD have supported the cholinergic hypothesis. Abnormalities of the noradrenergic and cholinergic locus ceruleus have been described in AD along with decreased norepinephrine levels in the cerebral cortex and hypothalamus *(7,13)*. Recently, Petrie et al. demonstrated an increase in sympathoadrenal response to cholinergic stimulation and concluded that plasma catecholamine responses to physostigmine do not appear to be useful peripheral neuroendocrine estimates of the severity of brain cholinergic deficits in AD *(14)*.

Another important factor, which modulates the activity of glucocorticoids at the brain level, is type 1 11β-hydroxysteroid dehydrogenase (HSD1) that has been described in the hippocampus, hypothalamus, and anterior pituitary. This isoform of the enzyme mainly acts as a reductase, thus amplifying the deleterious effects of excess of cortisol. It was hypothesized that 11HSD1 inhibitors could protect the hippocampus in aging *(15)*.

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Table 1
Median and (Range) of Age, Type I Corticosteroid Receptors, Type II Corticosteroid Receptors, Plasma Cortisol and DHEAS, DHEAS/Cortisol, Percentage of CD4+ and CD8+ Lymphocytes, and CD4+/CD8+ Ratio in Controls and Patients with Alzheimer's Disease

	Controls	Alzheimer's Patients
Age	66 (49–89)	67 (49–89)
Type I receptors (n × cell)	200 (90–420)	170 (29–258)
Type II receptors (n × cell)	1950 (1035–2760)**	1219 (1000–2700)
Cortisol (F) (μmol/L)	0.47 (0.36–0.77)**	0.74 (0.47–1.21)
DHEAS (μmol/L)	3.51 (1.35–9.07)*	1.81 (0.21–3.69)
DHEAS/F	6.8 (2.7–24)**	2.04 (0.3–5.8)
CD4+ (%)	45 (33–50)	42 (31–54)
CD8+ (%)	27 (18–40)	27 (15–41)
CD4+/CD8+	1.7 (1.1–3.1)	1.5 (0.9–3.6)

*$p < 0.05$; **$p < 0.01$.

The brain corticosteroid receptors are inaccessible in humans; therefore, their study must be indirect. It has been shown, in adrenalectomized rats, that corticosteroid receptor regulation is similar in the hippocampus and lymphoid tissue *(16)*. Nijhuis et al. in addition demonstrated that phytoagglutinin-induced proliferation and interleukin (IL)-2 and IL-4 synthesis by mononuclear leukocytes (MNL) from patients with AD are less sensitive to the inhibitory effect of dexamethasone compared to patients with other types of dementia *(17)*. These findings further support the hypothesis that the characteristics of MNL reflect related changes in the central nervous system and indicate that MNL may be a useful and accessible tool to obtain more insight into the pathogenesis of AD.

Recently, a number of studies have focused interest on the determination of dehydroepiandrosterone sulfate (DHEAS) in aging and in AD. DHEAS has antiglucocorticoid activity and neuroprotective properties and is considered to be a specific individual marker *(18,19)*. It has been demonstrated that DHEA is also a neurosteroid, being produced autonomously by some brain areas *(20–23)*. The decrease of DHEAS during aging is not the result of a change in the metabolism of DHEA and DHEAS, but of a reduced production by the adrenals *(17)*. DHEAS could modulate the effect of cortisol *(19)*, and its reduction is probably involved in the toxicity from glucocorticoids *(21)*. Patients with AD, who have higher levels of DHEAS and lower cortisol, can perform better than patients with low DHEAS and higher plasma cortisol levels *(24)*. A recent review *(25)* reports that a reduction in DHEAS levels in AD is only observed in some studies, suggesting that low DHEAS levels cannot be regarded as a key feature of this disease; similar to other factors like gender, medication, body composition, or peripheral glucose regulation. The DHEAS/cortisol ratio might be a more appropriate and sensitive measure and might help to discriminate between AD and age matched controls *(26)*.

The aim of this study was to evaluate several hormonal parameters, which are involved in the corticosteroid effector mechanism in patients with AD and to find a possible correlation with age and cognitive alterations.

Results

Cortisol was increased in the plasma of all but one of the subjects with AD (median 0.74, range 0.47–1.21 μmol/L) and the mean value was significantly higher ($p < 0.001$) than in healthy controls of the same age and sex (median 0.47, range 0.36–0.77 μmol/L). DHEAS and the DHEAS/cortisol ratio were lower in AD than in controls (DHEAS 1.81, 0.21–3.69 vs 3.51, 1.35–9.07 μmol/L; $p < 0.007$; DHEAS/cortisol ratio 2.04, 0.3–5.8 vs 6.8, 2.7–24; $p < 0.001$) (Table 1).

Mean number of type I corticosteroid receptors (Table 1) was not different in AD (170, 29–258 receptors per cell) than in control (200, 90–420 receptors per cell). The number of type II receptors was significantly lower in AD (median 1219, range 1000–2700 receptors per cell) than in controls (1950, 1035–2760 receptors per cell; $p < 0.001$). The affinity of the tracers for the receptors was similar in the two groups (type I: controls 2.0, 1.9–2.3; AD 2.1, 1.9–2.4 nmol/L; type II: controls 7.7, 7.2–7.9; AD 7.8, 7.2–7.8 nmol/L).

The median percentage of CD4 and CD8 lymphocytes was 42 (31–54) and 27 (12–41) in AD, and 45 (33–57) and 28 (16–40) in controls. The CD4/CD8 ratio was not different in the two groups (AD 1.5, 0.9–3.6 and controls 1.7, 1.1–2.5) (Table 1).

No significant correlation was found in AD between all parameters studied and age (Figs. 1 and 2) or length of the disease, or the minimental test score or the grade of cere-

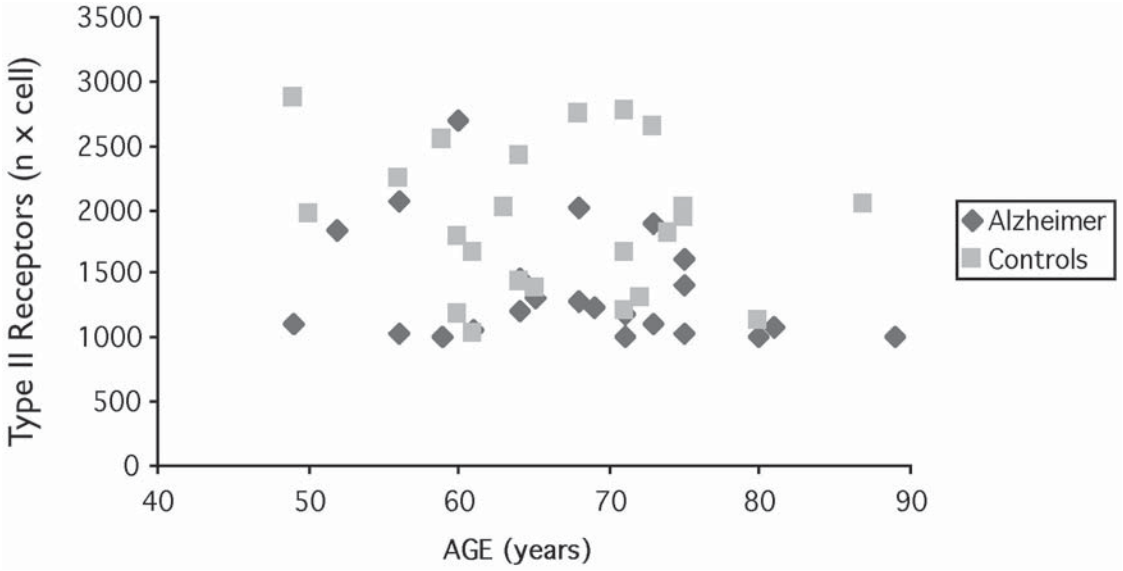

Fig. 1. Lack of correlation between age and type II receptors in Alzheimer's disease ($r = –0.29$, $p = 0.17$) and in controls ($r = –0.16$, $p = 0.44$).

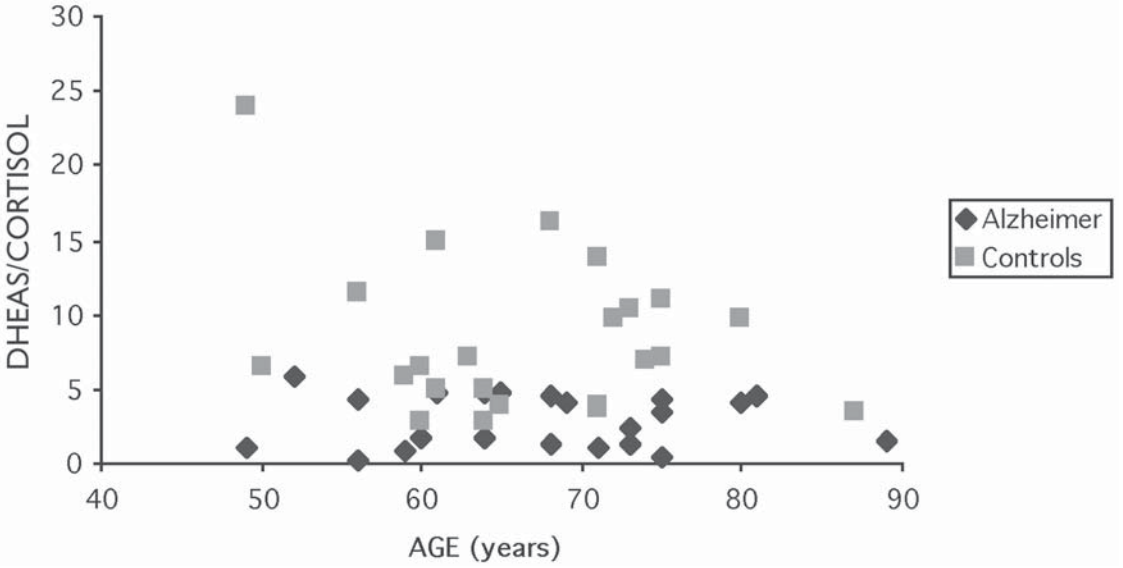

Fig. 2. Lack of correlation between age and DHEAS/cortisol in Alzheimer's disease ($r = –0.02$, $p = 0.92$) and in controls ($r = –0.24$, $p = 0.26$).

bral atrophy. In addition we could not demonstrate a correlation between the score of the minimental test and the hormonal parameters ($p > 0.05$ for all the parameters) (Table 2); the only parameter that is close to reaching significance is type II receptors (direct, $p = 0.054$).

Discussion

Our results are different from those of Rupprecht et al. *(27)*, who studied 12 subjects with the disease and found normal plasma cortisol and a normal number of corticosteroid type II receptors in MNL. Almost all our subjects, like most of those described in the literature *(28,29)*, had hypercortisolism and perhaps, for this reason, our data on corticosteroid receptors were different.

The number of type II receptors has been reported to be normal in MNL of almost all of the patients with Cushing's syndrome tested *(8,30,31)*, who do have much higher values of plasma cortisol. For that reason it is difficult to believe that type II receptors in AD are down-regulated by the increase in plasma cortisol. It is indeed more likely that the increase in plasma cortisol is the consequence of a disease-related decrease in corticosteroid type II receptors, also at the level of the centers that regulate the hypothalamic–

Table 2
Correlation between Hormonal Parameters and the Mini-Mental-State-Exam (MMSE) Score

Parameters vs MMSE in AD	*r*	*p*
Type I receptors (*n* × cell)	0.31	0.15
Type II receptors (*n* × cell)	0.41	0.05
CD4+/CD8+	0.10	0.64
DHEAS (μmol/L)	0.11	0.60
F (μmol/L)	0.14	0.52
DHEAS/F	0.17	0.43
Age	0.17	0.44

pituitary–adrenal axis. This hypothesis is consistent with the study of Linder et al. *(32)*, who found a decreased sensitivity to cortisol in AD and with the data of Lupien et al., who demonstrated a strong correlation between the degree of hippocampal atrophy and the degree of cortisol elevation in elderly humans *(7)*. In our study the degree of brain atrophy at magnetic resonance was not evaluated accurately enough to determine hippocampal volume, and for that rea-son we cannot exclude a correlation with hormonal parameters.

Another support for our hypothesis is the study of Masera et al., who found a correlation between mental deterioration and response of natural killer cell activity to physiological modifiers in patients with short-history AD, consistent with a partial glucocorticoid resistance at the immune level *(33)*. In our study the correlation between type II receptors and the score of minimental test was at the limit of significance in AD, and it is not excluded that with more cases the correlation would be significant.

We previously found a reduction in the number of corticosteroid receptors in MNL, also in a large group of healthy subjects up to 55 yr, when compared with younger subjects *(2)*. In AD the values of plasma cortisol were indeed higher, without any change in the affinity of dexamethasone nor of aldosterone for the respective receptor in vitro. A reduced number of type II receptors was also present in MNL of relatively young cases of AD. From all these considerations we can suggest that a slow and progressive neurodegeneration is physiological in the healthy aged subjects, while in AD this phenomenon begins earlier or is stronger and is accompanied by some disease-related alterations at the suprahypothalamic and lymphocytic level. Other data supporting this hypothesis is the normality of the number of type I receptors in MNL, which is usually very low in Cushing's syndrome *(8)*.

The range of normality of the number of corticosteroid receptors per leukocyte and of plasma DHEAS is wide, but it was found that the concentrations of these receptors and of DHEAS do not change in several determinations over one year (difference less than 10% from the mean value) *(22,34)*. These findings are consistent with the concept that both corticosteroid receptors and DHEAS are individual markers. It is thus possible that the actual corticosteroid receptor content in a leukocyte of an aged person is dependent on their number during adulthood and for this reason we were not able to find a correlation with the length of the disease. The mechanism for this acceleration of the aging process is not known, but surely some individual genetic and environmental factors may be involved. One of these factors is probably the repeated stress over a life span and damage at the suprahypothalamic and neuronal level. We did not evaluate the peripheral catecholamine regulation in our patients, but this model does not appear to provide useful peripheral neuroendocrine estimates of the severity of cholinergic deficits in AD *(14)*.

We were not able to find a correlation either between hormonal parameters, corticosteroid receptors, and minimental test. A positive correlation between DHEAS/cortisol and Gottfries Brane Rating Scale was indeed demonstrated by Masera et al. *(33)* in mild to moderate AD, and the discrepancy can be related to our cases, which also included patients with long-lasting and more severe disease.

The incidence of AD increases with age *(35)*, and it has also been demonstrated that the β-amyloid protein plaques occur to a lesser degree in normal aging; thus, it is possible that the progression of aging is more rapid in AD. The possibility that the aging process or age-related diseases are due to changes in the DNA has been considered recently and spontaneous mutations have also been hypothesized *(36, 37)*. These mutations could be involved in the production of proteases or enzymes, which are related to the cell membrane composition and accelerate cellular apoptosis.

We did not find a significant difference in the content of type I receptors in AD with respect to controls, in the presence of significantly lower content of type II receptors. It is interesting to note that we previously found a significant correlation between the two receptors in healthy subjects of all ages *(38)*, and our data in AD are consistent with a more severe involvement of type II than of type I receptors.

Another finding of our study is that the values of DHEAS and DHEAS/cortisol ratio were significantly lower in AD than in controls. The DHEAS/cortisol ratio might be a more sensitive and appropriate measure and might help in discriminating between AD and age-matched controls *(26, 33)*. In healthy subjects, DHEAS probably antagonizes the action of cortisol, thus limiting the stress-induced injury to the tissues that can be involved in AD. Our results are in agreement with those of Roberts *(39)*, Sunderland et al. *(40)*, and Masera et al. *(33)*; of interest is the observation of a decrease in serum amyloid component by administration of DHEAS in aged rats *(40)*.

Finally, the ratio of lymphocyte subpopulations in peripheral blood from patients with AD was not different than that in healthy controls, and this finding was also reported by Masera et al. *(33)* and Nijhuis et al. *(17)*. We cannot

exclude differences in production of cytokines by these T cells, and these findings could be important for understanding the role played by cortisol and DHEAS in AD. It is likely that the evaluation of lymphocyte subsets in peripheral blood is not an index of the corticosteroid effect, the blood lymphocytes comprising only 2% of the total body lymphocytes *(41)*.

The values of each parameter involved in the corticosteroid effector mechanism alone cannot completely distinguish patients with AD from controls, but the contemporaneous evaluation of corticosteroid receptors, cortisol, and DHEAS can perhaps predict a more severe neurodegeneration, each factor being implicated to a different extent. The implications of these endocrine alterations in the disease thus seem to be important, even if we cannot now say whether they are involved primarily or they are an indirect marker of the disease. The interpretation of these results is difficult owing to the wide range of normality of each parameter in adulthood, when the patient who could later develop AD is free of symptoms.

These data support the importance of longitudinal studies because hypercortisolemia appears related to the clinical progression of the disease *(42)*.

Patients and Methods

The design was a case-control study. The cases were 23 subjects with Alzheimer's type dementia. The diagnosis was made by the three criteria of the *Diagnostic and Statistical Manual of Mental Disorders IV* (*DSM IV-R*) *(43)*. All the patients satisfied the criteria of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS -ADRDA) for the probable diagnosis of AD *(44)*. The median score of the Mini-Mental-State-Exam was 20 (range 15–24). Patients were studied by physical examination, blood measurements, neuropsychological tests, and imaging techniques (electroencephalogram, computerized tomography scan, and magnetic resonance imaging). Imaging techniques showed a pronounced hippocampal and temporal, and a mild frontal, atrophy. Patients with other types of dementia were not considered. The age of the patients (18 female and 5 male) ranged from 49 to 83 yr, median 68, and the median length of the disease was 4.3 yr, range 3.9–5.9 yr. Blood pressure was lower than 141/91 mmHg, BMI was 24.2, range 20–26.1. The controls were a random group of healthy subjects comparable for age (65, range 49–89), sex, BMI (24.5, range 21.1–26.0), and blood pressure. The patients were free of other diseases that could affect the measurement of the parameters considered, and they were not taking hormonal therapy or other drugs prior to the study.

After informed consent of the patients and, when necessary, of the relatives, a blood sample was drawn at 9 AM in the sitting position, for the measurements of plasma cortisol and DHEAS, lymphocyte subpopulations, and corticosteroid receptors in MNL. The CD4 and CD8 lymphocyte subpopulations were measured by cytofluorimetry (Coulter Epix XL-MCL), using CD45, CD3, CD4, and CD8 antibodies (Beckman-Coulter). Plasma cortisol and DHEAS were measured by enzyme immunoassay using commercial kits. Corticosteroid receptors in MNL were measured by radioreceptor assay, as previously described *(45,46)*. MNL were separated from the blood by a Percoll gradient (Pharmacia, Uppsala, Sweden); MNL layer was removed and washed three times with saline at room temperature. The cells were resuspended in RPMI-1640 medium (Serva-Heidelberg, Germany) at a concentration of 10^6/mL, and a 500-µL aliquot incubated for 1 h at 37ºC with increasing amounts of ^{3}H-aldosterone (for the mineralocorticoid receptor assay, specific activity 80 Ci/mmol, from New England Nuclear, Boston, MA) or ^{3}H-dexamethasone (for the glucocorticoid receptors assay, specific activity 49 Ci/mmol), alone or with addition of an excess of the respective cold steroid. In the type I assay an excess of a pure glucocorticoid (RU 26988, Roussel, France) was added to prevent binding of aldosterone to glucocorticoid receptors. After incubation, the cells were washed three times with 2 mL of cold saline and the radioactivity of the cells was measured by a beta-counter (LKB 1216, efficiency 60%); the data on the affinity and capacity were derived from Scatchard analysis.

The results are presented as median and range. The data of patients with AD were compared to that of controls and the differences were calculated using the Mann–Whitney rank sum at a $p < 0.05$ level. The relationship between each parameter and age was calculated by linear regression analysis.

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