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CLINICAL STUDY

Allopregnanolone and dehydroepiandrosterone response to corticotropin-releasing factor in patients suffering from Alzheimer's disease and vascular dementia

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Abstract

Objective: Neurosteroids have been suggested to be involved in the regulation of cognitive performances. A major neurosteroid gamma-aminobutyric acid (GABA) agonist is allopregnanolone: the main source of circulating allopregnanolone is the adrenal cortex. Studies indicated that a disturbance of the central regulation of the hypothalamic–pituitary–adrenocortical axis occurs in both senile (Alzheimer's disease: AD) and vascular dementia (VD).

Design: The aim of the present study was to evaluate the levels of circulating allopregnanolone, dehydroepiandrosterone (DHEA) and cortisol and their response to corticotropin-releasing factor (CRF) test in AD and VD.

Methods: Three groups of 12 subjects were included in the study: AD, VD and age-matched control subjects. CRF test was performed in all subjects and allopregnanolone, DHEA and cortisol levels were measured every 15 min for 2 h.

Results: Mean ± S.E.M. allopregnanolone and DHEA basal levels were significantly lower in AD and VD than in controls, while cortisol levels were significantly higher than in controls ($P<0.01$). Allopregnanolone and DHEA levels increase in response to CRF test in all subjects but the area under curve (AUC) in patients was significantly lower than in controls ($P<0.01$). Cortisol secretion appeared to be very sensitive in response to CRF stimulation: in fact, cortisol response to CRF test in AD and VD subjects was higher (both as AUC and as % max increase) than in controls ($P<0.01$).

Conclusions: The present study firstly showed that allopregnanolone levels are reduced both in AD and in VD and that dementia has a preserved stimulated response of allopregnanolone to CRF. Overall, however, the total response of allopregnanolone to CRF remains reduced in respect to controls. Further studies are necessary for a better understanding of the role of neurosteroids in the regulation of cognitive function.

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Introduction

An increased vulnerability of neurons in Alzheimer's disease (AD) is hypothesized to result from an imbalance of excitatory and inhibitory inputs. An involvement of the gamma-aminobutyric acid (GABA)ergic system in Alzheimer-related neuropathological changes has been demonstrated (1, 2). In particular, different benzodiazepine receptor agonists or antagonists are able to influence cognitive function by modulating GABAergic function in the brain (3–5). Recently, the brain was shown to be the source of *de novo* synthesized steroid hormones, named neurosteroids. Some of these bind to GABA A receptors and seem to be involved in modulating stress, cognitive performances and, in particular, in aggressive behavior of AD (6).

Allopregnanolone is one neuroactive steroid which acts as a GABA A receptor agonist, modulating behaviors, stress and neuroendocrine functions in rats. The increased synthesis of allopregnanolone in rat brain following acute stress suggests that this steroid may play a role as an endogenous stress-protective compound (7–9). Pretreatment with high doses of allopregnanolone significantly decreased corticotropin-releasing factor (CRF)-induced behavioral manifestations of stress and anxiety, indicating an anxiolytic,

sedative–hypnotic and anti-aggressive effect similar to that produced by benzodiazepines (9). Conversely, dehydroepiandrosterone (DHEA) and its sulfated conjugated metabolite (DHEAS), both acting as GABA A antagonist neuroactive steroids, are able to improve well-being and cognitive function in aged subjects (10). While it is known that serum DHEA and DHEAS are reduced in AD (11, 12), no data are available concerning allopregnanolone circulating levels.

Previous studies showed a disturbance in the central regulation of the hypothalamic–pituitary–adrenocortical axis (HPA) in both senile and vascular dementia (VD). Some behavioral disturbances in dementia appear as a consequence of an increased stress-induced HPA activity. In fact, these patients fail to adapt to chronic stress owing to an insufficient feedback system (13, 14). CRF appears as the key neuropeptide in the interplay between stress and cognition (15). Conflicting data are present in the literature concerning CRF levels in cerebrospinal fluid of AD patients (16–19), while a dramatic reduction in CRF concentration and an increase in CRF receptor density in the brain cortex have been described (20, 21). Cortisol response to CRF test has been used in AD patients to clarify the activity of HPA axis, with conflicting results (16, 22). CRF increases serum allopregnanolone in healthy subjects (23) while no data are available on allopregnanolone and DHEA response to CRF test in patients suffering from AD or VD. The aim of the present study was to evaluate circulating allopregnanolone, DHEA and cortisol basal levels and their response to CRF test in AD and VD.

Materials and methods

Subjects

The present study included three groups of subjects ($n = 36$): group a, patients suffering from AD ($n = 12$: 5 men and 7 women, age range 64–84); group b, patients suffering from VD ($n = 12$: 6 men and 6 women, age range 65–82); and group c, control subjects ($n = 12$: 4 men and 8 women, age range 68–81).

Protocol

Before entering the study each subject had medical history, physical examination and routine laboratory tests performed. Subjects with a history of cancer were excluded. None of the subjects were taking psychoactive medications, hormonal drugs, mineral or vitamin supplements or anti-inflammatory drugs. In group c, a diagnostic interview did not show current or recent (within the past 2 years) medical or psychiatric illness nor pathological changes in mood and behavior in recent years. In group a, AD was diagnosed by two independent psychiatrists according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV). Patients with dementia of vascular origin (history of stroke, insufficiency of cerebral arteries, infatuate cerebral areas seen in computed tomography) were included in group b. After approval of the Local Ethical Committee, informed consent was obtained from each subject or from relatives after full description of the protocol.

In all patients a catheter was inserted into the antecubital vein and a slow infusion of 0.9% saline solution was started. A basal sample was drawn at time −30 min before beginning the CRF test. The CRF test consisted of an intravenous bolus injection of 100 μg hCRF (Clinalfa AG, Laufelfingen, Switzerland) and blood samples were taken at 0, 15, 30, 45, 60 and 90 min after injection of CRF. Blood samples were centrifuged and serum stored at −20 °C until assayed. Allopregnanolone, DHEA and cortisol were evaluated in each sample.

Methods

Allopregnanolone assay Analytical grade solvents were purchased from Merck (Darmstadt, Germany); C-18 Sep-Pak cartridges were obtained from Waters Corporation (Milford, CT, USA). Standard allopregnanolone was purchased from Sigma Chemical Co. (St Louis, MO, USA) and pregnan-3α-ol-20-one, 5α-[9,11,12,-3H(N)] (45 Ci/nmol) from Amersham (Amersham, Bucks, UK). The polyclonal antisera, raised in sheep against allopregnanolone carboxymethyl ether coupled to BSA, were kindly provided by Dr R H Purdy. Serum samples (1 ml) were thawed. The assay was performed as previously described (23). The sensitivity of the assay, expressed as a minimal amount of allopregnanolone distinguishable from the zero sample with 95% probability, was 15–20 pg/tube and the intra- and interassay coefficients of variation were 7.2% and 9.1% respectively.

DHEA assay Serum samples for the determination of DHEA were extracted with ether, purified through a C18 Sep-Pak cartridge and then assayed by RIA using a trade kit (Radim SpA, Pomezia, Italy); the sensitivity was 15 pg/ml and the intra- and interassay coefficients of variation were 3.1% and 6.9% respectively.

Cortisol assay Cortisol was assayed using RIA methods (Radim SpA). The sensitivity of the method was 0.9 nmol/l and the intra- and interassay coefficients of variation were 3.6% and 7.3% respectively.

Statistics

The statistical analysis of the results was performed with a Macintosh personal computer using Abacus Concepts, Stat-View 4.0 program. All results are reported as the mean ± S.E.M. Related measures ANOVA was used for comparison of hormone levels between times and Scheffé *F*-test was used to determine the presence of significant differences in mean values.

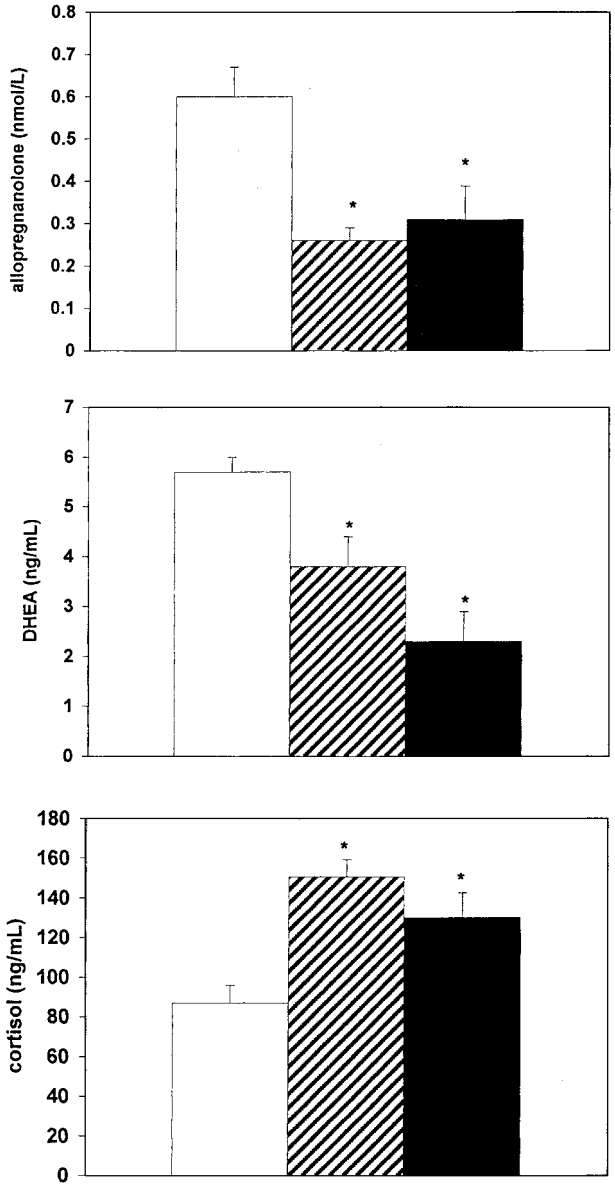

Figure 1 Mean ± S.E.M. allopregnanolone, DHEA and cortisol basal levels. White column, controls; striped column, VD; and black column, AD. *P<0.001.

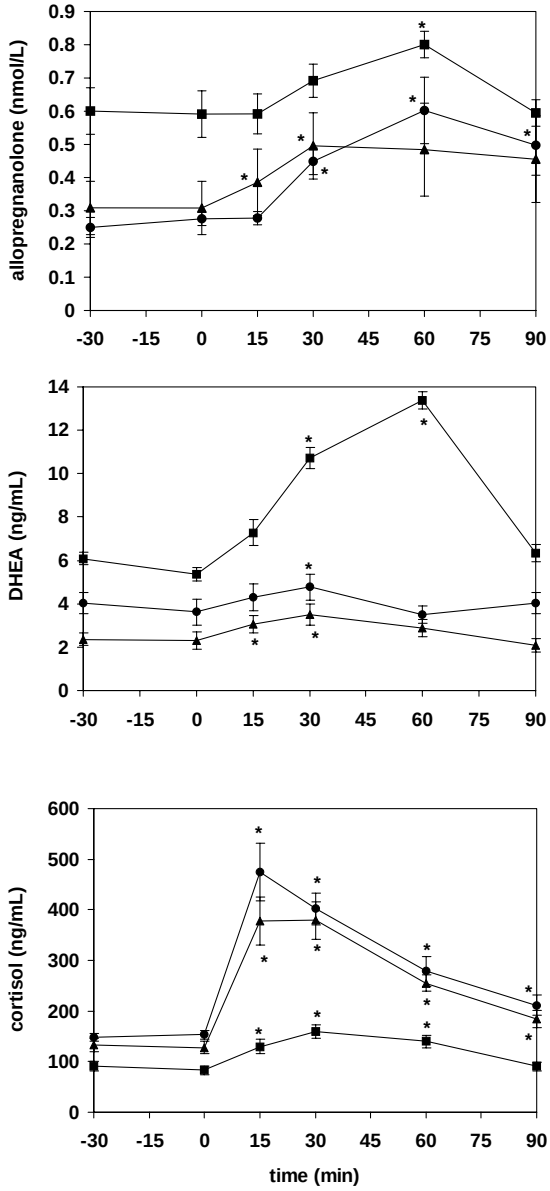

Figure 2 Mean ± S.E.M. allopregnanolone, DHEA and cortisol response to CRF test in control (■), AD (▲) and VD (●) subjects. *P<0.001.

Results

Patients suffering from AD or VD showed serum allopregnanolone and DHEA basal levels significantly lower than controls (P<0.001), while cortisol levels were significantly higher (P<0.001) (Fig. 1). Mean ± S.E.M. allopregnanolone and DHEA levels increased in response to CRF test (Fig. 2) but the area under curve (AUC) in patients with AD or VD was significantly lower than in controls (Fig. 3) (P<0.001). Serum cortisol increased in response to CRF (Fig. 2), and the AUC in AD and VD patients was significantly higher than in controls (Fig. 3). No significant difference either in basal levels or in response to CRF test between patients suffering from AD or VD was observed.

Discussion

The present study firstly showed that AD and VD patients have low serum allopregnanolone levels compared with age-matched healthy controls, confirming that serum DHEA levels are low in dementia (11, 12). It is known that DHEA and DHEAS improve long-term memory and diminish amnesia in mice and enhance neuronal and glial survival and differentiation in cultures of embryonic mouse brain cells (24). The

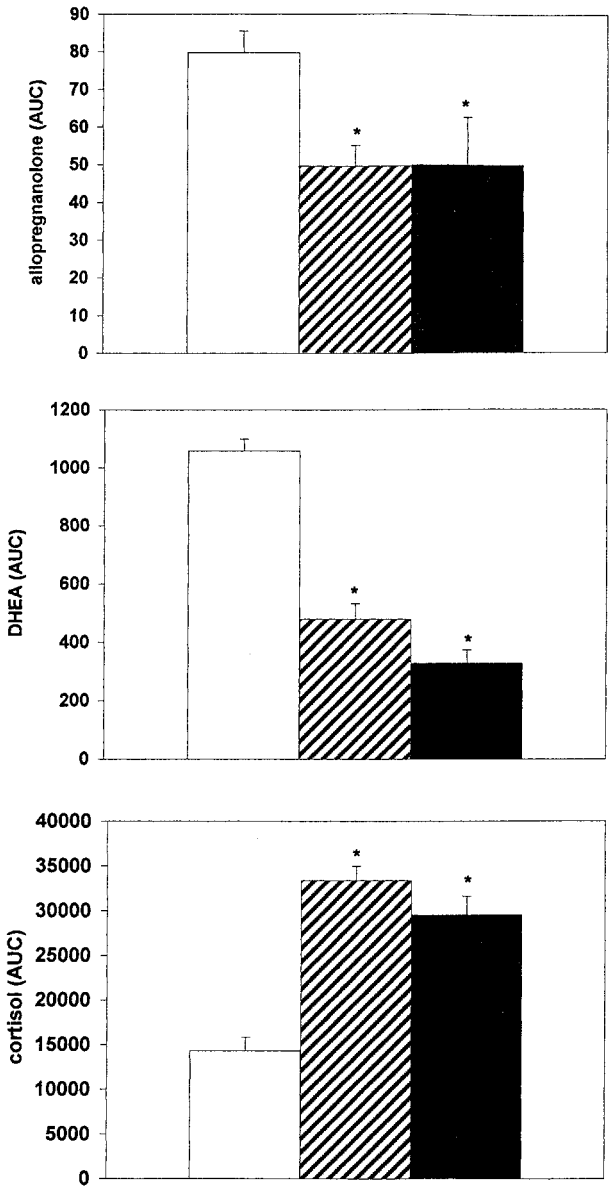

Figure 3 AUC of allopregnanolone, DHEA and cortisol response to CRF test. White column, controls; striped column, VD; and black column, AD. *$P < 0.001$.

administration of DHEA improves physical and psychological well-being and cognitive performances in aged subjects (10). The mechanism(s) of DHEA-mediated neuroprotection is unclear: it is probable that DHEA antagonizes some deleterious effects of cortisol which causes a progressive hippocampal damage and cognitive impairment in dementia (25–29). A low DHEA/cortisol ratio may be deleterious to hippocampal function (22). The hippocampus has a leading role in HPA axis regulation, but it is also an important brain site in memorization processing and in the connections between intellectual capacity and psychoaffective conditions; moreover, it is vulnerable to ipoxic–anoxic stimuli.

Experimental data in rats and primates showed that the increase in the glucocorticoid concentrations determines loss of specific receptors and neural damage in the hippocampus. Patients that underwent prolonged and high dose corticosteroid treatments showed memory and logical capacity deficits. It can be hypothesized that functional alterations of hippocampus glucocorticoid receptors for the negative feedback leads to a hyperactive HPA axis function, which in turn may cause further neural damage in the hippocampus; this could explain the correlation between cortisol levels and degree of brain vascular aging.

The finding that cortisol levels in AD and VD are higher than in control patients is in agreement with most of the literature (13, 22, 30–32) and a correlation between the severity of dementia and the increase of cortisol levels has been reported (12, 13, 33, 34). Nevertheless, this is the first paper describing a reduction in allopregnanolone circulating levels in patients affected by AD or VD. The hypothesis that allopregnanolone might be involved in modulating cognitive function is supported by the evidence that conditions characterized by modifications in behavior, mood and cognitive performance, such as menstrual cycle, pregnancy and aging, are associated with changes in allopregnanolone levels (23, 35, 36). It is possible to hypothesize that in dementia the low allopregnanolone levels are a consequence of the low CRF levels (23) that may explain also the low DHEA levels observed. On the other hand, both the AUC of DHEA and allopregnanolone response to CRF were reduced in dementia, indicating that these patients had an impairment in the total neuroendocrine balance capacity involving DHEA and allopregnanolone secretion. The different pattern observed between allopregnanolone and DHEA could reflect different metabolic pathways involved in their secretion and could indicate a major or more precocious involvement of DHEA secretion in dementia with respect to allopregnanolone.

The present study indicates that cortisol secretion is ultra-sensitive in response to CRF stimulation: in fact, cortisol response to CRF test was higher, both as AUC and as percentage maximum increase in AD and VD subjects, than in controls. Cortisol response to CRF test in AD have been described as similar (16, 37) or lower (30) than in controls; however, recent data are consistent with our findings of a higher cortisol response to CRF test (22) and adrenocorticotropin (ACTH) test (38) in AD patients. On the other hand, Dodt *et al.* (39) described a similar response of DHEA to CRF test in elderly and young subjects, regardless of their mental state; however, the simultaneous administration of vasopressin in association with CRF (half dose compared with the present study) means that the results are not comparable. The reason for the difference between cortisol and DHEA basal and stimulated levels in dementia remains unclear. The present study showed that in dementia an impairment of the DHEA/cortisol

ratio appeared more clearly in response to CRF test than at basal levels, indicating that in dementia, i.e. the reverse of the situation seen in controls, CRF determined a huge stimulation of cortisol secretion and an abatement of DHEA release. Since the adrenal androgen-stimulating factor and the alterations in adrenal androgen synthesis have been proposed for explaining the enhanced cortisol and reduced DHEA secretion in the elderly (39), such factors might play a role also in dementia.

In addition, the present study demonstrates that the kind of dementia did not influence either allopregnanolone, DHEA and cortisol levels or their response to CRF. Previous evidence regarding the differences in neuroendocrine function between AD and VD patients indicated contrasting results: while neuroendocrine regulation of growth hormone (GH) appeared more damaged in AD than in multi-infarct dementia patients (40), both AD and VD patients showed a similar behavior of plasma β-endorphin and cortisol circadian rhythms and similar abnormalities of dexamethasone suppression test (41, 42).

In conclusion, the present study firstly showed that serum allopregnanolone and DHEA levels are reduced both in AD and in VD and that allopregnanolone but not DHEA stimulated response to pharmacological doses of CRF test is maintained, although the low basal levels and the lower AUC showed an impairment of neuroendocrine balance involving allopregnanolone and DHEA in AD and VD patients. The present evidence supports the concept that dementia *per se*, independent of the nature, may be the result of an altered stress response, and the modifications in the circulating levels of allopregnanolone, a stress-related steroid hormone, might enter in this view. Therefore, it will be of interest to study the role of allopregnanolone in the complex way leading from stress to cognition, possibly via the interplay between brain CRF and CRF-related peptides.

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