THE LIMBIC - HYPOTHALAMIC -
PITUITARY - ADRENAL AXIS
IN ALZHEIMER'S DISEASE

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ABSTRACT

The limbic-hypothalamic-pituitary-adrenal axis in Alzheimer's disease

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Dysfunction of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis is a common finding in advanced dementia. In this study, the function of the LHPA axis at different levels was investigated in patients with dementia and in healthy elderly.

A subtle disturbance in the feedback regulation of the LHPA axis was found in patients with early (i.e., mild to moderate) Alzheimer's disease (AD). After 0.5 mg dexamethasone, serum cortisol levels were less suppressed in AD patients and plasma adrenocorticotropin (ACTH) levels were lower as compared with healthy elderly. After stimulation with human corticotropin-releasing hormone a blunted ACTH response was found in AD patients while relative serum cortisol, dehydroepiandrosterone, and androstenedione responses were increased. Significant correlations were found between low plasma ACTH levels and temporal lobe atrophy and between low peak plasma ACTH levels and hippocampal atrophy measured with computer tomography. Patients with advanced AD and multi-infarct dementia had lower basal levels of dehydroepiandrosterone sulphate in combination with no difference in cortisol levels, resulting in a high cortisol/DHAS ratio. The difference persisted after adjustments for age and sex in a multivariate analysis. In patients with early AD, basal serum levels of dehydroepiandrosterone and androstenedione were increased, and this increase was accentuated after stimulation with ACTH. Peripheral glucocorticoid sensitivity was examined by skin vasoconstrictor blanching tests. Patients with AD and patients treated with glucocorticoids showed skin blanching at higher clobetasol concentrations than healthy elderly.

These findings justify further investigations on the role of LHPA axis dysfunction in Alzheimer's disease and its possible importance for the pathophysiology of the disease.

Key words: Alzheimer's disease, multi-infarct dementia, hippocampus, temporal lobe, adrenal androgens, adrenocorticotropin, corticotropin-releasing hormone, cortisol, skin blanching.
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PITUITARY - ADRENAL AXIS
IN ALZHEIMER'S DISEASE

Birgitta Näsman

University of Umeå
Umeå 1994
"Vad tjänar vetandet till om vettet saknas"

Montaigne
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ABBREVIATIONS

ACTH  adrenocorticotropic hormone
AD    Alzheimer's disease
ADL   activities of daily living
ADRDA the Alzheimer's Disease and Related Disorders Association
ANOVA analysis of variance
BMI   body mass index (kg/m^2)
CBG   corticosteroid-binding globulin
CDR   clinical dementia rating
CNS   central nervous system
CRH   corticotropin-releasing hormone
CSF   cerebrospinal fluid
CT    computed tomography
DEX   dexamethasone
DHA   dehydroepiandrosterone
DHAS  dehydroepiandrosterone sulphate
DSM-III-R Diagnostic and Statistical Manual of Mental Disorders, 3rd ed. (revised)
DST   dexamethasone suppression test
EEG   electroencephalography
GABA  γ-Aminobutyric acid
GR    glucocorticoid receptor
5-HIAA 5-hydroxy-indoleacetic acid
5-HT  serotonin
HU    Hounsfield units
IGF-I insulin-like growth factor I
LHPA axis limbic-hypothalamo-pituitary-adrenal axis
MANOVA multiple analysis of variance
MMSE Mini-Mental State Examination
MID   multi-infarct dementia
MR    mineralocorticoid receptor
MRI   magnetic resonance imaging
NINCDS National Institute of Neurological and Communicative Disorders
NMDA  N-methyl-D-aspartate
17-OHP 17α-hydroxyprogesterone
St.c.  standardized regression coefficient
ABSTRACT

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Dysfunction of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis is a common finding in advanced dementia. In this study, the function of the LHPA axis at different levels was investigated in patients with dementia and in healthy elderly.

A subtle disturbance in the feedback regulation of the LHPA axis was found in patients with early (i.e., mild to moderate) Alzheimer's disease (AD). After 0.5 mg dexamethasone, serum cortisol levels were less suppressed in AD patients and plasma adrenocorticotropin (ACTH) levels were lower as compared with healthy elderly. After stimulation with human corticotropin-releasing hormone a blunted ACTH response was found in AD patients while relative serum cortisol, dehydroepiandrosterone, and androstenedione responses were increased. Significant correlations were found between low plasma ACTH levels and temporal lobe atrophy and between low peak plasma ACTH levels and hippocampal atrophy measured with computer tomography. Patients with advanced AD and multi-infarct dementia had lower basal levels of dehydroepiandrosterone sulphate in combination with no difference in cortisol levels, resulting in a high cortisol/DHAS ratio. The difference persisted after adjustments for age and sex in a multivariate analysis. In patients with early AD, basal serum levels of dehydroepiandrosterone and androstenedione were increased, and this increase was accentuated after stimulation with ACTH. Peripheral glucocorticoid sensitivity was examined by skin vasoconstrictor blanching tests. Patients with AD and patients treated with glucocorticoids showed skin blanching at higher clobetasol concentrations than healthy elderly.

These findings justify further investigations on the role of LHPA axis dysfunction in Alzheimer's disease and its possible importance for the pathophysiology of the disease.

Key words: Alzheimer's disease, multi-infarct dementia, hippocampus, temporal lobe, adrenal androgens, adrenocorticotropin, corticotropin-releasing hormone, cortisol, skin blanching.
This thesis is based on the following papers which will be referred to in the text by their Roman numerals.


INTRODUCTION

The nervous and endocrine systems regulate almost all metabolic and homeostatic activities of the organism. These two regulatory systems interact; endocrine secretion is influenced directly or indirectly by the brain, and many hormones can influence brain activity. Diseases affecting these systems may thus have profound effects. The focus of this thesis has been the possible linkage between dysfunction of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis and dementia.

Dementia

Dementia is one of the most severe mental health problems in the aged population. The prevalence rates for dementia increase steeply with advancing age. According to the Diagnostic and Statistical Manual (DSM-III-R) (1) of the American Psychiatric Association, the criteria for dementia include demonstrable evidence of impairment in memory and either impairment in one other intellectual function or personality change. These disturbances must be sufficient to interfere with usual activities or relationships. Another widely used definition proposes that dementia is "the decline of memory and other cognitive functions in comparison with the patient's previous level of function as determined by a history of decline in performance and by abnormalities noted from clinical examination and neuropsychological tests" (2).

Alzheimer's disease

The most frequent cause of dementia is Alzheimer's disease (AD) (3), a catastrophic neurodegenerative disorder that affects millions of individuals. AD accounts for about 50% of the dementing disorders, followed by vascular dementia (4). AD is probably an underestimated cause of death. Because of its high frequency, malignant natural course, and shortening of life expectancy, Katzman (1976) named the disease "a major killer" (5).

AD is a syndrome with an insidious onset with memory disturbances, striking loss of spontaneity and reduced initiative.
With progression of the disease, more pronounced focal symptoms appear, *i.e.*, aphasia, apraxia, agnosia. The patient also shows a decline in insight and judgement, and dysfunction in coping with complex tasks and new activities (6).

The diagnosis of Alzheimer's disease

The final diagnosis of AD is based on the combination of an appropriate clinical picture of progressive dementia and histopathological confirmation from brain tissue. The clinical diagnosis of AD depends on clinical criteria such as: a characteristic history, compatible findings on physical and mental status examination, and the exclusion of other disorders that mimic AD by appropriate information from history, examination, and laboratory tests (2). It is likely that AD will be found to have several causes, and it may therefore represent multiple disorders with clinical and neuropathological expressions in common (7, 8).

The clinical diagnosis of AD has to be made in the absence of valid biological markers. The clinical criteria for diagnosis have been defined largely due to the work group on the diagnosis of AD that was established by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA). In 1984 the work group presented criteria that would serve as a clinical guide for the diagnosis of "definite", "probable" and "possible" AD (2). Another leading set of diagnostic criteria is that of DSM-III-R (1) for "primary degenerative dementia of the Alzheimer type", senile and presenile type. These criteria require the insidious onset of dementia with a gradually progressive deteriorating course and the exclusion of all other specific causes of dementia.
Neuropathological changes

The neuropathological findings include atrophy of the brain, loss of neurons, decrease in the arborization of the dendritic tree, presence of neurofibrillary tangles, neuritic plaques, amyloid deposition and accumulation of lipofuscin (9). Senile plaques are the defining histological characteristics of AD, and the diagnosis is defined according to their numbers and distribution in the brain (10, 11). However, there is considerable heterogeneity, both in the criteria themselves and in the laboratory methods used to demonstrate the pathology (12-14).

The hippocampus is prominent among brain regions in showing degenerative changes with aging (15). However, there is a fundamental, qualitative difference between the regional pattern of neuron loss in the hippocampal formation associated with normal aging and that associated with AD (16). The typical pattern of neuropathological degeneration documented in AD is consistent with an interruption of the pathways linking the hippocampal formation and amygdala to the neocortex (17). The pyramidal cells of the entorhinal cortex are severely involved. These are the neurons which connect the cortical association areas with the hippocampal formation (17-19). The pattern of degeneration results in the "isolation" of the hippocampal formation from the neocortical association areas (17). Thus, the hippocampal formation seems to be among the earliest regions involved in AD, and AD has been described as a "hippocampal dementia" (20). Computed tomography (CT) (21, 22) and magnetic resonance imaging (MRI) (21, 23-25) studies describe volume loss in the hippocampus of patients with early AD as compared with healthy elderly.

Neurochemical changes

Multiple disturbances in neurotransmitter concentration and function in AD have been shown. One of the most consistent neurochemical changes in the AD brain is a profound loss of cortical and hippocampal cholinergic innervation (26). This is of interest because the cholinergic system has a specific relation to memory and cognitive function (27). There is a major depletion of cortical
cholinergic innervation in AD (28, 29). It remains unclear whether the damage to the cholinergic neurons occurs in a retrograde fashion as a result of events in the cortex, or as an early, independent phenomenon (30). Reductions in the serotonin (5-HT) system have been reported in AD (31, 32). It is assumed that the 5-HT system is important for mood and developing of anxiety (33), as well as for the control of neuroendocrine function (34). A correlation between 5-hydroxy-indoleacetic acid (5-HIAA) levels in cerebrospinal fluid (CSF) and symptoms of emotional disturbance has been found in AD patients (35). Furthermore, there are reductions in the concentrations of the glutamatergic neurotransmitters system, dopamine, noradrenaline, γ-aminobutyric acid (GABA), somatostatin and neuropeptide Y within the brain in AD (36, 37).

Aetiology and pathogenesis

To date, neither the aetiology, nor the pathogenesis of AD is fully understood. The most common hypothesis contends that β-amyloid, seen in neuritic plaque and in tangles, is the central factor in AD and a direct cause of dementia (38,39). A genetic component has long been suspected and autosomal dominance with age-dependent penetrance is usually proposed to be the best fitting mode of inheritance (40). Recent studies have shown that the inheritance of AD is a complex mixture of causative single genes and susceptibility genes. The genes perhaps need to aggregate to cause the disease. We now know of the existence of at least three AD loci on chromosomes 14, 19 and 21 (41) which are at least partly linked to amyloid. Therefore, AD should be considered an amyloidosis that specifically affects the brain, leading to loss of neuronal function and neuron loss (42). Furthermore, it has recently been shown that apolipoprotein E4 gene dosage correlates with increased risk for AD (43-45). The pathogenetic importance of this is unclear; neither plaques nor tangles are specific for AD as they also occur in non-demented aged individuals and neurological disorders.

Epidemiologic studies show that the prevalence ratio and incidence rates of AD increase steeply with age (46). Advanced age is the most important risk factor for AD, indicating that the
"normal" biology of aging may be related to increased susceptibility to factors which promote development and/or worsening of the disease. Some studies point out a higher prevalence of AD in women than in men (46-48), while others show no difference (49) or show a higher prevalence in men (50). The only consistent risk factors for AD are high age and family history of dementia (51). Data on head trauma are mixed (52), and the relationship to education is unclear (48, 49, 53).

The limbic-hypothalamic-pituitary-adrenal axis in old age

The limbic-hypothalamic-pituitary-adrenal (LHPA) axis (Fig. 1) is an integrated, multilevel system, regulated by endogenous and exogenous factors. The apex of this hierarchy is formed by the central nervous system (CNS) where neurotransmitters act to
modulate hypothalamic release of corticotropin-releasing hormone (CRH) into the portal circulation. CRH is released from neurons whose cell bodies are located in the paraventricular and supraoptic nuclei in the hypothalamus. CRH binds to membrane receptors on the pituitary corticotrophs and plays a key role in the response of the LHPA axis by stimulating the release of ACTH into the systemic circulation. ACTH stimulates the release of steroid hormones from the adrenal cortex. The major glucocorticoid hormone produced by the adrenal cortex in humans is cortisol, a hormone which plays a critical role in a variety of body functions and in adapting to stress. The most abundant adrenal androgens in human peripheral circulation are: dehydroepiandrosterone (DHA), its sulphate (DHAS) and androstenedione. 17α-hydroxyprogesterone (17-OHP) has a central position in adrenocortical steroid biosynthesis as a precursor for cortisol and androstenedione (Fig. 2).

Fig. 2  Biosynthesis of steroids
The activity of the LHPA system is determined by a complex interaction of endogenous and exogenous factors together with feedback loops. Several neurotransmitters, probably mainly serotonin, produce an episodic and circadian release of CRH (54). The cortisol axis is activated by a variety of physical and psychological stresses, ultimately mediated via the limbic and reticular activating systems and by various neurotransmitter systems impinging on the CRH-secreting neurons. As in all neuroendocrine systems, feedback control mechanisms maintain vital homeostatic functions. Traditionally, feedback functions in the cortisol axis have been localized to the pituitary and the hypothalamus. However, the hippocampus also contains a high density of glucocorticoid receptors (55) which indicates that this brain area may be of importance for the central regulation of the LHPA axis.

**Animal studies**

A vast literature suggests that there is an age-related dysfunction of the LHPA axis in rodents. Sapolsky (1991) (56) concluded that if rats are studied under truly basal conditions, there appears to be an age-related increase in circulating corticosterone levels. More limited data examining basal pituitary activity also suggest that ACTH levels increase with age (57, 58). Studies focusing on the initial stress response generally find no age-related differences (59, 60). By contrast, there are prominent age-related decreases in the ability to "turn off" the stress response; *i.e.*, a decreased feedback sensitivity. As a result, older animals tend to show a more prolonged elevation of corticosterone (61-63), less attenuation of the response to chronic stress (64-66), and reduced suppression of endogenous corticosterone secretion after administration of a synthetic glucocorticoid, dexamethasone (DEX) (64, 67, 68).

However, within the group of aged rats, there are distinct subgroups. Thus, one subgroup of rats behaves essentially like young rats with an efficient negative feedback function. In contrast, old rats that are cognitively impaired have less efficient "shut-off" of the corticosterone response to stress (63). Furthermore, neonatal handling, *i.e.*, removing rat pups from their mothers for 15 minutes daily during the first three weeks of life, results in an increase in
corticosteroid receptor binding in young rats. This prevents the senescent phenomenon of inability to terminate corticosterone secretion after stress, which is coupled with prevention of cognitive dysfunction in old rats (69).

Non-human primate studies using baboons and monkeys show important parallels to the rodent data. The rhesus monkey hippocampus has glucocorticoid receptors with a distribution similar to that in the rat (70). Lesions of the rhesus monkey hippocampus result in adrenal steroid hypersecretion and failure to shut-off the pituitary-adrenal stress response (71). Hypercortisolism and resistance to DEX are more common at high age in wild baboons (72), and chronic social stress in monkeys is associated with both hyperplastic adrenal cortices indicative of sustained glucocorticoid release, and hippocampal neuron loss (73).

**Human studies**

It is not yet known whether the hippocampus is a major glucocorticoid target site in human brain, though it plays this role in other mammalian species, including nonhuman primates. However, the human hippocampus is a key structure for neuron loss both in normal aging and AD, and the aged human hippocampus loses neurons in a pattern and magnitude quite similar to that seen in the aged rodent (15, 71). It has also been demonstrated that mRNAs for glucocorticoid receptors are highly expressed in the human hippocampus (74). Furthermore, electric stimulation of the hippocampus during neurosurgery inhibits adrenocortical secretion (75).

Increasing age does not have a major impact on basal serum cortisol and plasma ACTH levels (76-79), probably due to a combination of a reduction in cortisol secretion rate and a decreased metabolic clearence rate (76, 80). However, there is an age-related trend toward increased levels of evening plasma cortisol (77). There is also a consistent trend towards higher mean serum cortisol levels after overnight suppression with 1 mg DEX (78). Furthermore, increased peak ACTH and cortisol responses to stimulation with ovine CRH have been reported at higher ages (77), while no age-related difference in cortisol release after ACTH challenge is reported (79-83).
One of the most consistent findings in biological psychiatry is that of LHPA axis dysfunction in major depression (84), mainly abnormal negative feedback function, as tested by the dexamethasone suppression test (DST). Serum cortisol levels >138 nmol/l after overnight suppression with 1 mg DEX are found in 50-60% of the depressed patients. A number of studies have shown that non-suppressibility to DEX becomes far more common among aged depressed individuals (85-88). Interestingly, a close correlation has been shown between cognitive impairment and non-suppressibility to DEX in patients with depression (87, 89).

Other LHPA axis abnormalities have also been shown in depressed patients. Thus, a blunted ACTH response to exogenous CRH administration (90), and an augmented response of cortisol to exogenous ACTH, have been shown (91, 92). In some studies the changes are associated with adrenal gland hyperplasia (93, 94).

Early after ischemic stroke an increased cortisol secretion rate, DEX non-suppressibility, and increased sensitivity to exogenous ACTH are common findings (95-97). It is of major interest that cognitive impairment early after stroke has been shown to be associated with abnormalities of both cortisol secretion rate and feedback function (95-97).

The limbic-hypothalamic-pituitary-adrenal axis in Alzheimer's disease

Because the brain exerts multiple regulatory effects on hormone synthesis and release, endocrine functions have been studied to some extent in AD. Studies of thyrotropin response to thyrotropin releasing hormone, growth hormone response to growth hormone releasing hormone, and CSF somatostatin levels (98-100) have shown altered patterns in patients with AD, but the findings have been inconsistent. However, several studies have shown that LHPA axis dysregulation may be a main feature of this disease. Thus, increased circulating levels of cortisol are seen in patients with advanced AD (101, 102), while studies of the suppressibility of the cortisol axis have generated conflicting results (103-106). In most studies, a standard dose of 1.0 mg DEX has been given late in the evening with analysis of serum cortisol levels the following day, mainly at 4 P.M. (107). However, since the administration of only
0.5 mg DEX late one evening has been shown to be enough to suppress the subsequent early morning peak in serum cortisol in young and old healthy individuals (108, 109), the use of this lower dose of DEX might allow discovery of minor abnormalities in the LHPA axis feedback. Furthermore, it is important to consider other factors which may influence post-DEX cortisol levels, such as age, gender, body mass index (BMI) and basal serum cortisol levels (96, 110).

Cerebrospinal fluid (CSF) CRH levels are reported to be elevated (111), unchanged (112) or decreased (113, 114) in AD. A decrease in cortical CRH is associated with a subsequent increase in CRH receptor binding (115). It has recently been shown that the hypothalamus is considerably more affected in AD than has been assumed (116) and CRH mRNA levels in AD patients are markedly increased in the hypothalamus (paraventricular nucleus) at autopsy (117). Furthermore, CRH stimulation tests (118, 119) and CRH/vasopressin tests (120) have been reported to cause a blunted ACTH but normal cortisol response in hospitalized AD patients. In addition, serum cortisol levels after intravenous glucose loading were, in one study, correlated with the severity of cognitive impairment and hippocampal atrophy in AD patients (121). Finally, reduced adrenal acetylcholinesterase activity and adrenal medullary inclusion bodies have been reported in AD (122, 123).

These findings indicate possible disturbances at several levels of the LHPA axis in this disease. This is of major interest as disturbances in the LHPA axis have been proposed to be related to hippocampal degeneration and cognitive impairment in both animal models (124) and patients with AD (121). Increased glucocorticoid levels are associated with cognitive dysfunction in Cushing's syndrome and in healthy individuals given glucocorticoids (125-127). Evidence has accumulated for toxic effects of glucocorticoids on neurons, especially in the hippocampus where there is a high density of glucocorticoid receptors (71).

**Adrenal androgens**

Dehydroepiandrostosterone sulphate (DHAS) is the most abundant adrenal steroid in the circulation. Secretion of DHAS and DHA diminish progressively from the third decade onward (128), a state
named "adrenopause" (129). There is a decline in the serum DHA response to ACTH and CRH stimulation in old age (79, 80). The serum levels of 17-OHP in response to ACTH increase with age and reach a plateau level at 60 years of age (130, 131). Basal secretion of androstenedione and the response to ACTH infusion decrease with age in both men and women (130). In experimental studies, steroids that can be synthetized in the brain, such as DHA and DHAS, have been suggested to have memory-improving effects in animals (132). It is thus of interest that unaltered or decreased levels of serum DHAS have recently been reported in patients with moderate to severe dementia of various causes (133-138).

Insulin-like growth factor I (IGF-I) is an anabolic substance. IGF-I receptors have been identified in human adrenocortical cells (139) and are believed to be important in maintaining the specific functions of the adrenal cells (140). Serum levels of IGF-I decrease with increasing age (141) and this factor is thus of interest to study in patients with AD.

**Peripheral tissue sensitivity to glucocorticoids**

Despite the evidence of hypercortisolism and disturbed regulation of the LHPA axis in AD, typical features of hypercortisolism in peripheral tissues are lacking. This pattern may be explained by tissue-specific alterations in glucocorticoid sensitivity such that decreased negative feedback and hypercortisolism are only associated with increased glucocorticoid receptor activation in selected sites. It would therefore be of interest to study the effects of cortisol on peripheral tissues and the association between peripheral tissue sensitivity to glucocorticoids and negative feedback function in the LHPA axis.

**Summary of introduction**

Exposure to glucocorticoids seems to be of importance to loss of corticosteroid receptors and neurons in the hippocampus and for cognitive impairment in rodents and humans. Furthermore, there are indications of disturbances at different levels of the LHPA axis in stroke and depression, and these abnormalities are associated
with cognitive impairment. There is some evidence of dysfunction of the LHPA axis in advanced AD.

Most earlier studies on LHPA axis regulation in AD have involved small numbers of institutionalized patients with advanced dementia and hospitalized elderly with minor medical problems as controls. Therefore, there is an obvious need for detailed studies in the early stages of the disease in comparison with healthy, ambulatory elderly.

Finally, hormone levels are known to be potentially affected by a number of factors such as age, gender, and body mass index (BMI). In order to determine the independent influence of disease per se (i.e., AD) on hormone levels it is important to use modern statistical methods.

AIM OF THE STUDY

The aim of the present study was to examine the function of the LHPA axis in dementia with specific emphasis on the early phase of AD in a sample of well characterized patients.

The specific aims were:

• to evaluate the feedback function of the LHPA axis in AD;

• to examine the pituitary and adrenal responses to CRH in AD;

• to study adrenal androgens in AD and in multi-infarct dementia (MID);

• to examine the adrenal response to ACTH in AD; and

• to investigate peripheral sensitivity to exogenous glucocorticoids in AD.
SUBJECTS

The patients in studies I, II and IV were selected from patients remitted for investigation of their dementia to the Department of Psychogeriatric Medicine, University Hospital of Northern Sweden, Umeå, and of the Department of Geriatric Medicine, Huddinge University Hospital, Huddinge, Sweden, during a fourteen-month period 1990-1991. The patients were investigated as outpatients or admitted to the hospital for investigation of their dementia during three to ten days; none was hospitalized due to the disease per se. Two patients were treated with benzodiazepines in low doses, *i.e.*, 10 mg oxazepam daily. No other drugs were used. Exclusion criteria were: acute medical illness including infectious disease, endocrine disease, depression, liver disease, renal dysfunction, prostatic hyperplasia, gynaecological disorders, smoking, and excessive drinking. Patients who, after investigation, fulfilled the criteria of "probable" AD according to the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) (2) and did not fulfill any of the exclusion criteria were included in the study. Number of subjects, age, sex, severity of disease, Mini-Mental State Examination (MMSE) rating, and BMI in the studies are shown in Table 1. Some patients were included in more than one study (Fig. 3).

![Fig. 3. Number of patients studied in papers I, II and IV; some patients were included in more than one study as indicated.](image-url)
Table 1. Basic and clinical data on the subjects of papers I, II and IV.

<table>
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<th>Paper I</th>
<th>Paper II</th>
<th>Paper IV</th>
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Numbers are given as mean±SD
AD = Alzheimer's disease
dd = degree of dementia was evaluated according to Clinical Dementia Rating (CDR) (Hughes et al 1984);
mi = mild; mo = moderate
MMSE = Mini-Mental State Examination (Folstein et al 1975)
In study III, the patients were inpatients at the Department of Psychogeriatric Medicine, University Hospital of Northern Sweden, with mainly severe dementia and with a mean±SEM hospital stay of 18±1.9 months. Thirty-five were men and 51 women, with a mean±SEM age of 77.6±0.7 years. Forty-five had the diagnosis of AD and 41 MID. The dementia diagnoses were made according to DSM-III-R. MMSE was performed with a median score of 2 (range 0-23) in AD patients and 9 (range 0-23) in MID patients. Exclusion criteria for this study were: acute medical illness including infectious disease, thyroid disease, diabetes mellitus, malignant disease, smoking and treatment with estrogens or glucocorticoids.

In study V patients were 13 inpatients with probable AD according to the NINCDS-ADRDA criteria at the Department of Psychogeriatric Medicine, University Hospital of Northern Sweden, with a mean±SD age of 74.8±9.5 years. Nine were men and 4 women with a MMSE mean±SD of 18.1±3.4. Ten healthy elderly controls (5 women and 5 men, 73.5±6.1 years) participated. Nine young healthy volunteers were recruited among medical students (7 men and 2 women, mean±SD age 26.3±3.9). Eight patients with polymyalgia rheumatica (3 men and 5 women, mean age 71.5±7.5 years) who had been treated with prednisolone for a period of 13.4±3.1 months were included. Patients with polymyalgia rheumatica were treated with glucocorticoid medication. Otherwise, the same exclusion criteria as in studies I, II, and IV were used.

Healthy elderly in studies I, II, IV and V were recruited from subjects who had participated in The Umeå Longitudinal Study (142) and in endocrine studies among elderly (143). Exclusion criteria were the same as for the patients. Healthy elderly in study III were recruited from nonhospitalized individuals, participating in ongoing endocrine studies, and were 80 years old (143). None of the healthy elderly had known central nervous disease. The MMSE median in healthy elderly was 29 (range 27-30). None was taking any medicine.
METHODS

Diagnostic criteria including clinical investigations

All patients (papers I-V) were thoroughly investigated by a specialist in geriatric medicine to exclude acute or chronic illness that could influence the test results. Medical history was obtained from interviews with patients and their families or care givers. All patients were evaluated by physical examination. Laboratory tests were performed to rule out various infections, metabolic diseases, and other causes of dementia. Electroencephalogram (EEG) was performed in all patients. Cerebral CT scan was performed in 53/86 patients in study III and in all patients in the rest of the papers.

In papers I, II, IV and V, AD was diagnosed according to the exclusion and inclusion grid of McKhann and criteria for "probable" Alzheimer's disease, outlined by the NINCDS-ADRSA Work group (2). None of the patients with AD had any cerebral infarction and their white matter changes were only mild on CT scan. No patient had a medical history of cerebrovascular disease and the course of the disease displayed no evidence for vascular dementia. Maximum Hachinski score (range 0-18) was 2 points (144). The Montgomery-Åsberg Depression scale (145) (range 0-60) was used to exclude patients with concomitant depressive symptoms. Cognitive function was investigated by neuropsychological assessment including memory, orientation, visuospatial, logical and verbal tests (146, 147). Mini-Mental State Examination (MMSE) (148) was performed for all subjects in all papers. In papers I, II and IV staging of dementia severity was performed with the Clinical Dementia Rating Scale (CDR) (149). The CDR scale has five stages: not demented (CDR=0), questionable dementia (CDR=0.5), mild dementia (CDR=1), moderate dementia (CDR=2) and severe dementia (CDR=3). Functioning was judged in six areas: memory, orientation, judgement/problem solving, personal care, home/hobbies, and community affairs. Information was used from the physician's examination and information from family members or care givers. Patients with mild and moderate dementia according to CDR were regarded as in the early phase of Alzheimer's disease in papers I, II
and IV. BMI was calculated by dividing body weight in kilograms by the square of height in meters.

The diagnostic criteria used for AD and MID in paper III were those stated in Diagnostic and Statistical Manual of Mental Disorder, 3rd ed., revised (DSM-III-R). In paper III activities of daily living (ADL) were measured by the Multi-Dimensional Dementia Assessment Scale (150).

The healthy elderly were thoroughly investigated clinically by a specialist in geriatric medicine and underwent routine laboratory screening and cerebral CT scan.

The study was approved by the Ethics Committees of Umeå University and the Karolinska Institute, Stockholm.

**Dexamethasone suppression test (DST)**

In papers I and V, on the first day of study, blood was drawn with the patient in supine position after an over-night fast at 8 A.M for serum cortisol and plasma ACTH analyses. At 10 P.M. the same day 0.5 mg DEX was given orally (Decadron®, Merck Sharp & Dohme Internat., Rahway, NJ, USA). Blood was drawn on the following day at 8 A.M. for hormone analyses. In paper I the same procedure was repeated with 1.0 mg DEX for patients and control subjects after an interval of at least four days. Blood for the determination of serum cortisol was collected into plain glass tubes, let stand at room temperature to coagulate and then centrifuged within one hour; blood for plasma ACTH was collected into chilled tubes containing EDTA, and kept at 4°C before centrifugation. Tubes were centrifuged within 10 minutes and plasma samples were stored at -70°C until assayed.

**CRH stimulation test**

In paper II, an intravenous catheter was inserted into an antecubital vein at 2 P.M. after fasting since 10 A.M. One hour later each subject was injected with a single intravenous bolus dose of 1 μg/kg human CRH (Corticobiss®, Pharma, Bissendorf Peptide). Blood samples were obtained at -15, 0, 15, 30, 60, 90, and 120 minutes relative to the hCRH injection with the subject in supine position. Blood for determination of ACTH was immediately placed into prechilled tubes containing EDTA, kept on ice and
centrifugated within ten minutes at 4°C at 2000 x g for ten minutes. Plasma samples were frozen at -70°C until assayed. Blood for determination of cortisol, DHA, 17-OHP and androstenedione was centrifuged within one hour of collection, and serum was stored at -70°C until assayed.

**Adrenocorticotropic stimulation test**

In paper IV, after an over-night fast, an intravenous catheter was inserted at 8 A.M. into an antecubital vein. One hour later, serum samples were drawn at 15 minutes before and immediately before a bolus injection of 250 µg synthetic ACTH$_{1-24}$ (Synacthen®, CIBA-GEIGY AG, Basel, Switzerland). Blood samples were then obtained at 30, 60, 90 and 120 minutes after the injection with the patient in supine position throughout the test. Blood for the determination of serum cortisol, DHA, DHAS, 17-OHP, androstenedione, corticosteroid-binding globulin (CBG) and IGF-I was centrifuged within one hour of collection and the serum was stored at -70°C until assayed.

**Brain CT procedure**

In paper II, twelve patients and all healthy elderly underwent a special CT scan procedure (Siemens Somatom 2N, Erlangen, Germany). This was performed using four-mm thick slices parallel to the temporal lobes. The level and window settings were kept constant. With an electronic cursor the hippocampus was outlined bilaterally, and using Hounsfield units (HU) the pixel areas for CSF (0-18 HU) and for brain substance (19-50 HU) were calculated. The CSF/brain substance ratio was expressed as hippocampus substance index. Similar index calculations were performed for temporal lobes at the level of the lower part of the Sylvian fissures. In addition, general atrophy, temporal lobe atrophy, cortical atrophy, and the width of the third ventricle were estimated visually. These estimations were graded on a four-point scale (no, slight, moderate or severe atrophy). The two first groups were arbitrarily considered as within normal limits and the two other as abnormal. All CT scans were examined blinded by a neuroradiologist.
Skin blanching

In paper V a skin blanching assay was used, modified from that of Mc Kenzie and Stoughton (151, 152). A synthetic glucocorticoid, clobetasol, was dissolved in 95% ethanol to final concentrations of 0, 0.3, 1, 3, 10, 30 and 100 μg/ml. The test solutions were applied on the volar side of the subject's forearm during 15 hours. The degree of skin blanching was determined after another 3 hours to allow time for disappearance of any tape-induced skin irritation.

Assay of hormones, IGF-I and CBG

Radioimmunological or immunoradiometric (ACTH) techniques were used for determination of hormones, IGF-I and CBG. Serum concentrations of cortisol were determined in untreated samples using commercial kits from Diagnostic Products Corp., Los Angeles, CA (papers I, II, IV) and Orion Diagnostica (previously Farmos Diagnostica) OY, Finland (papers III, V). Plasma ACTH and serum CBG and IGF-I were determined using commercial kits from Eurodiagnostics Ltd, Apeldoorn, Holland (ACTH), Medgenix SA, Fleurus, Begium (CBG), and Nichols Institute Diagnostics Inc., San Juan Capistrano, CA (IGF-I). In papers II and IV serum concentrations of DHA, DHAS, androstenedione and 17-OHP were determined after extraction with diethyl ether with methods developed at the Hormone Laboratory, Department of Obstetrics and Gynaecology, Huddinge University Hospital (153-155). In the assay of DHAS the sulphoconjugate was cleaved by thermal hydrolysis prior to extraction. In paper III, DHAS was determined in untreated serum using an antibody from Endocrine Sciences, Tarzana, CA. The detection limits and within- and between-assay coefficients were as follows: for cortisol 11 nmol/l, 4.5 and 7% (papers I, II, IV) and 5 nmol/l, 7.2 and 8.1 % (papers III, V); for ACTH 0.8 ng/l, 4.5 and 5.4%; for CBG 0.25 mg/l, 5 and 7.7%; for IGF-I 0.6 μg/l, 6 and 10%; for DHA 0.8 nmol/l, 5 and 7%; for DHAS 200 nmol/l, 8 and 12% (papers II and IV), 13 and 4.9% (paper III); for androstenedione 0.6 nmol/l, 6 and 10% and for 17-OHP 0.1 nmol/l, 6.7 and 9.5%. Apparent concentrations of serum cortisol were
calculated from cortisol and CBG concentrations using an equation supplied by the manufacturer of the CBG kit.

**Statistical methods**

All analyses were made in a computerized statistical program, SYSTAT® (156). Due to skewed distribution of data, the statistical work-up was performed after natural logarithm transformation in papers II and III. Pearson correlation coefficients were used for the calculations of correlations in papers I, III, IV and Spearman correlation coefficients in papers II and V. One-way analysis of variance (ANOVA) was used to test mean differences in hormone levels between groups in papers I, III, and IV; Student's t-test was used in papers I and II, and the Mann Whitney U-test was used in paper V. A multiple general linear hypothesis program (156) was used in all papers for multivariate analyses of covariance and for the multiple linear regression studies (MANOVA) in papers II and III after factor analyses, using the principal component analysis model with varimax rotation. The multiple regression studies were performed with the use of dummy variables (0/1 corresponding to no/yes) when necessary. Two-tailed t-tests were utilized for testing of the regression coefficients of each independent variable against the dependent variable. Partial correlation coefficients were used in paper IV to estimate correlation between hormone levels. Fisher's exact test was used in paper I to test differences between groups. P-values of <0.05 were considered significant.

**RESULTS**

The major findings in this study are:

increased serum cortisol levels but decreased plasma ACTH levels in response to a "low-low" (0.5 mg) DEX dose in AD patients;

blunted ACTH response combined with relative increases in cortisol, DHA and androstenedione resposiveness to CRH stimulation in AD patients;
low basal serum levels of the adrenal androgen DHAS in advanced AD and MID, but increased basal levels and increased responsivity of the adrenal androgens, DHA and androstenedione, to ACTH stimulation test in AD patients in the early phase of AD;

decreased skin blanching in response to a synthetic glucocorticoid, clobetasol, in patients with AD.

Dexamethasone suppression test with different doses in early Alzheimer's disease (I)

Patients with early AD were compared with healthy elderly in their response to different doses of DEX. Basal serum cortisol levels did not differ between groups. After 0.5 mg DEX, serum cortisol levels were significantly higher in patients with AD (p=0.03). After 1.0 mg DEX, serum cortisol levels were higher in AD patients, although not significantly so (p=0.06) (Fig. 4A-B). When the conventional cut-off limit of a post-DEX level of 138 nmol/l at 8 A.M. was used (107), 14/35 patients with AD versus 2/20 healthy elderly were non-suppressors after 0.5 mg DEX (p=0.03). After 1.0 mg DEX, 2/33 were non-suppressors among AD patients and 0/20 among healthy elderly.

Basal plasma ACTH levels were somewhat lower in AD patients. After 0.5 mg and 1.0 mg DEX this difference was accentuated and significant (p=0.01 and p<0.001, respectively) (Fig 5).

In a multiple linear regression analysis serum cortisol levels after 0.5 mg DEX were best predicted by basal cortisol levels (standardized regression coefficient, St.c.=0.48, p<0.001), followed by AD (St.c.=0.23, p=0.10). After 1.0 mg DEX independent predictors for high cortisol levels were basal cortisol levels (St.c.=0.41, p=0.004), AD (St.c.=0.40, p=0.009), and male sex (St.c.=0.37, p=0.01). In similar multiple regression models, low plasma ACTH levels after 0.5 mg DEX were best predicted by AD and basal ACTH levels (St.c.=0.40, p=0.04 and St.c.=0.30, p=0.06, respectively). After 1.0 mg DEX the only significant independent predictor for low plasma ACTH was AD (St.c.=0.38, p=0.04).
**Fig. 4A.** Serum cortisol levels before and after 0.5 mg DEX in healthy controls and in AD patients.

**Fig. 4B.** Serum cortisol levels before and after 1.0 mg DEX in healthy controls and in AD patients.
CRH stimulation test of pituitary and adrenal function in early Alzheimer's disease (II)

The reactivity of the LHPA axis was evaluated by stimulation with human CRH (hCRH) in 23 patients with early, i.e., mild to moderate, AD and compared with 19 healthy elderly. hCRH-stimulated plasma ACTH levels were significantly lower in AD patients (Fig 6). This abnormality in ACTH increase persisted after adjustment for age, gender and BMI in a multivariate analysis. Women had significantly higher peak serum cortisol levels than men (p=0.001).
Fig. 6. Plasma ACTH levels before and after hCRH in healthy elderly and in AD patients.

AD patients had higher steroid responses relative to the amount of ACTH released during stimulation with hCRH. Thus, serum cortisol, DHA and androstenedione output per unit ACTH in the bloodstream were significantly increased in AD patients after adjustment for possibly confounding factors.

At CT scan, six AD patients had slight temporal lobe atrophy, three moderate, and three patients showed severe temporal lobe atrophy. Among healthy elderly, eleven had no temporal lobe atrophy, six slight, and in two subjects moderate atrophy was seen.

In a multiple regression analysis, low MMSE rating was independently associated with both temporal lobe atrophy (St.c.=0.73, p<0.001) and general brain atrophy (St.c.=0.68, p<0.001).

Temporal lobe atrophy was significantly correlated to basal plasma levels of ACTH when patients and healthy elderly were included in the analysis (r_S=0.46; p=0.02). Among AD patients, the hippocampus substance index was significantly correlated with peak plasma levels of ACTH after hCRH injection, i.e., pronounced hippocampal atrophy was associated with low ACTH responsiveness (r_S=0.75, p=0.01). No associations were found between steroid hormone levels and neuroradiological parameters.
DHAS in advanced Alzheimer's disease and multi-infarct dementia (III)

Patients with advanced AD and MID had significantly lower basal serum DHAS levels than healthy elderly (p=0.004 and p=0.009, respectively). In a multivariate analysis, high age and AD were independent predictors of serum DHAS levels. There was no significant difference in serum cortisol levels between demented patients and healthy elderly, resulting in a significantly higher cortisol/DHAS ratio for AD patients.

Adrenal androgens in early Alzheimer's disease (IV)

AD patients had significantly higher basal levels of serum DHA and androstenedione than healthy elderly. These differences were accentuated after ACTH stimulation (Fig. 7). In general linear regression models, AD had an independent influence on

![Graphs showing serum DHA and androstenedione levels before and after ACTH stimulation in healthy elderly and AD patients.](image)

Fig. 7. Serum DHA (left) and androstenedione (right) before and after ACTH in healthy elderly and in AD patients.
androstenedione and DHA basal levels (St.c.=0.42, p=0.003 and St.c.=0.36, p=0.04, respectively), on peak androstenedione levels (St.c.=0.64, p<0.001), and on peak DHA levels (St.c.=0.36, p=0.02).

Furthermore, in these models significant negative associations were found between age and peak DHA level (St.c.=-0.43, p=0.008), and positive association between male sex and basal androstenedione values (St.c.=0.59, p<0.001). BMI had an independent influence on basal serum cortisol level (St.c.=0.47, p=0.01), and on basal and peak androstenedione level (St.c.=0.32, p=0.03 and St.c.=0.38, p=0.01, respectively). There were no significant differences between patients and healthy elderly in circulating CBG, free cortisol concentrations or in serum concentrations of IGF-I.

In order to analyze if hormonal interrelationships were affected by AD, partial correlations were calculated between hormone levels in AD patients and healthy elderly. Positive correlations between androgens were seen in healthy elderly. In contrast, no significant associations were seen between hormones within the AD group after ACTH stimulation.

The increase in 17-OHP levels (Δ response) was negatively correlated to age in the AD patients (r=-0.60, p=0.008) but not in the healthy elderly (r=0.01). Serum levels of IGF-I and CBG were negatively correlated to age in the healthy elderly (r=-0.60 and r=-0.51; p=0.007 and p=0.02, respectively) but not in the patients (r=-0.02 and r=-0.19, respectively). A positive correlation between the ACTH-induced increase of androstenedione and basal IGF-I levels was found in the combined patient and control group (r=0.37, p=0.02). This correlation was mainly confined to the patient group (r=0.37 and r=0.01, for patients and healthy elderly, respectively).

Peripheral glucocorticoid sensitivity in Alzheimer's disease (V)

All healthy, young and elderly, showed skin blanching at a clobetasol concentration ≤3 μg/ml, whereas 4/13 patients with AD showed skin blanching at a clobetazol concentration ≥ 10 μg/ml. Two out of 8 patients with polymyalgia rheumatica showed skin blanching at a clobetasol concentration ≥ 10 μg/ml, thus showing the same blanching pattern as the patients with AD (Fig 8). There
was no correlation between the results from the skin tests and the post-dexamethasone serum cortisol levels.

Fig. 8. Lowest levels of clobetasol concentrations at which skin blanching could be detected in different groups of healthy controls and patients. Healthy old = Old volunteers; Healthy young = young volunteers; AD = patients with Alzheimer's disease; PMR = patients with polymyalgia rheumatica with ongoing glucocorticoid treatment.
DISCUSSION

There are abnormalities at several levels of the LHPA axis in patients with Alzheimer's disease (AD). However, in order to be able to discuss the general validity of these results it is important to discuss the diagnostic criteria used for AD and multi-infarct dementia in this thesis.

Diagnostic criteria

In four of the studies the NINCDS-ADRDA criteria for probable AD were used (2). These criteria have satisfying interrater reliability and validity (157-159). Kukull et al (1990) (160) compared the interrater reliability of three established criteria and found that the NINCDS-ADRDA criteria had the highest interrater reliability among clinicians. Lopez et al (1990) compared the interrater agreement between two neurologists and two psychiatrists and concluded the NINCDS-ADRDA enabled moderate levels of agreement among clinicians in general (161). The validity has been examined against Khachaturian neuropathological criteria (10) and has been found to have a high sensitivity (92%) but lower specificity (65%) (162). Recent data suggest that specificity increases to a major extent with more stringent inclusion criteria, especially in an older patient population (aged 64 or older) (163). In study III, DSM-III-R (1) was used for the diagnosis of AD and MID. It was used in this study on clinical grounds in consecutive patients and was used to be able to diagnose both AD and MID.

There are difficulties in staging dementia, as there is no scale available solely for studying the natural course of the disease. We used the first version of the CDR, a scale including cognitive dysfunction, social activities and ADL functions (149, 164) in papers I, II, IV. This scale has good interrater reliability and is a widely used instrument (165, 166). Patients in studies I, II and IV with mild and moderate dementia according to CDR were regarded as in the early phase of AD, due to the rating and the fact that they were ambulatory. In study III, the Multi-Dimensional Dementia Assessment Scale (150) was used to measure ADL-functions.

A dilemma is how to assess depressive symptoms in dementia as depression may manifest itself differently in patients with
dementia (167). The instrument used to measure depressive symptoms, the Montgomery-Åsberg Depression Scale, has been shown to have high reliablity (168, 169).

The limbic-hypothalamic-pituitary-adrenal axis

It is possible that the syndrome of hippocampal atrophy, LHPA axis dysfunction and cognitive disability that has been shown in rodents may be applicable to the human situation. This association is strengthened by a recent study of patients with Cushing's syndrome where a significant association between increased peripheral cortisol levels and hippocampal atrophy was shown (170). The primary and secondary events behind these symptoms and signs are not clear. It was originally suggested by Sapolsky et al (124) that elevation of glucocorticoids by various mechanisms could lead to a decreased central feedback function with ensuing hypersecretion of glucocorticoids. That would eventually lead to cell death in hippocampal neurons, as they have a high density of glucocorticoid receptors. This may be applicable to patients with Cushing's syndrome and certain patients that develop depressive disorders. However, it is possible that primary changes in the brain may explain most abnormalities of LHPA dysfunction in AD.

As the LHPA axis is a multilevel axis, it is necessary to evaluate both basal and dynamic aspects of the system's function. We have thus measured basal hormone levels, feedback sensitivity (the dexamethasone suppression test) and the sensitivity of the axis at the pituitary and adrenal levels to stimulation by CRH and ACTH.

The hippocampus in LHPA axis regulation in Alzheimer's disease

Our findings of a subtle defect in feedback regulation of the LHPA axis in patients with early AD emphasize the need to use graded doses of DEX to characterize feedback sensitivity.

The decreased sensitivity to DEX in AD patients may be due to functional alterations in glucocorticoid receptors at the hippocampus level, or more likely, at the pituitary level (171). Alternatively, the decreased sensitivity of the pituitary to DEX is related to an abnormal central control from higher brain centers,
including the hippocampus. The hippocampal region is an early and major target during the disease course. This has been shown by autopsy studies (19, 172) and by CT scan, as in our study, and by MRI (24, 25, 173). Connections from the hippocampus to various levels of the LHPA axis may influence glucocorticoid output, for review see (71). This may be mediated by direct connections to the hypothalamus, indirect or direct connections with the pituitary, and by direct connections to the adrenal glands through splanchnic nerves (71). A consistent finding after hippocampal lesions in both rats and primates is resistance to DEX-mediated lowering of endogenous corticosteroid levels (71, 72). However, hippocampal or fornix lesions do not produce complete resistance to corticosteroid feedback; only the glucocorticoid dose required to achieve inhibition increases, which seems well in agreement with our data (71).

Within the hippocampus both types of glucocorticoid receptors, mineralocorticoid receptors (MR) (type I) and glucocorticoid receptors (GR) (type II) are abundant. In rodents, MR receptors seem to be involved mainly in the basal activity of the LHPA axis and the threshold or reactivity of the neuroendocrine responses to stress, while GR mediate the negative feedback action. Hypercortisolism in the absence of abnormal feedback function may thus represent a type-I receptor defect. It would therefore be of interest to study cortisol production rates, metabolism and diurnal rhythms at various stages of AD. However, recent data indicate the importance of both glucocorticoid receptor types for control of basal and peak/stress-induced glucocorticoid levels (174, 175). Our data on an early defect in DEX suppressibility may be related to a defect in type II receptors, mainly located, at least in rodents, in the pituitary. However, chronic activation of type-I receptors can lead to down regulation of type-II receptors (176). It is thus not clear if feedback sensitivity to glucocorticoids in elderly humans is related to type-I or type-II receptor activation. Suppression tests with an agonist to type-I receptors, e.g. aldosterone, may shed further light on this issue (171).

A potential problem with the DST is non-compliance (177). In this study, careful instructions were given to the spouse; alternatively, when the patient was living alone, I visited the patient to administer the drug to avoid non-compliance.
Furthermore, it would be of interest to take into account plasma DEX measurements in further studies of this patient group as differences between patients and healthy elderly in DEX metabolism might influence the results. However, it has been shown repeatedly that plasma DEX is a minor contributor to cortisol levels after DEX in patients with major depression (110). Furthermore, two earlier studies of AD patients in which plasma DEX levels after 1.0 mg DEX were analyzed revealed no differences between patients and healthy elderly (104, 178).

A possible starting point for the vicious circle ending with high glucocorticoid levels damaging sensitive parts of the brain may be an abnormal neuronal input to the hippocampus, an input which affects glucocorticoid receptor expression. This is supported by several experimental findings. Depletion of brain serotonin attenuates feedback function (179). Furthermore, lesions of central serotonergic systems decrease the expression of GR and MR in subregions of the hippocampus (180). In contrast, medial septal cholinergic lesions increase hippocampal MR and GR mRNA expression (181). Thus, combined lesions of the serotonin and the acetylcholine systems, not unexpectedly, lead to unchanged glucocorticoid receptor expression in the hippocampus (Yau, personal communication). This is of interest in relation to recent findings of unaltered glucocorticoid receptor expression in the hippocampus in patients dying from advanced AD (182). In combination with a possible decrease in plasticity of receptors in old age, a failure of receptor down regulation in response to various stressors may result in more or less continuous over-exposure to glucocorticoids. This is again most dangerous for the glucocorticoid-sensitive neurons in the hippocampus.

An increase in the expression of glucocorticoid receptors in the hippocampus may be an interesting option. How can one counteract these abnormalities? Recent animal data suggest that this is indeed possible. Thus handling of infantile rats, as referred to earlier in this text, is associated with an increase in hippocampal GR expression and binding, leading in late life to preserved spatial memory and adequate termination of stress responses (69). The effect of handling is suggested to be mediated through the serotonin system (69). Furthermore, substantial increases in hippocampal glucocorticoid receptor expression have been shown
after treatment with antidepressant drugs in rats (183, 184), and this is associated with improvement in memory in young, but not old, animals (Yau, personal communication). Finally, changes in environment ("enriched" versus "isolated" environment) are associated with profound changes in glucocorticoid receptor expression; high GR mRNA expression in the hippocampus being associated with improved spatial memory after one month of environmental enrichment (185).

The association between cognitive function and temporal lobe atrophy is in line with earlier findings (186). Temporal lobe atrophy probably indirectly reflects the early involvement of the limbic system, mainly the hippocampal region in AD. It has been shown that CSF volumes adjacent to the hippocampus have the strongest correlation with memory, suggesting a connection between hippocampal atrophy and impaired memory in AD (187).

Adrenocorticotropic regulation in Alzheimer's disease

The blunted ACTH response to CRH stimulation agrees with earlier studies of small groups of AD patients with moderate to severe dementia (118-120). CRH hypersecretion may thus lead to an adaptive down regulation of CRH receptors in the anterior pituitary, as seen in studies of aged rats (188, 189). Possibly a cholinergic deficit may lead to hypersecretion of CRH in the early phase of AD with subsequent down regulation of corticotroph receptors (190). However, there are a number of other inputs to the pituitary including, e.g., vasopressin, that can theoretically influence ACTH secretion (191).

However, hypercortisolism also occurs in conjunction with normal or low levels of ACTH in depression and anorexia nervosa (192-194). An alternative explanation for this scenario may be that hypercortisolism may be a primary event in AD, at least in part a consequence of enhanced adrenocortical sensitivity to low circulating levels of ACTH. Thus, lowering of peripheral glucocorticoid levels by a steroid synthesis inhibitor could be undertaken to explore if a primary increase of glucocorticoid levels contributes to feedback insensitivity. An increase in circulating ACTH levels, including an increased responsiveness to CRH after blockage of steroid synthesis, would be consistent with this. If the
association between low circulating ACTH levels and temporal lobe/hippocampal atrophy can fit into this model of primary glucocorticoid excess remains unclear.

Earlier findings of an increased cortisol response to CRH stimulation in women (195) are confirmed in this study among healthy elderly. However, among AD patients there were no differences in cortisol response to CRH between men and women.

We have used human CRH. Ovine CRH has a prolonged effect, probably due to its delayed clearance. We find it more physiological to use human CRH in these studies. However, a confounding factor when using human CRH may be an increase in CRH-binding protein levels in AD patients, and this should be studied further (196).

**Adrenal function**

We confirm and extend earlier studies that show that there are low levels of serum DHAS in advanced AD and MID (135, 138). Furthermore, we have shown that this decrease is persistent after adjustment for age and sex. The interest in an interaction between steroid hormones other than glucocorticoids and central nervous functions has been renewed during recent years. Thus, both high and low levels of "neurosteroids" may be associated with profound effects on mood, behaviour, seizure susceptibility and memory (197), and a recent study showed a loss of the diurnal rhythm of DHA variation with increased cortisol/DHA ratios in major depression (198). Many effects of these steroids may be mediated by GABA and N-methyl-D-aspartate (NMDA) receptors after local conversion in the brain of androgens to estrogens (197). These "neurosteroids" have mainly excitatory effects on neurons (199). However, excessive stimulation may mediate neural degeneration through the activation of NMDA receptors (200). Interference with synthesis of/or supplementation with DHA(S) or other "neurosteroids" during different phases of the disease may merit future studies in AD and in major depression. It is of interest that oral administration of DHA for 6 months led to improvement in physical and psychological well-being in healthy elderly subjects (201).
The high cortisol/DHA ratio in advanced dementia may be of pathophysiological importance. As glucocorticoids have been suggested to have a toxic effect on neurons (202) and DHA has been proposed to act as an antiglucocorticoid (203, 204), a high cortisol/DHAS ratio may thus damage mainly hippocampal cells because these neurons seem to be preferentially sensitive to the toxic effects of glucocorticoids (205).

In early AD both basal and ACTH-induced levels of the C₁₉ steroids were elevated. This may fit with an increased adrenocortical sensitivity to ACTH. However, a highly abnormal, age-related decrease in the Δ₁₇-OHP was found in the patients, resembling an adrenarchal/pubertal pattern (130). This may indicate a redistribution of the steroid flux in order to keep the cortisol levels constant. Alternatively, an increased central nervous drive in early AD may lead to an alteration in the splicing of proopiomelanocortin to β-endorphin and β-lipotropin (206). An altered ratio of these products might contribute to abnormal adrenal stimulation with a change in zonation.

The expected age-related decrease in serum IGF-I levels was found in healthy elderly but not in AD patients. IGF-I acts synergistically with ACTH and is also capable of inducing steroidogenesis on its own (140). In this study a correlation was found between basal serum IGF-I levels and androstenedione peak levels after ACTH stimulation. This correlation was mostly confined to the AD patients. As there were no differences between IGF-I levels in patients and healthy elderly, this finding is of unclear importance.

The well-known and expected relationships between different adrenal steroids (130) were found in the healthy elderly but not in the AD patients. These results indicate abnormalities in either metabolism or secretion of adrenocortical C₁₉ steroids in early AD. The causes for this are not known.

In the ACTH stimulation test we used the standard dose, 250 µg synthetic ACTH. This is a "supraphysiologic" dose. It would be of interest to use a lower, more physiological dose, to evaluate the existence of a more subtle abnormality in the sensitivity to ACTH stimulation of the adrenal gland. A partial support for this hypothesis is given from our findings of an increased relative
steroid hormone output after CRH stimulation (paper II in this thesis).

Peripheral glucocorticoid sensitivity

The decrease in glucocorticoid responsiveness in patients with AD in the skin vasoconstrictor assay is comparable to that in patients undergoing long-term glucocorticoid treatment. This may be due to a generalized primary defect in glucocorticoid receptors in AD. A generalized alteration of steroid receptor function is in keeping with our findings of decreased responsiveness to topical glucocorticoids in the skin assay. This bears similarities to accumulating evidence of peripheral adrenal steroid receptor dysfunction in depressed patients, including decreased steroid receptors in peripheral blood mononuclear cells and reduced sensitivity of immune cells to the inhibitory effects of glucocorticoids, for review see (171). Alternatively, the decrease in peripheral sensitivity may be due to a down regulation of glucocorticoid receptors secondary to increased exposure to free hormone levels.

The lack of correlation between skin sensitivity and serum cortisol levels after DEX may indicate both a disturbance in peripheral sensitivity to glucocorticoids and a central glucocorticoid receptor abnormality in different subgroups of AD patients.

CONCLUSIONS

This thesis has given a basic description of the function of the LHPA axis at different levels in patients with AD. The results indicate multiple disturbances in the LHPA axis in AD:

- increased serum cortisol levels but decreased plasma ACTH levels in response to a "low-low" (0.5 mg) DEX dose in mild to moderate AD;
• blunted ACTH response combined with a relative increase in cortisol, DHA and androstenedione responsiveness to CRH stimulation in mild to moderate AD;

• low basal serum levels of the adrenal androgen DHAS in advanced AD and MID in combination with no differences in cortisol levels, resulting in a high cortisol/DHAS ratio;

• increased basal levels and increased responsiveness of the adrenal androgens, DHA and androstenedione, to ACTH stimulation test in AD patients in the early phase of AD;

• decreased skin blanching in response to a synthetic glucocorticoid, clobetasol, in patients with AD.

There are thus several lines of research, including the data presented in this thesis which point towards the possibility of major interactions between LHPA axis dysfunction and the development of cognitive dysfunction. The possible importance of this for the development and/or progression of neurodegenerative disease is intriguing.
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