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Glucocorticoid Receptors on Mononuclear Leukocytes in Alzheimer's Disease

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Abstract. Several lines of evidence suggest disturbances of the hypothalamic-pituitary-adrenal (HPA) system in Alzheimer's disease (AD). In an exploration of the potential role of the glucocorticoid receptor (GR) in AD, GR density and affinity were assessed on mononuclear leukocytes of 12 AD patients and 12 healthy controls. GR binding characteristics did not differ between patients and controls or between patients subdivided according to diagnosis or associated clinical features. These data suggest that the abnormalities of the HPA system in AD are not related to a GR deficiency.

Key Words. Glucocorticoid receptors, Alzheimer's disease, aging.

Abnormalities of the hypothalamic-pituitary-adrenal (HPA) system linked to Alzheimer's disease (AD) include insufficient cortisol suppression following dexamethasone administration (Raskind et al., 1982; Spar and Gerner, 1982; Davis et al., 1986) and hypersecretion of cortisol (Davis et al., 1986). A recent study noted elevated 36-hour cortisol levels in AD patients that tended to normalize during the second sampling night following adaptation to the experimental procedure, while 36-hour levels of adrenocorticotropic hormone (ACTH) and corticotropin releasing hormone (CRH)-stimulated ACTH values did not differ from those of nondemented controls (Heuser et al., 1989).

Although several lines of evidence suggest multiple disturbances of neurotransmitter function in the central nervous system (Hardy et al., 1985; Sofic et al., 1988) as responsible for the hypercortisolemia observed in AD (Davis et al., 1986), the exact origin of the HPA system disturbance is still to be resolved. As glucocorticoids exert most of their effects via specific intracellular receptors, which are known to decrease during aging independent of age-related cell loss (Sapolsky et al., 1986) and which may be down-regulated by glucocorticoids (Schlechte et al., 1982), the present study was designed to explore the potential role of the glucocorticoid receptor (GR) in the disturbance of the HPA system in AD in relation to age, duration, and severity of illness.

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Methods

Subjects. Twelve medication-free patients (5 men and 7 women) meeting *DSM-III-R* criteria (American Psychiatric Association, 1987) for primary degenerative dementia of the Alzheimer type (DAT) and NINCDS-ADRDA criteria (McKhann et al., 1984) for probable AD were studied at least 7 days following hospital admission. Their mean age and their mean body weight were 65.1 ± 9.7 (± SD) years and 72.5 ± 10.2 (± SD) kg, respectively.

Patients were evaluated by physical examination, routine laboratory screening, and various imaging techniques, including event related potentials, brain electrical activity mapping (BEAM), computed tomography (CT) or magnetic resonance imaging (MRI), and single photon emission computed tomography (SPECT) with HMPAO (hexamethylpropylenamine-oxime), all of which showed results compatible with DAT—e.g., decreased cerebral blood flow, atrophy, and electroencephalographic (EEG) slowing, especially in the parietotemporal brain regions. The mean duration of illness was 3.7 ± 1.6 (± SD) years.

Six patients suffered from presenile and six from senile DAT. Severity of dementia was assessed by Folstein's Mini-Mental State (MMS) examination (Folstein et al., 1975) and the Brief Cognitive Rating Scale (BCRS; Reisberg et al., 1983) as rating scales, and the Short Syndrome Test (SKT; Erzigkeit, 1986) as a psychometric test. The mean (± SD) scores were 19.3 ± 5.7 for the MMS, 35.6 ± 9.4 for the BCRS, and 19.1 ± 3.7 for the SKT. Six of the patients additionally met *DSM-III-R* criteria for DAT with depression.

Twelve healthy subjects (3 men and 9 women) served as controls. Their mean (± SD) age was 62.5 ± 12.6 years, and their mean (± SD) body weight was 67.6 ± 8.7 kg.

For determination of GR binding characteristics and hormone levels, 50 ml of blood were collected at 1600h as previously noted (Rupprecht et al., in press). The controls were free of any psychiatric history and had not taken any medication for at least 4 weeks. All female subjects were postmenopausal at the time of the study.

GR Assay. GR pharmacological characteristics were determined as described in detail elsewhere (Rupprecht et al., in press). In brief, a mononuclear cell fraction was prepared by sodium metrizoate-Ficoll density gradient centrifugation (Boyum, 1968). Cells were washed two times in PBS for 10 min, incubated for 60 min at 37 °C, and then washed again to allow sufficient dissociation of endogenous hormone. The final concentration of cells was determined using a Coulter Counter (Model S5, Coulter Electronics Ltd, England). Viability of cells exceeded 95%, as judged from their ability to exclude trypan blue. Contamination by erythrocytes was $< 10\%$, and contamination by granulocytes and monocytes was $< 8\%$. The cells were incubated with increasing amounts of ^{3}H-dexamethasone with 10 μM unlabeled dexamethasone added to determine nonspecific binding. After a 90-min incubation, bound ligand was separated from free ligand by rapid filtration through Scatron filters with a Titertek cell harvester. The filters were monitored for tritium in a Beckman LS 1801 counter at about 54% efficiency. All samples were assayed in triplicate with a variation within a single experiment of $< 7\%$.

Hormone Assays. ACTH was measured by a newly developed IRMA supplied by the Nichols Institute (San Juan Capistrano, CA), which does not require extraction procedures (Raff and Findling, 1989). The lower detection limit was 1.5 pmol/l, and the intra-assay and interassay coefficients of variation were 3% and 6.8%, respectively.

Cortisol was measured by a direct radioimmunoassay (Stalla et al., 1981). The lower detection limit was 25 nmol/l, and the intra-assay and interassay coefficients of variation were 5% and 9%, respectively.

Data Analysis. Preliminary estimates of binding parameters from saturation experiments were provided by the EBDA program (McPershon, 1983). Final estimates of binding parameters were determined with a computerized nonlinear, least-square regression analysis (Munson and Rodbard, 1980). The results are expressed as the mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA) followed by post hoc comparison with Student's t test and Pearson's product-moment correlation. All significance levels are two-tailed.

Results

No significant difference was found for ACTH or cortisol levels (Table 1). GR sites per cell ($F = 1.1$, $df = 23$, $p = 0.2$) and GR affinity ($F = 0.3$, $df = 23$, $p = 0.14$) did not differ between the groups (Table 2). Age failed to correlate with GR sites per cell or GR affinity in either patients or controls or in both groups combined (Table 3).

Table 1. ACTH and cortisol levels of DAT patients and controls

	ACTH (pmol/l)		Cortisol (nmol/l)	
Subjects	**Mean**	**SD**	**Mean**	**SD**
Patients ($n = 12$)	3.6	3.0	262.1	93.8
Controls ($n = 12$)	5.1	6.9	229.7	79.3

Note. DAT = dementia of the Alzheimer type. ACTH = adrenocorticotropic hormone.

GR density and GR affinity did not correlate with duration of illness, severity of DAT measured by MMS, BCRS, or SKT scores, or levels of ACTH or cortisol.

Table 2. GR sites/cell and *Kd* levels of DAT patients and controls

	GR sites/cell		*Kd* (n*M*)	
Subjects	**Mean**	**SD**	**Mean**	**SD**
Patients ($n = 12$)	4065	1249	14.3	3.2
Controls ($n = 12$)	3270	1300	11.1	6.1

Note. GR = glucocorticoid receptor. DAT = dementia of the Alzheimer type.

Moreover, no differences in GR binding characteristics were observed between men and women, senile and presenile DAT patients, or depressed and nondepressed DAT patients.

Table 3. Age: Correlations with GR sites/cell and *Kd*

	GR sites		*Kd*	
Subjects	*r*	*p*	*r*	*p*
Patients	0.02	NS	0.36	NS
Controls	0.03	NS	-0.03	NS
Both groups	0.06	NS	0.11	NS

Note. GR = glucocorticoid receptor.

Discussion

This study did not reveal disturbances of GR pharmacological parameters on mononuclear leukocytes in AD. In addition, neither GR density nor GR affinity were related to duration of illness, severity of DAT, or depressed/nondepressed subtype.

These findings support the hypothesis that the mild hypercortisolemia partly observed in DAT patients (Davis et al., 1986) is not capable of down-regulating the GR. Moreover, hypercortisolemia and insufficient suppressibility of cortisol by dexamethasone are not likely a consequence of a specific GR defect occurring in AD but may arise from an impaired ability of DAT patients to adapt to stressful events—e.g., hospital admission or a neuroendocrine test protocol (Heuser et al., 1989). In this study, ACTH and cortisol levels of the patients did not differ from those of controls. However, it should be noted that we studied GR binding characteristics only under baseline conditions, which may not detect a potential slight deficiency of the GR autoregulation capacity being highly sensitive also in humans (Rupprecht et al., in press). Furthermore, there may be differences in GR regulation between distinct brain regions and peripheral blood cells. Type I and Type II corticosteroid-receptors have been shown to underlie a more sensitive regulatory effect of corticosteroids in the hippocampus in comparison to other brain regions (McEwen et al., 1987). Moreover, an exaggerated cortisol reponse to a glucose tolerance test has recently been reported to be related to hippocampal atrophy in DAT patients (de Leon et al., 1988). Thus, the regulation of both subtypes of corticosteroid receptors in the brain with their behavioral and endocrinological implications is highly complex (De Kloet et al., 1987), and it would be premature to attempt to generalize from peripheral hormonal levels or receptor pharmacology to putative brain function.

In addition, GR autoregulation has recently been shown to be impaired in aged rats, possibly due to a deficit in GR biosynthesis (Eldridge et al., 1989). A decrease of GR autoregulatory plasticity comprising GR density with increasing age might therefore play a role in the changes in the activity of the HPA system observed during aging (Landfield et al., 1978).

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