

Oxidative stress-mediated DHEA formation in Alzheimer's disease pathology

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Abstract

An alternative pathway for dehydroepiandrosterone (DHEA) synthesis has been suggested by treating rat and human brain cells with ferrous sulfate and β -amyloid (A β). To determine if this pathway exists in human brain, levels of DHEA in hippocampus, hypothalamus and frontal cortex from Alzheimer's disease (AD) patients and age-matched controls were measured. DHEA is significantly higher in AD brain than control, and was highest in AD hippocampi. Cytochrome P450 17 α -hydroxylase, responsible for peripheral DHEA synthesis, is not present in hippocampus. DHEA levels in AD cerebrospinal fluid (CSF) were significantly higher than age-matched controls. AD serum DHEA levels are lower than CSF, and not significantly different from controls. Treatment of control hippocampus, hypothalamus and serum with FeSO₄ increases DHEA, suggesting that levels of precursor are higher in control than in AD brain. This suggests that (i) an alternative precursor is present in control brain, (ii) AD brain DHEA is formed by oxidative stress metabolism of precursor, and (iii) CSF DHEA levels and serum DHEA formation in response to FeSO₄ may serve as an indicator of AD pathology.

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1. Introduction

3 β -Hydroxy-5-androsten-17-one (DHEA) is a major adrenal steroid in humans [31]. Peripheral levels peak early in adulthood and gradually decline with aging [2,31]. The role of DHEA in the periphery is not well established, but it may serve as a precursor for androgens. Levels of DHEA in the brain exceed those seen in the periphery, and are maintained after the removal of peripheral steroidogenic endocrine glands [1,14]. DHEA and its sulfated form are considered neuroactive because they affect the GABA_A receptor complex and NMDA-mediated glutamate transmis-

sion via sigma receptors [5,26]. DHEA and DHEA sulfate also modulate learning and memory [3,15]. While the role of DHEA as a neurosteroid is well established [4], the mechanism by which DHEA is produced in the brain has been debated. Evidence for the existence of active cytochrome P450 17 α -hydroxylase (P450c17), the peripheral enzyme that catalyzes the formation of DHEA in the adrenal gland, has been contradictory. One group of investigators have provided some evidence for the expression of P450c17 mRNA early in development [13], but were not able to detect message in the adult rodent [29]. Another report describes the presence of P450c17 mRNA in the adult rodent [35], but evidence for either protein or enzymatic activity has not been forthcoming [11,24]. The presence of a P450c17-independent pathway for DHEA synthesis in rat brain extracts [32] and in rat and human cells [8,11,12] was recently demonstrated. Intracellular levels of reactive oxygen species (ROS) induced by treatment with ferrous ions or β -amyloid peptide (A β) regulate this pathway [8] by acting on an as yet unidentified precursor, probably a C-17 or C-20 oxygenated steroid [12].

Recent evidence suggests that inflammation and the production of free radicals may play a large role in the

Abbreviations: DHEA, 3 β -hydroxy-5-androsten-17-one or dehydroepiandrosterone; P450c17, cytochrome P450 17 α -hydroxylase; A β , β -amyloid peptide; AD, Alzheimer's disease; FeSO₄, ferrous sulfate; ROS, reactive oxygen species; CSF, cerebrospinal fluid

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pathogenesis of Alzheimer's disease (AD) [27]. Increased lipid peroxidation and mitochondrial energy metabolism defects are now known to be hallmarks of the disease [27]. A β , a major component of AD neuritic plaques [20], increases free radicals in neurons [28,30,36] and glia [8], and directly produces hydrogen peroxide through metal ion reduction [19]. Application of A β in vitro causes an increase both in ROS and DHEA in human glioma cells; this increase can be blocked by co-treatment with Vitamin E [8].

There has been much speculation on the role of DHEA in aging [2,31]. Although serum DHEA levels decrease with age in the human there are no available studies on the levels of DHEA in the brain. A number of groups have tried to correlate serum levels of DHEA and DHEA sulfate with cognitive function [17,37,38] or future development of AD [6]. These studies have failed to find a connection between DHEA levels and cognitive ability, or to demonstrate serum DHEA levels as a predictor of future AD development.

In this study, we measured levels of DHEA in the hypothalamus, hippocampus and frontal cortex specimens from AD patients and age-matched controls, and looked for evidence of P450c17-mediated DHEA synthesis. In addition, we measured DHEA levels in sera and in the cerebrospinal fluid (CSF) of AD patients and age-matched controls. We found that in contrast to decreasing serum levels of DHEA in patients with AD, brain DHEA is significantly higher in AD patients than in age-matched controls. P450c17 protein and mRNA were not found in the hippocampus. In agreement with the brain tissue DHEA levels, DHEA levels in the CSF of AD patients are significantly higher than in controls. Furthermore, there is evidence for alternative pathway activity in specimens from both AD and control patients although DHEA precursor availability was much higher in control samples. Treatment of control, but not AD, sera with ferrous sulfate (FeSO₄) causes an increase in DHEA levels. We propose that measurement of CSF-DHEA levels, in conjunction with serum DHEA levels and alternative pathway activity, examined in the presence of FeSO₄, can be used as a predictive diagnostic measurement of AD neuropathology.

2. Methods

2.1. Tissue samples

All human tissue samples and CSF were obtained from the Harvard Brain Tissue Resource Center in Belmont, MA. Samples for steroid measurements were either snap frozen or passively frozen in liquid nitrogen. Samples for RNA extraction were snap frozen. Brain tissue samples were obtained for 19 patients, 12 AD (6 men and 6 women) and 7 age-matched control patients (4 men and 3 women). Tissues from all patients for all three areas (hypothalamus, hippocampus and frontal cortex) were not available. CSF samples were obtained from 9 AD (4 men and 5 women) and 5 age-matched control patients (3 men and 2 woman). AD

patients were classified by the Harvard Brain Tissue Resource Center as having "severe AD". Mean age for all patients was 74.6 ± 7.2 years for AD patients and 73.4 ± 10.5 years for controls. Mean post-mortem interval was 10.2 h for AD patients and 14.7 h for controls. Serum samples (5 AD patients, all men, mean age = 80.0 ± 6.9 years, and 6 control subjects, 2 men and 4 women, mean age = 78.8 ± 4.8 years) were obtained from Dr. J.R. Rapin (CEB, Mont Saint-Agnes, France). Protocols for the use of human tissue were approved by the Georgetown University Internal Review Board.

2.2. Endogenous steroid measurements

Tissue samples were homogenized in 0.25 M sucrose buffer and split in half. One-half was stored at -80°C and used for later immunoblots. The other half was split again; one aliquot was used to measure endogenous steroid levels. The other aliquot was kept for analysis of alternative pathway activity. Endogenous steroid levels were determined as previously described [8]. Samples were extracted twice with 4 volumes of ether:ethyl acetate (v/v) and taken to dryness. Extracts were purified on a C-18 reverse phase HPLC column using a Beckman System Gold HPLC, running a methanol gradient. HPLC-purified fractions were collected for DHEA and assayed using specific RIA as previously described [8]. The identity of DHEA was confirmed by gas chromatography coupled to mass spectrometry [12]. Serum samples were split and treated with and without 10 mM FeSO₄. Serum and CSF samples were extracted as described for the brain samples, and purified by HPLC. DHEA levels were measured by specific RIA.

2.3. Histochemistry

Five micrometer sections were cut, deparaffinated and hydrated. Sections were treated with 10% H₂O₂ for 10 min at room temperature. Polyclonal antibodies to P450c17 (1:200; donated by Dr. A. Payne, Stanford University Medical Center, Stanford, CA [18]) and cytochrome P450 side chain cleavage (1:200; [16]) or normal rabbit serum in 10% fetal bovine serum in PBS were added to the sections at 4°C and incubated overnight. Secondary antibody reactions were performed using horseradish peroxidase coupled goat anti-rabbit secondary antibody diluted 1:500 in PBS. The substrate 3-amino-9-ethyl carbazole was used to detect the peroxidase reaction. Slides were counterstained with hematoxylin. Sections were also examined for the presence of Campbell-Switzer silver staining, indicative of AD pathology, performed by Neuroscience Associates Inc. (Knoxville, TN).

2.4. RNA extraction and RT-PCR

Total RNA was extracted as previously described [8] using the guanidinium-phenol chloroform extraction method (RNAzol B, Tel-Test, Friendswood, TX). mRNA was

amplified using the Perkin-Elmer GeneAmp RT-PCR Kit, according to the manufacturer's instructions. Specific primers were used to amplify message for P450c17 from three control and four AD subjects [8]. After 35 cycles of PCR, products were run on 1.5% agarose gels, transferred to nylon membranes and incubated with specific probes radiolabeled with ^{32}P . After incubation overnight at 42°C , membranes were washed and exposed to X-OMAT autoradiography film (Eastman Kodak, Rochester, NY).

2.5. Immunoblots for A β

Levels of A β species in AD and control brain and CSF samples were analyzed using 4G8, a monoclonal antibody that recognizes amino acids 17–24 of A β (Senetex PLC,

Napa, CA). Samples in 0.25 M sucrose buffer were run on precast 4–20% gradient gels (Novex, San Diego, CA). Gels were transferred to nitrocellulose membranes and incubated in primary antibody for 1 h at room temperature at a dilution of 1:2000. Membranes were incubated in secondary antibody (1:1000) for 1–1.5 h at room temperature and blots were visualized using enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Pisquataway, NJ). Blots were exposed to X-OMAT autoradiography film.

2.6. Alternative pathway measurements

Samples were treated as previously described [8,11,12]. Aliquots of homogenates were incubated with a final concentration of 30 mM (brain) or 10 mM (serum and CSF)

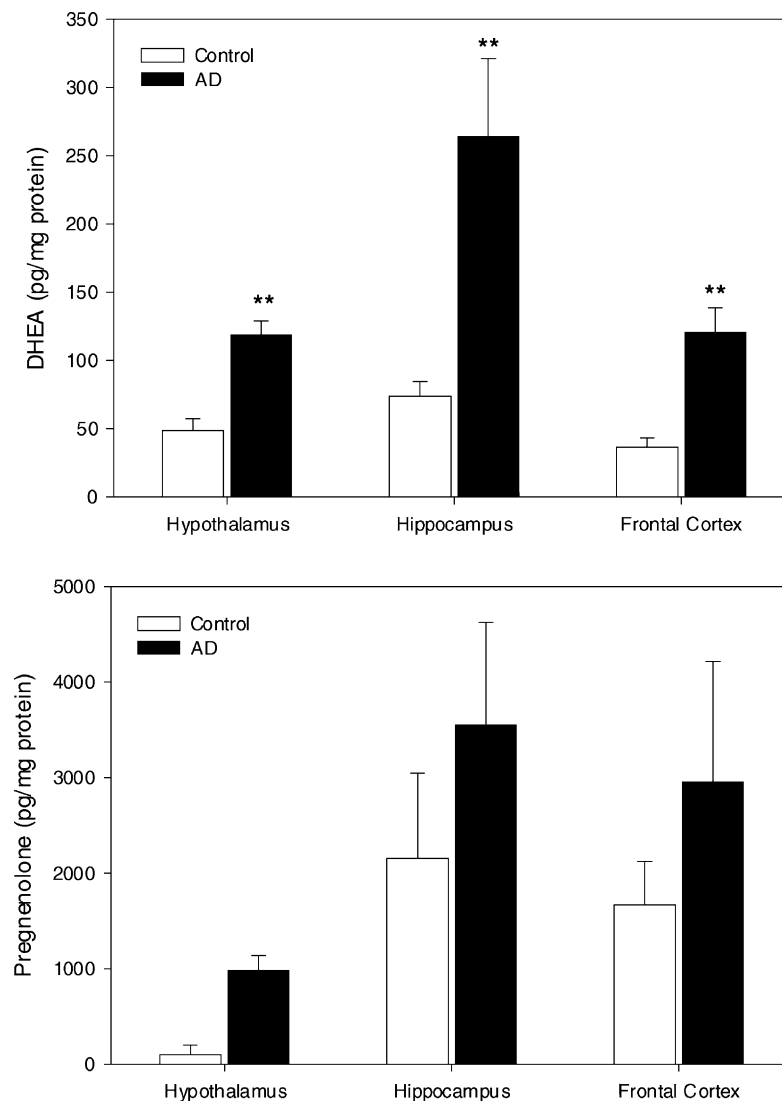


Fig. 1. DHEA and pregnenolone levels in AD and control brain. Endogenous DHEA and pregnenolone levels in human brain were measured by specific radioimmunoassay after HPLC purification. All three regions examined show a significantly higher level of DHEA in AD patients (** $P < 0.01$). There is a trend towards higher pregnenolone levels in all three areas as well, although these results are statistically significant only for hypothalamus. Data presented is means \pm S.E.M. for duplicate measurements from 3 to 10 samples, except for control hypothalamus (multiple measurements from $n = 1$) and control hippocampus ($n = 2$).

FeSO₄ at 37 °C for 2 h. After 2 h, samples were extracted and purified as described for the endogenous levels, and assayed by a specific RIA.

2.7. Statistics

All data is shown as means \pm S.E.M. from 3 to 15 measurements. In cases where only one patient was available, data is presented as mean \pm S.E.M. for multiple measurements from that patient. Data were analyzed using unpaired Mann–Whitney *U*-test, Student's *t*-test, Welch's *t*-test with normality tests, or by one-way ANOVA, as indicated.

3. Results

Considering that the presence of an alternative pathway for the formation of DHEA from an unknown precursor has recently been described in acellular and cellular studies using rodent and human material [8,11,12,32]. We investigated the potential of normal and pathologic (AD) human brain tissue to form DHEA. We measured DHEA levels in samples from hypothalamus, hippocampus and frontal cortex from AD patients and age-matched controls. There is a significant increase in DHEA levels in all three areas of the brain ($P < 0.01$ versus control levels, Fig. 1). We also measured levels of pregnenolone, the precursor for DHEA, in these same three regions and found that pregnenolone was higher in AD than in control brain, although significantly increased only in AD hypothalamus (Fig. 1). We investigated the mechanism of DHEA formation in AD specimens. Immunohistochemical studies in sections from frontal cortex and hippocampus from brains from AD patients and age-matched controls failed to demonstrate the presence of P450c17 immunoreactivity (Fig. 2E–H), the enzyme responsible for DHEA formation from pregnenolone in the endocrine glands, although DHEA levels were very high in these regions. P450 side chain cleavage immunostaining was detected indicating the presence of this enzyme, responsible for pregnenolone formation from cholesterol, in sections from AD and age-matched controls (Fig. 2A–D). Campbell–Switzer silver staining, characteristic of the neuritic plaques in AD brains, was seen in sections from both the cortex and hippocampus specimens used (Fig. 2I and J). Age-matched controls did not react to this stain (Fig. 2K and L).

We looked for mRNA for P450c17 in the frontal cortex and hippocampus using RT-PCR followed by Southern blot with specific P450c17 probes. Although the mRNA for the P450c17 enzyme appears to be in the frontal cortex of controls and in one AD patient (in a second AD patient it was absent), it was not detected in the hippocampus (Fig. 2, bottom). We looked for the presence of A β as a potential trigger for the previously described alternative pathway for DHEA formation using the monoclonal antibody 4G8, which recognize A β complexes of various sizes as well as the amyloid precursor protein from which A β is derived.

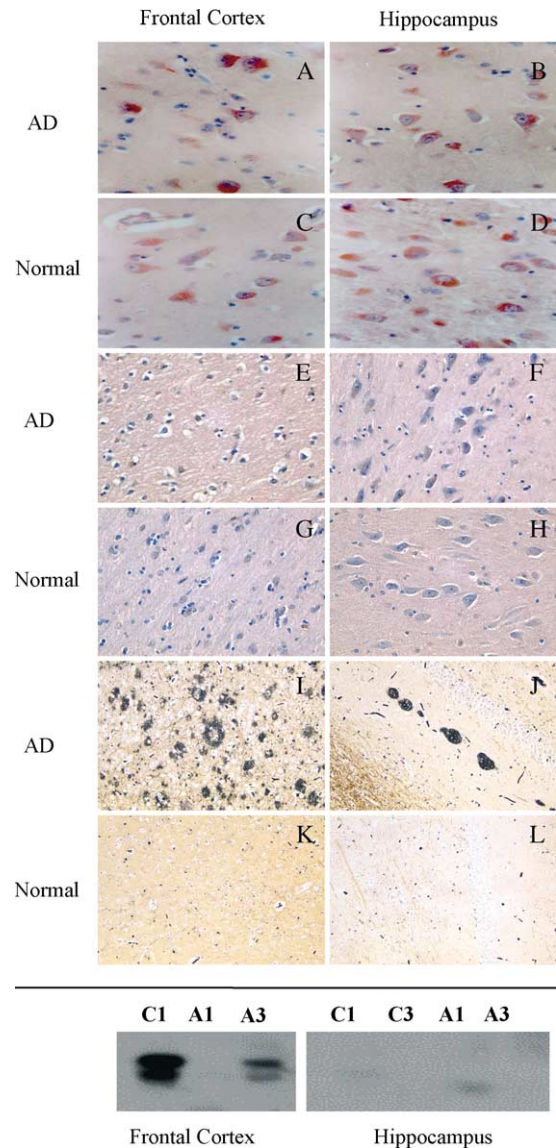


Fig. 2. (Top) Absence of P450c17 immunoreactivity in frontal cortex (E and G) and hippocampus (F and H) from AD and control brain specimens. P450 side chain cleavage enzyme immunolocalization in both frontal cortex (A and C) and hippocampus (B and D) from AD and control brain specimens serves as a positive control for brain immunostaining. Campbell–Switzer silver staining (I–L) confirms the AD pathology in the specimens examined. Magnification: 400 \times . (Bottom) mRNA expression of P450c17 in AD and control brain. RT-PCR amplification of P450c17 and DNA blot indicates that P450c17 is expressed in the cortex of both control and AD patients, although expression is only seen in one AD patient samples. There is no detectable expression of P450c17 in the hippocampus, indicating that DHEA in this area is not made via the peripheral enzyme-mediated pathway. Lanes are labeled by arbitrary patient identification number.

We found that in the hippocampus there is more A β immunoreactivity per total amount of protein as compared to control samples (Fig. 3). These proteins have molecular weights ranging from 45 to 80 kDa. Control samples have very little detectable A β immunoreactivity. Samples from AD hypothalamus and frontal cortex also contained higher

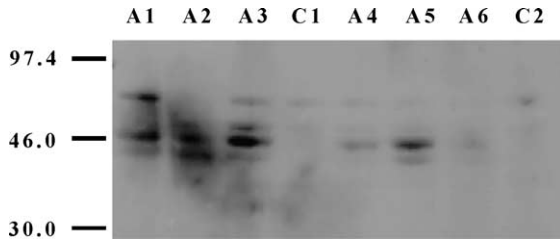


Fig. 3. A β immunoreactivity in hippocampus from AD and control brain. Homogenates were separated by SDS-PAGE and blotted with 4G8, a monoclonal antibody to amino acids 17–24 of the A β peptide. A β immunoreactivity is undetectable in the hippocampus of control patients, but is present in the hippocampus of AD patients. Control, $n = 2$; AD, $n = 6$. Representative gels are shown. Lanes are labeled by arbitrary patient identification number.

levels of A β immunoreactivity compared to controls (data not shown).

We measured serum DHEA levels in five AD and six control subjects to see if the increase in DHEA in the CNS was reflected in the periphery. There was no significant difference between AD and control serum DHEA levels (AD = 688 ± 174 pg/ml serum, control = 891 ± 159 pg/ml serum;

unpaired Student's t -test, $P = 0.4122$). In agreement with the data obtained using the brain tissue specimens however, we found in CSF from patients with AD that DHEA levels were higher than those in CSF from age-matched controls (Fig. 4A, unpaired Mann–Whitney U -test, $P = 0.05$). The exact values obtained are shown in Fig. 4B. A β immunoreactive protein levels, as detected by immunoblots, were also greater in CSF from all patients with AD as compared to all control patients (Fig. 4C).

We then examined the ability of AD and control brains to make DHEA via the alternative pathway, by treating each with 30 mM FeSO₄. FeSO₄ treatment caused a significant increase in DHEA levels in the hippocampus and frontal cortex of control patients, but had no effect in the hypothalamus (Table 1). Treatment with FeSO₄ of tissues from AD patients showed a significant increase in DHEA in the frontal cortex, but no significant change in the hippocampus or hypothalamus.

Lastly, we examined the effects of 10 mM FeSO₄ on serum DHEA levels to determine if the alternative precursor is present in serum. FeSO₄ caused a 1.5- to 3-fold increase in DHEA levels in control serum, but had little effect on AD serum (Fig. 5). There is a wide patient-to-patient variability

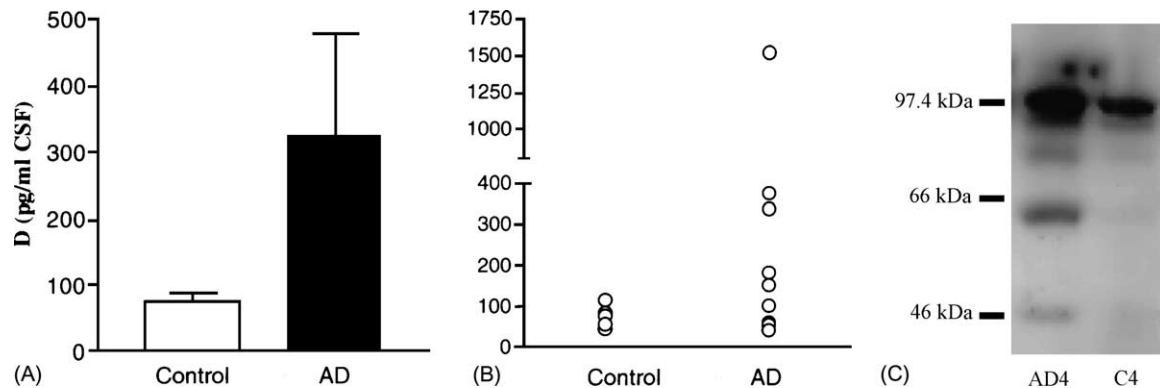


Fig. 4. DHEA and A β levels in cerebrospinal fluid from AD and control patients. (A) DHEA was purified from CSF extracts by HPLC and measured by specific radioimmunoassay. DHEA levels are much higher ($P = 0.05$) in AD subjects than in control. Control, $n = 5$; AD, $n = 9$. (B) Exact values of DHEA in the specimens examined. (C) Both AD and control patients have immunoreactive A β in the CSF. Control patients have only 98 kDa species, while AD patients have A β immunoreactivity occurring at a wide range of molecular weights from 46 to 98 kDa. Representative blots are shown for one AD and one control patient. Lanes are labeled by arbitrary patient identification number. Similar results were obtained using CSF from all AD patients and controls.

Table 1

DHEA levels (pg/mg protein) in brain samples from AD and control patients with and without treatment with 30 mM FeSO₄

| Patients | Control | Control | AD | AD |
|----------------|--------------------|--------------------|-----------------------|-------------------------|
| Treatment | –FeSO ₄ | +FeSO ₄ | –FeSO ₄ | +FeSO ₄ |
| Hippocampus | 73.8 ± 10.9 | $128.3 \pm 17.0^*$ | $321.2 \pm 77.9^{##}$ | $241.7 \pm 48.2^{\#}$ |
| Hypothalamus | 48.8 ± 8.6 | 61.4 ± 14.2 | $118.9 \pm 10^{##}$ | $180.8 \pm 23.9^{##}$ |
| Frontal cortex | 36.4 ± 7.0 | $70.0 \pm 11.2^*$ | $131.4 \pm 19.0^{##}$ | $219.3 \pm 23.3^{##\#}$ |

Samples were extracted, DHEA was purified by HPLC and measured using specific radioimmunoassay. Results shown are means \pm S.E.M. of duplicate measurements from 3 to 10 samples. Statistical analysis of DHEA levels was done between treated and untreated samples, and AD and control samples within the same brain area. C, control patients; AD, Alzheimer's disease patients. * $P < 0.05$ vs. untreated patient, ** $P < 0.01$ vs. untreated patient, # $P < 0.05$ vs. control patient, ## $P < 0.01$ vs. control patient.

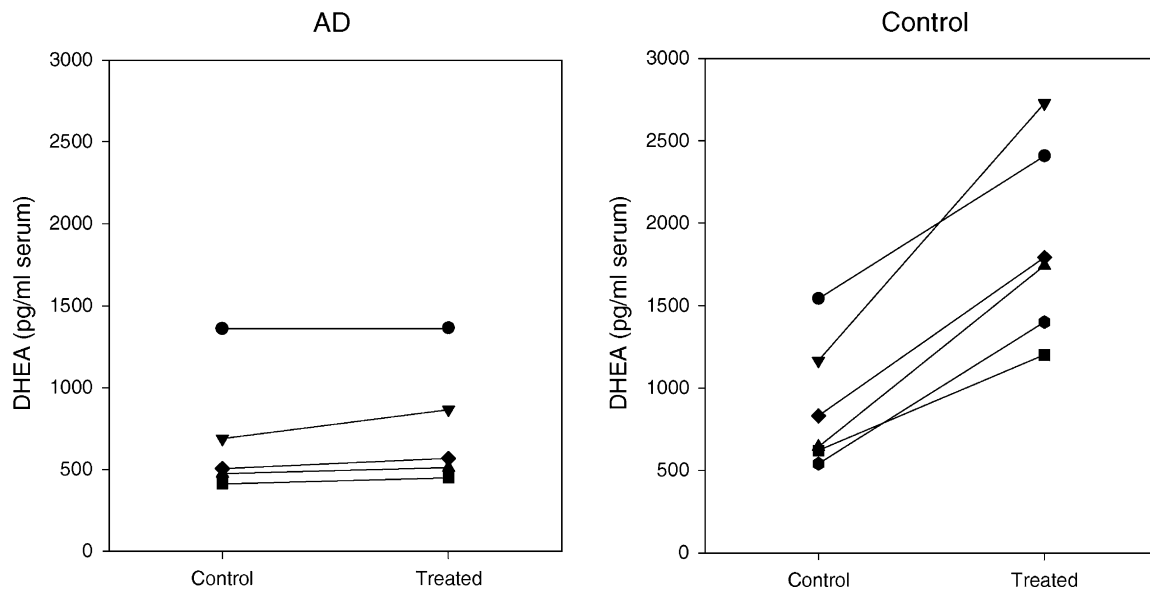


Fig. 5. Serum response to FeSO_4 in AD and control patients. When serum from AD patients is treated with 10 mM FeSO_4 there is no change in DHEA levels, indicating the lack of an alternative precursor in these samples. However, when serum from control patients is treated with 10 mM FeSO_4 , there is a significant increase in DHEA levels (one-way ANOVA, $F = 8.785$, $P < 0.001$), indicating the presence of an alternative DHEA precursor that can be acted upon by oxidizing agents. Although there is wide patient-to-patient variability, the relative change after FeSO_4 treatment is consistent across samples. AD patients do not have this precursor present, potentially because it has already been converted by disease-mediated oxidative stress.

in serum DHEA levels, but the increase in DHEA in control patients in response to FeSO_4 occurred in all samples. One-way ANOVA analysis indicates a significant effect of FeSO_4 treatment in control serum, but not in AD serum ($F = 8.785$, $P < 0.001$).

4. Discussion

The role of the neurosteroid DHEA in aging is unknown, although a number of attempts have been made to link low serum DHEA with dementia and memory disorders, particularly AD [2,6,17,37,38]. The presence of an alternative pathway for the formation of DHEA from an unknown precursor was recently suggested by the results of treatment of rat and human glioma cells with agents inducing ROS and oxidative stress, such as ferrous sulfate and $\text{A}\beta$ peptide [8,11,12]. Recent evidence also suggests that $\text{A}\beta$ and oxidative stress play a large role in the pathogenesis of AD [27]. Furthermore, there is iron accumulation in AD plaques and neurofibrillary tangles that can act as a source of redox-generated free radicals [33]. These findings prompted us to hypothesize that if the alternative pathway of DHEA formation is present in human brain tissue, formation of DHEA should be increased in AD brain tissue due to the presence of increased $\text{A}\beta$ and oxidative stress levels.

We measured levels of DHEA in AD and control brain. DHEA levels are significantly increased in AD brain in all three areas examined, and are maximal in AD hippocampus. This may be a reflection of increased oxidative stress in the

AD brain, potentially due to the actions of $\text{A}\beta$. Other studies have shown increased levels of carbonyls in neuronal cytoplasm and in nuclei of neurons and glia from AD brain [34], as well as increases in lipid peroxidation, protein peroxidation, disruption of mitochondria energy metabolism in AD and increased RNA oxidation [27,28,30,36], suggesting a role for ROS in the development of AD.

In order to determine the source of DHEA production in the human brain, we looked for P450c17, the peripheral enzyme responsible for DHEA formation. We found that, while there is mRNA for P450c17 in the frontal cortex of control and AD patients, there is no detectable P450c17 mRNA or immunoreactivity in the hippocampus, and no immunoreactivity in frontal cortex. Interestingly, P450 side chain cleavage was found in all specimens, suggesting that these tissues have the ability to form pregnenolone from cholesterol. AD pathology in the specimens used was confirmed using the Campbell–Switzer silver stain that indicates neuritic plaques, a hallmark of AD. These results indicate that the high level of DHEA in the hippocampus is either derived from peripheral sources and accumulated and stored in the hippocampus, or it is derived from local activity of the alternative pathway. The variation in P450c17 expression in the frontal cortex may be indicative of between patient variation, or may reflect a greater loss of the cells in the frontal cortex that contain P450c17 in one AD patient versus the other. While we do not know the serum DHEA levels from these specific patients, published reports indicate levels of serum DHEA from 0.3 to 2 ng/ml (age 60–90 years; [3]), 0.52 ng/ml (age 76–93; [22]) in healthy aged people and

~0.06–17 ng/ml (age 3–85 years; [6]). The measured serum levels of DHEA in AD patients and age-matched controls are similar to those presented above, and the levels of DHEA found in brain tissues from control and AD subjects are comparable to those previously reported [23]. The higher level of DHEA in AD hippocampus, frontal cortex and hypothalamus as compared to controls indicates that DHEA can possibly be derived by alternative pathway activity triggered by increased oxidative stress. Increased oxidative stress induced by either ferrous ions or A β was previously shown to induce DHEA formation by human brain cells in vitro [8]. Thus, the presence of increased levels of A β in AD specimens compared to controls could explain the increased oxidative stress [19,28,30,36]. Indeed, the AD specimens used were found to contain both neuritic plaques and increased A β immunoreactivity as compared to controls.

In agreement with the tissue data, CSF from AD patients has higher levels of DHEA as compared to controls. In some instances, the level of DHEA in AD CSF is as much as 35 times than seen in control CSF. This is in stark contrast to serum DHEA levels, where although DHEA is lower in AD serum as compared to control serum, the differences seen are not significant, in agreement with previous findings [2,6,22]. This observation is contrary to previous hypotheses on the importance of DHEA in memory and AD. Many studies have tried to use age-related decreases in serum DHEA as indicative of mental degradation in dementia. Although DHEA is known to affect NMDA receptors [5] and to potentiate both memory formation [15] and hippocampal long-term potentiation [39], there is no in vivo evidence for a role for DHEA in memory and dementia. Considering the data presented herein, where DHEA in AD CSF is significantly higher than DHEA in control CSF, a conclusion on this issue would require a larger sample than the nine CSF patients examined in the present report.

We examined the potential for alternative pathway activity in AD and control brain (Table 1). Treatment with the reducing reagent FeSO₄, causes a significant increase in DHEA in the hippocampus and cortex of control patients, possibly indicating the presence of an alternative precursor in these areas. There was no effect in the control hypothalamus. AD patients show a significant increase in DHEA only in the frontal cortex, indicating that an alternative precursor is still present there. However, it appears not to be present in the AD hypothalamus or hippocampus. The higher levels of DHEA present in the hypothalamus and hippocampus of AD brain before FeSO₄ treatment suggest that the precursor of the alternative pathway has already been converted to DHEA by endogenous oxidative stress due to the disease process. This results in the higher endogenous level of DHEA measured in the AD brain. The identity of the precursor of DHEA in brain is unknown although there has been evidence indicating that this precursor is probably an oxygenated metabolite of cholesterol or pregnenolone [12,32]. In support of these findings, it was recently reported that AD and vascular demented patients appear to have higher

plasma levels of 24(S)-hydroxycholesterol [25], a product of cholesterol oxidation in the brain [7]. Although the results presented are obtained from a small number of samples, the correlation between increased levels of DHEA in hippocampus, hypothalamus and frontal cortex seen in AD brain, and the levels found in CSF suggests a consistent phenomenon.

One of the major problems with AD diagnosis and treatment is the inability of clinicians to determine the onset of the disease. Currently, this is done by a combination of MRI scans to measure generalized shrinkage of the brain, and cognitive tests to determine the state of dementia. Typically symptoms do not occur until very late in the disease process. If the changes in DHEA in the CSF are indeed regulated by oxidative conditions within the brain, there may be alterations in CSF–DHEA levels very early in the progression of the disease. Furthermore, presence of the alternative precursor can be detected in the serum of control patients, but not in AD serum. Further analysis of the data in Table 1 and Fig. 5 indicates that the serum shows a greater response to FeSO₄ treatment than does any brain area tested, indicating that this may be a useful compartment for determining the progression of brain oxidative stress in AD, since there is an increase in all control serum after FeSO₄ treatment, and no change in AD serum. Therefore, by determining (at present an indirect determination using FeSO₄) whether or not the alternative precursor is present in the blood, and measuring CSF–DHEA levels, it may be possible to determine the conditions of oxidative stress in the brain. The availability of the alternate precursor for DHEA in serum could be used as a diagnostic tool to determine the progression of AD. One might expect to see higher serum levels of DHEA in AD patients if all available alternative precursor has already been converted. However, serum DHEA derived from this alternative pathway activity may be preferentially sequestered into other compartments rather than in the serum.

It has been long speculated that DHEA may be important in the aging process, particularly in modulating memory formation. The results presented here demonstrate that, contrary to current hypotheses, levels of DHEA are much higher in AD brain than in normal brain. It has been shown that DHEA and its sulfate are neuroprotective against excitatory amino acid-induced toxicity [21] and A β toxicity in the hippocampus [10] but little has been done on the neuroprotective effects of DHEA on glia. We have proposed that DHEA produced by glial cells in response to A β may act as a glial protective agent [8]. In response to A β , astrocytes become reactive and produce extracellular matrix molecules and growth factors that may help to potentiate amyloid deposition [9]. In this case, the increase in DHEA could potentially be a harmful event, helping to protect reactive astrocytes and potentiate neuronal damage and plaque formation. It remains to be seen whether DHEA plays an important role in AD pathology or is simply an epiphenomenon of the disease process.

Regardless of whether DHEA plays an important role in the pathogenesis of AD, it may serve as a useful marker of

the disease. Although a larger sampling is required to validate the results presented herein, we speculate that by measuring DHEA levels in the serum and CSF of aging patients, and looking for evidence of alternative pathway activity in these compartments, it may be possible to determine early changes in the levels of oxidative stress in the brain. These changes may reflect early damage in AD or even in other neurodegenerative disorders involving oxidative stress.

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