



In vitro metabolism of dehydroepiandrosterone (DHEA) to 7 α -hydroxy-DHEA and Δ 5-androstene-3 β ,17 β -diol in specific regions of the aging brain from Alzheimer's and non-demented patients

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Abstract

The description of dehydroepiandrosterone (DHEA) as a neuroactive neurosteroid has raised the important question of whether the steroid itself and/or its metabolite(s) are active in the brain. Classical transformations of DHEA in brain and peripheral tissues include its conversion to testosterone and estradiol. In the human brain, the metabolism of DHEA to other metabolites is still poorly understood, particularly in aging people and Alzheimer's patients. The present study describes the in vitro transformation of DHEA into 7 α -hydroxy-DHEA and Δ 5-androstene-3 β ,17 β -diol, for the first time in the aging brain of patients with Alzheimer's disease in comparison with non-demented controls. Formal identification of DHEA metabolites is provided by gas chromatography–mass spectrometry, thus indicating the presence of NADPH-dependent 7 α -hydroxylase and 17 β -hydroxysteroid oxidoreductase activities. Under our experimental conditions, the synthesis of 7 α -hydroxy-DHEA and Δ 5-androstene-3 β ,17 β -diol occurs in the frontal cortex, hippocampus, amygdala, cerebellum and striatum of both Alzheimer's patients and non-demented controls. In both groups of patients, the pattern of DHEA metabolism is similar, but significant higher synthesis of 7 α -hydroxy-DHEA in the frontal cortex and Δ 5-androstene-3 β ,17 β -diol in the cerebellum and striatum were observed compared with those in other brain regions. In addition, a trend toward a significant negative correlation is found between the density of cortical amyloid deposits and the amount of 7 α -hydroxy-DHEA formed in the frontal cortex and that of Δ 5-androstene-3 β ,17 β -diol in the hippocampus. Therefore, the biosynthesis of 7 α -hydroxy-DHEA and/or Δ 5-androstene-3 β ,17 β -diol is likely to regulate DHEA cerebral concentrations and may contribute to the control of DHEA activity in the aging brain including in Alzheimer's disease.

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1. Introduction

Some steroids have been named 'neurosteroids' because they are synthesized in the nervous system independently of the activity of steroidogenic glands, hence differentiat-

ing them from steroids derived from gonads and adrenal glands [12,20,21,52,56]. By definition, 'neuroactive steroids' are active upon the nervous system, irrespective of their peripheral or central origin (for recent reviews, see Refs. [2,10,19,33,54,55]). Some neurosteroids are neuroactive, such as dehydroepiandrosterone (DHEA) and its sulfated form (DHEAS) which regulate neurotransmission, various aspects of behavior [1,13,23,26,61,64], and neuro-

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nal viability [6,9,18,30,37,40,68]. Particularly relevant to the aging process and Alzheimer disease (AD), may be the beneficial effects of DHEA on impaired memory performance in aged [28] or β -amyloid peptide-injected mice [41], and neuroprotection against the β -amyloid peptide-induced neurotoxicity in vitro [17].

In humans, contrary to rodents, the adrenals secrete large amounts of DHEA and its ester sulfate DHEAS whose blood concentrations decline asymptotically during normal aging [34,47,48]. In AD patients in comparison to non-demented controls, measurements of circulating levels of DHEA and its sulfate are rather contradictory [27,36,67], but the brain concentrations tend to be lower [63]. Causal relationships between the DHEA levels and impaired cerebral functions in aging or AD are still difficult to assert, and mixed results have been observed with DHEA replacement therapy in the elderly [7,11,43,51,65]. Classical DHEA metabolism in peripheral and brain tissues includes the formation of biologically active androgens and estrogens, such as testosterone and estradiol. The question has then been raised of whether some of the activities of DHEA in the central nervous system (CNS) are performed by the steroid per se or other metabolic products.

Numerous studies, including our own, have demonstrated that the adult rodent brain is the site of an extensive metabolism of DHEA to 7α -hydroxy-DHEA (7α -OH-DHEA) [3,4,24,45,58]. The formation of Δ^5 -androstene- $3\beta,17\beta$ -diol (ADIOL) from DHEA in the rodent brain has also been documented [31,32,58]. To date, reports on the metabolism of neurosteroids including DHEA in the adult human brain have been rare [59]. However, by using

temporal lobe biopsies from patients suffering from chronic epilepsy, 7α -hydroxylation of DHEA and a 17β -hydroxysteroid oxido-reductase activity that form 7α -OH-DHEA and ADIOL from DHEA, respectively, have been recently described [58]. In AD patients, evidence for an aromatase activity has been shown in postmortem frontal and temporal cortices [66].

7α -OH-DHEA and ADIOL have been found to up-regulate immunity, increase resistance against lethal infection and counteract glucocorticosteroid immune suppressive properties in peripheral tissues [29,35,39,46,49]. Their biological significance in the CNS remain to be fully elucidated. The possible importance of 7α -OH-DHEA in brain has been raised by the observation of a shift in DHEA metabolism to 7α -OH-DHEA in rat cultured astrocytes at high cell density, i.e., in a situation resembling inflammation [4]. This is in accordance with increased 7α -hydroxylase activity at the site of inflammation in other tissues [44].

One report has found that plasma levels of free 7α -OH-DHEA remained unchanged in AD patients compared to aged-matched controls [8]. Our recent findings indicated that DHEA concentrations in several brain regions of AD patients tend to be lower compared with those of aged non-demented controls [63]. However, the biosynthesis of 7α -OH-DHEA in the aged or AD brain is unknown. Therefore, in the present study, we investigated the in vitro metabolism of DHEA in the CNS of those patients, with the reduced nicotinamide adenine dinucleotide phosphate (NADPH) as cofactor. The possible biochemical pathways of DHEA metabolism are depicted in Fig. 1. Here, we report the conversion of DHEA to both 7α -OH-DHEA and

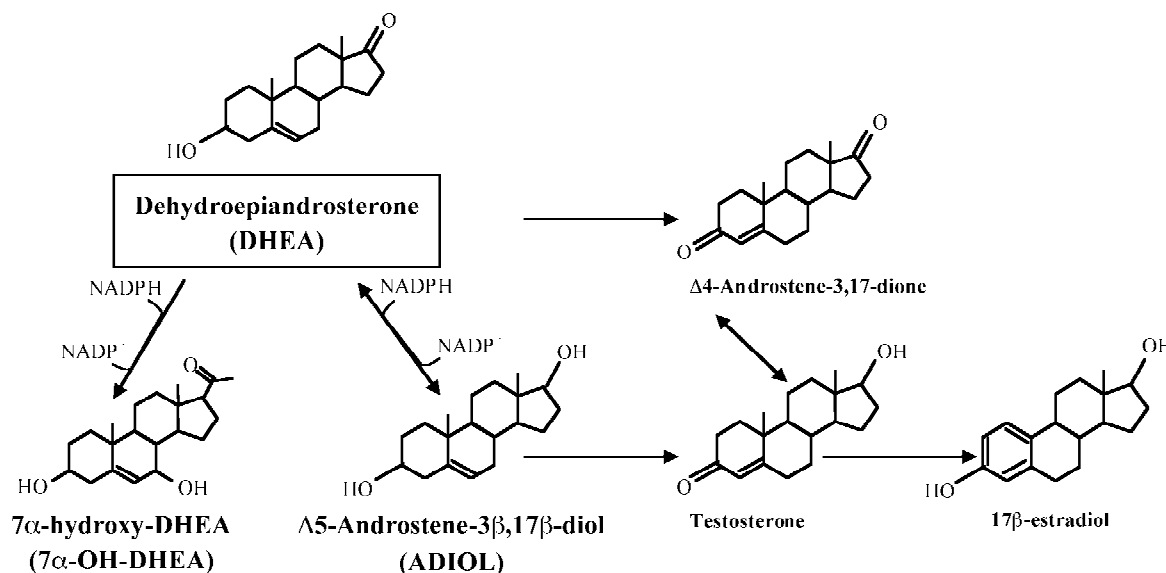


Fig. 1. Biochemical pathways of DHEA metabolism. 7α -Hydroxylase catalyzes the synthesis of 7α -OH-DHEA. 17β -hydroxysteroid oxidoreductase (17β -HSOR) catalyzes reversibly both the conversion of DHEA to Δ^5 -Androstene- $3\beta,17\beta$ -diol (ADIOL) and of Δ^4 -androstene- $3,17$ -dione to testosterone. 3β -Hydroxysteroid dehydrogenase activity leads to the formation of Δ^4 -Androstene- $3,17$ -dione from DHEA and of testosterone from ADIOL in the presence of cofactor NAD⁺. Aromatase transforms testosterone to estradiol in the presence of cofactor NADPH.

ADIOL in each of the five brain structures evaluated, i.e., frontal cortex, hippocampus, amygdala, cerebellum and striatum. DHEA metabolites were characterized by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), and formally identified by gas chromatography–mass spectrometry (GC–MS).

2. Materials and methods

2.1. Subjects

Subjects were those described in a recently published study [63]. Eleven patients (75.6–91.5 years), who were hospitalized and died in a geriatric unit (Service de G erontologie 3, H opital Emile Roux, Limeil-Br evannes, France), were selected. Exclusion criteria were the opposition by family for autopsy, postmortem delay beyond 24 h, steroid or benzodiazepine administration during the month before death, and prolonged hypoxemia at the time preceding death. Six patients were classified as non-demented controls and five as AD patients on the basis of their clinical and neuropathological examination. Criteria for dementia were based on the Mental Diagnosis and Statistical Manual Disorders, 3rd edition, revised [5], and criteria of AD were those proposed by the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association [42]. Classification of patients was ascertained by post-mortem neurological examination of cortical amyloid plaques and neurofibrillary tangles and the levels of β -amyloid peptide and PHF- τ in the brain as indicated in previous studies [22,63]. Detailed characteristics of all patients, including age, gender and neuropathological features (density of cortical amyloid plaques and neurofibrillary tangles), have been recently published [63] and are summarized in Table 1. All clinical investigation was approved by the local committee and conducted in accordance of the Declaration of Helsinki.

2.2. Chemicals

[4- 14 C]DHEA (55.2 mCi/mmol) was purchased from New England Nuclear (Boston, MA, USA). 7 α -OH-DHEA and ADIOL were generously donated by Roussel-Uclaf (Romainville, France). DHEA, 17 β -estradiol, NADPH and primuline were from Sigma–Aldrich (St. Louis, MO, USA). 5 β -Androstane-3 β -ol-17-one and 5 β -pregnane-3 α ,6 α -diol-20-one were purchased from Sigma–Aldrich and Steraloids (Newport, RI, USA), respectively. The solvents of analytical grade were purchased from Merck (Darmstadt, Germany) and Carlo Erba (Milano, Italy).

2.3. Tissue preparation

Upon thawing of one cerebral hemisphere at -10°C ,

Table 1

Neuropathological features of the aged non-demented and Alzheimer’s patients

Group	Gender	Age (years)	Histological classification	
			Amyloid plaques	Neurofibrillary tangles
Non-demented control	Woman	87.9	0	III
	Woman	90.3	A	III
	Man	75.6	0	II
	Man	80.9	0	I
	Man	86.3	A	II
	Man	91.5	A	I
Alzheimer	Woman	81.4	C	V
	Woman	85.5	C	IV
	Woman	88.0	C	VI
	Woman	91.1	B	IV
	Man	86.0	C	V

The neuropathological features for each patient were indicated as in a previous study [63]. Amyloid plaques and neurofibrillary tangles were classified in cortical areas according to Braak and Braak’s classification with respect to their location and the severity of their density: 0–C (amyloid plaques) and I–VI (neurofibrillary tangles) [14,15].

representative fractions (100 mg) of frontal cortex, hippocampus, amygdala, cerebellum and striatum were dissected out and immediately stored at -80°C . One hippocampus and two amygdala from controls, and three hippocampi and two amygdala from AD patients were not included because of difficulties for definite identification. Each of the 47 brain regions collected was homogenized in 10% of 0.32 M sucrose (w/v) and homogenates were assayed for protein concentration as described by Bradford [16].

2.4. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analysis

Homogenates (0.5 mg proteins) of 47 individual brain regions were incubated with 0.5 nmol [14 C]DHEA in a total volume of 0.5 ml containing the buffer $\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ M/15, 1 mM EDTA pH 7.4 and 0.5 mM final concentration of NADPH. Control incubations were performed with homogenate boiled for 15 min or buffer alone. In some incubations, 5 μM estradiol was added to inhibit 7 α -hydroxylation of DHEA, as demonstrated previously in rat brain microsomes [3]. All incubations were carried out at 37°C for 6 h in a shaking water bath (70 rpm). On the basis of preliminary experiments, the 6-h time period was chosen in order to ensure sufficient formation of DHEA metabolites because of limited amounts of available brain material. Incubations were stopped by addition of 1 ml of acetone and tubes were stored at -20°C . After thawing, radioactive steroids were extracted then three times with 4 vol of ethyl acetate. The organic phases were pooled and dried in vacuum centrifuge (Speed-vac concentrator, Savant Instrument, Hicksville, NJ, USA). Recoveries in the organic phases were in the 84–91% range.

Radiosteroids were first analyzed by one-dimensional TLC. Dried extracts and authentic reference steroids were applied to silica-gel F₂₅₄ thin-layer plates (Macherey-Nagel, Düren, Germany) that were developed in chloroform–ethyl acetate (4:1, v/v). Standards were located by spraying on the plates a solution of primuline in 50% acetone (w/v). The R_F values of reference steroid standards were: for 7 α -OH-DHEA, 0.028 ± 0.004 ($n=6$) and for ADIOL, 0.301 ± 0.019 ($n=4$). Autoradiography of chromatograms was performed by exposure of Kodak Biomax XLS X-ray films (Kodak, Rochester, NY, USA) for 5 days. In each experiment, the radioactive areas shown on the films were located on the chromatograms, silica gel was scraped into glass vials and eluted with 3 \times 2 ml methanol. Eluates corresponding to each metabolite were pooled, dried and re-dissolved in 50% methanol.

Reversed-phase HPLC was then performed on a 5- μ m Hibar Lichrospher 100 RP-18 (125 \times 4 mm, Merck, Darmstadt, Germany) using methanol–water as a mobile phase, at a flow rate of 1 ml/min. The chromatograph (Model P100, Thermo Separation Product (TSP), San Jose, CA, USA) was equipped with an auto-injector (AS100XR, TSP). The elution system consisted of an isocratic mode of 50% methanol for 10 min, followed by a linear gradient of 50–100% methanol for 10 min. Fractions of 1 ml were collected using a fraction collector (model 202, Gilson, Villiers-le-Bel, France). The retention times of reference steroids were: for 7 α -OH-DHEA, 7–8 min and for ADIOL, 18–19 min.

2.5. Gas chromatography–mass spectrometry (GC–MS) analysis

Homogenate of frontal cortex (1 mg protein) from a control patient was incubated with DHEA (1 μ g) in a total volume of 0.5 ml containing the buffer KH₂PO₄/Na₂HPO₄ M/15, 1 mM EDTA, pH 7.4, and NADPH (1 mM final concentration), at 37 °C for 6 h in a shaking water bath (70 rpm). At the end of the incubation period, 10 ng of internal standards 5 β -androstane-3 β -ol-17-one and 5 β -pregnane-3 α ,6 α -diol-20-one (for ADIOL and 7 α -OH-DHEA, respectively) which are not endogenous steroids, were added. Incubation was stopped by addition of 5 ml of methanol 100%.

The GC–MS method used for the identification of DHEA metabolites was adapted from the one previously described [38]. Briefly, the methanolic extracts were submitted to chromatographic ‘clean-up’ procedure using silica minicolumns C18 (500 mg, International Sorbent Technology, Mid Glamorgan, UK). Free steroids were eluted with MeOH (85:15, v/v), dried, re-dissolved in MeOH–H₂O (20:80, v/v), and deposited on the same minicolumn that was previously washed with H₂O. After successive elutions with H₂O and MeOH–H₂O (50:50) that were discarded, free steroids were collected by elution with MeOH–H₂O (90:10) and purified by filtration before

HPLC analysis. The HPLC system from Thermoquest (San Jose, CA, USA) consisted of a P1000XR quaternary pump and an AS 100 XR TSP auto-injector. HPLC was achieved with a Lichrosorb Diol column (25 cm \times 4.6 mm, 5 μ m). The solvent system consisted of hexane and mixture A (90:10, v/v), the latter composed of hexane–isopropanol (85:15, v/v). The elution was performed at a flow rate of 1 ml/min. Two fractions were collected: the first fraction, containing ADIOL and 5 β -androstane-3 β -ol-17-one, was collected from 20 to 33 min. The second fraction, containing 7 α -OH-DHEA and 5 β -pregnane-3 α ,6 α -diol-20-one, was collected from 40 to 50 min. Retention times for authentic ADIOL and 5 β -androstane-3 β -ol-17-one were 30 and 24 min, respectively, and for authentic 7 α -OH-DHEA and 5 β -pregnane-3 α ,6 α -diol-20-one were the same i.e., 44 min. The HPLC fractions were then dried, dissolved in acetone prior to derivatization. The first fraction was derivatized with heptafluorobutyric anhydride (HFBA, Pierce, Rockford, IL, USA) while the second one was derivatized with *N*-methyl, *N*-trimethylsilyltrifluoroacetamide (MSTFA, Pierce). Ditrimethylsilyl ethers (TMS₂) and diheptafluorobutyrate (HFB₂) of biological sample and reference steroids were then injected into the GC–MS system. GC was performed in the splitless mode with a GC 8000 Top gas chromatograph (Carlo Erba, Milan, Italy). The mass spectrometer (model 150, Finnigan Automass, Argenteuil, France) was operated in the electron impact mode. Identification was achieved with full scan monitoring. Measurement was made in duplicate. For the identification of 7 α -OH-DHEA-TMS₂, GC was performed with a 1-min splitless-time. The temperature in the oven was initially 50 °C for 1 min and further increased to 320 °C at 10 °C/min. Temperatures of injection chamber and line transfer were 250 and 300 °C, respectively, and the flow rate of helium (carrier gas) was maintained constant at 0.7 ml/min. For the identification of ADIOL-HFB₂, the GC protocol used was the same as the one described for 7 α -OH-DHEA-TMS₂ identification except that the temperature of the oven was increased from 50 to 175 °C at 30 °C/min, and further increased to 320 °C at 10 °C/min. Under these conditions, the retention times for authentic 7 α -OH-DHEA-TMS₂ and ADIOL-HFB₂ were 24.9 and 13.59 min, respectively.

2.6. Quantification of DHEA metabolites

The relative amounts of ¹⁴C-labeled metabolites were measured by scanning the thin-layer plates with an automatic TLC linear analyzer (Tracemaster 20 model LB 284–285, Berthold Analytical Instruments, Nashua, NH, USA). Other radioactive measurements were carried out in 5 ml of scintillation liquid (Picofluor 15, Packard Bioscience, Groningen, The Netherlands) with a Tri-carb 2100TR (Packard Instruments, Warrenton, RI, USA) equipped with quench correction.

2.7. Statistics

Metabolic conversion rates are given as mean \pm S.E.M. The Kruskal–Wallis test was used for overall comparison between regions, followed, when significant, by the Mann–Whitney test. The Wilcoxon test was used for comparison between regions. Significance was defined at $P < 0.05$.

3. Results

3.1. Identification of 7α -OH-DHEA and ADIOL

Radioactive metabolites formed after the incubations of each brain region from all patients were first characterized by one-dimensional TLC, scanning and autoradiography. Only two radioactive areas, other than that of the substrate, with R_F values corresponding to 7α -OH-DHEA and ADIOL were located on autoradiograms. A representative radiochromatogram of [14 C]DHEA metabolism in the frontal cortex is shown in Fig. 2. In control incubations of [14 C]DHEA with boiled homogenates or buffer alone, no other radioactive peak than that of the substrate was detected by TLC.

Labeled metabolites with the same R_F values as authentic 7α -OH-DHEA and ADIOL were further characterized by HPLC as reported in the Section 2. Their retention times were found identical to those of respective reference steroids (for 7α -OH-DHEA, 7–8 min and for ADIOL, 18–19 min). The recovery after HPLC analysis was in the 82–90% range for both metabolites.

Finally, 7α -OH-DHEA and ADIOL were formally identified by GC–MS analysis. To ascertain the identity of DHEA metabolites, a frontal cortex homogenate from a non-demented patient was incubated with unlabelled DHEA and NADPH, and metabolites were purified, separated by HPLC as described in Section 2. GC–MS analysis

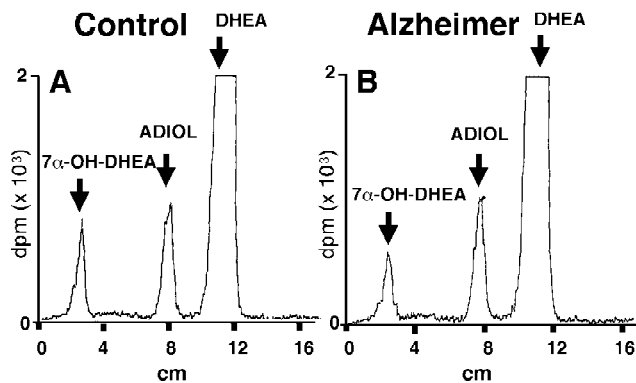


Fig. 2. Representative radiochromatograms of DHEA metabolism in the frontal cortex from non-demented control (A) and Alzheimer (B) patients. After the incubation of 1 μ M [14 C]DHEA with 0.5 mg frontal cortex homogenate proteins and 0.5 mM NADPH in phosphate buffer, steroids were extracted and analyzed by TLC in chloroform–ethyl acetate (4:1, v/v). Radioactivity was measured with an automatic TLC linear analyzer.

of the TMS₂ derivative of the HPLC fraction eluted at 40–50 min revealed the presence of one compound with the same diagnostic ions of the TMS₂ derivatives of authentic 7α -OH-DHEA. Similarly, the HFB₂ derivatives of the HPLC fraction eluted in the 20–33 min and of authentic ADIOL showed identical characteristic ion fragment patterns. Based on specific diagnostic ions, i.e., ions with high molecular mass at m/z 358 and m/z 468, for 7α -OH-DHEA-TMS₂ and ADIOL-HFB₂, respectively, GC analysis showed that the derivatized DHEA metabolites had nearly identical retention times (within 0.3%) as those of authentic 7α -OH-DHEA-TMS₂ and ADIOL-HFB₂. The respective reconstructed ion chromatograms analyzed in the full scan mode by GC–MS are represented in Fig. 3.

3.2. Metabolism of [14 C]DHEA in specific brain regions

3.2.1. Biosynthesis of [14 C] 7α -OH-DHEA

The synthesis of 7α -OH-DHEA was found in all the brain regions evaluated. An overall significant difference was found between structures in both groups ($P < 0.0001$) (Fig. 4A). In the non-demented controls, the amount of 7α -OH-DHEA formed in the frontal cortex was significantly higher compared with that found in all the other brain regions ($P < 0.05$). Similarly, in the AD group, the highest amount of 7α -OH-DHEA formed was observed in

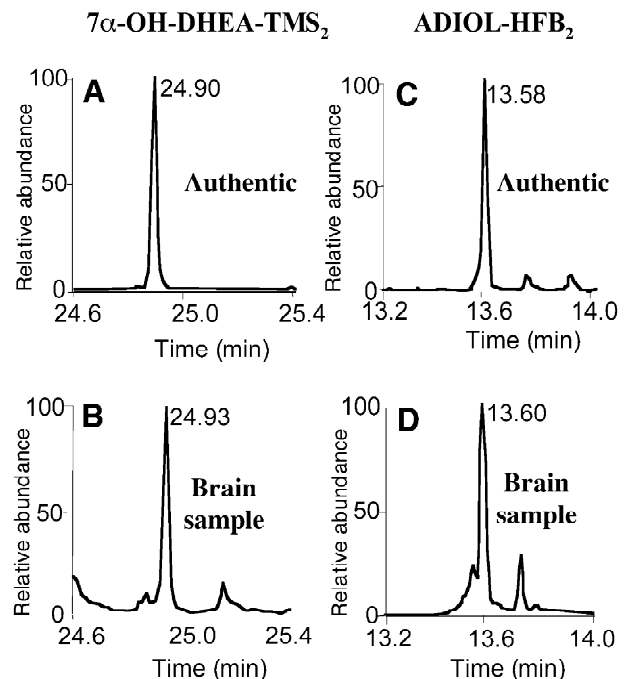


Fig. 3. Reconstructed ion chromatograms of steroid compounds in the full scan mode by GC–MS. (A) Authentic 7α -OH-DHEA-TMS₂ (m/z 358). (B) Ditrimethylsilyl ether of brain metabolite (m/z 358) after incubation of DHEA and NADPH with frontal cortex homogenate and subsequent HPLC analysis, as described in Section 2. (C) Authentic ADIOL-HFB₂ (m/z 468). (D) Diheptafluorobutyrate of brain metabolite (m/z 468) after incubation of DHEA and NADPH with frontal cortex homogenate and subsequent HPLC analysis, as described in Section 2.

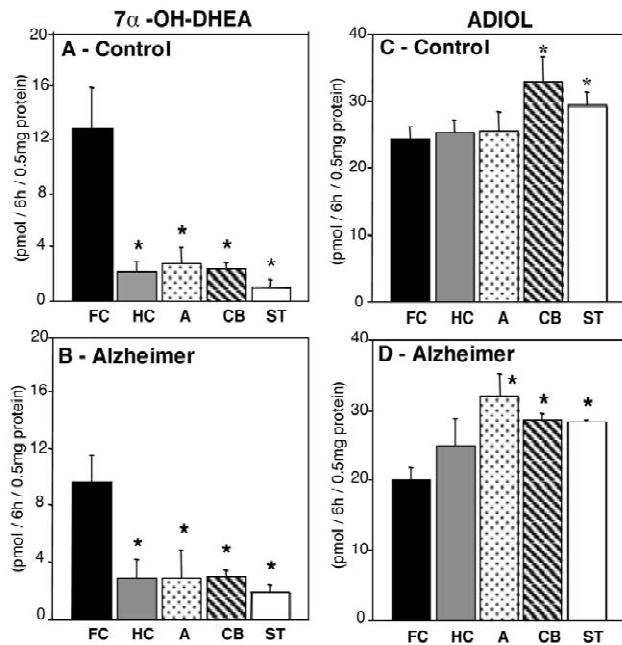


Fig. 4. Metabolism of DHEA in brain regions of non-demented control (A,C) and Alzheimer (B,D) patients. After the incubation of $1 \mu\text{M}$ [^{14}C]DHEA with 0.5 mg homogenate proteins and 0.5 mM NADPH in phosphate buffer, steroids were extracted and analyzed by TLC in chloroform–ethyl acetate (4:1, v/v). Radioactivity was measured with an automatic TLC linear analyzer. FC, frontal cortex; HC, hippocampus; A, amygdala; CB, cerebellum; ST, striatum. Metabolic conversion rates are expressed as mean \pm S.E.M. * $P < 0.05$ compared with frontal cortex.

the frontal cortex, and was significantly different from that found in all the other brain areas (Fig. 4B). The overall pattern of $7\alpha\text{-OH-DHEA}$ synthesis was similar between controls and AD with no significant regional brain difference ($P = 0.27$).

3.2.2. Biosynthesis of [^{14}C]ADIOL

The synthesis of ADIOL was also observed in all the brain structures studied. In each group, an overall significant difference was observed between regions ($P < 0.0001$). In the non-demented controls, the cerebellum and striatum had significantly higher conversion rates of DHEA to ADIOL compared to the frontal cortex, hippocampus and amygdala ($P < 0.05$) (Fig. 4C). In the AD group, the production of ADIOL was significantly higher in the amygdala, cerebellum, and striatum compared with that found in the frontal cortex and hippocampus ($P < 0.05$) (Fig. 4D). As for $7\alpha\text{-OH-DHEA}$, the overall pattern of ADIOL synthesis was similar between controls and AD with no significant regional brain difference ($P = 0.86$).

3.2.3. Effect of estradiol on [^{14}C]DHEA metabolism

As predictable from studies with rodent brain [3], when homogenates of all brain regions from AD and control patients were incubated with [^{14}C]DHEA in the presence of $5 \mu\text{M}$ estradiol, the formation of $7\alpha\text{-OH-DHEA}$ was

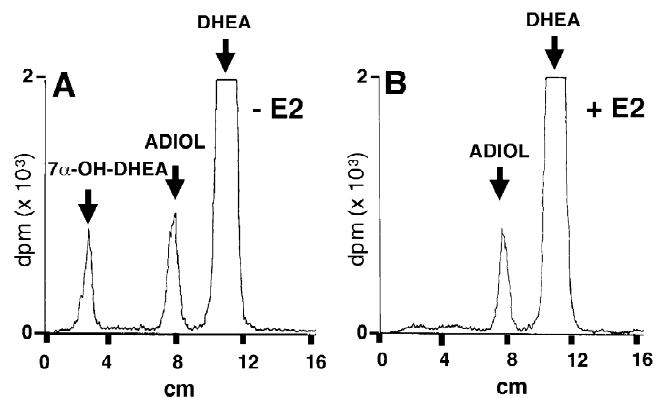


Fig. 5. Representative radiochromatograms of DHEA metabolism in the frontal cortex in the absence (A) or presence (B) of $5 \mu\text{M}$ of estradiol (E2). After the incubation of $1 \mu\text{M}$ [^{14}C]DHEA with 0.5 mg frontal cortex homogenate proteins and 0.5 mM NADPH in phosphate buffer, steroids were extracted and analyzed by TLC in chloroform–ethyl acetate (4:1, v/v). Radioactivity was measured with an automatic TLC linear analyzer.

completely inhibited, whereas that of ADIOL was not affected (Fig. 5).

3.3. Relationship between AD neuropathology and formation of [^{14}C]DHEA metabolites

The density of amyloid plaques was previously evaluated in the cortex of all patients [63]. The search for a relationship between AD neuropathology and the in vitro synthesis of [^{14}C]DHEA metabolites revealed a trend towards a negative correlation ($\rho = -0.598$) between the density of cortical amyloid deposits and the synthesis of $7\alpha\text{-OH-DHEA}$ in the frontal cortex, that was close to significance ($P = 0.059$). In addition, the density of cortical amyloid deposits tended to be negatively correlated with the ADIOL synthesis in the hippocampus ($\rho = -0.750$, $P = 0.066$). No relationship was found between the density of neurofibrillary tangles and the cerebral production of DHEA metabolites.

4. Discussion

The present study describes the in vitro transformation of DHEA to $7\alpha\text{-OH-DHEA}$ and ADIOL, for the first time in the aging brain of both AD and non-demented patients. Identification of DHEA metabolites was based on: (i) identical R_F values with that of the respective reference steroids on TLC, (ii) identical retention times with that of the respective reference steroids on HPLC, (iii) nearly identical retention times on GC and identical fragmentation patterns in mass spectra with that of authentic of $7\alpha\text{-OH-DHEA-TMS}_2$ and ADIOL-HFB $_2$. In control incubations conducted with [^{14}C]DHEA and boiled homogenate or with buffer alone, no significant amounts of metabolites

with R_F values identical to 7α -OH-DHEA and ADIOL were detectable.

These results indicate that the aging brain of AD and aged non-demented patients contains an NADPH-dependent 7α -hydroxylase and 17β -hydroxysteroid oxidoreductase activity upon DHEA. This is in agreement with results obtained from experiments using rodent brain tissue [3,25,31,32,53,58] or temporal lobe biopsies obtained from patients suffering from epilepsy [58]. Our data cannot suggest any specific isoform(s) in the aged brain of AD and non-demented patients, to be involved in DHEA metabolic activities. The 7α -hydroxylase isozyme CYP7B1, which has been shown to in vitro metabolize DHEA and oxysterols in the rodent and human brain [53,58] may be implicated. The 17β -hydroxysteroid oxidoreductase types 1, 3 and 5, which catalyze androgen reduction at position 17 [50] and which mRNA are expressed in human temporal lobe [57,60] may also be implicated.

The in vitro synthesis of 7α -OH-DHEA in the aged human brain was also verified by its complete inhibition by estradiol, as was previously demonstrated in the rat brain [3]. In contrast to what was previously observed in the rodent brain [3,25], no formation of 7β -OH-DHEA could be detected in the human brain tissues evaluated here, neither after TLC, HPLC nor GC–MS analysis. This corroborates the lack of detectable in vitro formation of 7β -OH-DHEA in the brain of patients suffering from chronic epilepsy [58].

The synthesis of both 7α -OH-DHEA and ADIOL was demonstrated in several brain regions, such as the frontal cortex, hippocampus, amygdala, cerebellum and striatum. Under described experimental conditions, regional differences were observed with significantly higher production of 7α -OH DHEA in the frontal cortex compared to other brain regions, and of ADIOL in cerebellum and striatum compared to frontal cortex, in both groups of patients. The activity of 17β -hydroxysteroid dehydrogenase activity (involved in the reduction of DHEA to ADIOL) in patients with temporal lobe epilepsy has also been found significantly higher in the subcortical white matter than the neocortex of freshly delivered biopsies [58]. In the present work, all brain structures were collected within a 24-h delay following autopsy and standardized measurements revealed similar amounts of total proteins in homogenates of all areas, thus making doubtful differential effects of postmortem delay on cell protein content in those specific brain areas. It is noteworthy that cells in postmortem adult human brain tissue, including material from neurological patients such as Alzheimer's, remain viable in culture for several weeks and can be used for functional studies [62].

The cerebral metabolism of DHEA to 7α -OH DHEA and ADIOL in aged AD patients may be of local importance. Indeed, a trend toward a negative correlation was found between the density of cortical amyloid deposits and the synthesis of both 7α -OH-DHEA in the frontal cortex

and ADIOL in the hippocampus, suggesting a possible neuroprotective role of these steroid metabolites in the AD brain. The demonstration of 7α -OH-DHEA or ADIOL as modulators of brain function, as has been demonstrated in experimental animals for their steroid parent DHEA, awaits further investigation.

In conclusion, the present study demonstrated that several structures of the aged human brain, including in AD, are capable of generating 7α -OH-DHEA and ADIOL from DHEA. While the significance of the production of 7α -OH-DHEA and ADIOL in the human brain is still unclear, it is likely to regulate cerebral DHEA availability and therefore of biologically active androgens/estrogens derived from DHEA, and may contribute to the control of DHEA activities in the CNS.

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