DEHYDROEPIANDROSTERONE 7-HYDROXYLASE CYP7B:
PREDOMINANT EXPRESSION IN PRIMATE HIPPOCAMPUS AND
REDUCED EXPRESSION IN ALZHEIMER’S DISEASE

J. L. W. YAU, a, S. RASMUSON,b R. ANDREW,a M. GRAHAM,a J. NOBLE,a T. OLSSON,b E. FUCHS,d R. LATHEc and J. R. SECKL a1

aMolecular Medicine Centre, Western General Hospital, Edinburgh EH4 2XU, UK
bDepartment of Medicine, Umea University Hospital, Umea, Sweden
cDivision of Biomedical Sciences, University of Edinburgh, George Square, Edinburgh EH8 9XD, UK
dDivision of Neurobiology, German Primate Research Centre, Kellnergeweg 4, 37077 Göttingen, Germany

Abstract—Neurosteroids such as dehydroepiandrosterone (DHEA), pregnenolone and 17β-estradiol are synthesized by cytochrome P450s from endogenous cholesterol. We previously reported a new cytochrome P450 enzyme, CYP7B, highly expressed in rat and mouse brain that metabolizes DHEA and related steroids by hydroxylation at the 7α position. Such 7-hydroxylation can enhance DHEA bioactivity in vivo. Here we show that the reaction is conserved across mammalian species: in addition to mouse and rat, DHEA hydroxylation activity was present in brain extracts from sheep, marmoset and human. Northern blotting using a human CYP7B complementary deoxyribonucleic acid (cDNA) probe confirmed the presence of CYP7B mRNA in marmoset and human hippocampus; CYP7B mRNA was present in marmoset cerebellum and brainstem, with lower levels in hypothalamus and cortex. In situ hybridization to human brain revealed higher levels of CYP7B mRNA in the hippocampus than in cerebellum, cortex, or other brain regions. We also measured CYP7B expression in Alzheimer’s disease (AD). CYP7B mRNA was significantly decreased (approximately 50% decline; P < 0.05) in dentate neurons from AD subjects compared with controls. A decline in CYP7B activity may contribute to the loss of effects of DHEA with ageing and perhaps to the pathophysiology of AD. © 2003 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: glucocorticoids, neurosteroids, ageing, cognition, cytochrome P450, pregnenolone.

Dehydroepiandrosterone (DHEA), the most abundant steroid in human blood, is not only an intermediate in the biosynthesis of testosterone and estrogen, but also has a broad range of physiological effects that are independent of the sex steroids. This includes a major role in the CNS where it increases neuronal excitability, has neuroprotective properties and enhances memory in rodents (Wolf and Kirschbaum, 1999a; Vallee et al., 2001). Circulating levels of DHEA in primates, including humans, fall with age (Orentreich et al., 1994; Belanger et al., 1994; Lane et al., 1997; Morley et al., 1997). Because DHEA improves hippocampus-associated learning and memory in rodents (Flood and Roberts, 1988; Flood et al., 1988, 1992) and perceived well-being in middle-aged human volunteers (Morales et al., 1994), a causal link between falling DHEA levels and age-related cognitive decline, including Alzheimer’s disease (AD), has been discussed (Vermeulen, 1995; Baulieu, 1996; Hinson and Raven, 1998; Lathe and Seckl, 2002). However, the molecular basis for the actions of DHEA and other sex steroid precursors, such as pregnenolone, in the CNS and even peripheral tissues is unclear. Such hormones may be precursors for local activation to more potent forms. Indeed, intracellular metabolism is emerging as a key regulator of the cellular activity of steroids and related hormonal ligands for nuclear receptors. Thus 11β-hydroxysteroid dehydrogenase type 2 blocks and type 1 amplifies glucocorticoid access to mineralocorticoid and glucocorticoid receptors, respectively, and 5α-reductase and aromatase perform similar roles for testosterone access to androgen and estrogen receptors. Thus the local metabolism of DHEA and other precursor sex steroids in the brain may be key to their biological activity.

In brain and other extra-hypothalamic tissues, hydroxylation at the 7α position represents the major metabolic fate of DHEA and pregnenolone (Warner et al., 1989; Akwa et al., 1992; Doostzadeh and Morfin, 1996; Rose et al., 1997). CYP7B, originally cloned on the basis of hippocampal enrichment of expression (Stapleton et al., 1995), hydroxylates DHEA, pregnenolone and related sex steroid precursors, as well as 25-hydroxycholesterol, at the 7α-position. The enzyme also exhibits some activity at the 7β, 6α and 2 positions (Rose et al., 1997; Schwarz et al., 1997); 6α-hydroxylation is the predominant pathway for 5α-reduced substrates (Warner et al., 1989). Tissue extracts (with the exception of liver) from mice homozygous for targeted disruption of the gene encoding CYP7B fail to generate hydroxylated products of DHEA, 25-hydroxycholesterol or 5α-androstane-3β-,17β-diol at any of these positions (Rose et al., 2001), demonstrating that a single enzyme is responsible. In liver, hydroxylation of steroids, but not steroids, continues due to the expression of a related but distinct hepatic enzyme, CYP7A (Schwarz et al., 1997; Li-Hawkson et al., 2000). While CYP7B is particularly well...
expressed in rodent hippocampus, liver and kidney, enzyme activity and mRNA are present in diverse brain and body regions of mouse and rat (Stapleton et al., 1995; Rose et al., 2001).

The biological role of 7-hydroxylation is not known. 7α-hydroxylation of DHEA may be on a metabolic pathway to more potent derivatives (Lardy et al., 1995). Indeed, the immunostimulatory effects of DHEA and related steroids are boosted by 7-position hydroxylation (Morfín and Courchay, 1994; Loria and Padgett, 1998; Padgett and Loria, 1998). Intriguingly, 7-oxoDHEA is more active than DHEA in promoting memory function in mice, and effect more marked in aged animals (Shi et al., 2000). If 7-hydroxylation is a key activating step for action of DHEA and other sex steroid precursors, perhaps a loss of this pathway might contribute to the cognitive decline seen with ageing and age-related pathologies.

Sequences encoding CYP7B have been cloned from human (Setchell et al., 1998; Wu et al., 1999), but little is known of the distribution and role of the enzyme in primates, nor of its involvement in disease states including degenerative conditions affecting the CNS. If 7-oxygenated derivatives of DHEA have stimulatory activity in the brain, it is possible that alterations in DHEA metabolism might contribute to diseases such as AD. Here we studied CYP7B expression in the hippocampus of a range of mammalian species including non-human primates and humans. We also examined its expression in AD.

**EXPERIMENTAL PROCEDURES**

**Human CYP7B cloning**

The sequence of human CYP7B was obtained independently by the authors and by Drs. David W. Russell and Eric G. Lund, University of Texas Southwestern Medical School, Dallas, TX, USA. A human genomic clone encompassing exons 3 and 4 of the CYP7B gene was obtained by screening a size-selected 3.8 kb BamHI sub-library of human DNA using the mouse CYP7B cDNA as a hybridization probe. cDNA clones encompassing the remaining coding sequence were obtained by screening a human liver cDNA library (this work, a kind gift of Transgene SA, Strasbourg, France) or by screening human spleen (Dallas; obtained from Stratagene, La Jolla, CA, USA) or liver (Dallas; obtained from Clontech, Palo Alto, CA, USA) cDNA libraries. Positive signals were obtained at a frequency of one per 10^5 – 10^6 clones screened. The hybridization probe employed here was a 0.5 kb segment of human CYP7B genomic DNA encompassing 293 nt of exon 3 and 194 nt of intron 4 (R. Lathe, unpublished data). Preliminary human genomic sequence data were presented previously (Setchell et al., 1998).

**Tissues**

Mouse and rat brain tissues were from C57BL/6 and Fisher strains respectively. Sheep (Scottish blackface, 5 year; Roslin Institute, Edinburgh, UK), marmoset (F, 3 year; German Primate Research Centre, Gottingen, Germany), rat (n=2) and mouse (n=1) tissues and brain regions were excised immediately following terminal anesthesia (≤ 0.5 h postmortem). Human material (Harvard Brain Tissue Resource Center, Massachusetts, USA) used in the bioactivity assays and northern blots was from three patients (one female and two males aged 69–81 years, 14–25.5 h postmortem) dying of a cause unrelated to brain disease. The in situ hybridization in AD tissue employed sections from human brain adjacent to and representative of sections previously employed to study receptor mRNA expression in brain (Seckl et al., 1993). Brains were from five patients with typical clinical signs of dementia of the Alzheimer’s type (mean age 81 years; two males, three females, mean postmortem interval 12.5 h) and six age-matched control subjects (mean age 81 years; three males, three females, mean postmortem interval 11.8 h) without evidence of neurodegenerative disease. For the mapping of CYP7B mRNA in control human brain regions by in situ hybridization, postmortem sections (n=4/region, two females and two males aged 66–86 years, 24–48 h postmortem) of human hippocampus, frontal cortex, raphe, hypothalamus and cerebellum were obtained with ethical approval and relatives’ consent from the Edinburgh Brain Bank. All procedures were carried out in strict accordance with the UK Animals (Scientific Procedures) Act. All efforts were made to minimize the number of animals used.

**Steroid conversions**

Extracts of tissue samples were prepared by homogenization as described (Rose et al., 1997); standard DHEA hydroxylation assays employing [14C]-DHEA (Perkin Elmer Life Sciences, Hounslow, UK) substrate were performed and analyzed by thin-layer chromatography (TLC) and autoradiography as described (Rose et al., 1997, 2001). Samples were run alongside products obtained with recombinant CYP7B enzyme expressed from vaccinia virus (Rose et al., 1997) or in yeast (Vico et al., 2002). Unlabelled steroid standards were purchased from Steraloids (Newport, USA).

**Northern blotting**

RNA was extracted by a standard guanidinium protocol, resolved by denaturing gel electrophoresis, transferred to nylon, and hybridized with the human CYP7B probe under high SDS conditions (Virca et al., 1990; Rose et al., 2002).

**In situ hybridization**

This used 10 µm hippocampal sections previously reported (Seckl et al., 1993) cut at the level of the lateral geniculate and thalmounted onto gelatin-subbed, poly-L-lysine-coated slides. Blocks from cerebellum, raphe nuclei, frontal cortex, hypothalamus, locus coeruleus, parietal cortex and prefrontal cortex were also examined. The hybridization probe (human CYP7B, 0.5 kb) was radio-labelled by transcription in the presence of [35S]-substituted UTP. Brain sections were fixed in 4% paraformaldehyde, followed by acetylation (0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0), washed in phosphate-buffered saline, dehydrated through graded alcohols and air dried. Hybridization used 10^5 d.p.m./ml antisense probe as described (Yau et al., 1997); after RNase A treatment and washing (final stringency 0.1× SSC, 60 °C) slides were emulsion dipped (NTB-2; Kodak Ltd., UK) and exposed for 4 weeks, 4 °C. The density of silver grains was assessed over individual neurons by computer-assisted grain counting using an image analysis system (Seescan plc, Cambridge, UK). Silver grains were counted (using ×40 microscope objective under bright-field illumination) per pyramidal cell in the cornu ammonis (∼0.5 h postmortem). Human material (Harvard Brain Tissue Resource Center, Massachusetts, USA) used in the bioactivity assays and northern blots was from three patients (one female and two males aged 69–81 years, 14–25.5 h postmortem) dying of a cause unrelated to brain disease. The in situ hybridization in AD tissue employed sections from human brain adjacent to and representative of sections previously employed to study receptor mRNA expression in brain (Seckl et al., 1993). Brains were from five patients with typical clinical signs of dementia of the Alzheimer’s type (mean age 81 years; two males, three females, mean postmortem interval 12.5 h) and six age-matched control subjects (mean age 81 years; three males, three females, mean postmortem interval 11.8 h) without evidence of neurodegenerative disease. For the mapping of CYP7B mRNA in control human brain regions by in situ hybridization, postmortem sections (n=4/region, two females and two males aged 66–86 years, 24–48 h postmortem) of human hippocampus, frontal cortex, raphe, hypothalamus and cerebellum were obtained with ethical approval and relatives’ consent from the Edinburgh Brain Bank. All procedures were carried out in strict accordance with the UK Animals (Scientific Procedures) Act. All efforts were made to minimize the number of animals used.

**Steroid conversions**

Extracts of tissue samples were prepared by homogenization as described (Rose et al., 1997); standard DHEA hydroxylation assays employing [14C]-DHEA (Perkin Elmer Life Sciences, Hounslow, UK) substrate were performed and analyzed by thin-layer chromatography (TLC) and autoradiography as described (Rose et al., 1997, 2001). Samples were run alongside products obtained with recombinant CYP7B enzyme expressed from vaccinia virus (Rose et al., 1997) or in yeast (Vico et al., 2002). Unlabelled steroid standards were purchased from Steraloids (Newport, USA).

**Northern blotting**

RNA was extracted by a standard guanidinium protocol, resolved by denaturing gel electrophoresis, transferred to nylon, and hybridized with the human CYP7B probe under high SDS conditions (Virca et al., 1990; Rose et al., 2002).

**In situ hybridization**

This used 10 µm hippocampal sections previously reported (Seckl et al., 1993) cut at the level of the lateral geniculate and thaw-mounted onto gelatin-subbed, poly-L-lysine-coated slides. Blocks from cerebellum, raphe nuclei, frontal cortex, hypothalamus, locus coeruleus, parietal cortex and prefrontal cortex were also examined. The hybridization probe (human CYP7B, 0.5 kb) was radio-labelled by transcription in the presence of [35S]-substituted UTP. Brain sections were fixed in 4% paraformaldehyde, followed by acetylation (0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0), washed in phosphate-buffered saline, dehydrated through graded alcohols and air dried. Hybridization used 10^5 d.p.m./ml antisense probe as described (Yau et al., 1997); after RNase A treatment and washing (final stringency 0.1× SSC, 60 °C) slides were emulsion dipped (NTB-2; Kodak Ltd., UK) and exposed for 4 weeks, 4 °C. The density of silver grains was assessed over individual neurons by computer-assisted grain counting using an image analysis system (Seescan plc, Cambridge, UK). Silver grains were counted (using ×40 microscope objective under bright-field illumination) per pyramidal cell in the cornu ammonis (∼0.5 h postmortem). Human material (Harvard Brain Tissue Resource Center, Massachusetts, USA) used in the bioactivity assays and northern blots was from three patients (one female and two males aged 69–81 years, 14–25.5 h postmortem) dying of a cause unrelated to brain disease. The in situ hybridization in AD tissue employed sections from human brain adjacent to and representative of sections previously employed to study receptor mRNA expression in brain (Seckl et al., 1993). Brains were from five patients with typical clinical signs of dementia of the Alzheimer’s type (mean age 81 years; two males, three females, mean postmortem interval 12.5 h) and six age-matched control subjects (mean age 81 years; three males, three females, mean postmortem interval 11.8 h) without evidence of neurodegenerative disease. For the mapping of CYP7B mRNA in control human brain regions by in situ hybridization, postmortem sections (n=4/region, two females and two males aged 66–86 years, 24–48 h postmortem) of human hippocampus, frontal cortex, raphe, hypothalamus and cerebellum were obtained with ethical approval and relatives’ consent from the Edinburgh Brain Bank. All procedures were carried out in strict accordance with the UK Animals (Scientific Procedures) Act. All efforts were made to minimize the number of animals used.
RESULTS

Conservation of DHEA hydroxylation activity across species

We previously reported hydroxylation of DHEA in ex vivo brain extracts from rat and mouse. To determine whether the reaction is conserved across species, homogenates from sheep and marmoset brain and body regions were incubated with DHEA substrate and appropriate cofactors and the products analyzed by chromatography. A single major product was produced in different regions of sheep and marmoset brain (Fig. 1). We previously reported that mouse and rat brain metabolism of DHEA yields a molecule formally identified as 7α-hydroxyDHEA. The sheep and marmoset brain molecule co-migrates on two different TLC systems (Fig. 1 and not presented) with both the CYP7B and rodent brain product 7α-hydroxyDHEA and also with reference standard; we infer that the major metabolite of DHEA in ex vivo sheep and marmoset brain is 7α-hydroxy DHEA. Conversion was also seen in marmoset kidney, spleen, prostate and liver, but not at significant levels in muscle or heart (Fig. 1D). In these tissues, with the exception of mouse brain (Fig. 1B), there was little conversion to the 17β-HSD product of DHEA, androstenediol, that migrates immediately below the DHEA substrate on this TLC system (Rose et al., 1997; Rose et al., 2001; Vico et al., 2002).

CYP7B activity in postmortem samples of human hippocampal tissue

Despite variable levels of activity, we observe conversion of DHEA to a molecule that co-chromatographs with 7α-hydroxyDHEA on two different solvent systems (Fig. 1E and not shown), and which we assume to be identical to 7α-hydroxyDHEA; significant 17β-HSD conversion of DHEA to androstenediol was observed in these postmortem human hippocampal samples.

CYP7B mRNA is expressed in marmoset and human brain

To address whether CYP7B enzyme is responsible for this ex vivo hydroxylation activity we obtained human CYP7B exon sequences from a genomic library (materials and methods). An exon III+IV probe was used to explore mRNA from marmoset and human brain (Northern blotting). CYP7B mRNA (approximately 2 kb and approximately 6 kb) was readily detected in brainstem, cerebellum and hippocampus of marmoset brain (Fig. 2A); levels were lower in cortex and hypothalamus than in hippocampus, while mRNA was not detected in the kidney, spleen or lung samples, tissues that displayed significant DHEA hydroxylation activity, attesting to the insensitivity of this technique. In human brain, CYP7B mRNA (approximately 2 kb and >7 kb) was readily detected in all three hippocampal samples analyzed (Fig. 2B).

Abbreviations used in the figures

Bs brainstem
Cb cerebellum
Cx cortex
DG dentate gyrus
G granular
Hp hippocampus
Ht heart
Ki kidney
Li liver
Lu lung
M molecular layer
m’set marmoset
Mu muscle
Pr prostate
Sp spleen
V0 control vaccinia virus with no insert
Distribution of CYP7B mRNA in human brain

To refine our analysis of the distribution of CYP7B mRNA in human brain, in situ hybridization was performed upon postmortem brain sections. Abundant CYP7B transcripts were detected in hippocampal neurons, including cells of the dentate gyrus and cornu ammonis (Fig. 3), with lower expression in the cerebellum (CYP7B mRNA expression was found in the granular and Purkinje layer but not molecular layer of the cerebellum at the microscopic level in contrast to the sense control), but there was little expression in other regions examined (prefrontal cortex, frontal cortex, parietal cortex, hypothalamus, raphe nuclei, and locus coeruleus; not shown). Control sections hybridized with positive strand RNA showed no specific signal and low background (Fig. 3B, D).

CYP7B activity is abundant in primate hippocampus

To assess the relative abundance of DHEA hydroxylase enzyme in different brain regions, steroid conversion autoradiographic signals were quantitated and the reaction extent was determined (Fig. 4). In rat and sheep brain,
similar levels of substrate conversion were seen in hippocampus and cortex extracts. In marmoset, activity was enriched in hippocampus but the level of activity appeared lower than in rat or sheep. In human, we were only able to access one series of control brain samples, for comparative purposes, where hippocampus and cortex originated from a single individual. Here, overall activity was much reduced over that seen in marmoset possibly due to enzyme denaturation during the postmortem period. However, this sample pair confirmed enrichment of CYP7B activity in hippocampus (Fig. 4). In other human samples (e.g. Fig. 1E) the conversion rates were higher, estimated to be between 8 and 27% substrate conversion (not presented), suggesting that the human pattern may more resemble that found in marmoset. Furthermore, conversion of DHEA to androstenediol (by 17β-hydroxysteroid dehydrogenase activity) appears to be more effective in human brain than in marmoset (see Fig. 1), further depressing the yield of 7-hydroxyDHEA and thereby apparent CYP7B activity.

CYP7B expression is reduced in AD brain

Because DHEA and its metabolites may have neuroprotective properties, we surmised that CYP7B levels could be altered in AD. Postmortem brains were obtained from five patients with typical clinical signs of dementia of the Alzheimer’s type were compared with six age-matched controls. These same sections were previously analyzed for changes in glucocorticoid receptor (GR and MR) expression levels; none were observed (Seckl et al., 1993). Parallel sections were analyzed by in situ hybridization to CYP7B and control probes; mRNA levels were assessed by semi-automatic quantitation of film grains at high resolution. CYP7B mRNA expression per neuron was found to be significantly reduced in AD samples, and preferentially in dentate granule neurons (approximately 50% decrease, \( P<0.01 \) compared with controls) and CA1 pyramidal neurons (\( P<0.05; \) Fig. 5) but not in other subfields examined.

**DISCUSSION**

We report that sheep, marmoset and human brain extracts convert DHEA to 7α-hydroxyDHEA. In sheep, as in rat, levels of conversion were similar in cortex and hippocampus. Primate brain showed considerable enrichment in hippocampus: in marmoset, conversion levels were more than five times greater in hippocampus than in cortex, a similar difference was observed in human brain despite reduced activity that is likely due to the extended postmortem interval. This reinforces our previous reports of enrichment in rodent hippocampus (Stapleton et al., 1995; Rose et al., 2001) and is consistent with recent reports showing 7α-hydroxylase activity in various human brain regions, including hippocampus (Steckelbroeck et al., 2002; Weill-Engerer et al., 2003). The presence of DHEA-hydroxylating activity in all species we have analyzed, including mouse and rat (Rose et al., 1997), sheep, marmoset and human (this work) demonstrates that the hydroxylation reaction is well conserved across mammalian species.
2002) is consistent with our finding of CYP7B mRNA in human brain. In mice, cyp7b gene inactivation abolishes DHEA hydroxylation in brain, demonstrating that a single enzyme is responsible in this species (Rose et al., 2001). Although we cannot be certain that CYP7B is responsible for this conversion in primates, no other brain enzyme capable of catalyzing this reaction has been reported. We suggest that, as in mice, CYP7B is responsible for brain 7-hydroxylation of DHEA and other substrates in primates including human.

We also report that per neuron levels of CYP7B mRNA are reduced in AD hippocampal sections, specifically in dentate gyrus and CA1, compared with age-matched controls. This is not due to a general decline in mRNA in the AD sections because previous studies with these specimens showed other transcripts (glucocorticoid receptor and mineralocorticoid receptor) to be unaltered in surviving neurons in AD (Seckl et al., 1993). Our data suggest that the AD brain may be selectively impaired in its ability to synthesize 7α-hydroxyDHEA from DHEA. In contrast, a recent study found no significant difference in the metabolism of DHEA to 7α-hydroxyDHEA in AD brain samples and age-matched non-demented controls (Weill-Engerer et al., 2003). However, in contrast to the usual stability of mRNAs in postmortem brain, there are well-documented difficulties in measuring the function (activity) of labile proteins such as enzymes in postmortem brain, due to the likely variable enzyme denaturation during postmortem delays, which might well obscure differences between groups—measuring 7α-hydroxyDHEA levels in the brain tissues would be more useful and informative but is unreported. In addition, while our data showed the decline in CYP7B mRNA expression to be most prominent in the dentate gyrus of AD brains, gross tissue studies used to measure enzyme activity may miss such changes. Weill-Engerer et al. (2003) also showed significantly higher 7α-hydroxylation activity in frontal cortex homogenates than in any other brain area tested (including hippocampus). This contrasts with our observation that conversion to 7α-hydroxyDHEA is highest in hippocampus in both marmoset and human. One factor which may underlie the different findings could be the reaction time used in the enzyme activity assays. Weill-Engerer et al. (2003) incubated substrate with homogenate for 6 h while we used much shorter times (15 min; Rose et al., 2001). Extended incubation could lead to differential decay of cofactors such as NADPH, of the enzyme itself, or favor conversion to 17β-hydroxysteroid dehydrogenase products. These could depress apparent CYP7B activity in hippocampus versus cortex. Moreover, our in situ hybridization study on human brain confirms more prominent expression of CYP7B mRNA in hippocampus versus overlying cortical regions and cerebellum. Furthermore, we have recently also found a decrease in hippocampal CYP7B enzyme activity selectively in cognitively impaired aged rats, compared both to aged-matched rats with preserved memory and to young rats (Graham et al., 2001). Consistent with this, 7α-hydroxylation of DHEA by whole mouse brain microsomes decreases in older mice (Doostzadeh and Morfin, 1996). Although care needs to be taken in extrapolating from animal data to humans, not least because of the much lower DHEA levels in rodents, the clear link between hippocampal CYP7B (activity and mRNA) and age-related cognitive impairments in rodents supports the relationships we have found in AD patients.

DHEA and its sulfate ester (DHEAS) have been shown to improve memory in mice (Flood and Roberts, 1988; Flood et al., 1988) and show neuroprotection against the β-amyloid peptide-induced neurotoxicity in vitro (Mao and Barger, 1998; Cardounel et al., 1999). In particular, the 7-hydroxylated metabolites of DHEA have been proposed to exert anti-glucocorticoid effects in vivo (Kalimi et al., 1994; Starka et al., 1998; Lathe and Seckl, 2002). The mechanism whereby 7-hydroxyDHEA counters glucocorticoid actions is not known but a recent study showed that 7-hydroxy-DHEA could prevent the nuclear uptake of [3H]-dexamethasone-activated GR in brain cells in vitro (Morfin and Starka, 2001). Since AD is accompanied by elevated plasma cortisol (Davis et al., 1986; Swaab et al., 1994; Rasmussen et al., 2002) which may attenuate cognitive function, reduced hippocampal CYP7B activity in AD (and thus a decreased contribution of DHEA’s potential neuroprotective role via 7α-hydroxyDHEA) would be anticipated to promote the disease processes (Seckl and Olsson, 1995). Moreover, both plasma DHEAS and intracerebral DHEA levels are reduced in patients with severe AD (Sandland et al., 1989; Nasman et al., 1991; Weill-Engerer et al., 2002). Thus, reduced CYP7B metabolism of DHEA and perhaps other sex steroid precursors to their 7-hydroxy forms in the hippocampus might contribute to unopposed anti-cognitive effects of glucocorticoids in human ageing and in AD patients. Whatever the precise mechanism, this work suggests that the lack of response of the more elderly (Wolf and Kirschbaum, 1996) to the central effects of DHEA may reflect a lack of CYP7B to activate this precursor in critical CNS regions.

Acknowledgements—We thank R. G. M. Morris, J. Webster and B. Whitelaw for expert assistance in providing ovine material. We are grateful for human brain material provided by the Harvard Brain Tissue Resource Centre, supported in part by PHS grant number MH/NS31862-25 and the Edinburgh Brain Bank, University of Edinburgh. This work was supported by a Medical Research Council grant (to R.L. and R. G. M. Morris, Centre for Neuroscience, Edinburgh), a Wellcome Trust project grant (J.R.S. and R.L.), a BBSRC science into ageing (SAGE) initiative grant (J.L.W.Y., R.L. and J.R.S.), and by European Commission (BIO4-CT98-0311) funding (R.L. and J.R.S., with J. A. Gustafsson, Stockholm, and E. Degryse, Strasbourg) and by The Foundation Gamla Tjänningar (S.R.).

REFERENCES


and unconjugated steroids in 40- to 80-year-old men. J Clin Endo-
crinol Metab 79:1086–1090.

protects hippocampal neurons against neurotoxin-induced cell

Davis KL, Davis BM, Greenwald BS, Mohs RC, Mathe AA, Johns CA,
Horvath TB (1986) Cortisol and Alzheimer’s disease: I. Basal stud-

Doostzadeh J, Morfin R (1996) Studies of the enzyme complex re-
 sponsible for pregnenolone and dehydroepiandrosterone 7α-

Flood JF, Roberts E (1998) Dehydroepiandrosterone sulfamate im-

Flood JF, Smith GE, Roberts E (1988) Dehydroepiandrosterone and
its sulfamate enhance memory retention in mice. Brain Res 447:269–
278.

Flood JF, Morley JE, Roberts E (1992) Memory-enhancing effects in
male mice of pregnenolone and steroids metabolically derived from it. Proc Natl Acad Sci USA 89:1567–1571.


Lane MA, Ingram DK, Ball SS, Roth GS (1997) Dehydroepiandro-
sterone sulfate: a biomarker of primate aging slowed by calorie

thermogenic enzymes in liver of rats treated with steroids derived from dehydroepiandrosterone. Proc Natl Acad Sci USA 92:6617–
6619.

ics of steroid biosynthesis and function. Modern genetics, Vol 6

Li-Hawkins J, Lund EG, Turley SD, Russell DW (2000) Disruption of
the oxysterol 7α-hydroxylation gene in mice. J Biol Chem 275:
16536–16542.

Loria RM, Padgett DA (1998) Control of the immune response by

Mao X, Barger SW (1998) Neuroprotection by dehydroepiandro-
sterone-sulfate: role of an NFkappaB-like factor. Neuronoreport
9:759–763.

Morales AJ, Nolan JJ, Nelson JC, Yen SSC (1994) Effects of replace-
ment dose of dehydroepiandrosterone in men and women of ad-

Morfin R, Courchay G (1994) Pregnenolone and dehydroepiandro-
sterone as precursors of native 7-hydroxylated metabolites which
increase the immune response in mice. J Steroid Biochem Mol Biol
50:91–100.

Morfin R, Starka L (2001) Neurosteroid 7-hydroxylation products in the

Morley JE, Kaiser F, Raum WJ, Perry HM III, Flood JF, Jensen S,
blood serum correlates of aging in the healthy human male: pro-
gressive decreases in bioavailable testosterone, dehydroepiandro-
sterone sulfate, and the rate of insulin-like growth factor 1 to growth

Nasman B, Olsson T, Backstrom T, Eriksson S, Grankvist K, Viitanen
M, et al. (1991) Serum dehydroepiandrosterone sulfhate in Alzhei-
mer’s disease and multi-infarct dementia. Biol Psychiatry 30:684–
690.

Long-term longitudinal measurements of plasma dehydroepiandro-
sterone sulphate in normal men. J Clin Endocrinol Metab 75:1002–
1004.

Padgett DA, Loria RM (1998) Endocrine regulation of murine macro-
phage function: effects of dehydroepiandrosterone, androstenediol,

levels of adrenocortical and gonadal hormones in mild to moderate


Rose K, Allan A, Gauldie S, Stapleton G, Dobbie L, Dott K, Martin C,
Neurosteroid hydroxylase CYP7B: vivid reporter activity in dentate
gyrus of gene-targeted mice and abolition of a widespread pathway of
23944.

Rose KA, Stapleton G, Dott K, Kiency MP, Best R, Schwarz M, Russell
DW, Bjorkhem I, Seckl J, Lathe R (1997) Cyp7b, a novel brain cytochrome P450, catalyzes the synthesis of neurosteroids 7α-
hydroxy dehydroepiandrosterone and 7α-hydroxy pregnenolone. Proc Natl Acad Sci USA 94:4925–4930.

Schwarz M, Lund EG, Lathe R, Bjorkhem I, Russell DW (1997) Iden-
tification and characterization of a mouse oxysterol 7α-hydrox-

Seckl JR, Olsson T (1995) Glucocorticoids and the age-impaired
hippocampus: cause or effect? J Endocrinol 145:201–211.

(1993) Glucocorticoid receptor gene expression is unaltered in
hippocampal neurons in Alzheimer’s disease. Mol Brain Res 18:
239–245.

Setchell KD, Schwarz M, O’Connell NC, Lund EG, Davis DL, Lathe R,
Identification of a new inborn error in bile acid synthesis: mutation
of the oxysterol 7α-hydroxylase gene causes severe neonatal

memory in young and old C57BL/6 mice. Steroids 65:124–129.

Stapleton G, Steel M, Richardson M, Mason JO, Rose KA, Morris
RGM, Lathe R (1995) A novel cytochrome P450 expressed primar-

Starka L, Hill M, Hampl R, Malewiak M, Benalycherif A, Morfin R,
Kolena J, Scsukova S (1998) Studies on the mechanism of anti-
glucocorticoid action of 7 α-hydroxydehydroepiandrosterone.

Steckelbroeck S, Watzka M, Lufjohann D, Makicola P, Nassen A, Hans
VH, Clusmann H, Reissinger A, Ludwig W, Siekmann L, Klingmuller
D (2002) Characterization of the dehydroepiandrosterone (DHEA)
metabolism via oxysterol 7α-hydroxylation and 17-kerasteroid

Sunderland, T, Merrill, CR, Harrington, MG, Lawlor, BA, Molchan, SE,
Martinez, R, Murphy, DL (1989) Reduced plasma dehydroepi-
androsterone concentrations in Alzheimer’s disease. Lancet 2
(8662):570.

Swaab DF, Raadsheer FC, Endert, E, Hofman MA, Kamphorst W,
Ravìd R (1994) Increased cortisol levels in aging and Alzheimer’s
disease in postmortem cerebrospinal fluid. J Neuroendocrinol

Valleja M, Mayo W, Le Moal M (2001) Role of pregnenolone, dehydro-
epiandrosterone and their sulfate esters on learning and memory in


androsterone (DHEA) metabolism in Saccharomyces cerevisiae
expressing mammalian steroid hydroxylase CYP7B: Ayr1p and
Fox2p display 17beta-hydroxysteroid dehydrogenase activity.

Simplified northern blot hybridization using 5% sodium dodecyl


(Accepted 30 May 2003)