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

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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 the loss of effects of DHEA with ageing and perhaps to the pathophysiology of AD. © 2003 IBRO. Published by Elsevier Ltd. All rights reserved.

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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., 1992; 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, 1999; 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-hepatic 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 sterols, but not steroids, continues due to the expression of a related but distinct hepatic enzyme, CYP7A (Schwarz et al., 1997; Li-Hawkins et al., 2000). While CYP7B is particularly well

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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 (Morfin 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 *Bam*HI 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, F, 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* hybridiza-

tion 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 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 [^{14}C]-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 coerulus, parietal cortex and prefrontal cortex were also examined. The hybridization probe (human CYP7B, 0.5 kb) was radiolabelled by transcription in the presence of [^{35}S]-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 10^7 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 (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 (6–10 cells/subregion for each specimen) and in the dentate gyrus (eight to 12 cells in total per specimen). Background, counted over areas of neutrophil (equivalent to approximately 10% of total expression for CYP7B mRNA per subregion) was subtracted from the mean counts per subregion for each specimen. All grain counting was performed blind to the diagnosis. Data were assessed by ANOVA followed by the Scheffe post hoc test. Significance was set at P<0.05. Values are means±S.E.M.
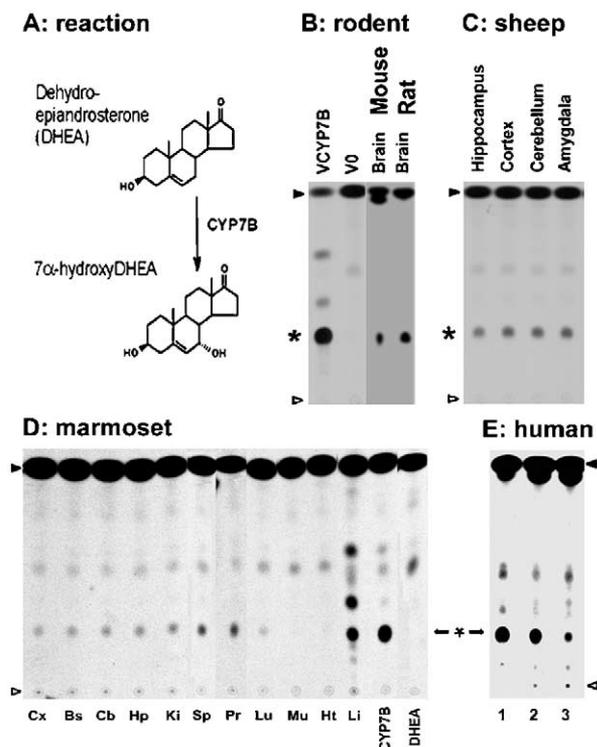
Fig. 1. DHEA hydroxylation activity in brains of different species including primates. For rodents, total brain extracts were employed. Sheep brain regions were as indicated. M'set brain and body regions were Cx, Bs, Cb, Hp, Ki, Sp, Pr, Lu, Mu, Ht, Li, CYP7B (recombinant rat CYP7B enzyme expressed from vaccinia virus), DHEA (substrate alone). Human samples were hippocampus from three subjects. The product marked (*) comigrates with 7α-hydroxyDHEA on two different solvent systems and fails to comigrate with 7β-hydroxyDHEA; other analyses previously confirmed 7α-hydroxyDHEA (Rose et al., 1997; Vico et al., 2002). The product migrating immediately below DHEA on the chromatogram, and which is prominent in mouse and human but not in rat, sheep or m'set, has previously been shown to be the product of 17β-HSD activity, androstenediol (Vico et al., 2002).

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., 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

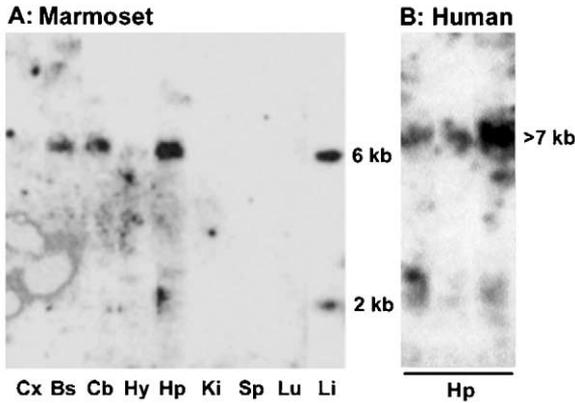|			
Bs	brainstem	Lu	lung
Cb	cerebellum	M	molecular layer
Cx	cortex	m'set	marmoset
DG	dentate gyrus	Mu	muscle
G	granular	Pr	prostate
Hp	hippocampus	Sp	spleen
Ht	heart	V0	control vaccinia virus with no insert
Ki	kidney		
Li	liver		

Fig. 2. Northern analysis of m'set and human brain for CYP7B sequences. Marmost brain regions were as in Fig. 1; highest levels of expression were seen in Li (this one track has been underexposed) with next most prominent expression in Hp.

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).
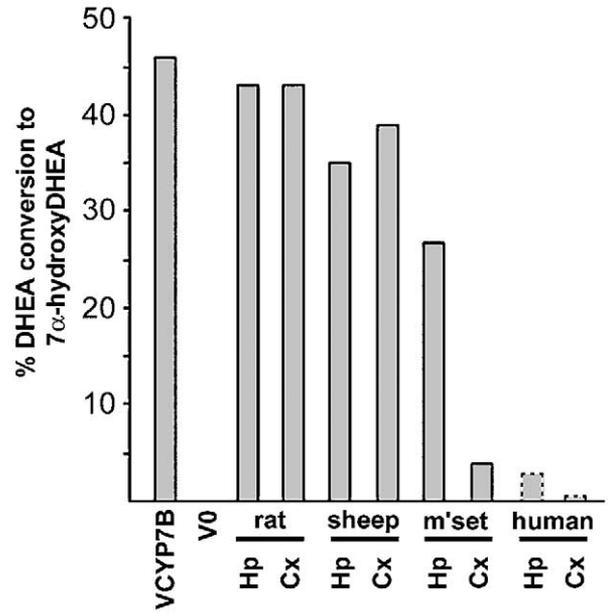

Fig. 4. Conversion of DHEA to 7α-hydroxyDHEA assessed by autoradiogram signal quantitation (constant protein concentration; single samples). VCYP7B, extracts of Hela cells infected with vaccinia virus expressing rat CYP7B enzyme (Rose et al., 1997). The rat, sheep and m'set samples were immediately frozen; the human samples (dashed) were from an individual (postmortem interval, 24 h) may underestimate the extent of conversion *in vivo*.

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,
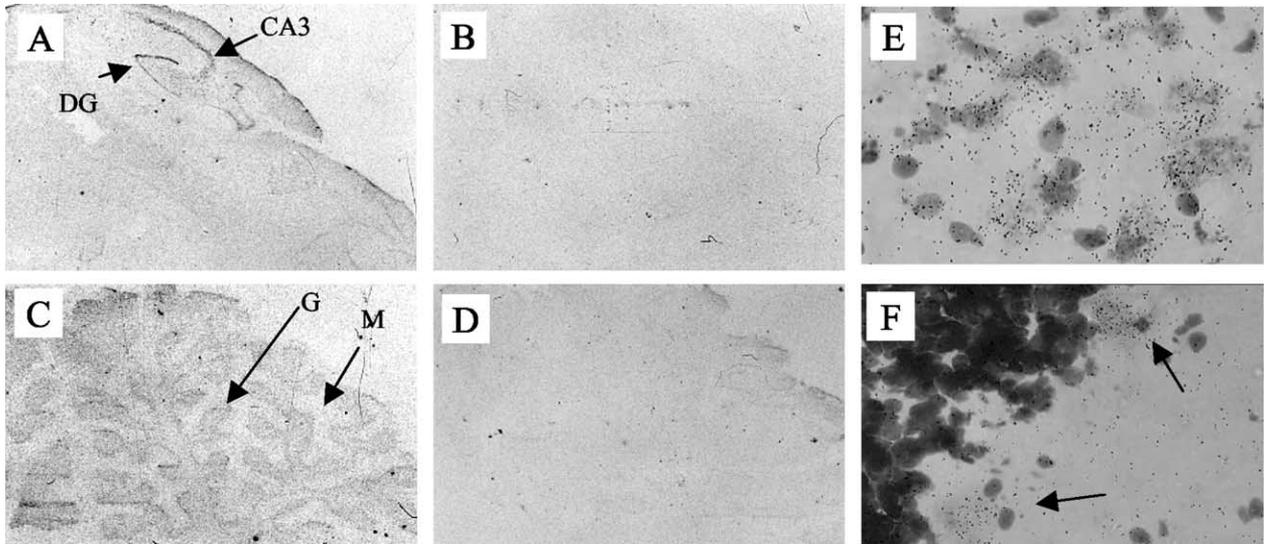

Fig. 3. CYP7B mRNA expression in human hippocampus and cerebellum (representative *in situ* on a specimen from one patient with no CNS disorder). At the autoradiograph level note high CYP7B mRNA expression using antisense CYP7B probe in DG and CA3 regions of the Hp (A) with lower-level CYP7B mRNA expression in Cb, found in the G but not M (C). No significant signal was detected above background on duplicate sections (B, D) using the corresponding sense probes. At the microscopic level under bright-field illumination (×40 objective), an example of CYP7B mRNA expression is shown over CA3 pyramidal cells (E) and in the Cb, high expression of CYP7B mRNA is over Purkinje cells (arrows, F).

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-hydroxylation 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.
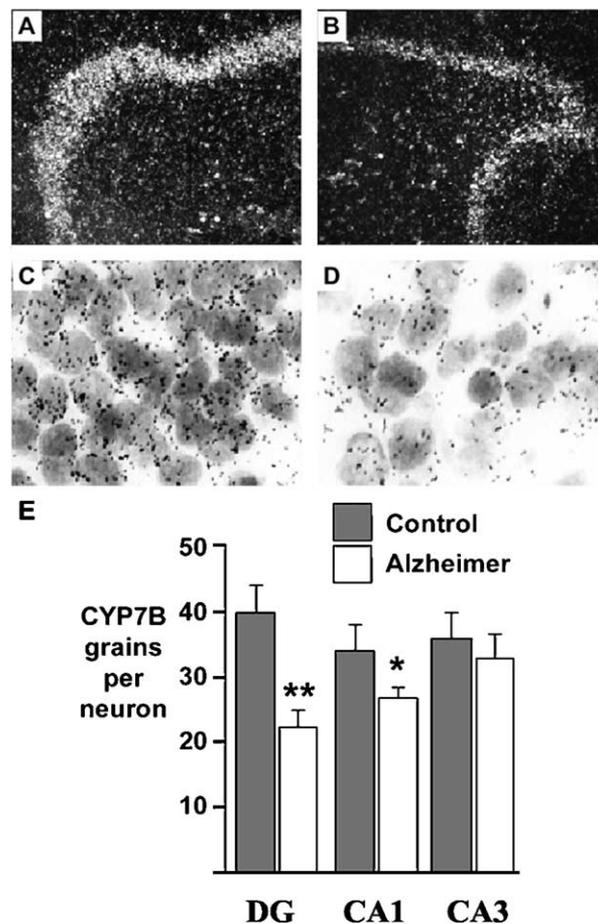

Fig. 5. *In situ* hybridization assay of per-neuron levels of CYP7B mRNA in hippocampus (DG, CA1 and CA3) of AD and age-matched control brain. A, B: Representative dark-field sections of control (A) and Alzheimer (B) Hp showing photographic grains over neurons expressing CYP7B in the cell layer of the DG. C, D: High power light-field analysis of grain counts over individual neurons of control (C) and Alzheimer (D) brain. E: Quantitation of per-neuron levels of expression of CYP7B in normal and Alzheimer brains showing significant reductions in expression in DG and region CA1 from AD brain. Data were analyzed by ANOVA followed by the Newman-Keuls post hoc test. Significance was set at $P<0.01$ (**, DG) and $P<0.05$ (*, CA1).

Hybridization studies point to CYP7B as the enzyme responsible. We obtained a human cDNA for use as a hybridization probe: consistent with a recent report of the cloning of human CYP7B (Wu et al., 1999) the human CYP7B sequence is 64% identical to mouse and rat CYP7B (Stapleton et al., 1995; data not shown). The full-length coding region of CYP7B specifies an enzyme capable of 7α-hydroxylating DHEA and 25-hydroxycholesterol (D. W. Russell, personal communications). Northern blotting revealed abundant CYP7B mRNA in marmoset hippocampus, with lower levels elsewhere. Northern blotting also detected CYP7B mRNA in human hippocampus, while *in situ* hybridization to brain sections demonstrated CYP7B mRNA expression particularly in CA1-3 and dentate gyrus neuronal layers. The presence of high levels of CYP7B mRNA expression in temporal lobe biopsies obtained from patients with epilepsy (Steckelbroeck et al.,

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 [^{3}H]-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; Rasmuson 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 (Sunderland 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, 1999b) to the central effects of DHEA may reflect a lack of CYP7B to activate this precursor in critical CNS regions.

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