cyp7b1 catalyses the 7α-hydroxylation of dehydroepiandrosterone and 25-hydroxycholesterol in rat prostate

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Dehydroepiandrosterone (DHEA) is the most prominent circulating steroid in humans, and it is a precursor for sex-steroid synthesis in peripheral tissues, including the prostate. Recently, enzyme-mediated pre-receptor metabolism has been recognized as a key step in determining steroid action *in vivo*. Hydroxylation of 3β-steroids at the 7α-position has been reported in rat and human prostate to be a major inhibitory pathway to sex-steroid synthesis/action. However, the molecular identity of the enzyme responsible is so far unknown. We recently described a novel cytochrome P450 enzyme, cyp7b1, strongly expressed in the hippocampus of rodent brain, which catalyses the metabolism of DHEA, pregnenolone and 25-hydroxycholesterol to 7α-hydroxy products. In the light of this new enzyme, we have examined its possible role in 7α-hydroxylation conversion in rat prostate. NADPH-dependent 7α-hydroxylation was confirmed for 3β-hydroxysteroids including DHEA and androstenediol, as well as 25-hydroxycholesterol. Kinetic analysis yielded an apparent K_m of 14 ± 1 μM for 7α-hydroxylation of DHEA in the prostate gland, a value similar to that recorded for recombinant cyp7b1 enzyme [13.6 μM; Rose, Stapleton, Dott, Kieny, Best, Schwarz, Russell, Bjoorkheim, Seckl and Lathe (1997) Proc. Natl. Acad. Sci. U.S.A. **94**, 4925–4930]. The V_{max} value of the prostate was 46 ± 2 pmol/min per mg, and this activity was inhibited by clotrimazole, a P450-enzyme blocker. Moreover, RNA analysis (reverse-transcription PCR, Northern blotting and *in situ* hybridization) revealed a high expression of cyp7b1 mRNA in the rat prostate, restricted to the epithelium, suggesting that cyp7b1 catalyses oxysterol 7α-hydroxylation in the prostate gland.

Key words: brain, cholesterol, cytochrome P450, epithelium.

INTRODUCTION

Hyperplasia and subsequent neoplastic transformation of the prostate gland constitutes a significant health risk, in particularly in elderly males. Much research has been directed towards an understanding of the factors governing cell proliferation in this tissue. Growth, development and function of the prostate are subject to hormonal control, in particular by androgens. *In vivo*, steroids with androgenic activity originate primarily from two sources: from the endocrine gonads via the circulation, and via local synthesis in target tissues from the adrenal precursor dehydroepiandrosterone (DHEA). DHEA and its sulphate ester are the most abundant steroids in human blood [1], and together they provide the substrates for testosterone and oestradiol biosynthesis in accessory reproductive organs such as the mammary gland and the prostate, and also in brain [2] (Scheme 1). DHEA itself may have anti-glucocorticoid, neuroprotective and immunoprotective properties [3–7]. Intriguingly, DHEA is also reported to inhibit carcinogenesis in a variety of tissues, such as mammary gland, skin, lung, liver and prostate [8–13]. However, despite many studies suggesting that DHEA or related molecules may have considerable therapeutic potential, the mechanism of action of DHEA remains unclear, and no dedicated receptor has yet been identified.

Local metabolism in target tissues is an important determinant of steroid hormone action. A major pathway of DHEA metabolism is via hydroxylation, principally at the 7α position. Such activity has been reported in the human, mouse and rat, and in a number of tissues and organs, such as skin, brain, prostate and liver [14–17]. The enzyme(s) that are responsible for this in most tissues are unknown. In liver, three different 7α-hydroxylation pathways, each with distinct roles, are thought to regulate cholesterol homoeostasis by producing 7α-hydroxylated bile acids. In one pathway, cholesterol is converted by cholesterol 7α-hydroxylase (cyp7a) into 7α-hydroxycholesterol (7α-HOChol) [18–20]. cyp7a shows a marked preference for cholesterol, and is not active against DHEA. In another pathway, cholesterol is first converted into 24-HOChol before being 7α-hydroxylated by cyp39A1 [21]. Both cyp7a and cyp39A1 are primarily expressed in the liver, and appear to play primary roles in bile-acid synthesis and cholesterol degradation.

We recently identified a new enzyme, cyp7b1, which is highly expressed in brain, particularly in the hippocampus, but which is also expressed in kidney and liver, although at a much lower level [22,23]. cyp7b1 is a dual-acting steroid and sterol 7α-hydroxylase. Whereas in the liver it appears to contribute to bile acid synthesis via 7α-hydroxylation of HOChol [24,25], cyp7b1 (unlike cyp7a and cyp39A1) is also active against circulating 3β-hydroxysteroids, including DHEA [23–25].

In the prostate, 7α-hydroxylation of 3β-hydroxysteroids has also been described, but the enzyme responsible for the 7α-hydroxylation of DHEA and related molecules has not been identified [14,26,27]. In the present study, we used a combination of RNA analysis [reverse-transcription (RT) with PCR, Northern blotting and *in situ* hybridization] and biochemical techniques to investigate the 7α-hydroxylase enzyme in rat prostate. We

Abbreviations used: A/enedione, 4-androstene-3,17-dione; A/anediol, 5α-androstane-3β,17β-diol; A/anetriol, 3β,7α,17β-androstanetriol; A/enediol, 5-androstene-3β,17β-diol; A/enetriol, 3β,7α,17β-androstenetriol; cyp7a, cholesterol 7α-hydroxylase; DHEA, dehydroepiandrosterone; E_2, 17β-oestradiol; HOChol, hydroxycholesterol; RT, reverse transcription. The term cyp7b1 is used in this paper to refer to mRNA and/or protein corresponding to P450 cyp7b1, irrespective of the species of origin.

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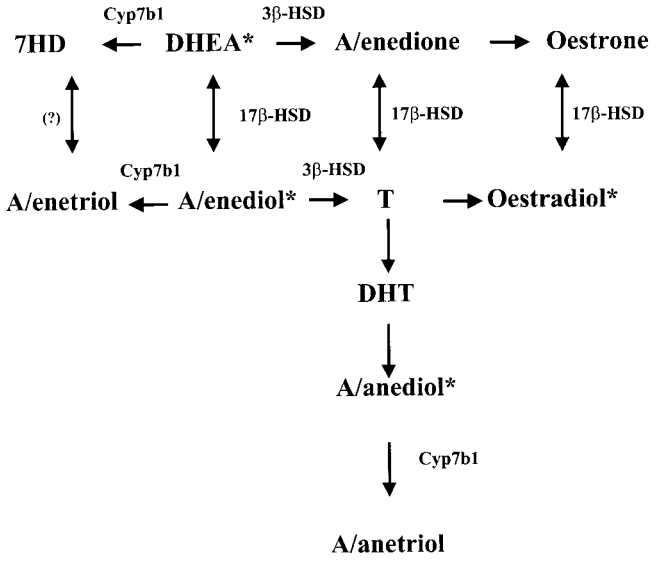

Scheme 1 Steroid pathway of DHEA

Abbreviations: 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase. The asterisks show the steroid substrates for cyp7b1.

demonstrate that cyp7b1 mRNA is expressed in rat prostate, and may be the predominant enzyme responsible for local steroid 7α-hydroxylation. We discuss the possible role of cyp7b1 expression in the prostate.

EXPERIMENTAL

Animals

Male Wistar rats (1 to 8 months old) were used for experiments. Animals were killed by cervical dislocation. Brain, liver and ventral prostate were rapidly removed and directly frozen in liquid nitrogen. All tissues were stored at -70 °C for the subsequent preparation of RNA or protein extracts.

Steroids

[1,2,6,7-^{3}H]DHEA (60 Ci/mmol), [9,11-^{3}H]5α-androstane-3β,17β-diol (A/anediol; 50 Ci/mmol), [1,2-^{3}H]3β,7α,17β-androstenetriol (A/enediol; 42 Ci/mmol) and [26,27-^{3}H]25-HOChol (78.5 Ci/mmol) were purchased from NEN Life Science Products, Boston, MA, U.S.A. [2,4,6,7,16,17-^{3}H]17β-Oestradiol (153 Ci/mmol) was purchased from Amersham Pharmacia Biotech. (Little Chalfont, Bucks., U.K.). Non-radioactive steroids and clotrimazole were obtained from Sigma–Aldrich (Poole, Dorset, U.K.). 7α-Hydroxy-DHEA was purchased from Steraloids Inc. (Wilton, NH, U.S.A.).

7α-Hydroxylase activity

7α-Hydroxylase activity was determined in homogenates of pooled rat prostate. Rat brain and protein extracts from HeLa cells transfected with recombinant cyp7b1 (vaccinia virus-cyp7b1) were used as a positive control [23]. Liver, a tissue expressing cyp7a, was included for comparison purposes. Frozen tissue was homogenized in ice-cold PBS containing 20 % (w/v) glycerol, 0.25 M sucrose, 1 mM EDTA, 1 μg/ml pepstatin and 0.1 mM PMSF. The homogenate was centrifuged (4000 ***g***, 5 min, 4 °C) and the supernatant was separated into aliquots and stored at -70 °C. Ethanol solutions of the radiolabelled steroid substrates were dried under nitrogen in glass tubes. Substrate stock solutions were diluted in ethanol or acetone/ethanol, and reactions were performed using substrate concentrations in the range of 5–20 μM, as described previously [23]. Incubations were carried out in a total vol. of 1 ml. KPO_4 buffer (0.1 M, pH 7.4) containing 1 mM EDTA was added, followed by the protein extract; this was then mixed and incubated at 37 °C in a shaking water bath. Reactions were started by addition of NADPH to a final concentration of 1 mM. A 15 min incubation time was employed within the linear part of the relationship between product formation and time. Steroids were extracted with ethyl acetate, dried and resuspended in 10 μl of ethyl acetate. Recovery was typically around 90 %. Reaction products were applied to silica-gel TLC plates and developed in ethyl acetate/n-hexane/acetic acid (16:8:1, by vol.). In this buffer, 7α-hydroxy-DHEA and 7β-hydroxy-DHEA differ in terms of their migration on TLC plates, with a mobility relative to DHEA of 0.32 and 0.5 for 7α-hydroxy-DHEA and 7β-hydroxy-DHEA respectively [23]. Quantification of chromatograms was accomplished by using a Phospho-Imager (FLA-2000; Fujifilm, Straubenhardt, Germany). Initial reaction velocities were expressed in pmol/min per mg of protein. Linear regression analysis was computed using the 'Sigma Plot' program and a double reciprocal plot (Lineweaver–Burk) was used to determine V_{max} and K_m values. Experiments were repeated three times.

HPLC analysis

A Waters HPLC system and an on-line scintillation counting system (Berthold, Pforzheim, Germany) were used to verify the identity of 7α-hydroxylated products of DHEA. The column utilized was a reversed-phase C_{18} column (Phenomenex, Hösbach, Germany) using a mobile phase [water/methanol/acetonitrile (55:25:20, by vol.)]. This mobile phase gave retention times for 7α-hydroxy-DHEA and DHEA of 7 and 24 min respectively.

RNA extraction

Total RNA from the prostate gland was isolated as described previously [28], resuspended in RNase-free water and stored at -70 °C. All the samples had intact 18 S and 28 S RNAs, as assessed by ethidium bromide staining after agarose gel electrophoresis.

Oligonucleotide primers, RT and PCR amplification

5′ and 3′ primers for PCR (Oswel DNA Service, Southampton, U.K.) were 5′-dGTTCAGCCGAAGATTATCAGCG-3′ and 5′-dTCACGCAGGACTTCCATAGC-3′, corresponding to nt 54–75 and 655–674 of the rat cyp7b1 cDNA [22]. RT was performed (in a reaction volume of 20 μl) in 10 mM Tris/HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 % (w/v) Triton X-100, 1 mM each of dCTP, dGTP, dTTP and dATP, 10 units of RNasin (Promega, Southampton, U.K.), 1–2 μg of total RNA, 12 units of avian myeloblastosis virus ('AMV') reverse transcriptase (Pharmacia, Milton Keynes, U.K.) and 0.1 nmol of 3′ PCR primer. Reactions were incubated for 10 min at room temperature, followed by 30 min at 42 °C, then 95 °C for 5 min (to inactivate the reverse transcriptase). Subsequent PCR amplification of specific cDNAs was achieved by transferring the RT reaction to 80 μl of buffer containing 50 mM KCl, 10 mM Tris/HCl (pH 9.0), 2.5 mM $MgCl_2$, 0.2 mM each of dCTP, dGTP, dTTP and dATP, 0.1 nmol of 5′ PCR primer and 2.5 units

of *Taq* polymerase (Promega). Following a 'hot start' (5 min at 96 °C), samples were subjected to 30 cycles, as follows: 10 cycles of 96 °C for 30 s, 50 °C for 1 min and 72 °C for 1 min, followed by a further 20 cycles of 96 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min. A final extension step was carried out at 72 °C for 10 min. Amplified products were analysed by electrophoresis on 1 % (w/v) agarose gels. The identity of the PCR product was checked by restriction enzyme digestion using *Dra*I and *Hin*fI.

Cloning and sequencing of RT-PCR products

PCR products were subcloned into the vector pGEM-(T) easy (Promega), which was then sequenced on both strands.

Northern blot analysis

RNA (20 μg) was size-fractionated by electrophoresis through a 1 % (w/v) agarose gel in the presence of formaldehyde, followed by capillary transfer to a nylon membrane and UV cross-linking. Pre-hybridization was performed in pre-hybridization buffer [0.13 M NaH_2PO_4/0.4 M Na_2HPO_4/3.3 mM EDTA/7 % (w/v) SDS/0.05 mg of denatured herring testes sperm DNA] for 2 h at 55 °C. The [^{32}P]P_i-labelled cDNA probe, synthesized using a random prime DNA-labelling kit (Boehringer Mannheim, Mannheim, Germany), was added to the pre-hybridization buffer, and hybridization (55 °C) was performed overnight. Blots were washed twice with 1 × SSC buffer (0.15 M NaCl/0.015 M sodium citrate)/0.1 % (w/v) SDS for 20 min at room temperature, and then once with 0.3 × SSC/0.1 % (w/v) SDS for 20 min at 55 °C. The membranes were subjected to autoradiography. The cDNA template for the synthesis of the cyp7b1 probe was a 1.2 kb cDNA fragment [22]. The loading control probe was 7 S cDNA [29]. The ratio of cyp7b1 to 7 S RNA in the prostate was arbitrarily set at 1.0 for prostate RNA from 1-month-old rats, and results for older rat prostate RNA were expressed relative to this.

cyp7b1 probe for *in situ* hydridization

A 700 bp *Eco*RI–*Pst*I fragment of cyp7b1 cDNA was subcloned into pGEM-3 (Promega) [22]. Linearized plasmid was used as a template for SP6 or T7 RNA polymerase to generate the antisense and sense RNA probes respectively. Probes were purified using Nick columns (Sephadex G50 DNA grade; Amersham Pharmacia, Uppsala, Sweden).

mRNA *in situ* hybridization

Cryostat sections (10 μm) from prostate glands of 3-month-old adult rats were taken; sections from five different animals were thaw-mounted on to 3-aminopropyltriethoxysilane-coated slides and stored at −80 °C. Fixing, hybridization (to 10^7 c.p.m./ml ^{35}S-UTP-labelled cRNA probe at 50 °C for 14–16 h) and washing were as described previously [30]. After RNase A treatment, the slides were dehydrated, dried, placed against film (β-Max Hyperfilm; Amersham, Herts., U.K.), and stored at room temperature for 2 days. Films were developed in D19 developer and fixed in a 1:5 dilution of Amfix fixative. Non-specific hybridization was determined by incubating adjacent sections with a ^{35}S-UTP-labelled sense probe under identical conditions. Following autoradiography, slides were first dipped in photographic emulsion, and then counterstained with haematoxylin and eosin. Quantification was performed using a Seescan Imager (Seescan, Cambridge, U.K.).

Statistics

Values shown are means ± S.E.M. Statistical comparisons (using the 'Sigma Stat' program) were made by analysis of variance ('ANOVA') and the Rank Sum Test. Significance levels were set at $P < 0.05$.

RESULTS

Rat prostate homogenate catalyses the production of 7α-hydroxy-DHEA from DHEA

First, we examined extracts of rat prostate for 7α-hydroxylation of DHEA, a preferred substrate for recombinant cyp7b1 *in vitro* [23]. NADPH-dependent 7α-hydroxylation of DHEA was clearly detectable in extracts of this tissue. No products were obtained in the absence of NADPH (results not shown). The rat prostate homogenate generated a major product with a mobility on TLC plates identical with that of the major product of recombinant vaccinia virus-cyp7b1, which was also similar to the major rat brain product [23] (Figure 1A). The unlabelled commercial reference compound, 7α-hydroxy-DHEA, co-migrated in an identical manner with the major product of prostatic DHEA,

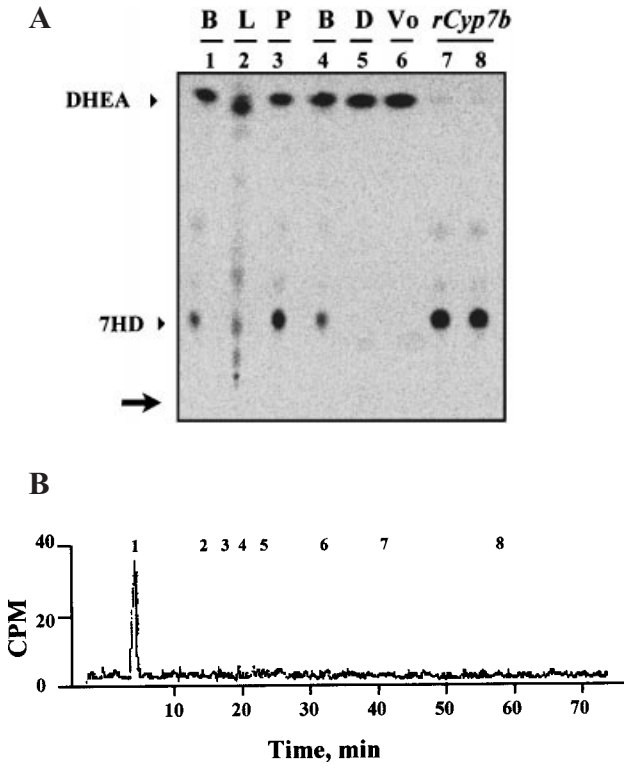

Figure 1 Metabolism of DHEA in rat tissues

(**A**) TLC resolution of products generated by incubation of rat tissues homogenates with [^{3}H]DHEA. Lane markers: B (lanes 1 and 4), whole brain; L (lane 2), liver; P (lane 3), prostate. Control with no membrane (D) is shown in lane 5. DHEA products generated by incubating control vaccinia-transfected cell extracts (lane 6) or extract of cells infected by vaccinia virus expressing cyp7b1 (lanes 7 and 8) are run in parallel for the tissue extracts. The horizontal arrow indicates the origin of the gel. (**B**) HPLC profile of the radioactive spot removed from the TLC plate showing a single peak at 7 min. The mobile phase was methanol/water/acetonitrile (55:25:20, by vol.). The elution profile of the known reference compound was: 1, 7α-hydroxy-DHEA; 2, A/enediol; 3, testosterone; 4, A/enedione; 5, DHEA; 6, 5α-dihydrotestosterone; 7, 3α-5α-androstenediol; and 8, androsterone.

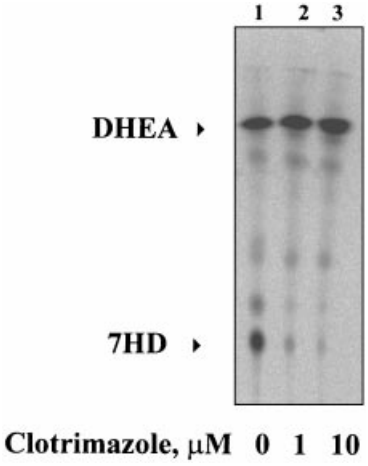

Figure 2 Effect of clotrimazole on DHEA 7α-hydroxylation in the prostate

TLC of DHEA 7α-hydroxylation in rat prostate in the absence (lane 1) or in the presence of 1 μM (lane 2) or 10 μM (lane 3) clotrimazole.

Table 1 Catalytic properties of 7α-hydroxylation in prostatic homogenate

Each value represents the mean ± S.E.M. for three determinations.

Compound	V_{max} (pmol/min/mg)	K_m (μM)
A/enediol	97 ± 7	4 ± 0.2
25-HOChol	55 ± 3	1 ± 0.05
DHEA	46 ± 2	14 ± 1
E_2	4 ± 1	11 ± 1
A/anediol	3 ± 2	22 ± 2

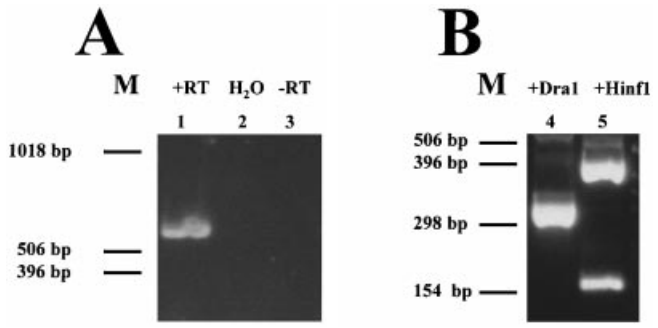

Figure 3 RT-PCR analysis of cyp7b1 mRNA in rat prostate

(**A**) RT-PCR was performed on total RNA from rat prostate (3 months old) (lane 1). No product was detected in a PCR reaction lacking RNA (lane 2). RNA sample without reverse transcriptase confirmed the absence of genomic DNA contamination (lane 3). (**B**) The identity of the PCR product (620 bp) was checked by enzymic restriction with *Dra*I (lane 4) and *Hin*fI (lane 5), which cut the PCR product at 308 bp and 160 bp respectively. The nucleic-acid-size markers are shown on the left of the gels (M).

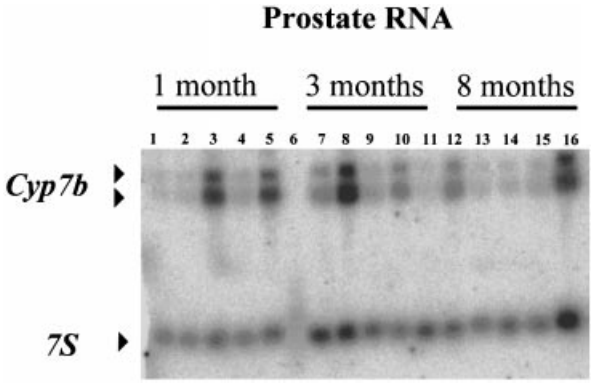

Figure 4 Northern blot analysis of cyp7b1 expression in the rat prostate

Three different groups of animals were used for RNA extraction: 1-month-old (lanes 1–5), 3-months-old (lanes 7–11) and 8-months-old (lanes 12–16) rats. RNA from each prostate was resolved by gel electrophoresis, and the hybridization probe was a fragment of the rat cyp7b1 cDNA. cyp7b1 probe hybridized to two transcripts in the rat prostate at 1.8 and 2.1 kb. The probe for the loading control corresponds to ribosomal protein 7 S.

and was clearly distinct from 7β-hydroxy-DHEA, which migrates differently under the same conditions [23]. The major metabolite of DHEA, eluted from the TLC plates with ethyl acetate and subjected to HPLC chromatographic analysis, emerged from the column as a single peak at a retention time identical with that of the commercial 7α-hydroxy-DHEA obtained by chemical synthesis (Figure 1B). These data confirm that the major product of DHEA is 7α-hydroxy-DHEA. The extent of the conversion of [^{3}H]DHEA into 7α-hydroxy-DHEA, normalized to protein content, was greater in rat prostate extracts than in total rat brain homogenate. These data indicate that rat ventral prostate, like recombinant cyp7b1, predominantly hydroxylates DHEA at the 7α position. Furthermore, the conversion of DHEA into 7α-hydroxy-DHEA in the prostate was blocked, as reported for the recombinant cyp7b1 enzyme [23], by the P450 inhibitor clotrimazole, at concentrations of 1–10 μM (Figure 2).

Kinetic parameters of 7α-hydroxylase activity in the prostate gland: A/enediol, DHEA and 25-HOChol are the preferred substrates

Kinetic characterization of the enzyme was performed in homogenates of pooled rat prostate glands. K_m and V_{max} values were determined for DHEA, 17β-oestradiol (E_2), A/anediol, A/enediol and 25-HOChol. The substrate specificity of the enzyme is summarized in Table 1. K_m values obtained ranged from 1–22 μM (in order of decreasing K_m, A/anediol > DHEA > E_2 > A/enediol > 25-HOChol). The V_{max} value for A/enediol was approximately twice that obtained for DHEA, and 30-fold higher than that for A/anediol (3 pmol/min per mg protein; in decreasing order of V_{max}, A/enediol > 25-HOChol > DHEA > E_2 > A/anediol). On the basis of catalytic-efficiency constants (V_{max}/K_m) for this enzyme, 25-HOChol is the best substrate in the prostate gland.

The effect of A/anediol and A/enediol on 7α-hydroxylation of DHEA in prostate homogenate was examined. Unlabelled steroids (2 and 10 μM) were co-incubated with 10 nM [^{3}H]DHEA under standard assay conditions. Both steroids inhibited the formation of 7α-hydroxy-DHEA in a dose-dependent manner (results not shown).

cyp7b1 mRNA is present in the prostate

To address whether the prostatic activity could be due to the presence of the cyp7b1 enzyme, RT-PCR was performed on total RNA from three different rat prostates. The amplified fragment of 620 bp (Figure 3A) was cleaved with *Dra*I and *Hin*fI, producing

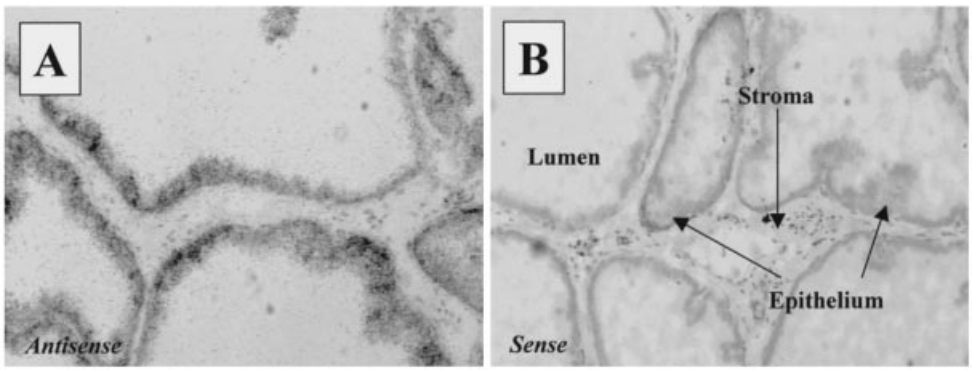

Figure 5 Distribution of cyp7b1 in rat prostate

High-resolution views of mRNA-encoding cyp7b1 distribution in rat prostate (**A**). Representative sense control sections are shown (**B**).

sub-fragments of 308 and 160 bp respectively, as expected for cyp7b1 cDNA (Figure 3B). The PCR product was subcloned and sequenced; the nucleotide sequence thus obtained was identical with that previously reported for cyp7b1 from rat hippocampus [22] (results not shown).

cyp7b1 mRNA in the rat prostate is differentially expressed with aging

To examine the expression of cyp7b1 mRNA during postnatal developmental of the rat prostate, we performed Northern blot analysis on rat prostatic RNA obtained at three different ages (1, 3 and 8 months). The cyp7b1 cDNA probe identified two mRNA transcripts corresponding in size to the cyp7b1 transcripts reported previously in rat brain [22] (Figure 4). Some inter-individual variability in the levels of cyp7b1 mRNA expression was noted, which was not reflected in the intensity of the signal obtained with the 7 S RNA loading control. Moreover, the cyp7b1/7S mRNA ratio in the rat prostate appeared to decrease with age: the ratio was 1.0 ± 0.2 at 1 month, 0.7 ± 0.2 at 3 months and 0.6 ± 0.1 at 8 months; nevertheless, these differences have failed to achieve statistical significance at the $P = 0.05$ level.

In situ hybridization analysis of cyp7b1 mRNA in the rat prostate shows a high expression in the epithelium

To determine the site of cyp7b1 mRNA expression in the adult rat prostate, *in situ* mRNA hybridization was performed on cryosections of ventral prostate. cyp7b1 mRNA was restricted to the epithelium, with no expression detected in the stroma (Figure 5A). Control sections hybridized to sense RNA probe revealed only low background levels of hybridization (representative sections are shown in Figure 5B); the level of cyp7b1 expression was 15-fold greater than that of background (sense).

DISCUSSION

We present several lines of evidence to support the hypothesis that oxysterol 7α-hydroxylase (cyp7b1) is expressed in the rat prostate and is responsible for 7α-hydroxylation of steroids and oxysterols.

We show that rat prostatic homogenates catalyse NADPH-dependent hydroxylation of 3β-steroids such as DHEA, A/enediol and 25-HOChol at the 7α position. Remarkably, in our study, the best steroid substrate was A/enediol. A/enediol is derived directly from DHEA by one of several 17β-hydroxysteroid dehydrogenases predominantly acting in the reductase direction, and can itself be converted into testosterone by widespread 3β-hydroxysteroid dehydrogenase activity. To aid comparison of the substrates, we used only tritiated isotopes. However, the apparent V_{max} obtained with DHEA is almost certainly an underestimate. [^{3}H]DHEA is substituted at the 7α-position, and it is known that the hydroxylation of tritium-substituted sites is slower than that of unsubstituted sites. However, the K_m value obtained in our study is identical with the one reported previously for the recombinant vaccinia virus-Cyp7b protein using [^{14}C]DHEA (13.6 μM; [23]). 25-HOChol also proved to be an effective substrate, with a catalytic efficiency that was double than of A/enediol.

These kinetic parameters demonstrate clearly the presence of a steroid 7α-hydroxylase activity in the prostate that catalyses the 7α-hydroxylation of A/enediol, DHEA, E_2, A/anediol and 25-HOChol. High concentrations of DHEA and A/enediol (6–7 times as high as plasma values) have been found in the human prostate gland [31]. Moreover, the prostatic DHEA concentration is 10-fold higher than the concentration of A/anediol in the same tissue [31]. These data suggest that 3β-steroids, such as DHEA and A/enediol (both substrates for cyp7b1), may selectively accumulate in the prostate.

Previous studies have shown that only the novel brain-enriched cytochrome P450 cyp7b1 exhibits both steroid and oxysterol 7α-hydroxylase activity [23]. Other 7α-hydroxylases show different substrate specificities: cyp7a is active against cholesterol and 24-HOChol, but is largely inactive against steroids such as DHEA. A second hepatic enzyme, cyp39A1, primarily metabolizes HOChols [19,21]. Both cyp7a and cyp39A1 enzymes are expressed predominantly, if not exclusively, in the liver. cyp7b1 is the only enzyme identified to date with the relevant substrate preference and kinetic properties to account for the 7α-hydroxylase activity in the prostate. Moreover, recent data show that DHEA 7α-hydroxylation is dramatically reduced to baseline levels in prostatic homogenates of mice homozygous for targeted disruption of the cyp7b1 gene, suggesting that cyp7b1 is likely to be the enzyme predominantly responsible for steroid 7α-hydroxylation in this tissue [31a]. However, this does not mean that cyp7b1 is the sole enzyme with 7α-hydroxylase activity in the prostate. An enzyme possessing 7α-hydroxylase activity has previously been described in both rat and human prostates

[14,26,27], which appears to be specific for A/anediol and fails to metabolize DHEA. Interestingly, DHEA and A/enediol can inhibit the 7α-hydroxylation of A/anediol in the prostate [14]. Whereas this might reflect competition among substrates, these data raise the possibility that additional enzymes with 7α-hydroxylase activity may exist in the prostate, notably in species other than mouse.

We report here that cyp7b1 mRNA is prominently expressed in the rat prostate, and is exclusively expressed in the epithelium. Although cyp7b1 was originally described as a brain-enriched enzyme, expression was also detected in the liver and kidney [22]. Our more recently undertaken study performed in mouse has suggested that cyp7b1 mRNA is quite widely expressed during fetal development, including in the prostate, but the expression becomes highly restricted in the immediate post-natal period [32]. In another recent study, cyp7b1 mRNA was detected in a variety of human tissues, particularly in tissues active in steroid metabolism (testes, ovary, prostate, kidney and liver), but also in tissues involved in bile-acid synthesis and absorption (liver, small intestine, kidney and colon) [23,33]. Of course, the enzyme is well-placed to determine the fate of DHEA in the epithelium, and hence perhaps growth, during development and in adult life.

Finally, we found a considerable sample-to-sample variability in cyp7b1 mRNA expression in the prostate, suggesting possible regulation by factor(s) as yet unknown. Our data might allude to the fact that there is a general decrease in mRNA expression with age of the rat and/or the size of the prostate. A similar relationship among prostate size, androgen levels and 7α-hydroxylase activity has been reported previously [14,26]. Levels of cyp7b1 mRNA and/or 7α-hydroxylase activity might directly reflect the androgen status and/or the degree of cellular proliferation in the prostate gland. Indeed, prostate development and morphology are known to be dependent on androgen regulation and the balance between cell proliferation and apoptosis.

What is the function of cyp7b1 in the prostate? One possibility, suggested by the substrate preference of the enzyme, is that the enzyme might protect against hyperplasia. DHEA stimulates cell proliferation, and might serve either as a substrate for the local synthesis of active androgens and/or oestrogens, or, possibly, by facilitating androgen and oestrogen receptors by an unknown mechanism [34–37]. A/enediol also appears to have intrinsic oestrogenic and androgenic activity at physiological concentrations [1,38]. It is possible that, by metabolizing DHEA and A/enediol, cyp7b1 could protect the prostate against sex-steroid-induced proliferation and hyperplasia. In rat brain astrocytes, which also metabolize DHEA and other neuroactive steroids to androgens and oestrogens, 7α-hydroxylation is dependent upon cell contact, such that a large degree of contact among cells shifts steroid metabolism towards 7α-hydroxylated products at the expense of the 3β-hydroxysteroid dehydrogenase oxidation pathway to A/enedione [39]. It is possible that a similar mechanism exists in the prostate, increasing the production of 7α-hydroxysterols during the proliferative phase of the gland. This might explain a rather controversial report that DHEA exhibits anti-proliferative properties, inhibiting prostate carcinogenesis [40]. Moreover, some cyp7b1 products could act locally as active steroids responsible for at least some of the effects ascribed to DHEA and A/enediol.

In the liver, cyp7b1 catalyses the 7α-hydroxylation of 25- and 27-HOChol as a part of the process of the cholesterol degradative pathway [24]. Although cholesterol metabolism has been studied extensively in the liver, the prostate gland also contains high levels of lipid [41]. Indeed, synthesis of cholesterol in the prostate epithelium can be several times greater than that in the liver [42]. Furthermore, dramatic changes in feedback regulation of cholesterol synthesis have been noted in malignant tissues, including prostatic adenoma (for a review, see [42]). Androgens, and, more specifically, testosterone, appear to regulate cholesterogenesis and, intriguingly, oxysterol 7α-hydroxylation, in the prostate, as they also do in fibroblasts or liver [43].

In summary, we have demonstrated in the present study that cyp7b1 mRNA is highly expressed in the epithelial cells of the rat prostate, and is the enzyme responsible in both brain and prostate for the 7α-hydroxylation of oxysterols. The function of oxysterol metabolism in the prostate is still unknown and merits further investigation.

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