Human Type 3 3α-Hydroxysteroid Dehydrogenase (Aldo-Keto Reductase 1C2) and Androgen Metabolism in Prostate Cells

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Human aldo-keto reductases (AKRs) of the AKR1C subfamily function in vitro as 3-keto-, 17-keto-, and 20-ketosteroid reductases or as 3α-, 17β-, and 20α-hydroxysteroid oxidases. These AKRs can convert potent sex hormones (androgens, estrogens, and progestins) into their cognate inactive metabolites or vice versa. By controlling local ligand concentration AKRs may regulate steroid hormone action at the prereceptor level. AKR1C2 is expressed in prostate, and in vitro it will catalyze the nicotinamide adenine dinucleotide (NAD⁰) dependent oxidation of 3α-androstanediol (3α-diol) to 5α-dihydrotestosterone (5α-DHT). This reaction is potently inhibited by reduced NAD phosphate (NADPH), indicating that the NAD⁰ : NADPH ratio in cells will determine whether AKR1C2 makes 5α-DHT. In transient COS-1-AKR1C2 and in stable PC-3-AKR1C2 transfectants, 5α-DHT was reduced by AKR1C2. However, the transfected AKR1C2 oxidase activity was insufficient to surmount the endogenous 17β-hydroxysteroid dehydrogenase (17β-HSD) activity, which eliminated 3α-diol as androstenedione. PC-3 cells expressed retinol dehydrogenase/3α-HSD and 11-cis-retinol dehydrogenase, but these endogenous enzymes did not oxidize 3α-diol to 5α-DHT. In stable LNCaP-AKR1C2 transfectants, AKR1C2 did not alter androgen metabolism due to a high rate of glucuronidation. In primary cultures of epithelial cells, high levels of AKR1C2 transcripts were detected in prostate cancer, but not in cells from normal prostate. Thus, in prostate cells AKR1C2 acts as a 3-ketosteroid reductase to eliminate 5α-DHT and prevents activation of the androgen receptor. AKR1C2 does not act as an oxidase due to either potent product inhibition by NADPH or because it cannot surmount the oxidative 17β-HSD present. Neither AKR1C2, retinol dehydrogenase/3α-HSD nor 11-cis-retinol dehydrogenase is a source of 5α-DHT in PC-3 cells. (Endocrinology 144: 2922–2932, 2003)

The human prostate is an androgen-dependent target tissue and requires the potent androgen 5α-dihydrotestosterone (5α-DHT) for its development, growth, differentiation, and function. Overproduction of 5α-DHT can lead to the development of benign prostatic hyperplasia (BPH) and prostate cancer (1, 2).

5α-DHT is formed in this gland from testosterone by the action of 5α-reductase type 2 (2, 3). As the most potent androgen in man, 5α-DHT binds to androgen receptor with a very high affinity (Kd = 10⁻¹³ M) and modulates gene expression (2). Within the prostate 5α-DHT can be inactivated by 3α-hydroxysteroid dehydrogenase (3α-HSD) to form 3α-androstanediol (3α-diol) or by 3β-HSD to form 3β-androstanediol (3β-diol). Of these two pathways, prostatic 3α-HSDDs play a dominant role in 5α-DHT reduction (4–7). Subsequent inactivation of the 3α- and 3β-diols is achieved by glucuronidation, followed by excretion (6, 8) (Fig. 1).

Mechanisms that could result in an excess of 5α-DHT in the prostate include 1) increased 5α-DHT synthesis due to elevated expression of 5α-reductase type 2, 2) elevated expression of the oxidative 3α-HSD isoforms that convert 3α-diol to 5α-DHT, and 3) decreased inactivation of 5α-DHT due to the down-regulation of 3-ketosteroid reductases.

Oxidative 3α-HSD activity, which converts 3α-diol to 5α-DHT, has been reported in human prostatic hypertrophy (9) and in the ventral prostate of castrated rats (10). 3α-Diol stimulated prostate growth in castrated dogs (11, 12) and induced virilization of the urogenital tract both in the fetal rat and in the fetus found in the pouch of the female wallaby (13, 14). These androgenic effects of 3α-diol can be explained if it is oxidized to the potent androgen 5α-DHT.

Because of the importance of 5α-DHT in prostate growth, androgen ablative therapy of prostatic disease involves the use of the 5α-reductase type 2 inhibitor finasteride (15–17). This drug will reduce plasma 5α-DHT levels by 90%, but reduces prostatic volume by only 30%, indicating that other sources of 5α-DHT exist (15). This has led to the development of dual inhibitors, i.e., compounds that inhibit 5α-reductase types 1 and 2. This approach will eliminate 5α-DHT production throughout the body, but the preferred approach would be to abolish 5α-DHT formation locally in the prostate.

Oxidative 3α-HSD isoforms may be an important source of 5α-DHT formation in the prostate. These enzymes require a source of 3α-diol substrate that is probably produced by...
hepatic metabolism of androgens via 5α-reductase type 1 and the hepatic-specific type 1 3α-HSD [aldo-keto reductase 1C4 (AKR1C4)] (18). Identification of the major oxidative 3α-HSD isoform in the prostate may reveal a tissue-specific enzyme that should be targeted for inhibition.

Multiple 3α-HSD isoforms exist in human prostate, and we are interested in identifying the major oxidative enzyme. These enzymes are either members of the short-chain dehydrogenase (SDR) or AKR superfamilies.

Potential candidates for oxidative 3α-HSD from the SDR superfamily are microsomal human RoDH/3α-HSD (7, 19), 11-cis-retinol dehydrogenase (RDH5) (20, 21), and mitochondrial 1,3-hydroxyacyl coenzyme A dehydrogenase (ERAB; 17β-HSD type 10) (22).

Potential candidates for the oxidative 3α-HSD from the AKR superfamily are AKR1C1 to AKR1C4. In vitro these enzymes function as 3-keto-, 17-keto-, and 20-keto-steroid reductases or as 3α-, 17β-, and 20α-hydroxysteroid oxidases to varying degrees (18). By acting as ketosteroid reductases or hydroxysteroid oxidases, these AKRs can convert potent sex hormones (androgens, estrogens, and progestins) into their inactive metabolites, or they can form potent hormones by catalyzing the reverse reaction (18). In this manner they may regulate occupancy and trans-activation of steroid hormone receptors (18). Of the four human AKR1C isozymes, only AKR1C2 (formerly referred to as type 3 3α-HSD and bile acid-binding protein) and AKR1C3 (type 2 3α-HSD and type 5 17β-HSD) are highly expressed in human prostate (18, 23). We focused our attention on AKR1C2, because in vitro this was the only peripheral isoform that could oxidize 3α-diol to 5α-DHT (18).

We transiently transfected AKR1C2 (pcDNA3-AKR1C2) into COS-1 cells and stably transfected pcDNA3-AKR1C2 and pLNX-AKR1C2 constructs into PC-3 and LNCaP cells, respectively. COS-1 cells are monkey kidney cells, and PC-3 and LNCaP cells are androgen receptor-negative and -positive human prostate adenocarcinoma cell lines. We found that in a cellular context AKR1C2 preferentially acts as a 3-ketosteroid reductase and diminishes 5α-DHT levels.

1 The nomenclature for the AKR superfamily is found at the AKR superfamily homepage at www.med.upenn.edu/akr (34).

unidirectionality of the enzyme is in part attributed to the potent inhibition of the nicotinamide adenine dinucleotide (NAD⁺)-dependent oxidation of 3α-diol by reduced NAD phosphate (NADPH). AKR1C2 was found to be highly expressed in prostate epithelial cells from cancerous, but not normal, tissue, suggesting that AKR1C2 depletes the androgen receptor from androgen excess in this disease. Our data also indicate that AKR1C2, RoDH/3α-HSD or 11-cis-retinol dehydrogenase, have insufficient oxidase activity to convert 3α-diol to 5α-DHT in PC-3 cells.

Materials and Methods

Materials

[4,14C]5α-DHT (57.3 mCi/mmol) and [9,11-N-3H]androstane-3α,17β-diol (40.0 Ci/mmol) were obtained from NEN Life Science Products (Boston, MA). Recombinant AKR1C9 (1.6 μmol androsterone oxidized/min/mg) and recombinant AKR1C2 (2.5 μmol 1-acenaphthene oxidized/min/mg) were expressed and purified from Escherichia coli as previously described (24). The cytomegalovirus promoter (pCMV)-AKR1C9 construct was prepared as described previously (25).

Construction of eukaryotic expression plasmids

AKR1C2 cDNA was amplified from total RNA extracted from a human hepatoma cell (HepG2) line via RT-PCR using isoform-specific amplifiers (24). The purified single product (1.2 kb in size) was subcloned into the pCRII vector, and its fidelity was confirmed by dyeoxy sequencing. The cDNA was excised from the pCRII vector using KpnI and Apol digestion and was directionally subcloned into compatible sites in the linearized pcDNA3 vector to yield the pcDNA3-AKR1C2 construct. To obtain the pLNX-AKR1C2 construct, the full-length AKR1C2 cDNA was excised from the pcDNA3 vector using XhoI and partial BamHI digestion and blunt end-ligated into the pLNX vector (CLONTECH Laboratories, Inc., Palo Alto, CA) previously linearized with HindIII.

Cell culture

COS-1 cells and parental PC-3 and LNCaP cells were obtained from American Type Culture Collection (Manassas, VA). COS-1 cells (monkey kidney fibroblast cells) were maintained at 37 C and 5% CO2 in DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM l-glutamine, and 10% FBS. They were passaged at 1:4 to 1:8 dilutions.

PC-3 cells (bone metastatic site of adenocarcinoma; American Type Culture Collection, CRL10995) were maintained in Ham’s F-12 medium with the same concentrations of penicillin, streptomycin, and l-

FIG. 1. Androgen metabolism in human prostate.
glutamine as described above and 7% FBS. They were passaged at 1:3 to 1:6 dilutions. 
LNCaP cells (lymph node metastatic site of prostate carcinoma, epithelial; American Type Culture Collection, CRL1435) were grown in RPMI 1640 medium with penicillin, streptomycin, l-glutamine, and 10% FBS and passaged at 1:3 to 1:6 dilutions. 
Primary cultures of prostate epithelial cells were prepared and established as previously described (26).

**Transient transfection**

COS-1 cells were plated (3.5 × 10^5 cells/well in six-well plates) 24 h before transfection. Cells were transfected using FuGene (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer’s protocol. The total amount of plasmid DNA (pcDNA3, pcDNA3-AKR1C2, or pcRcCMV-AKR1C9) was 1 μg/well. Medium was changed 24 h after transfection. In each of these constructs AKR expression is driven by the pCMV.

**Stable transfection**

For stable transfection, plasmid constructs were first purified through cesium chloride (CsCl) gradients containing ethidium bromide. PC-3 cells were transfected with pcDNA3-AKR1C2 using the PerFect transfection reagent (Invitrogen, San Diego, CA). Briefly, trypsinized 1 × 10^6 cells were incubated with a mixture of 10 μg pcDNA3 or pcDNA3-AKR1C2 plasmid DNA and 24 μg PerFect transfection lipid in 2 ml OptiMEM at room temperature for 30 min and plated into 60-mm tissue culture plates. Transfectants were selected for resistance to 600 μg/ml Genetin (G418). Positive clones were expanded and screened for AKR expression using a [α-32P]ATP randomly primed AKR1C2 cDNA probe by Northern analysis. The PC-3 cells stably transfected with AKR1C2 were designated PC-3-AKR1C2.

The retroviral expression construct was first transfected into the RetroPack PT67 cell line using Lipofectamine reagent (Invitrogen), and the retroviral expression constructs were first purified through cesium chloride (CsCl) gradients containing ethidium bromide. PC-3 cells were transfected with the pCMV vector, the AKR1C2 cDNA and neomycin gene were packed into cesium chloride (CsCl) gradients containing ethidium bromide. PC-3/AKR1C2 transfectants were selected in 250 μg/ml G418 and were designated LNCaP-AKR1C2.

**Enzyme assay**

Five micrograms of recombinant AKR1C2 (18) were incubated with either 40 μM [14C]5α-DHT or 40 μM [3H]α-diol in the presence of 2.3 mM NADPH or NAD +, respectively, in a reaction volume of 100 μl at 37 C. For the estimation of steady-state kinetic constants, the steroid concentration was varied from 3–40 μM, and the data were analyzed using ENZFITTER as previously described (18). For product inhibition studies, the NADPH-dependent reduction of 5α-DHT was performed in the presence of increasing concentrations of NAD + (1.0 mM to 1.0 mM). In addition, the NAD +–dependent oxidation of 3α-diol was performed in the presence of increasing concentrations of NADPH (1.0 mM to 1.0 mM).

Enzyme activity was measured in lysates (55 μg) from either COS-1-mock or COS-1-AKR1C2-transfected cells prepared using Reporter Lysis Buffer (Promega Corp., Madison, WI) 48 h after transfection. Lysates were incubated with either 5 μM [14C]5α-DHT or 5 μM [3H]α-diol in the presence of 2.3 mM NADPH or NAD + in a reaction volume of 100 μl for 15 min at 37 C.

In each instance samples were extracted with ethyl acetate (400 μl), dried, resuspended in 40 μl methanol, and applied to Whatman LkD silica gel TLC plates. Chromatograms were developed in chloroform/ethyl acetate (4:1, vol/vol), followed by autoradiography. Bands were identified by comigration with authentic standards and quantified by scintillation counting.

**Androgen metabolism**

Transient and stable transfectants were plated (3.5 × 10^5 cells/well in six-well plates) and grown for 24 h in their respective media in the absence of phenol red and in the presence of charcoal-stripped FBS. Medium was changed after 24 h and either 5 μM [14C]DHT or 5 μM [3H]3α-diol was added to medium. Media were collected at selected time intervals, aliquots were extracted into ethyl acetate, and the extracts were analyzed as described above using TLC and autoradiography.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primer pairs used to amplify HSD isoforms</th>
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<td><strong>Target</strong></td>
<td><strong>Forward</strong></td>
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**RT-PCR analysis of HSD isoforms in transfected cells and primary cultures of prostate epithelial cells**

Total RNA was extracted from COS-1, PC-3-AKR1C2, LNCaP-AKR1C2, and mock-transfected cells with TRIzol (Life Technologies, Inc., Gaithersburg, MD). Total RNA was extracted from primary cultures by phenol at acidic pH. The first strand cDNA synthesis and isoform-specific RT-PCR amplification for AKR2C2 were performed as previously described (18). β-Actin was also amplified from the same samples using a β-actin amplier set (CLONTECH Laboratories, Inc.) to serve as an internal control. Primers used to detect the expression of different HSD isoforms are listed in Table 1.
Radioactivity that remained in the aqueous phase after ethyl acetate extraction was dried, redissolved in 1 ml 0.1 m phosphate buffer, pH 6.8, containing 200 U β-glucuronidase (type VII-A, Sigma-Aldrich Corp.), and incubated for 24 h at 37 C. The samples were reextracted with ethyl acetate and analyzed for androgens as described.

**Results**

**In vitro characterization of AKR1C2**

AKR1C2 is a dual pyridine nucleotide-specific HSD (18, 24). Steady-state kinetic parameters for the NAD(P)H-dependent reduction of 5α-DHT catalyzed by homogeneous recombinant AKR1C2 and steady-state kinetic parameters for the NAD(P)H-dependent oxidation of 3α-diol are given in Table 2. Examination of the bimolecular rate constants (kcat/Km) show that they are similar for all four reactions where the NAD+ dependent oxidation of 3α-diol may be slightly favored. Therefore, it is not possible based on kinetic parameters alone to determine the preferred direction of the enzyme.

Product profiling showed that recombinant AKR1C2 converted 5α-DHT to 3α-androstanediol and that AKR1C2 converted 3α-diol back to 5α-DHT, (Fig. 2). This profiling confirms that AKR1C2 is a bidirectional oxidoreductase. It will catalyze the reduction of 5α-DHT to 3α-diol using the major reduced (NADPH) cofactor found in cells, and it will catalyze the oxidation of 3α-diol using the major oxidized cofactor (NAD+ ) found in cells; thus, in vitro AKR1C2 will function as a reductase or as an oxidase.

When the NADPH-dependent reduction of 5α-DHT catalyzed by recombinant AKR1C2 was examined in the presence of increasing concentrations of NAD+ (1.0 μm to 1.0 mm), there was no effect on the formation of the 3α-diol product. By contrast, when the NAD+ dependent oxidation of 3α-diol catalyzed by recombinant AKR1C2 was performed in the presence of increasing concentrations of NADPH (1.0 μm to 1.0 mm), there was marked inhibition of 5α-DHT formation by this cofactor (Fig. 3). Thus, androgen flux through AKR1C2 in a cellular context will depend upon the prevailing ratio of NAD+/NADPH, necessitating transfection studies.

The homogeneous recombinant AKR1C2 used in these studies had the same kinetic parameters for the oxidation of androstenedione as that reported for the enzyme purified from human liver cytosol (27). It also maintained a constant specific activity for the oxidation of 1-acenaphthenol after storage at −80 C (24). Thus, there is no evidence that the recombinant enzyme was either labile or denatured as previously suggested (28).

**TABLE 2. Kinetic parameters for 5α-DHT reduction and 3α-diol oxidation catalyzed by recombinant AKR1C2**

<table>
<thead>
<tr>
<th></th>
<th>Km (μM)</th>
<th>Vmax (nmol/min/mg)</th>
<th>kcat (min⁻¹)</th>
<th>kcata/Km (min/mmol)</th>
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</thead>
<tbody>
<tr>
<td>5α-DHT + NADPH</td>
<td>26 ± 6</td>
<td>6.24 ± 0.82</td>
<td>0.23</td>
<td>9</td>
</tr>
<tr>
<td>5α-DHT + NADH</td>
<td>20 ± 3</td>
<td>6.58 ± 1.24</td>
<td>0.0 .0 .29</td>
<td>11</td>
</tr>
<tr>
<td>3α-diol + NADPH</td>
<td>42 ± 4.06</td>
<td>33.0 ± 1.86</td>
<td>1.0</td>
<td>29</td>
</tr>
<tr>
<td>3α-diol + NAD</td>
<td>22 ± 7.5</td>
<td>6.58 ± 1.24</td>
<td>0.24</td>
<td>11</td>
</tr>
</tbody>
</table>

a Radiometric assay performed at pH 7.0, with 2.3 mM NAD(P)H. From Ref. 18.

b In this reaction the kinetics were pseudo-first order because Km ≫ [S] under these conditions; the Michaelis-Menton equation simplifies to v([S]) = Vmax/Km. Vmax was then converted to kcat by Vmax/Km.

C Spectrophotometric assay performed with 2.3 mM NADPH. From Ref. 18.

**Transient transfection of AKR1C2 into COS-1 cells**

To determine the favored direction of AKR1C2 in mammalian cells, we transiently transfected AKR1C2 (pcDNA3-AKR1C2) into monkey COS-1 kidney cells. Control transient transfectants were prepared with empty pcDNA3 vector (negative control) or the construct pRCMV-AKR1C9 coding for rat 3α-HSD (AKR1C9 positive control) (25). We then examined the ability of COS-1-mock, COS-1-AKR1C9, and COS-1-AKR1C2 cell lysates to reduce 5α-DHT and oxidize 3α-diol in the presence of NADPH and NAD+, respectively. We found that the transiently transfected AKR1C9 and AKR1C2 expressed in COS-1 cell lysates catalyzed the reduction of [14C]5α-DHT to 3α-diol (Fig. 4A) and the oxidation of [3H]3α-diol to 5α-DHT in the presence of reductive (NADPH) and oxidative (NAD+) coenzymes, respectively (Fig. 4B). Thus, in mammalian cell lysates, transfected AKR1C2 had the same properties as the recombinant enzyme expressed in vitro.

**AKR1C2 acts as a reductase in intact COS-1 cells**

To determine the directionality of AKR1C2 in intact mammalian cells we next examined the metabolism of 5α-DHT and 3α-diol in intact COS-1-mock and COS-1-AKR1C2 transiently transfected cells. COS-1-AKR1C9-transfected cells were included as a positive control (29). When [14C]5α-DHT was incubated with mock-transfected COS-1 cells, the fol-
amounts were converted to 5α-HSD activity (Fig. 5B). These results indicate that in intact monkey COS-1 kidney cells, AKR1C2 functioned preferentially as a 3-ketosteroid reductase. A similar conclusion holds for AKR1C9, which supports our earlier findings (29).

**AKR1C2 acts as a reductase in PC-3 cells**

COS-1 cells have been used by other investigators to determine the role of steroid hormone-transforming enzymes within a cellular context; however, they are not endocrine target cells. Knowing that the directionality of AKR1C2 may be driven by the cofactor ratio NAD+/NADPH, we next examined the directionality of AKR1C2 in human prostate cells. We stably transfected AKR1C2 (pcDNA3-AKR1C2) and the empty pcDNA3 vector (negative control) into the androgen-independent human prostate cancer cell line PC-3. Three clones were chosen for further study based upon Northern analysis. This showed that the expression of the transcript was highest in clone 3. The metabolism data presented were obtained in clone 3, but similar data were obtained in the remaining clones. The AKR1C2 transcript was only detected by RT-PCR in PC-3-AKR1C2 transfectants, not in PC-3-mock transfectants (Fig. 6).

When [14C]5α-DHT was incubated with intact PC-3-mock transfectants, 3α-diol, 3β-diol, androsterone, and 5α-androstan-3,17-dione were all formed in the incubation medium. The two steroids dominantly produced were 5α-androstan-3,17-dione and androsterone, indicating a high level of endogenous oxidative 17β-HSD and reductive 3α-HSDs, respectively (Fig. 7A). When [14C]5α-DHT was incubated with intact PC-3-AKR1C2 transfectants, the amount of 5α-androstan-3,17-dione was suppressed, and significantly more 3α-diol was produced, demonstrating that the expressed AKR1C2 again functioned as a reductase. Formation of the 3α-diol in the AKR1C2 transfectants was inhibited by the bile acid ursodeoxycholate, a potent inhibitor of the enzyme (data not shown). Thus, in both COS-1 and PC-3 cells the level of AKR1C2 expression is sufficient to convert 5α-DHT into 3α-diol and direct this androgen away from the endogenous 17β-HSD activity.

When 3α-diol was incubated with PC-3-mock and PC-3-AKR1C2 transfectants, there was no significant difference in the amount of 5α-DHT formed. By contrast, 3α-diol was oxidized to androsterone as a result of the endogenous 17β-HSD activity. Interestingly, in PC-3-AKR1C2 transfectants the oxidation of 3α-diol to androsterone catalyzed by the endogenous 17β-HSD activity was greatly suppressed compared with that in the mock-transfected controls (Fig. 7B). Thus, in the androgen-independent prostate cancer cell line PC-3, AKR1C2 catalyzed the inactivation of 5α-DHT, but not its formation.

**Identification of endogenous HSD isoforms in PC-3 cells**

RT-PCR was next conducted to identify the major oxidative 17β-HSD isoforms present in PC-3 cells. Transcripts were found for human type 2 and human type 7 17β-HSDs in PC-3 cells (Fig. 8A). Of these, human type 2 17β-HSD is the probable oxidative isoform. Similarly, RT-PCR was con-

![Fig. 3. Effects of opposing cofactors on the NADPH-dependent reduction of 5α-DHT and the NADPH-dependent oxidation of 3α-diol catalyzed by recombinant AKR1C2. NADPH-dependent reduction of 40 μM [14C]5α-DHT (A) and NADPH-dependent oxidation of 40 μM [3H]3α-diol (B) catalyzed by recombinant AKR1C2 (5 μg) in the presence of either increasing concentrations of NADPH (1.0–1000 μM) or NAD+ (1.0–1000 μM) as inhibitor.](https://academic.oup.com/endo/article-abstract/144/7/2922/2888890)
ducted to determine whether these cells expressed any endogenous oxidative 3α-HSD activity. PC-3 cells were found to express retinol dehydrogenase (microsomal 3α-hydroxysteroid dehydrogenase RoDH/3α-HSD) and, to a lesser extent, RDH5. The former enzyme has been previously implicated as being important in the conversion of 3α-diol to 5α-DHT in prostate (7). PC-3 cells were unable to convert 3α-diol to 5α-DHT, suggesting that insufficient oxidase activity exists in these cells for the back reaction.

**Function of AKR1C2 in LNCaP cells**

As the androgen-dependent prostate cancer cell line LNCaP is considered a model for studying human prostate cancer (30), we next stably transfected AKR1C2 (pLNCX-AKR1C2) and the empty pLNCX vector (negative control) into LNCaP cells. The retroviral constructs were preferred over the CMV vectors due to the higher transfection efficiency that can be achieved. RT-PCR verified that the AKR1C2 transcript could only be detected in the LNCaP-AKR1C2 transfected cells, but not in mock transfectants (Fig. 6).

When [14C]5α-DHT was incubated with intact LNCaP-mock and LNCaP-AKR1C2 stable transfectants, 3α-diol, 3β-diol, and 5α-androstane-3,17-dione were all formed (Fig. 9A).

Unlike COS-1 and PC-3 cells, the amount of 5α-androstane-3,17-dione was barely detectable in LNCaP cells, indicating that the major endogenous activities were 3α/3β-HSDs rather than 17β-HSD. When [3H]3α-diol was incubated with intact LNCaP-mock and LNCaP-AKR1C2 stable transfectants no metabolism was apparent (the level of androsterone formed was barely detectable), and no 5α-DHT was formed (Fig. 9B).

When the reduction and oxidation metabolic pathways were studied in the LNCaP and LNCaP-AKR1C2 transfec-
tants, the recovery of 14C- and 3H-labeled steroids in the organic phase of the medium decreased dramatically over the time course of the incubations, indicating that androgens were metabolized to water-soluble conjugates. Treatment with β-glucuronidase led to the recovery of the radioactivity, demonstrating that androgens were converted to glucuronide conjugates. Using [14C]5α-DHT, a large portion was recovered as 3α-diol after β-glucuronidase treatment of medium from either the intact LNCaP-mock or LNCaP-AKR1C2 stable transfectants. In both cell types there was no significant difference in the amount of 3α-diol recovered (~60% of the total radioactivity) from the aqueous phase, suggesting that the expressed AKR1C2 activity was unable to increase the amount of 3α-diol that was glucuronidated.
Thus, transfected AKR1C2 was unable to alter the reductive metabolism of 5α-DHT in LNCaP cells (Fig. 9A).

Using [3H]3α-diol as substrate for either the LNCaP-mock or LNCaP-AKR1C2 stable transfectants, about 40% of the steroid was recovered after β-glucuronidase treatment. This suggests that the amount of AKR1C2 transfected was also unable to influence oxidative metabolism of 3α-diol in LNCaP cells. Thus, AKR1C2 does not affect 5α-DHT reduction or 3α-diol oxidation in LNCaP-AKR1C2 transfectants.

Elevated expression of AKR1C2 transcripts in primary cultures of epithelial cells derived from prostate cancer

Using Northern analysis, we previously showed that AKR1C isoform transcripts showed elevated expression in primary cultures of epithelial cells derived from BPH and prostatic cancer compared with normal prostate cells (23). These earlier analyses were unable to distinguish between the AKR isoforms present. To determine levels of AKR1C2 expression in cells cultured from normal and malignant prostate tissues, an isoform-specific semiquantitative RT-PCR assay was used (18). The primer set designed was specific for AKR1C2 and will not detect other related isoforms, AKR1C1, AKR1C3, and AKR1C4, under the same conditions.

![Diagram](https://academic.oup.com/endo/article/144/7/2922/2888890)
PCR conditions. β-Actin mRNA was also PCR amplified from each individual sample as an internal control (Fig. 10). It was found that epithelial cells derived from normal prostate (peripheral zone with no BPH and no cancer) had low levels of AKR1C2 transcripts, with only 3 of 12 (25%) showing elevated expression of the mRNA. In contrast, elevated expression of AKR1C2 transcripts was observed in 14 of 14 samples obtained from epithelial cells cultured from prostate cancer.

Discussion

3α-Androstanediol exerts its androgenic effects in the prostate by being converted to 5α-DHT. Interest exists in identifying the major oxidative 3α-HSD in human prostate responsible for this transformation. Recombinant AKR1C2 (type 3 3α-HSD) catalyzes this reaction in vitro and is a candidate enzyme for catalyzing this reaction in vivo. Using transient and stable expression strategies, we determined whether AKR1C2 functions as a 3-ketosteroid reductase or a 3α-hydroxysteroid oxidase in a whole cell context. We selected three different cell systems: monkey COS-1 kidney cells, with an AKR1C null environment, where we expected less interference by endogenous steroid-metabolizing enzymes; the androgen-independent human prostate cancer cell line PC-3; and the androgen-dependent human prostate cancer cell line LNCaP, which is considered a model for studying human prostate cancer (30). AKR1C2 (human type 3 3α-HSD) acted as a reductase in both monkey COS-1 kidney cells and the hormone-independent prostate cancer cells PC-3. In both instances the presence of the back-reaction, in which 3α-diol is oxidized to 5α-DHT, was not detectable. AKR1C2 was unable to influence androgen metabolism in LNCaP cells, which displayed a high level of glucuronidation.

The oxidase that converts 3α-diol to 5α-DHT in the prostate may not have to be robust, as the androgen receptor is activated by subnanomolar concentrations of 5α-DHT. We cannot exclude the possibility that AKR1C2 could make these low concentrations of 5α-DHT. We also cannot exclude the possibility that enzymes from the SDR superfamily are the source of the major oxidative 3α-HSD activity in the prostate. Transcripts for both RoDH/3α-HSD and RDH5 were detected in PC-3 cells, but these cells failed to convert 3α-diol to 5α-DHT, suggesting that they have insufficient oxidase
primers to detect 17\(^{-}\)HepG2 cells, was subjected to RT-PCR using forward and reverse
lanes 14 blank (primers only) RDH5 in PC-3 and COS-1 cells, respectively;
HepG2 cells; lanes 5 COS-1 cells, respectively. absence of RoDH in COS-1 cells, respectively; lanes 11 7, 8, and 10, no RNA blank (primers only), RoDH in PC-3 cells, and prostate, PC-3 cells, HepG2 cells, and COS-1 cells, respectively; lanes 2–4, no RNA blank (primers only) and type 2 17\(^{-}\)HSD enzymes in COS-1 and PC3 cells.

FIG. 8. RT-PCR of 17\(^{-}\)HSD isoenzymes in COS-1 and PC3 cells. Total RNA from the source indicated, e.g. COS-1 cells, PC3 cells, or HepG2 cells, was subjected to RT-PCR using forward and reverse primers to detect 17\(^{-}\)HSD isoenzymes. A: Lane 1, 1-kb ladder; lanes 2–4, no RNA blank (primers only) and type 2 17\(^{-}\)HSD in PC-3 and HepG2 cells; lanes 5–7, no RNA blank (primers only) and type 7 17\(^{-}\)HSD in PC-3 and HepG2 cells, respectively. RT-PCR was also conducted using reverse and forward primers to detect the major oxidative 3\(\alpha\)-HSD isoforms and ERAB. B: Lane 1, 1-kb ladder; lane 2, no RNA blank (primers only); lanes 3–6, \(\beta\)-actin transcript in prostate, PC-3 cells, HepG2 cells, and COS-1 cells, respectively; lanes 7, 8, and 10, no RNA blank (primers only), RoDH in PC-3 cells, and absence of RoDH in COS-1 cells, respectively; lanes 11–15, no RNA blank (primers only) RDH5 in PC-3 and COS-1 cells, respectively; lanes 14–16, no RNA blank (primers only) and ERAB in PC-3 and COS-1 cells, respectively.

AKR1C2 fails to act as an oxidase in COS-1 or PC-3 cells because it is either potently inhibited by NADPH, or it cannot surmount the high endogenous oxidative 17\(^{-}\)HSD activity that is present or both.

Examination of the in vitro properties of the recombinant AKR1C2 showed that it was potently inhibited in the oxidation direction by NADPH and that this may account for the unidirectionality of the enzyme in cells. When the NADPH-dependent reduction of 5\(\alpha\)-DHT catalyzed by AKR1C2 was conducted in the presence of increasing concentrations of NAD\(^+\), the reduction reaction occurred unimpeded. However, when the NAD\(^+\)-dependent oxidation of 3\(\alpha\)-diol catalyzed by AKR1C2 was conducted in the presence of increasing concentrations of NADPH, the oxidation reaction was potently inhibited by this cofactor. Thus, the directionality of this dual pyridine nucleotide-specific HSD in a cellular context will be governed by the NAD\(^+\)/NADPH ratio. It is perhaps unappreciated that only low micromolar concentrations of NADPH are required to inhibit the oxidase activity. We suggest that product inhibition by the opposing cofactor may be an unrecognized phenomenon that regulates flux through dual pyridine nucleotide-specific HSDs in cells.

The expression of oxidative 17\(^{-}\)HSD isoforms may also mask the oxidase activity of transfected AKR1C2. A candidate oxidative 17\(^{-}\)HSD that is present in COS-1 cells is the type 2 17\(^{-}\)HSD, which can be detected by RT-PCR (Rizner, T. L., and T. M. Penning, unpublished results). A candidate oxidative 17\(^{-}\)HSD isoform in PC-3 cells could also be type 2 or type 7 17\(^{-}\)HSDs, as their transcripts were detected by RT-PCR. These results are in agreement with others who reported the expression of type 2 17\(^{-}\)HSD in PC-3 cells (31).

AKR1C2 was unable to influence androgen metabolism in androgen-dependent prostate cancer cells, LNCaP. Instead, a high rate of endogenous glucuronidation was noted. Glucuronides have been reported as the major androgen conjugates formed in LNCaP cells (31). About 60% of 5 \(\mu\)M \([\text{\textsuperscript{14}C}]\)5\(\alpha\)-DHT and 40% of 5 \(\mu\)M \([\text{\textsuperscript{3}H}]\)3\(\alpha\)-diol were recovered as glucuronides, and the presence of AKR1C2 had no effect on this disposition (Fig. 9). The higher percentage of glucuronides formed from 5\(\alpha\)-DHT by LNCaP cells corroborates the preferred kinetic parameters of UDP glucuronosyl transferase (32). Thus, in the androgen-dependent human prostate cancer cell line LNCaP, high UDP-glucuronosyl transferase activity made it difficult to access the effects of AKR1C2 on 5\(\alpha\)-DHT and 3\(\alpha\)-diol metabolism.

Taken together, our results demonstrate that in the cell lines chosen, AKR1C2 acts as a reductase and eliminates 5\(\alpha\)-DHT, thereby preventing the trans-activation of the androgen receptor by this hormone. These studies are in agreement with those published earlier by Dufort et al. (28) that showed that AKR1C2 functioned as a 3-ketosteroid reductase after transfection into HEK-293 cells. These earlier studies, however, were flawed in several respects. First, the steroid concentration used was 0.1 \(\mu\)M, which is substantially
lower than either the $K_m$ for 5α-DHT reduction or the $K_m$ for 3α-diol oxidation catalyzed by AKR1C2. Therefore, the transfected enzyme was not assayed under optimal conditions. Second, the investigators provided no details of the background rate of steroid transformation in their studies. Third, no evidence was provided that the transiently transfected AKR1C2 was expressed in HEK cells. Fourth, transient transfection was not performed in a relevant prostate cell line. Fifth, Dufort et al. (28) maintained that AKR1C2 was labile when expressed in HEK-293 cells. By contrast, our study used steroid concentrations in the $K_m$ range, we corrected for background rates of steroid transformation, we provided RT-PCR evidence that the transcript was expressed, we conducted transfection studies in prostate cells, and we found no evidence that the recombinant enzyme is labile.

As AKR1C2 plays a role in the reductive elimination of 5α-DHT in human prostate, we next examined its expression by RT-PCR in primary cultures of epithelial cells from normal and malignant prostate tissues. We found that AKR1C2 transcripts were markedly overexpressed in epithelial cells from prostate cancer compared with cells from normal tissues. This suggests that AKR1C2 would eliminate 5α-DHT from diseased prostate and thus deprive the androgen receptor of its ligand. As prostate cancer progresses, most tumors become refractory to androgen ablative therapy, in part because activation of the androgen receptor becomes growth factor mediated (33). Overexpression of AKR1C2 may contribute to this progression toward androgen independence.

The role for AKR1C2 in prostate androgen action may be
dictated by epithelial cell type. Barbier and colleagues (7) proposed a model in which testosterone and 5α-DHT are produced in the basal epithelial cells, while the luminal epithelial cells make 5α-DHT and contain the androgen receptor. In this model there could be paracrine and intracrine regulation of the androgen receptor (7). It is now of interest to determine AKR1C2 expression levels in different populations of prostatic epithelial cells.

Note Added in Revision

While this manuscript was undergoing revision, Stolz et al. [Prostate (2003) 54:275–289] showed that AKR1C2 will reduce 5α-DHT to 3α-diol after transfection into PC-3 cells. It was also found using real-time RT-PCR that AKR1C2 expression was reduced in prostate cancer. However, whole prostate tissues were used in this work, and no attention was given to the prostate cell type analyzed.

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