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Structural and biochemical properties of cloned and expressed human and rat steroid 5α-reductases

(cDNA/androgen metabolism/dihydrotestosterone/benign prostate hyperplasia/4-azasteroid inhibitors)

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ABSTRACT **The microsomal enzyme steroid 5α-reductase is responsible for the conversion of testosterone into the more potent androgen dihydrotestosterone. In man, this steroid acts on a variety of androgen-responsive target tissues to mediate such diverse endocrine processes as male sexual differentiation in the fetus and prostatic growth in men. Here we describe the isolation, structure, and expression of a cDNA encoding the human steroid 5α-reductase. A rat cDNA was used as a hybridization probe to screen a human prostate cDNA library. A 2.1-kilobase cDNA was identified and DNA sequence analysis indicated that the human steroid 5α-reductase was a hydrophobic protein of 259 amino acids with a predicted molecular weight of 29,462. A comparison of the human and rat protein sequences revealed a 60% identity. Transfection of expression vectors containing the human and rat cDNAs into simian COS cells resulted in the synthesis of high levels of steroid 5α-reductase enzyme activity. Both enzymes expressed in COS cells showed similar substrate specificities for naturally occurring steroid hormones. However, synthetic 4-azasteroids demonstrated marked differences in their abilities to inhibit the human and rat steroid 5α-reductases.**

The enzyme steroid 5α-reductase (EC 1.3.99.5) is a microsomal protein that plays a central role in human sexual differentiation and androgen physiology. Interest in this protein arises from several distinguishing characteristics. First, steroid 5α-reductase catalyzes the conversion of testosterone to the more potent androgen dihydrotestosterone (1). This latter steroid induces a program of differentiation during fetal development that leads to the development of the male external genitalia (2). Second, mutations in the gene for steroid 5α-reductase give rise to a rare form of male pseudohermaphroditism in which affected males develop normal internal urogenital tracts but fail to develop external male structures (3). Third, the expression of the gene is regulated by androgens in tissues such as the prostate and liver (4). A fourth distinguishing feature of steroid 5α-reductase is its role in several endocrine abnormalities including benign prostate hyperplasia, male pattern baldness, acne, and hirsutism (5–7).

The central role played by steroid 5α-reductase and its product dihydrotestosterone in these disorders has been underscored by the development of inhibitors of this enzyme (8) and their recent use as therapeutic agents (9). These drugs include 4-azasteroid derivatives, such as 17β-(*N*-*t*-butyl)carbamoyl-4-aza-5α-androst-1-en-3-one (MK-906, Finasteride) and 17β-(*N*,*N*-diethyl)carbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA) (8, 9), that function as competitive inhibitors of the enzyme (10). The exact mechanism by which these compounds act *in vivo* has not been elucidated, and the development of additional inhibitors of the human steroid 5α-reductase has been hampered by the absence of knowledge of the structure of the protein and by the very low levels of expression of this enzyme, even in androgen-responsive tissues (10–12).

To gain insight into steroid 5α-reductase, we recently employed a *Xenopus* oocyte expression cloning strategy to isolate a full-length cDNA encoding the rat enzyme (4). The characterization of this cDNA revealed that a single mRNA encodes steroid 5α-reductase in both the liver and the prostate, that the enzyme is a hydrophobic protein of M_r 29,000, and that hormonal control of steroid 5α-reductase gene expression may be exerted at the transcriptional level (4). In the current studies, we have used the rat clone to isolate and characterize a full-length cDNA encoding the human steroid 5α-reductase.†

MATERIALS AND METHODS

Radiolabeled steroids were obtained from DuPont/New England Nuclear and steroid standards were from Sigma and Steraloids (Wilton, NH). The 4-azasteroids 4-MA and MK-906 were gifts of Merck Sharp & Dohme Research Laboratories. Inhibitors were subjected to chemical-ionization mass spectrometry to confirm their identity.

cDNA Cloning. Two cDNA libraries were constructed from human prostate mRNA. For the first, cDNA kindly provided by M. J. McPhaul of this institution (13) was ligated into the bacteriophage λgt10 vector as described (4). For the second, prostate tissue from a subject undergoing surgery for prostatic hyperplasia was obtained from J. McConnell (this institution) and used for the isolation of poly(A)$^+$ RNA (14). A size-fractionated cDNA library was subsequently prepared (4) in λgt10 (14). Clones from these libraries were screened by standard methods using hybridization conditions of reduced stringency (14). DNA sequence analysis was carried out using automated methods on an Applied Biosystems model 370A DNA sequencer. RNA blotting was performed as described (14).

Expression Vector Construction. A rat steroid 5α-reductase cDNA corresponding to nucleotides (nt) 1–1962 (4) was ligated into the pCMV4 expression vector (15). A human cDNA corresponding to nt 1–842 of Fig. 1 was initially ligated into pCMV4. To modify this poorly expressed human cDNA (see text), two oligodeoxynucleotides derived from the 5′ end of the cDNA (5′-ATAGATCTACCATGGCAACGGCGACGGGGGTGGCGG-3′) and from the 3′ untranslated region (5′-AAAGTCCATAGAGAAGCGCCATTGG-3′) were used in a polymerase chain reaction (16) to alter the human cDNA as described below. After amplification, the product was ligated into pCMV4.

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Abbreviations: 4-MA, 17β-(*N*,*N*,-diethyl)carbamoyl-4-methyl-4-aza-5α-androstan-3-one; MK-906, 17β-(*N*-*t*-butyl)carbamoyl-4-aza-5α-androst-1-en-3-one; nt, nucleotide(s).

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M32313).

Expression of Steroid 5α-Reductase cDNAs in COS Cells. Simian COS-M6 cells were transfected as described (15). Steroid 5α-reductase activity in intact cells was assayed as described (4) except that ^{14}C-labeled steroids dissolved in ethanol were added to the transfected cell medium and subsequent organic extractions were carried out with dichloromethane. Thin-layer chromatography and liquid scintillation counting were performed as described (4). To determine IC_{50} values for the 4-MA and MK-906 inhibitors, a mixture of [^{14}C]testosterone and inhibitor in ethanol was added to transfected cell medium, incubated at 37°C for 2 hr, and treated as above.

To assay steroid 5α-reductase activity *in vitro*, cells were harvested 48 hr after transfection, washed once with phosphate-buffered saline, and either frozen in liquid N_2 or homogenized directly with a Polytron (Brinkmann) at a protein concentration of 2 mg/ml in 10 mM potassium phosphate, pH 7.4/150 mM KCl/1 mM EDTA. A typical assay mixture contained 10–50 μg of cell homogenate protein in 0.5 ml of 0.1 M potassium phosphate buffer (pH 6.6, rat enzyme; pH 7.0, human enzyme). Steroids were added in 5 μl of ethanol, and the reaction was initiated by the addition of NADPH to a final concentration of 2–5 mM. Incubations were carried out for 10 min at 37°C and terminated by the addition of 5 ml of dichloromethane. Organic extractions and thin-layer chromatography were as described above. The formation of 5α-reduced steroid products was linear with respect to protein over a 10- to 50-μg range and with respect to incubation time over a 20-min period.

RESULTS

Identification and Analysis of Human Steroid 5α-Reductase cDNAs. To isolate clones encoding the human steroid 5α-reductase, cDNA libraries constructed from prostate mRNA were screened at reduced stringency with a radiolabeled fragment corresponding to the coding region of the rat cDNA. Five cDNA clones were isolated after screening of 3 × 10^6 recombinants from two different cDNA libraries. Each of these cDNAs was subjected to restriction enzyme mapping and DNA sequencing and represented one species of mRNA.

The sequence of the longest cDNA insert and the predicted amino acid sequence of the human steroid 5α-reductase are shown in Fig. 1. The DNA sequence predicts a prostate mRNA of at least 2.1 kilobases (kb) having a 3′ untranslated region of ≈1.3 kb. Within the 3′ untranslated sequence, a polyadenylylation signal (AATAAA) is located 15 nt 5′ to a poly(A) tract, suggesting that the 3′ end of this cDNA is authentic. A 5′ untranslated region of 30 nt precedes a translation reading frame of 780 nt encoding the steroid 5α-reductase protein.

RNA blotting experiments indicated that this cDNA hybridized to a single species of human prostate mRNA of about 2.3 kb (Fig. 2). Southern blot analysis and screening of human genomic DNA libraries have similarly revealed the presence of only one functional gene homologous to this steroid 5α-reductase cDNA (E. Jenkins and D.W.R., unpublished observations).

Structure of Human Steroid 5α-Reductase and Comparison to the Rat Enzyme. The amino acid sequence of the human steroid 5α-reductase was deduced from the cDNA insert by comparison to that of the functional rat enzyme (4). The human enzyme is 259 residues long with a predicted molecular weight of 29,462. Over 40% of the amino acids in this sequence are hydrophobic, and only 16% have positively or negatively charged side chains (Fig. 1). These observations are consistent with the intracellular membrane location of the enzyme (17).

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                              1                          10                                  20                              30
                              MetAlaThrAlaThrGlyValAlaGluGluArgLeuLeuAlaAlaLeuAlaTyrLeuGlnCysAlaValGlyCysAlaValPheAlaArg
GGGCATGGAGCACGCTGCCCAGCCCTGGCGATGGCAACGGCGACGGGGGTGGCGGAGGAGCGCCTGCTGGCCGCGCTCGCCTACCTGCAGTGCGCCGTGGGCTGCGCGGTCTTCGCGCGG   120

31                          40                                50                                60                          70
AsnArgGlnThrAsnSerValTyrGlyArgHisAlaLeuProSerHisArgLeuArgValProAlaArgAlaAlaTrpValValGlnGluLeuProSerLeuAlaLeuProLeuTyrGln
AATCGTCAGACGAACTCAGTGTACGGCCGCCACGCGCTGCCCAGCCACAGGCTCCGAGTGCCGGCGCGGGCCGCCTGGGTGGTGCAGGAGCTGCCCTCGCTGGCCCTGCCGCTCTACCAG   240

71                          80                                90                                100                        110
TyrAlaSerGluSerAlaProArgLeuArgSerAlaProAsnCysIleLeuLeuAlaMetPheLeuValHisTyrGlyHisArgCysLeuIleTyrProPheLeuMetArgGlyGlyLys
TACGCCAGCGAGTCCGCCCCGCGTCTCCGCAGCGCGCCCAACTGCATCCTCCTGGCCATGTTCCTCGTCCACTACGGGCATCGGTGCTTAATTTACCCGTTTCTGATGCGAGGAGGAAAG   360

111                         120                               130                               140                        150
ProMetProLeuLeuAlaCysThrMetAlaIleMetPheCysThrCysAsnGlyTyrLeuGlnSerArgTyrLeuSerHisCysAlaValTyrAlaAspAspTrpValThrAspProArg
CCTATGCCACTGTTGGCATGTACAATGGCGATTATGTTCTGTACCTGTAACGGCTATTTGCAAAGCAGATACTTGAGCCATTGTGCAGTGTATGCTGATGACTGGGTAACAGATCCCCGT   480

151                         160                               170                               180                        190
PheLeuIleGlyPheGlyLeuTrpLeuThrGlyMetLeuIleAsnIleHisSerAspHisIleLeuArgAsnLeuArgLysProGlyAspThrGlyTyrLysIleProArgGlyGlyLeu
TTTCTAATAGGTTTTGGCTTGTGGTTAACAGGCATGTTGATAAACATCCATTCAGATCATATCCTAAGGAATCTCAGAAAACCAGGAGATACTGGATACAAAATACCAAGGGGAGGCTTA   600

191                         200                               210                               220                        230
PheGluTyrValThrAlaAlaAsnTyrPheGlyGluIleMetGluTrpCysGlyTyrAlaLeuAlaSerTrpSerValGlnGlyAlaAlaPheAlaPhePheThrPheCysPheLeuSer
TTTGAATACGTAACTGCAGCCAACTATTTTGGAGAAATCATGGAGTGGTGTGGCTATGCCCTGGCCAGCTGGTCTGTCCAAGGCGCGGCTTTTGCTTTCTTCACGTTTTGTTTTTTATCT   720

231                         240                               250                          259
GlyArgAlaLysGluHisHisGluTrpTyrLeuArgLysPheGluGluTyrProLysPheArgLysIleIleIleProPheLeuPheEnd
GGTAGAGCAAAAGAGCATCATGAGTGGTACCTCCGGAAATTTGAAGAGTATCCAAAGTTCAGAAAAATTATAATTCCATTTTTGTTTTAAGTGCGTTTTTCATGAAATTATCTTCAACTT   840

GAAGCTTTCCAATGGCGCTTCTCTATGGACTTTGTAAATAAGTTATATCTTTGTAATTTTCCTGCTACTTTATCATTTTCAAGATGTCCTCTAGGAATTTTTTTTCTAGTAATTTTGCAA   960

TCTACCTAATAAGTACCTAAATACGCTGAAATGGAGGTTGAATATCCTACTGTGTAACAGGTCAGAATTTCAAGCTCTGGGTAATAACTGCTGATATTTTTTCTAATTTCAAATTTACCT   1080

CTTTTGGCTATGTCTTGCCAAGTGTGTATGAGACTAGACTTTACAACTGTCTTTGATGGCATTTTCAGAACAATAAATGTCACAATCCCTTCTATAGCCCCCTACAGTGATCTCTTCAAG   1200

GTCAACTGCAGTGTTGCTTCCCTCCCCCTATAGGGCTGGAATCTGTCTAGGAGCCCTCTCTCGGAGGCCACAGAGGCTGGGGGTAGCCATTGTGCAGTCATGGCCCGGGGGAAACTTGCC   1320

AACCTTCGTGTCAGGTGCTGTGTGTAAGTGGAGAACTTGGGGATAGAGGAGGAAGCTCCTCGTGGCCCTTCCAAGGTGAGGCAAAGGCATCTGGACTTGTTCCAGCCCAGCCCACCGGGT   1440

GACATCACCGGGCAGGGAGGGGTGCTGGTGGTGGTTCATACGGAGTAAGCTGCTCTGCCTGTGTGAGTGGCTCCTGGGCCCTAAACAGGCACCTTTAGGCCATGGGTCACTCACCGTGAG   1560

CCATCAATGTGCTCTGGTCTGACATGGTTTCTCTCTGTCTTCTAGTCTAGACCTAGTTTTTTTGTTCTGTTCCCCACGTATGGATATAGTAGAGATTGTTGTCTGTGAAATTTCTCTTTT   1680

GTAGATTTTGAGTTTTCCCTTGTAGTGTAAAGAATGATCACTTTCTGTAACAATAACAAGACCACTTTTTAAGATTTATCCTGTTTGTTCTTTGTTGATTGAAACATAATAATTGTTAAA   1800

ATTCTCTACAGCCTTCTTTTTCTTCCATAGCTAATCTTCCTTCTAATAGTTTTTGCTTTCTGTTTTGCTGTTGTTGCTTTGCAAAGCTTTCCCCTCATAGCCTGTACCTGTTATCAATAT   1920

AAAATAATCTTCCTGTTGAATGCTTCATGACTTGAATTCTACTTTGATAAAAACATTGCCATACTGCTTTTTATCTTGATGAATTCATCTGGCATTGCTTTGCCTTATCATCTCATCTGG   2040

AGTTTTTAAATGCCATTTGTTTCAGTTGTCTTTAACAACATAATAAATAGACTTTGCCATTTAAAAA...2107
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FIG. 1. cDNA sequence and predicted amino acid sequence of human steroid 5α-reductase. Nucleotides are numbered at right with dots placed below the sequence every 10th nucleotide. Amino acid residues are numbered above the protein sequence. A potential polyadenylylation signal (AATAAA) is overlined.

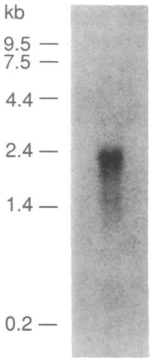

FIG. 2. Blot hybridization of RNA from human prostate. Poly(A)$^+$ RNA (25 μg) from prostate was analyzed by blotting as described (14). The filter was hybridized with ^{32}P-labeled probes (5 $\times$ 10^6 cpm/ml) complementary to three different regions of the human steroid 5α-reductase cDNA. Autoradiography was for 16 hr at −70°C. The positions to which standards of known size migrated in an adjacent lane on the gel are indicated at left.

A lineup of the human and rat steroid 5α-reductase protein sequences is shown in Fig. 3. The human enzyme is 4 amino acids longer at the amino terminus than the rat enzyme, and surprisingly, the overall identity between these two proteins is only 60%. There is a single methionine residue in the first 89 amino acids of the human protein, whereas there are three methionines in the first 19 residues of the rat protein (Fig. 3). A comparison of the human protein sequence to those present in the Genentech data bank and the Protein Identification Resource data bank (National Biomedical Research Foundation, release 59.0) did not reveal any extensive homologous sequences.

Expression of Human and Rat Steroid 5α-Reductases in COS Cells. To determine whether the observed sequence differences between the human and rat steroid 5α-reductase proteins affected their biochemical properties, the two cDNAs were expressed in simian COS cells. For the rat cDNA, a fragment corresponding to nt 1–1962 was ligated into the pCMV4 expression vector. For the human cDNA, a fragment corresponding to nt 1–842 of Fig. 1 was initially ligated into pCMV4. Subsequent transfection studies revealed that expression of this human cDNA yielded a 10-fold lower amount of steroid 5α-reductase enzyme activity than that obtained from the rat cDNA (data not shown). Inspection of the sequence at the 5′ end of the human cDNA revealed an upstream ATG at position 5 (Fig. 1) that could conceivably

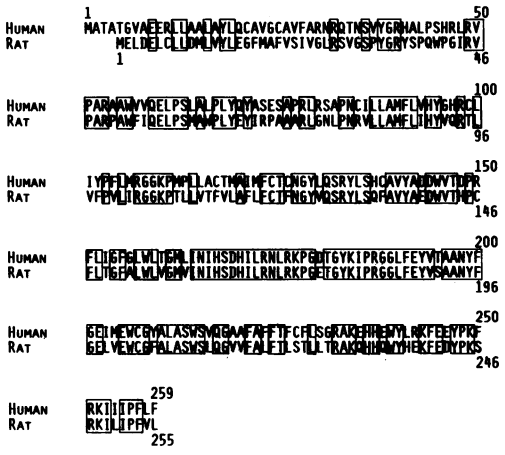

FIG. 3. Amino acid sequences of the human and rat (4) steroid 5α-reductase aligned to indicate homology between the two proteins. Identical residues are boxed. The single-letter amino acid code is used, with residues numbered on the right of the lineup.

result in spurious translation initiation, leading to the observed reduction in expression. To test this hypothesis, the polymerase chain reaction was used to (*i*) introduce a unique *Bgl* II restriction enzyme site in the 5′ untranslated region of the cDNA, (*ii*) remove the upstream ATG, and (*iii*) re-create an optimal context (18) for the ATG of steroid 5α-reductase. Transfection of this modified human cDNA into COS cells led to the expression of levels of steroid 5α-reductase enzyme activity that equaled those obtained with the rat cDNA construct (see below).

Fig. 4 shows the results of an *in vivo* time course experiment in which COS cells were transiently transfected with expression vectors harboring the rat or human steroid 5α-reductase cDNA, or with the pCMV4 vector alone. Forty-eight hours after transfection, [^{14}C]testosterone (2.5 μM) was added to the cell medium and conversion of this substrate into 5α-reduced steroid products was monitored at the indicated times by thin-layer chromatography. Cells transfected with either the rat or the human steroid 5α-reductase cDNA converted half of the starting substrate into product in 1 hr (Fig. 4). The background conversion in the cells that were transfected with vector alone was low, with only 0.5% conversion occurring after 1 hr.

This high level of expression of the cDNAs made it possible to assay steroid 5α-reductase activity *in vitro* in homogenates derived from the transfected cells. Homogenates were prepared and various biochemical parameters were first optimized to obtain maximum steroid 5α-reductase activity. Both the rat and human enzymes demonstrated a broad pH optimum centering around 7.0 (data not shown). The inclusion of NADPH in the COS cell homogenization buffer did not affect the stability of either enzyme. The specific activities of the expressed enzymes were in the nmol/(min·mg) range and were thus equal to that reported for liver homogenates of female rats (19).

Table 1 shows the apparent K_m and V_{max} values determined *in vitro* for both the rat and human steroid 5α-reductases with five different steroid substrates. The kinetic constants were determined from a Lineweaver–Burk plot of steroid 5α-reductase activity in the presence of 0.6–20 μM substrate and the apparent K_m and V_{max} values were determined by linear regression analysis. The apparent K_m values obtained for these three substrates are in good agreement with those

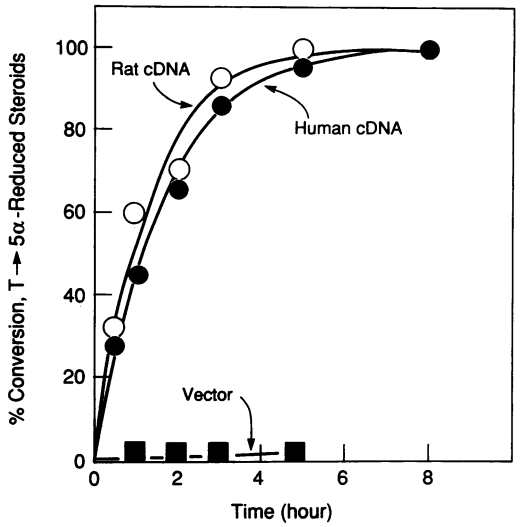

FIG. 4. Expression of rat and human steroid 5α-reductase cDNAs in transfected COS cells. On day 2 after transfection, [^{14}C]testosterone (2.5 μM) was added to the medium. At the indicated times, medium was removed from duplicate dishes and extracted with dichloromethane. Steroids were subjected to thin-layer chromatography and scintillation counting. T, testosterone.

Table 1. Characterization *in vitro* of rat and human steroid 5α-reductases expressed in transfected COS cells

Compound	Kinetic constants			
	Rat enzyme		Human enzyme	
Substrates	K_m, μM	V_{max}, nmol/(min·mg)	K_m, μM	V_{max}, nmol/(min·mg)
Testosterone	2.5	1.4–2.5	3.6	0.7–3.6
Androstenedione	2.8	1.3–2.2	1.7	1.1–5.3
Progesterone	0.5	1.2–1.8	0.8	1.1–5.0
Cortisol	—	<0.1	—	<0.1
Corticosterone	—	<0.1	—	<0.1
Inhibitors		K_i, nM		K_i, nM
4-MA		5.0, 7.0		7.0, 8.0
MK-906		3.0, 4.0, 5.0		340, 380, 620

Each K_m value represents the average of at least two experiments carried out on different days with cell lysates prepared from different transfections. Although both enzymes were active against cortisol and corticosterone, the amounts of 5α-reduced products formed were too small to obtain accurate kinetic constants.

reported in the literature (20). Similarly, both enzymes demonstrated very low activities towards 11β-substituted steroids such as cortisol or corticosterone (Table 1).

We next determined the apparent K_i values for two 4-aza-substituted steroids (4-MA and MK-906) that are inhibitors of both the human and rat steroid 5α-reductases (10). Experiments were initially carried out *in vitro* following a protocol in which two concentrations of [^{14}C]testosterone substrate were employed in the presence of various concentrations of a given inhibitor. The data obtained were analyzed using Dixon plots to determine the type of inhibition and the apparent K_i value (21). Fig. 5 illustrates the results obtained in a typical experiment with extracts prepared from COS cells transfected with the human cDNA. The results for both inhibitors with the rat and human enzymes are summarized in Table 1. The 4-MA compound was found to inhibit both the rat and human enzymes in a competitive fashion with an apparent K_i in the low nanomolar range, in accord with previously reported values (10). Surprisingly, MK-906 was a much less potent inhibitor of the human enzyme (K_i = 340–620 nM) than of the rat enzyme (K_i = 3–5 nM).

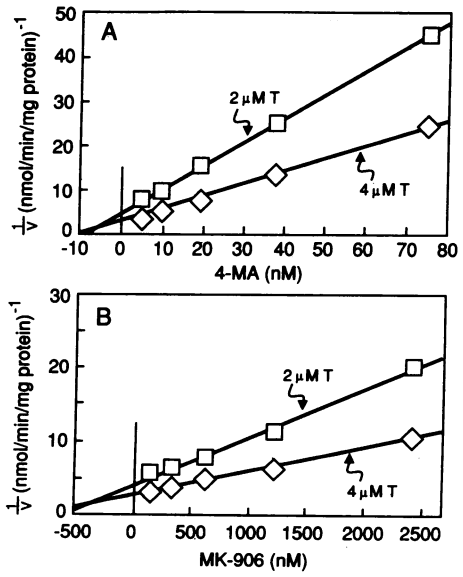

FIG. 5. Inhibition of human steroid 5α-reductase activity *in vitro* by 4-MA and MK-906. COS cells were transfected with a human cDNA expression plasmid, and cell homogenates were assayed *in vitro* for steroid 5α-reductase activity. Forty micrograms of cellular protein was assayed in the presence of the indicated concentrations of 4-MA (*A*) or MK-906 (*B*) and 2 or 4 μM [^{14}C]testosterone (T). Data were plotted using an Apple IIe program. In each panel, the intersection of the two lines defines the K_i (21).

To confirm these *in vitro* results, IC_{50} values were determined for the MK-906 and 4-MA inhibitors, using intact COS cells transfected with the human and rat steroid 5α-reductase cDNAs. The two compounds were equipotent in inhibiting the rat enzyme, but 4-MA was ≈10-fold more potent than MK-906 in inhibiting the human enzyme (Fig. 6).

DISCUSSION

We describe the isolation and characterization of a human steroid 5α-reductase cDNA. RNA blot analysis indicated that a single species of mRNA was present in prostate. DNA sequence analysis showed that the human enzyme contained 259 amino acids that were 60% identical to those of the rat enzyme. Transfection of the human and rat cDNAs into simian COS cells led to a high level of steroid 5α-reductase activity in both intact cells and cell homogenates. The reaction constants calculated for various steroid substrates

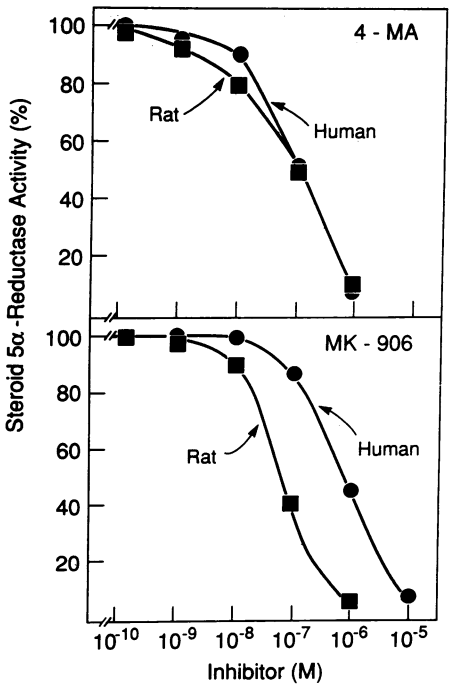

FIG. 6. Inhibition of steroid 5α-reductase in transfected COS cells. COS cells were transfected on day 0 with an expression plasmid containing the human or rat steroid 5α-reductase cDNA. On day 2, a mixture consisting of 1 μM [^{14}C]testosterone and 4-MA (*Upper*) or MK-906 (*Lower*) at the indicated concentration was added in ethanol to the medium of duplicate dishes. Conversion of testosterone into 5α-reduced products was monitored.

and inhibitors revealed similarities and differences between the human and rat enzymes.

Of the differences observed between these two proteins, several are notable. First, the two enzymes are quite different in their amino acid sequences (Fig. 3). This lack of conservation is most striking in the amino-terminal 130 residues, in which only 50% of the amino acids are identical. A 75% conservation in the carboxyl-terminal half leads to an overall identity of 60%. With the exception of a 4-amino acid extension at the amino terminus of the human protein, maximum identity by alignment did not require the introduction of any gaps into the two sequences. The hydropathy plots of the human and rat (4) enzymes, as calculated by the algorithm of Kyte and Doolittle (22), are almost identical (data not shown). Thus, even though only 60% of their amino acids are shared, the two proteins may have retained similar secondary structures. Interestingly, at the nucleic acid level the two cDNAs are 70% identical in their coding regions, a value that is commonly derived from comparison of other rat and human cDNA homologues (23).

The biochemical behavior of the rat and human enzymes expressed in COS cells is also indicative of conservation in the presence of disparate structures. Thus, the two proteins show a preference for progesterone as a substrate over testosterone and androstenedione (Table 1). Similarly, although both enzymes would reduce Δ^4, 11β-substituted steroids, measurement of activity required extensive incubation times with these substrates. Similar apparent K_m values were measured for naturally occurring steroid substrates for the rat and human enzymes, suggesting that the two cDNAs may encode homologues and not different isozymes.

Both the rat and human enzyme activities in COS cell homogenates were highest at physiological pH values. This result was unexpected for the human enzyme, as previous reports determined a pH optimum of 5.0–5.5 in cell homogenates prepared from prostate (10), epididymides (11), or genital skin fibroblasts (12). The existence of a steroid 5α-reductase activity with an alkaline pH optimum has also been reported in human fibroblasts (12, 24). The relationship between these acidic- and alkaline-optimum enzymes and the protein encoded by the human cDNA is unknown. It is possible that there are two steroid 5α-reductase genes in humans; however, current genetic evidence supports the existence of only one gene encoding steroid 5α-reductase in the human genome (25).

Differences in the biochemical behavior of the rat and human proteins were revealed with the use of synthetic 4-azasteroid inhibitors (Table 1). Thus, 4-MA was a potent competitive inhibitor of both the rat and human enzymes, while MK-906 was a 10- to 100-fold better inhibitor of the rat protein than the human protein in both intact-cell and *in vitro* assay systems (Figs. 5 and 6). A profound difference between the inhibitory capacities of these two steroids was not detected previously in a study comparing the biochemical behavior of the rat, human, and dog prostate steroid 5α-reductase enzymes (10). The reason for this discrepancy is not known but may be related to differences in the specific activities of the enzymes assayed [nmol/min per mg of protein (Table 1) versus pmol/min per mg (10)] or to the different cellular environments of the two enzymes [simian kidney cells (this study) versus human prostate (10)].

MK-906 and 4-MA differ in their functional groups at the 4 and 17 positions and by the presence of a Δ^1 double bond in MK-906 (10). For this reason, additional structure–activity studies will be required to determine the chemical basis of the observed differential inhibition of the human enzyme. Future studies will also be required to determine how results obtained with the enzymes expressed in COS cells relate to the efficacy of these drugs in the whole animal. Nevertheless, the ability to express high levels of the human steroid 5α-reductase in mammalian cells should facilitate the design of more powerful and specific inhibitors of this enzyme for therapeutic use (26). In addition, the availability of the human cDNA should allow the characterization of mutations in steroid 5α-reductase that reduce enzyme activity and lead to male pseudohermaphroditism (3).

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