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Cloning, Expression and Characterization of Rhesus Macaque Types 1 and 2 5Alpha-Reductase: Evidence for Mechanism-Based Inhibition by Finasteride

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The rhesus macaque types 1 and 2 5alpha-reductase (5aR1 and 5aR2) were cloned and expressed in COS cells to facilitate comparison of rhesus and human 5aRs. The deduced protein sequences of the rhesus 5aRs shared 94% and 96% identity with the human type 1 and 2 isozymes, respectively. Despite a four amino acid insertion at the N-terminal region of rhesus 5aR1, the biochemical properties of rhesus and human homologs are very similar with respect to pH optimum, K_m values for testosterone and progesterone, and inhibition by a variety of inhibitors. As expected, the biochemical properties of the human and rhesus 5aR2 are also very similar. The mechanism of inhibition of the rhesus 5aR1 and 5aR2 by finasteride was investigated in more detail. Finasteride displays time dependent inhibition of the rhesus 5aR1 and 5aR2 with second order rate constants of $4 \times 10^3\ M^{-1}\ s^{-1}$ and $5.2 \times 10^5\ M^{-1}\ s^{-1}$. Inhibition of rhesus 5aR2 with ^{3}H-finasteride resulted in ^{3}H bound to the enzyme which is not released by dialysis. Heat denaturation of the [rhesus 5aR2:inhibitor] complex releases dihydrofinasteride, a breakdown product presumably related to the NADP$^+$-adduct previously identified with the human 5aRs (Bull *et al.*, Mechanism-based inhibition of human steroid 5α-reductase by finasteride: Enzyme catalyzed formation of NADP-dihydrofinasteride, a potent bisubstrate analog inhibitor. *J. Amer. Chem. Soc.*, 1996, 118, 2359–2365). Taken together, these results provide good evidence that the rhesus macaque is a suitable model to evaluate the pharmacological properties of finasteride and other 5aR inhibitors. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

The enzyme steroid 5alpha-reductase (5aR[2]) catalyzes the 5α-reduction of androstenedione, testosterone (T), progesterone (P) and other 4-ene-3-keto-steroids to the corresponding 5α-dihydro-3-keto-steroids. This enzyme plays an important role in the generation of the more potent androgen, dihydrotestosterone (DHT) [1]. Finasteride (1, Fig. 1), an inhibitor of 5aR, has been found effective for the treatment of benign prostatic hyperplasia [2] and may also be useful for the treatment of other androgen-sensitive conditions such as male pattern hair loss and hirsutism [3–5].

Two isozymes of human 5aR, defined here as 5aR1 and 5aR2, have been identified [6, 7]. Defects in 5aR2 are responsible for a developmental condition in which the internal genitalia (epididymus, seminal vesicle, vas deferens) at birth are normal, but the external genitalia resemble those of female [8, 9]. Furthermore, as a consequence of life long decreases in DHT, these subjects do not develop an enlarged prostate, have less acne and do not experience male

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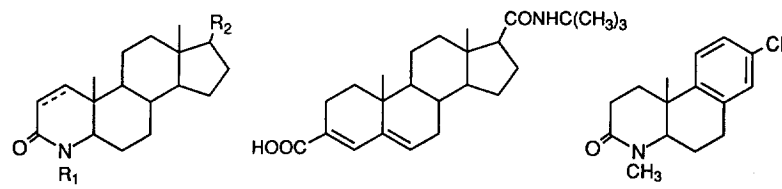
[2]*Abbreviations*: include 5aR, 5alpha-reductase; T, testosterone; DHT, dihydrotestosterone; P, progesterone.



		R_1	R_2
(1)	Δ^1	H	$CONHC(CH_3)_3$
(2)		H	$CONHC(CH_3)_3$
(3)	Δ^1	H	$2,5\text{-}(CF_3)_2C_6H_3$
(4)		CH_3	$CON(C_2H_5)_2$
(5)	Δ^1	CH_3	CO_2CH_3

(6) (7)

Fig. 1. Inhibitors of 5aR. Chemical structures of (1) finasteride, (2) dihydrofinasteride, (3) GG745 [29, 30], (4) 4MA [31], (5) methyl-4-methyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxylate [32], (6) epristeride [33] and (7) LY191704 [27, 34]

pattern hair loss [10, 11]. Inhibitors of human 5aR2 such as finasteride have been shown to lower serum DHT levels about 70% [2] while inhibitors of the 5aR1 isozyme decrease DHT in men about 30% [12]. Finasteride is approved for use in the treatment of benign prostatic hyperplasia and more recently has been under investigation for treatment of male pattern hair loss [5, 13]. The pharmacological effects of a 5aR1 inhibitor, other than effects on serum DHT, have not yet been fully investigated, in part, because of the lack of a suitable animal model.

The rat has been used to evaluate the potential risks of human fetal exposure to finasteride during pregnancy [14, 15]. However, it is difficult to directly extrapolate the rat findings to human in light of the significant developmental differences between primates and rodents. Moreover, there are species differences in the inhibition by finasteride [16, 17]. Recently, the effects of finasteride on fetal development were explored in rhesus macaque (*Macaca mulatta*) in an attempt to define the suitability of this species for testing 5aR inhibitors [18]. The choice of rhesus macaque was based on preliminary results which indicated that the tissue distribution and biochemical properties of the isozymes of 5aR were similar in rhesus macaque and human [18]. The studies described in this manuscript were initiated in order to clone, express and characterize rhesus 5aR1 and 5aR2 to confirm similarities with respect to protein sequence, kinetic characteristics and mechanism of inhibition by finasteride. These studies strive to confirm that the rhesus macaque is a suitable model for evaluating the safety of 5aR inhibitors.

MATERIALS AND METHODS

[1,2-^{3}H(N)]-P (47.5 Ci mmol^{-1}), [7-^{3}H(N)]-testosterone (22.5 Ci mmol^{-1}) were purchased from New England Nuclear. Rhesus liver tissue was obtained from the Department of Safety Assessment, Merck Research Laboratories. PCR primers were obtained from Life Technologies, Inc. (Gaithersburg, MD).

Cloning and expression of rhesus 5aR1

Total RNA was extracted from male rhesus liver tissue using RNAzol (Cinna/Biotecx, Houston, TX). PolyA$^+$ mRNA was isolated by oligo dT cellulose (FastTrack mRNA isolation kit by Invitrogen, San Diego, CA). Synthesis of cDNA was accomplished using the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA).

A specific fragment corresponding to the gene encoding the rhesus 5aR1 was amplified via polymerase chain reaction (PCR). Gene-specific primers were designed from similarities in comparing the known 5aR1 sequences of human and *Cynomolgus* monkey [19]. The 5′ primer [AGATCTCATGGA GCACGCTACCCAGC] contains a Bgl II site and the 3′ primer [GCTCTAGAGCCCTAGAGAAA CACCATTGGAAAG] contains an Xba 1 site; the restriction sites are underlined. The reaction conditions for PCR amplification in 0.05 ml were as follows: 2.5 units of PFU DNA polymerase (Stratagene, La Jolla, CA), 2.5 mM each of the four dNTPs, 20 pmol of each primer and one tenth volume of the cDNA reaction as described above. Cycle conditions were 95°C for 1 min, 50°C for 30 s, 72°C for 1.5 min for 30 cycles and a final extension period at 72°C for 10 min. The reaction products were analyzed on a 1% agarose gel containing ethidium bromide and the bands of interest were excised and the DNA eluted using the Wizard PCR prep kit (Promega, Madison, WI). A second round of PCR amplification was carried out using a nested primer (GAAGATCTT CGATGGCAACGGCGACGGGGG) and the 3′ primer described above. The reaction product was digested with Bgl II and Xba I, and ligated into pCMV 5 (a generous gift from Dr. David Russell's

Lab [20]). The plasmids were transformed into *E. coli* DH5α (Gibco BRL, Gaithersburg, MD). Positive clones were verified by restriction mapping of miniprep DNA (Wizard miniprep kit, Promega). DNA sequence analysis was performed by Sequetech Corp. (Mountain View, CA).

Cloning and expression of the rhesus 5aR2

Thesame strategy as described above for the type 1 isozyme was used to clone rhesus 5aR2. Gene specific primers were designed to regions of similarity between human and *Cynomologus* monkey in the 5′ and 3′ regions. The 5′ primer [TTTGAATTCGGCACGAGGCGCGATG] and the 3′ primer [TTTGAATTCAGTTTCAGCAGCCTTGACAG] contain EcoR1 sites (underlined) which were used for cloning into pCMV5. Plasmids were transformed into *E. coli* DH5α. Positive clones were verified by restriction digestion and sequence analysis performed by Sequetech Corp., Mountain View, CA.

Transfection of COS-7 cells with rhesus 5aR1 and 5aR2

COS (African green monkey kidney) cells were grown in DMEM (Delbecco's modified eagle medium), 10% fetal bovine serum and 0.05 mg ml^{-1} streptomycin/penicillin (Gibco BRL, Gaithersburg, MD) at 37°C in an atmosphere of 5% carbon dioxide. Trypsinized cells (10^7 cells per transfection) were transiently transfected with 0.03 mg of rhesus 5aR1 or 5aR2 expression plasmid DNA by electroporation and grown for an additional three days. Cells were harvested by trypsinization and collected by centrifugation.

Preparation of rhesus 5aR1 and 5aR2 from COS-7 cells

Trypsinized cells were resuspended in 10 mM potassium phosphate, pH 6.5, 150 mM KCl, 1 mM EDTA, 0.05 mM NADPH, and 20% glycerol. Cells were lysed by subjecting them to three cycles of freeze/thaw in a dry ice/methanol bath. Lysed cells were centrifuged at 100 000 × g for 60 min at 4°C. Supernatant was decanted and the pellet resuspended in the same buffer.

Enzyme assay

The reaction mixture for the 5aR1 contained 33 mM succinic acid, 44 mM imidazole, 33 mM diethanolamine (SID), pH 6.5, 100–700 nM ^{3}H-P, 1 mM DTT and 0.5 mM NADPH in a final volume of 0.1 ml. Conditions were similar for the 5aR2, except the pH of the buffer was adjusted to pH 5.5. The assay was initiated by the addition of enzyme and incubated at 37°C for 20–30 min. The reaction was quenched with cyclohexane:ethyl acetate (65:35 v/v) and P separated from dihydroprogesterone by normal phase HPLC (25 cm Whatman Partisil 5 silica column equilibrated in 1–2 ml min^{-1} cyclohexane:ethyl acetate, 78:22 v/v). The elution profile of steroids was monitored using a radioactivity flow detector. Under these conditions, P chromatographed at 11.3 min and dihydroprogesterone at 6.9 min. When T replaced P as substrate, HPLC separations were carried out as previously described [21]. For inhibitor studies, P was kept at $\sim K_m$ concentrations and the inhibitors were resuspended in 100% ethanol. Diluted stocks were prepared in 10% ethanol and the final concentration of ethanol in the assay was 0.5%. IC_{50} values for the compounds depicted in Fig. 1 were determined by using a 5 point titration where the concentration of inhibitor was varied from 0.1–1000 nM.

Mechanism of inhibition

The mechanism of action of finasteride was determined using the reaction conditions described above with varying concentrations of inhibitor. Reaction conditions employed for rhesus 5aR1 were 40 mM potassium phosphate, pH 6.5, 300 nM ^{3}H-P, 0.5 mM NADPH and 0–1000 nM finasteride. Reactions conditions for rhesus 5aR2 were 40 mM potassium phosphate, pH 7.0, 150 nM ^{3}H-P, 0.5 mM NADPH and 0–5 nM finasteride. Aliquots of the mixture were withdrawn, quenched at various times and product formation analyzed by HPLC. Time-dependent inhibition of the rhesus 5aRs was analyzed using the method previously described for rat 5aR2 [16]. Given that the formal kinetic mechanism of 5aR with substrate is ordered with the cofactor adding first and leaving last [22], a similar model (Scheme 1) was used to describe the inhibition of the rhesus 5aRs by finasteride where E represents enzyme with nicotinamde-bound cofactor rather than free enzyme. The time courses were fit by non-linear regression to an integrated first-order rate Equation 1, where v_0 is the initial velocity, v_s is the infinite-time velocity, and k is the rate constant for progression of the enzyme between these two steady-states. The infinite-time velocities were set equal to zero, since the inhibition generally appeared to go to completion over the entire range of inhibitor concentrations. The calculated initial velocities (v_0) were fit to Equation 2, where A represents P concentration. NADPH was included at 0.5 mM, a concentration which greatly exceeds the K_m, therefore the contribution of NADPH can be ignored in this analysis. The observed pseudo-first order rate constants (k_{obs}) were then fit to Equation 3, using this value for K_i. The value for k_4 is virtually zero since the inhibition is effectively irreversible.

$$y = v_s t + (v_0 - v_s)(1 - e^{-kt})/k + y_0 \quad (1)$$

$$E + I \underset{}{\overset{Ki}{\rightleftharpoons}} E{:}I \underset{k4}{\overset{k3}{\rightleftharpoons}} E{:}I^*$$

Scheme 1.

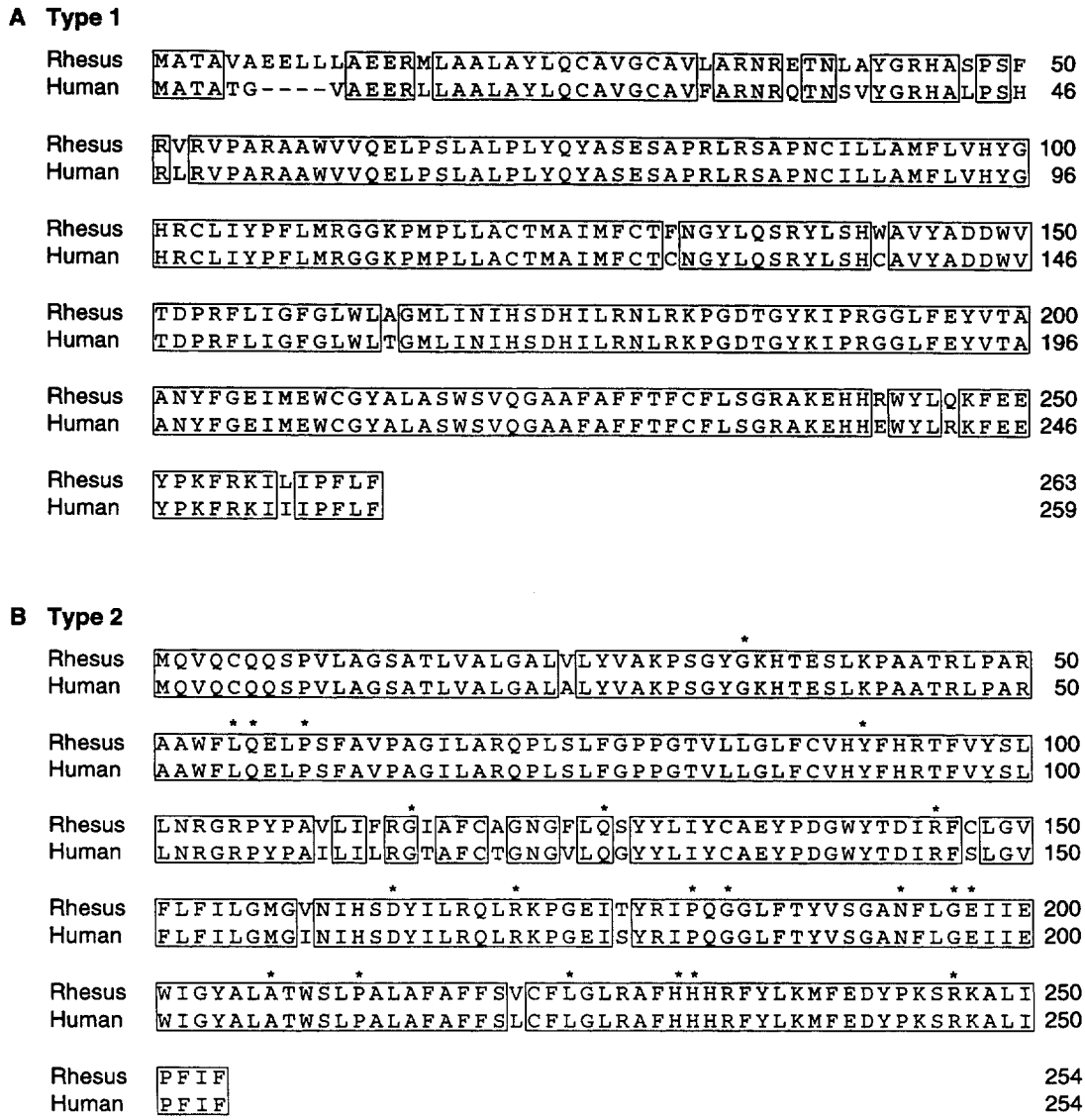


Fig. 2. (a) Amino acid alignment of the rhesus monkey and human 5aR1. (b) Amino acid alignment of the rhesus monkey and human 5aR2. * indicates amino acids changes which give rise to 5aR2 deficiency [1, 23]. Numbers indicate amino acid position

$$v_0 = v_{max}A/(K_a(1 + (I/K_i)) + A) \quad (2)$$

$$k_{obs} = k_4 + k_3[(I/K_i)/(1 + (A/K_a) + (I/K_i))] \quad (3)$$

Isolation of dihydrofinasteride

The rhesus [5aR2:^{3}H-finasteride] complex was formed in a reaction containing 0.02 ml of the recombinant enzyme homogenate (26.7 mg ml^{-1}; specific activity, 3.9 nmol min^{-1} mg^{-1}), 1 mM NADPH, and 134 pmol ^{3}H-finasteride (specific activity = 20 dpm f-mol^{-1}) and buffer in a total volume of 1 ml. The buffer consisted of 0.1 M MOPS, pH 7.2, 1 mM EDTA, and 0.1% BSA. The suspension was incubated for 2 h at 37°C. Afterwards, the excess ^{3}H-finasteride was removed by dialysis overnight against 0.001 M MOPS buffer at pH 7.2 at room temperature. The dialyzed enzyme solution represented an incorporation of 63% of the original ^{3}H-finasteride.

This labeled [enzyme-inhibitor] complex was heat denatured to bring about the release of the expected NADPH-^{3}H-dihydrofinasteride adduct and to catalyze its decomposition to ^{3}H-dihydrofinasteride. To this end, the pH of the dialyzed protein solution (1.4 ml) was adjusted to 4.0 by the addition of HCl, and then incubated in a boiling water bath for 30 min. The solution was clarified by centrifugation at 10,000 × g in a microfuge and then analyzed by reverse phase chromatography on a Whatman C-18 column employing 60% aqueous methanol as the mobile phase. Under these conditions dihydrofinasteride emerges at ~16.1 min. Dihydrofinasteride is

Table 1. Comparison of substrate specificity for rhesus and human 5aRs

Substrate	pH*	K_m (nM) Rhesus 5aR1	Rhesus 5aR2	Human 5aR1	Human 5aR2
Progesterone	Neutral	330	43	310	73
	Acidic	ND	160	ND	160
Testosterone	Neutral	3230	11	7700†	47
	Acidic	ND	330	ND	300†

*Neutral: pH 6.5-7.0; acidic: pH 5.0-5.5.
ND = not determined.
Standard errors were less than 30% for all determinations.
†Values reported previously [21]. Human 5aR1 and 5aR2 were expressed as described previously [21, 28].

easily distinguished from finasteride by co-injection of finasteride as an internal standard.

RESULTS AND DISCUSSION

Cloning and expression of the rhesus 5aR1 and 5aR2

Rhesus 5aR1 was cloned by RT/PCR methodology by taking advantage of the assumed sequence homology in the 5′ upstream and 3′ end of the gene of rhesus, human and cynomolgus monkey [6, 7, 19]. Sequence analysis revealed considerable identity at the DNA (95%) and amino acid levels (94%, Fig. 2) of rhesus and human 5aR1. One difference between rhesus and human 5aR1 is an additional 4 amino acids near the N-terminus of the monkey protein which encodes a total of 263 amino acids. The identical sequence is present in the cynomolgus monkey 5aR1 [19]. Like the human 5aR1 protein, the rhesus homolog shares considerably lower sequence homology with the rat type 1 enzyme, about 61% identity.

A similar cloning strategy was used for the rhesus 5aR2. As expected, high homology was found at the DNA (96%) and amino acid levels (Fig. 2, 96%). Both rhesus and human homologs encode proteins of 254 amino acids. The rhesus 5aR2 protein shares 76% homology with the rat isozyme of which is the also the same length. Of the 21 amino acids identified as point mutations in human 5aR2 deficiency [23], all are conserved between rhesus and human 5aR2 reinforcing their importance for enzyme function. The NADPH-binding site has been mapped by mutation analysis throughout the last half of the human 5aR2 protein [1, 23]. Of the 8 amino acid changes leading to altered cofactor binding, all are conserved in the rhesus 5aR2 sequence. However, the putative steroid binding site of four amino acids of rat 5aR identified by Thigpen *et al.* [24] near the N-terminus differs in the rhesus homolog. This finding calls into question the importance of this sequence in substrate binding and in turn led to the biochemical characterization and comparison of rhesus 5aRs.

Characterization of rhesus monkey 5aR1 and 5aR2

In order to characterize the biochemical properties of the rhesus monkey 5aRs, both enzymes were transiently expressed in mammalian COS cells. Enzyme activity was localized in the membrane fraction. As expected based upon previous results with the human, cynomolgus monkey and rat 5aRs, the pH dependence of the enzyme activity differed for rhesus 5aR1 and 5aR2 [6, 19, 21]. The 5aR1 isozyme exhibited a broad pH optimum of about 6.5–8.0, while maximal activity for the 5aR2 enzyme occurred in a sharp peak at pH 5.0 (pH dependence data not shown). These findings are in good agreement with results obtained previously using crude homogenates of rhesus skin and prostate as sources of 5aR1 and 5aR2 [18].

A comparison of substrate specificity of the human and rhesus 5aRs was investigated using P and T. As

Table 2. Inhibition of rhesus and human 5aRs

Inhibitor	IC_{50} (nM) Rhesus 5aR1	Rhesus 5aR2	Human 5aR1	Human 5aR2
1	75	0.2	82	<0.1
2	270	6.5	560	3.4
3	13	0.2	24	0.9
4	13	5.4	10	3.8
5	122	90	39	510
6	>1000	1.0	>1000	1.0
7	29	510	16	>1000

Inhibitor structures presented in Fig. 1. Standard error for all IC_{50} values were less than 25%. Numbers are averages of at least two determinations.

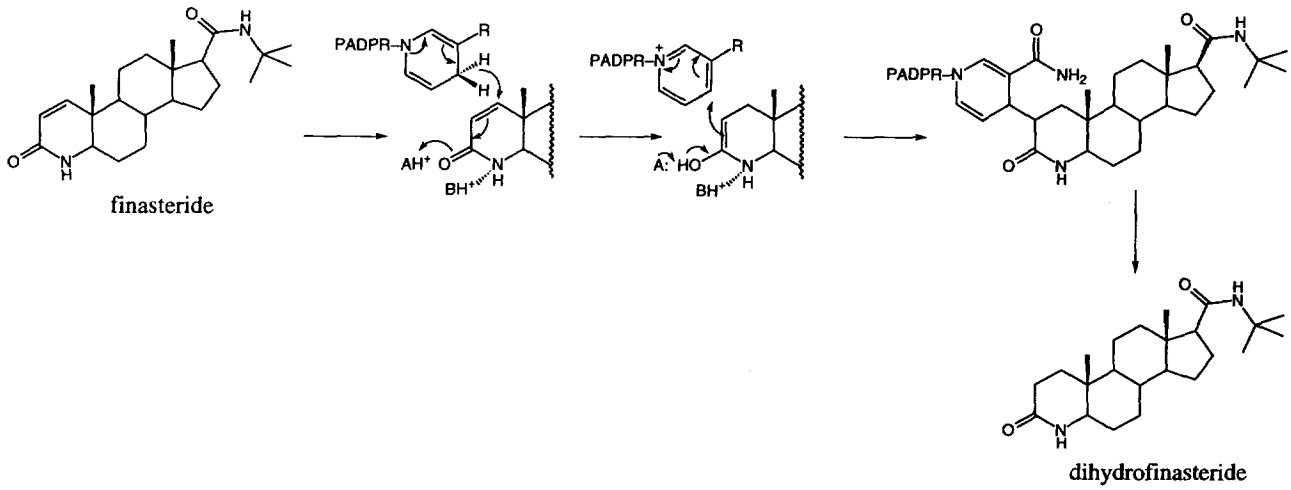


Fig. 3. Mechanism-based inhibition by finasteride. The scheme illustrates how mechanism-based inhibition by finasteride proceeds through an enolate intermediate. The resulting adduct undergoes acid catalyzed decomposition to produce dihydrofinasteride. PADPR = phosphoadenosine diphosphoribose

shown in Table 1, K_m values for T as compared to P are ~10–25 fold higher for both human and rhesus 5aR1. In contrast, similar K_m values were obtained for P and T using either human or rhesus 5aR2 as source of enzyme. As reported previously for the human, the K_m value for both substrates of the rhesus 5aR2 homolog are considerably lower at pH 7.0 as compared to pH 5.5 [26].

Over the past few years it has become clear that there are considerable species differences in sensitivity to known 5aR inhibitors [19, 25, 27]. In light of the fact that this property is a discriminating characteristic of the 5aRs, it is essential to compare the inhibitor sensitivities of the human and rhesus monkey isozymes. Comparative results of 7 selected compounds are included in Table 2. There is good agreement in the IC_{50} values of 5aR1-selective (7), 5aR2-selective (**1**, **2**, **6**) and dual 5aR1/2 inhibitors (**3**, **4**) with the human and rhesus 5aRs. Similar agreement was reported previously with the cynomolgus monkey and human 5aRs [19]. It is unclear whether the modest difference in the IC_{50} values for **5** represents a divergence in the structure activity relationship for rhesus and human 5aRs. Additional studies are necessary to explore this possibility.

Mechanism of inhibition of rhesus 5aRs by finasteride

In addition to differences in sensitivity to inhibitors, the rat and human 5aRs differ with respect to mechanism of inhibition by Δ^1-NH-azasteroids. Finasteride (**1**) displays reversible inhibition of rat 5aR1, but remarkably exhibits mechanism-based inhibition of human 5aR1 and 5aR2 as well as rat 5aR2. As depicted in Fig. 3, mechanism-based inhibition by finasteride results from enzyme-catalyzed reduction of the A-ring and subsequent adduct formation with $NADP^+$ [16, 17]. Information concerning the mechanism of inhibition of cynomolgus monkey 5aRs by (**1**)

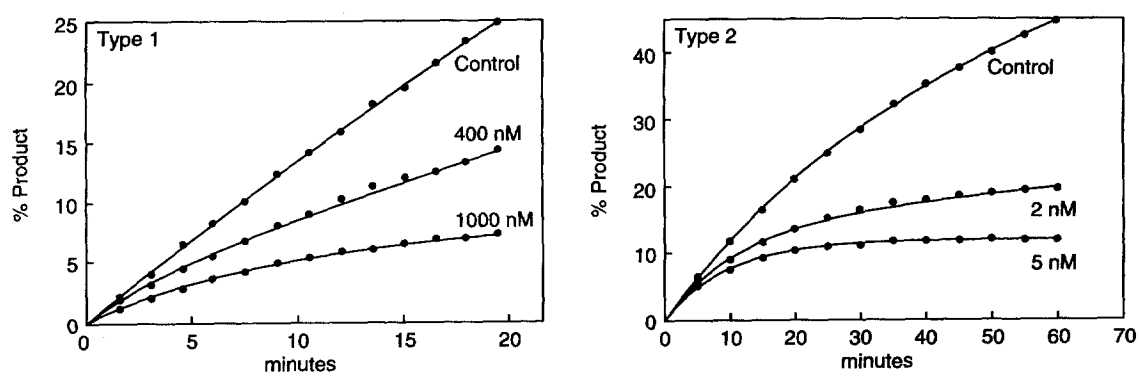


Fig. 4. Slow-binding inhibition by finasteride. Progress curves for inhibition of rhesus 5aR1 and 5aR2 by finasteride are shown. The solid lines represent theoretical fit of the experiment results (—●—) to Equation 1 by nonlinear regression

is not available; however, it was previously reported that finasteride displays time dependent inhibition of the 5aR in rhesus prostate [18]. Consequently, it is important to closely examine the mechanism of inhibition of rhesus 5aRs by this compound. Progress curves of product formation were obtained in the presence and absence of inhibitor. As presented in Fig. 4, finasteride displays time dependent inhibition of both rhesus 5aR1 and 5aR2. A fit of these time courses to Fig. 1 indicates this compound forms a preliminary Michaelis complex with the 5aR1 isozyme and NADPH with K_i=600 ± 1 nM, which is converted to the high-affinity complex (EI^*) with a rate constant k_3=2.4 ± 0.75 × 10^{-3} s^{-1}. The second-order rate constant for development of inhibition is k_3/K_i=4 × 10^3 M^{-1} s^{-1}. This rate constant is comparable to the value of 1 × 10^3 M^{-1} s^{-1} previously reported for the human type 1 isozyme [17]. Similar results were obtained with the rhesus 5aR2 (Fig. 4). In this case, the K_i for formation of the initial complex is 1.5 ± 0.8 nM and conversion to EI^* occurs at a rate of k_3=7.8 × 10^{-4} s^{-1}. The second order rate constant for inactivation of rhesus 5aR2 is 5.2 × 10^5 M^{-1} s^{-1}. These findings are in good agreement with results obtained previously with the human 5aR2 [17, 26].

Additional studies were conducted with rhesus 5aR2 to probe the mechanism of inhibition. Incubation of rhesus 5aR2 with ^{3}H-finasteride resulted in an [enzyme:^{3}H-inhibitor] complex that was not released by dialysis, a finding consistent with the pseudo-irreversible inhibition previously demonstrated with the human 5aRs. Heat denaturation of the [rhesus 5aR:inhibitor] complex at acidic pH produced a ^{3}H-product which did not elute with the retention time expected for authentic finasteride. Rather, the [^{3}H]-product eluted at 16.1 min, the retention time of dihydrofinasteride (Fig. 5). Co-injection with authentic ^{3}H-finasteride (retention time 14.0 min) established that the product did not comigrate with untransformed inhibitor (Fig. 5). On the basis of this result, it is reasonable to propose that rhesus 5aR2 is inactivated by finasteride according to the model presented in Fig. 3. We suggest that the enzyme catalyzes reduction of finasteride which collapses to form an adduct with $NADP^+$. Presumably, heat denaturation of the [enzyme:inhibitor] complex leads to release of inhibitor from the enzyme and decomposition of the adduct to yield dihydrofinasteride. Although additional studies are necessary to definitively establish that inhibition of rhesus 5aR2 proceeds by the mechanism in Fig. 3, it is worth noting there is good agreement with findings reported with human 5aRs where extensive characterization of the NADP adduct was conducted [17].

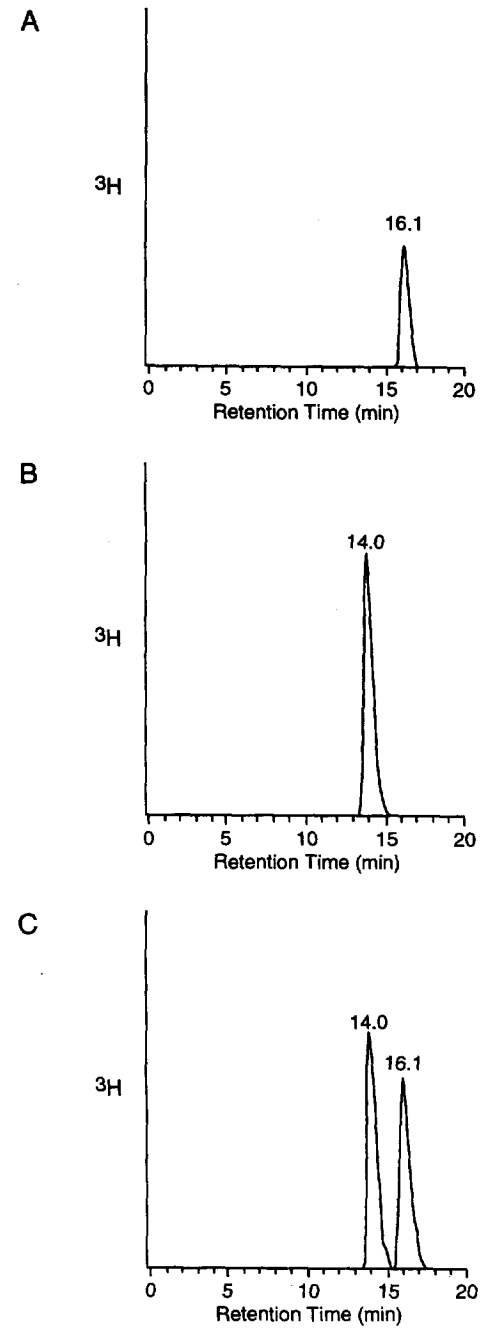


Fig. 5. Chromatographic identification of dihydrofinasteride. Top panel: Chromatographic profile of authentic ^{3}H-finasteride. Middle panel: Chromatographic profile of material released from [rhesus 5aR2:^{3}H-inhibitor] complex. Bottom panel: Co-injection of authentic ^{3}H-finasteride (14.0 min) and material released from the [enzyme:inhibitor] complex (16.1 min)

CONCLUSIONS

The cloning and expression of the rhesus 5aRs has afforded a more thorough study of this species and comparison to the human and other published isozyme sequences including rat and cynomolgus

monkey. The amino acid sequences and biochemical properties of the cloned rhesus 5aRs are very similar to the human homologs. These studies show very high homology (>93%) in the primary sequences of 5aR1 and 5aR2 from rhesus and human. Yet the extra four amino acids at the N-terminus differences of rhesus 5aR1 and the differences in the putative steroid binding site of 5aR2 warranted a closer examination of kinetic properties of these enzymes. There is good agreement in the biochemical properties of rhesus and human homologs with respect to pH optimum, K_m values for T and P, and inhibition by a variety of known 5aR inhibitors including finasteride Importantly, it appears that isoenzyme selectivity and mechanism of inhibition by finasteride are very similar in the rhesus macaque and human. Both human and rhesus 5aRs display time-dependent, mechanism-based inhibition with finasteride with selectivity for the 5aR2 [17]. It can be concluded that rhesus monkey is an appropriate species for evaluation of novel inhibitors of the human steroid 5aR1 and 5aR2.

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