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# Cloning, Expression and Functional Characterization of Type 1 and Type 2 Steroid 5α-Reductases from *Cynomolgus* Monkey: Comparisons with Human and Rat Isoenzymes

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**The *Cynomolgus* monkey may provide an alternative pharmacological model in which to evaluate the efficacy of novel inhibitors of the two known human steroid 5α-reductase (SR) isoenzymes. To evaluate the suitability of this species at the level of the molecular targets, a *Cynomolgus* monkey prostate cDNA library was prepared and screened using human SR type 1 and 2 cDNAs as hybridization probes. Two distinct cDNA sequences were isolated encoding the monkey type 1 and 2 SR isoenzymes. These sequences share 93 and 95% amino acid sequence identity with their human enzyme counterparts, respectively. Difference in monkey type 1 SR, however, was found within the contiguous four amino acids corresponding to the regions in the human and rat sequences that have been proposed previously to influence steroid and inhibitor affinities. Subsequently, both monkey cDNAs were individually expressed in a mammalian cell (CHO) line. Enzyme activities of both monkey SRs were localized to the membrane fractions of CHO cell extracts. Like the human and rat enzymes, the monkey type 1 and type 2 SRs were most active at neutral and low pH, respectively. The results of inhibition studies with over 30 known SR inhibitors, including epristeride, 4MA, and finasteride, indicate that the monkey SR isoenzymes are functionally more similar to the human than the rat homologues. The results from initial velocity and inhibition studies as functions of pH with the human and monkey type 2 SRs also compare favorably. These results, together, suggest that the monkey SR isoenzymes are structurally and functionally comparable on a molecular level to their respective human counterparts, supporting the relevance and use of the *Cynomolgus* monkey as a pharmacological model for *in vivo* evaluation of SR inhibitors.**

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## INTRODUCTION

5α-Dihydrotestosterone (DHT) appears to bear primary responsibility for the androgen stimulated component of prostate growth [1, 2]. Preclinical studies focusing on steroid 5α-reductase (SR), the enzyme that converts the classical androgen testosterone to DHT, have demonstrated selective growth retardation of DHT responsive organs such as prostate and seminal vesicles coincident with suppression of DHT biosynthesis [3–5]. Consequently, selective inhibition of SR has been pursued as a novel mechanism for controlling, managing and treating diseases such as benign prostatic hyperplasia (BPH), male pattern baldness, and acne where DHT is responsible for cellular response [for review, see reference 6]. Such specific androgen blockade would be expected to maintain testosterone supported androgenic stimulation such as increased muscle mass and growth of facial hair thereby

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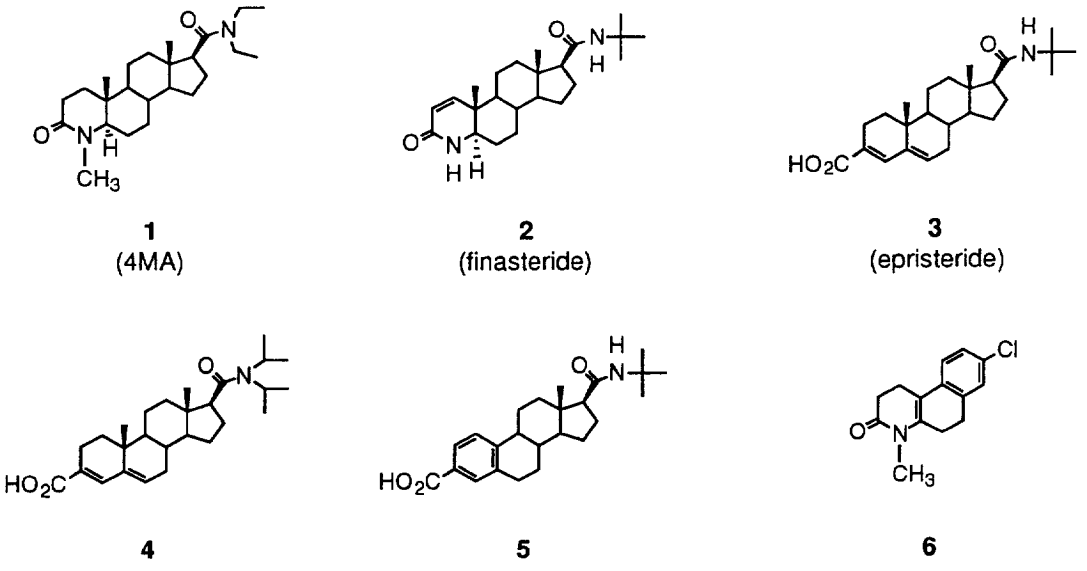


Scheme 1

avoiding potential undesirable effects associated with total androgen withdrawal.

Two distinct proteins, termed type 1 and type 2, which can catalyze the SR reaction have been identified in both rat [7, 8] and man [8–10]. Intraspecies and interspecies comparisons of the predicted primary amino acid sequences between the type 1 and type 2 enzymes show only moderate homologies, suggesting differing forms, functions, and/or regulation. For example, the type 1 human and rat isoenzymes have approx. 61% amino acid sequence identity while the two corresponding type 2 SRs are 75% homologous. Homology between the human types 1 and 2 SRs is 50% [7]. The differences in primary sequences result in unique functional characteristics of the two isoenzymes. Originally, the type 1 enzymes from man and rat were shown to preferentially catalyze the SR reaction at neutral pH, while both type 2 SRs were most active in a narrow pH range of 4.5–5.5 [7–9]. Functional differences of the same isoenzyme between species have also been demonstrated: while 4MA (compound 1) demonstrates essentially equivalent inhibition potency with both species' type 1 enzyme, the rat type 1 enzyme is at least 50-fold more susceptible to inhibition by finasteride (compound 2) than is its human counterpart. Tissue distributions of the two isoenzymes between rat and human also differ. For example, the activities of both type 1 and 2 SRs can be monitored in rat prostate, while activity of the type 2 isoenzyme predominates in tissue from the mature human organ.

Such differences between man and rat in functional characteristics and tissue distribution of the SRs would seem to make the rat a poor choice as a model for comparative *in vivo* pharmacological assessment of novel human SR isoenzyme inhibitors. Based on our previous observations [11] that prostatic SR activity in the non-human primate was similar to that found in man (e.g. exclusively low pH activity characteristic of type 2 SR), the *Cynomolgus* monkey presented an alternative model more closely related to the human condition in which to evaluate the *in vivo* effects of inhibitors.

As a model for pharmacological response, the effect upon oral administration of SR inhibitors on plasma DHT concentrations in the *Cynomolgus* monkey has been used as an alternative to monitoring pharmacological effects in the rat [12]. In order to validate the ability of this model to project the effectiveness of SR inhibitors in man, we have undertaken a set of comparative studies between the known human type 1 and type 2 enzymes and their counterparts in this non-human primate. The results of experiments demonstrating the presence of two monkey SR isoenzymes and the kinetic characterizations of their catalytic activities are reported in this paper.

## MATERIALS AND METHODS

### *Materials*

[4-$^{14}$C]Testosterone (57 mCi/mmol), [1,2,6,7-$^{3}$H]-testosterone (85 Ci/mmol), [4-$^{14}$C]androstenedione (51 mCi/mmol), [1,2,6,7-$^{3}$H]progesterone (107 Ci/mmol), [4-$^{14}$C]progesterone (57 mCi/mmol), [1,2-$^{3}$H]20α-hydroxyprogesterone (42 Ci/mmol), and [1,2,6,7-$^{3}$H]-corticosterone (73 Ci/mmol) were purchased from New England Nuclear. Glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Company (St Louis, MO). Unlabeled steroid substrates and 5α-reduced products were purchased from Steraloids. *Cynomolgus* monkey prostate tissue was provided by the Department of Laboratory Animal Sciences at SmithKline Beecham.

*Cloning of 5α-reductase enzymes*

Total RNA was prepared from *Cynomolgus* monkey prostate tissue by the guanidinium isothiocyanate method [13] and poly $(A)^+$RNA was isolated using a mRNA purification kit (catalog number 1353616) supplied by Boehringer Mannheim Corp. (Indianapolis, IN). A custom cDNA library cloned into lambda Uni-ZAP XR arms was prepared by Stratagene Cloning Systems. Based on the previously reported DNA sequence of the human SR type 1 and type 2 cDNAs [8, 9], oligonucleotide primers were prepared and used to obtain DNA fragments approx. 0.8 kb in length from 1 ng of plasmids harboring the human 5α-reductase type 1 (generously provided by Dr David Russell) and type 2 [14] cDNAs using the polymerase chain reaction (PCR) [15]. These DNA PCR fragments, which encompassed the coding regions of the human SR type 1 and type 2 cDNAs, were $^{32}$P-labeled using a TAG-IT kit (BIOS, New Haven, CT) and used to separately probe the monkey prostate cDNA library (one half million plaques per each probe) by *in situ* plaque hybridization as previously described [16]. More than a dozen cDNAs of each clone were identified. Positive clones were plaque purified and cDNAs were excised to yield recombinant Bluescript plasmids in *E. coli* XL1-Blue cells according to the method recommended by Stratagene Cloning Systems (La Jolla, CA). The cDNA inserts of recombinant clones were analyzed by DNA sequencing using an Applied Biosystems 373 DNA sequencer according to the manufacturer. Two clones were identified, termed pBmSRtyl, pBmSRty2, that contained cDNAs sharing a high degree of homology with the human SR type 1 and type 2 cDNAs, respectively. To verify sequence fidelities, both DNA strands of each isotype were completely sequenced at least twice.

*Plasmid construction and CHO cell expression*

In order to prepare the monkey SR type 1 cDNA for expression in CHO cells, PCR primers were synthesized that corresponded to the amino and carboxy termini. The 5′ PCR primer (5′-AGC GGA ATT CGC CAC CAT GGC TAC GGC TGT GGC GGA G-3′) included the following sequences: an Eco RI restriction site, a consensus Kosak sequence for optimal expression [17], an ATG initiation codon, and 6 subsequent codons of the human SR type 1 protein. The 3′ PCR primer (5′-TGG AAG CTT GCA CTT AAA ACA AGA ATG G-3′) contained (5′ to 3′-inverse complement) a Hind III restriction site followed by a stop codon and 4 carboxyl-terminal codons. These primers were used to obtain a cDNA fragment encoding the monkey SR type 1 from plasmid pBmSRtyl DNA using the polymerase chain reaction [15]. The resulting 820 bp DNA fragment was subcloned into the Eco RI, Hind III sites of the mammalian expression vector pCDN [18], producing clone pCDNmSRtyl. Comprehensive details describing the expression vector have been described by Aiyar *et al.* [18]. Since PCR was employed in its generation, the DNA sequence fidelity of the SR type 1 cDNA insert was confirmed by complete DNA sequence analysis. For the expression of the monkey SR type 2 gene, clone pBmSRty2 was digested with Eco RI and Hind III, releasing a 1019 bp fragment encompassing 13 bp of 5′ untranslated region (UTR), the complete coding region, and 235 bp of 3′ UTR sequence. This DNA fragment was subcloned into the cloning cassette of plasmid pCDN, forming plasmid pCDNmSRty2. To produce stable CHO cell transformants, pCDNmSRtyl or pCDNmSRty2 DNA were linearized and independently introduced into CHO cells by electroporation and populations of stable transformants expressing SR enzymatic activities were selected using G-418 media as described [19].

*RNA blotting and hybridization*

For use as a hybridization probe, the Eco RI–Hind III fragment of pCDNmSRtyl (820 bp) was isolated following agarose gel electrophoresis and radiolabeled with $^{32}$P-dCTP using a TAG-IT kit (BIOS, New Haven, CT). Total cellular RNA was extracted from monkey prostate tissue and the poly $(A)^+$ mRNA fraction was purified as described above. 10 μg of poly $(A)^+$ RNA was separated by electrophoresis through a 1.5% agarose/formaldehyde gel and blotted onto nitrocellulose [20]. The blot was baked at 80°C, prehybridized, hybridized with the $^{32}$P-labeled SR type 1 probe, and washed as described previously [16]. The filter was air dried and exposed to Kodak XAR-5 X-ray film at −70°C with an intensifying screen. Following radiolabel removal (confirmed by 1 week exposure of the blot to film with an intensifying screen at −70°C), the blot was hybridized to a $^{32}$P-labeled SR type 2 probe (Eco RI–Xho I fragment of pBmSRty2 (1680 bp), washed, and exposed to film as above.

*Preparation of cellular particulates used for SR activity assays*

Membrane fractions from rat epididymus used for type 1 activity assays were prepared as described [7]. Rat prostate microsomes for the type 2 activity were isolated as published [21]. Membrane fractions from CHO cells expressing the human and monkey isoenzymes 1 and 2 were prepared as previously outlined for the isolation of SR activity from human prostatic tissues [11, 22]. All membrane or microsomal preparations were stored in small aliquots at −80°C; enzyme activities were maintained for greater than 6 months under these storage conditions.

*Steroid 5α-reductase assays*

SR assays were conducted using the respective membrane or microsomal preparations described above. Protocols for enzyme activity assays were essentially as described previously [11, 14, 21]. For routine analysis,

assays for type 1 SR activity were evaluated in 50 mM sodium phosphate, pH 7.5, while 50 mM sodium citrate, pH 5.0, was used for assays of the type 2 SRs. Except as noted for individual experiments, a cofactor regenerating system ($NADP^+$ to NADPH) consisting of 1 mM glucose-6-phosphate and 0.5 units/ml glucose-6-phosphate dehydrogenase was included in each assay. The reaction was initiated by addition of an aliquot of enzyme preparation to a final volume of 0.5 ml.

*Inhibition studies*

Apparent inhibition constants ($K_{i,app}$) were estimated by Dixon analysis [11, 23] with 200–400 mM NADPH and the initial concentrations of steroid substrate equivalent to the experimentally determined $K_m$ values in the presence of the cofactor regenerating system to eliminate inhibition by $NADP^-$, except as noted. All other procedures for enzyme inhibition were the same as previously outlined [11, 14, 22].

*Dependence of kinetic parameters on pH*

Initial velocity pH profiles with recombinant human and monkey type 2 SRs were determined in constant ionic strength ($\mu = 0.05$ M) buffer solutions consisting of succinic acid, imidazole and diethanolamine [11]. Effects of pH upon the potency of recombinant type 2 SRs inhibition by compounds 2 and 4 were determined at concentrations of testosterone near $K_m$ values with the cofactor regenerating system.

*Data analysis*

Initial velocity and dead-end inhibition data were fit to appropriate rate equations with the COMP, NONCOMP and UNCOMP programs described by Cleland [11, 24]. Linear data evaluations were fit to the equation $y = mx + b$ using the LINE program. Data from the pH profiles were fit to the equations 1 (HABELL), 2 (HBBELL), 3 (BELL), or 4 (WAVL) according to the criteria established by Cleland [24]:

$$\log y = \log\left[\frac{c}{(1 + H/K_a)}\right] \quad (1)$$

$$\log y = \log\left[\frac{c}{(1 + K_b/H)}\right] \quad (2)$$

$$\log y = \log\left[\frac{c}{(1 + H/K_a + K_b/H)}\right] \quad (3)$$

$$\log y = \log\left[\frac{YL + YH(K/H)}{1 + H/K}\right] \quad (4)$$

where $K_a$, $K_b$, and $K$ are the pK of the group(s) whose ionization or protonation decreases activity, $c$ is the pH independent values of the parameter $y$ being evaluated, $YL$ is the value of $y$ at low pH, and $YH$ is the value of $y$ at high pH. Curve fitting of experimental data model equations [24] was accomplished with a nonlinear regression procedure using the Marquardt algorithm in the SAS statistical analysis software package (SAS Institute, Inc., Cary, NC) as outlined [11]. In the figures, displayed points are experimental values and the curves are computer calculated best fits.

## RESULTS AND DISCUSSION

*Cloning cDNAs encoding Cynomolgus monkey SR type 1 and 2 isozymes*

The coding region of the human SR type 1 and type 2 isozymes were separately used to probe a *Cynomolgus* prostate cDNA library under low stringency screening conditions. A number of hybridizing clones were discovered; clones which contained the largest cDNA inserts were chosen for complete characterization. The nucleotide and deduced protein sequences of clones pBmSRty1 and pBmSRty2, which encode complete proteins that closely resemble the human SR type 1 and 2 isozymes [8, 9], respectively, are shown in Figs 1 and 2.

The cDNA insert of plasmid pBmSRty1 is 1852 bp in length, containing an open reading frame of 789 bp encoding a protein of 263 amino acid residues. The cDNA is terminated at its 3′ end with a poly (A) tract, positioned 20 bp downstream from a potential eukaryotic polyadenylation signal, AATAAA [25], suggesting that the 3′ end of this cDNA is authentic. When aligned to maximize nucleotide sequence homology, the monkey cDNA sequence is 94.5% identical to the human SR type 1 cDNA across a linear span of 1750 bp, suggesting that they are species counterparts.

The cDNA insert of plasmid pBmSRty2 is 1668 bp in length, encoding a protein of 254 amino acid residues. Across a linear alignment of 1965 bp, the monkey cDNA inserts shares 95.3% nucleotide sequence identity with the human SR type 2 cDNA [9], suggesting that the two cDNAs are species homologues, with clone pBmSRty2 encoding the monkey SR type 2 enzyme. The cDNA insert of clone pBmty2 likewise ends with a poly (A) tract; however, the cDNA lacks a canonical polyadenylation signal that typically precedes poly (A) tails of eukaryotic mRNA transcripts [25]. It is quite possible that the poly (A) stretch of nucleotides that terminate this cDNA are a result of a cloning artifact in the construction of the cDNA library: a consequence of oligo d(T) priming within the 3′ untranslated region of the mRNA transcript. Indeed, Northern blot analysis (see below) indicates that the mRNA transcript size of the monkey SR type 2 gene is more than 50% larger than the size of the cloned cDNA, which suggests that the cloned cDNA does not represent the entire gene mRNA transcript.

*Primary sequence comparison of the SR type 1 and type 2 enzymes*

The deduced monkey proteins, together with the human [8, 9] and rat [7, 26] orthologs, are compared in Fig. 3. Although 4 and 8 amino acids larger than the

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-87  tagccgcccgtcccgcccccgcgctgccgccttatatgttgcctgccgcggcctctgggg
-27  catggagcacgctacccagccccggcgATGGCGACGGCGGTGGCGGAGGAGCTCCTGCTG
                              M  A  T  A  V  A  E  E  L  L  L     11
 34  GCCGAGGAGCGCATGCTGGCCGCGCTCGCCTACCTGCAGTGCGCCGTGGGCTGCGCGGTC
      A  E  E  R  M  L  A  A  L  A  Y  L  Q  C  A  V  G  C  A  V  31
 94  TTAGCGCGGAATCGTGAGACGAACTTGGCGTACGGCCGCCACGCGTCGCCCAGCTTTAGG
      L  A  R  N  R  E  T  N  L  A  Y  G  R  H  A  S  P  S  F  R  51
154  GTCCGAGTGCCGGCGCGGGCCGCCTGGGTGGTGCAGGAGCTGCCCTCGCTGGCTCTGCCG
      V  R  V  P  A  R  A  A  W  V  V  Q  E  L  P  S  L  A  L  P  71
214  CTCTACCAGTATGCCAGCGAGTCCGCCCCGCGCCTCCGCAGCGCGCCCAACTGCATCCTC
      L  Y  Q  Y  A  S  E  S  A  P  R  L  R  S  A  P  N  C  I  L  91
274  CTGGCCATGTTCCTCGTCCACTACGGGCATCGGTGCTTAATTTACCCATTTCTGATGCGA
      L  A  M  F  L  V  H  Y  G  H  R  C  L  I  Y  P  F  L  M  R  111
334  GGAGGAAAGCCTATGCCACTGTTGGCGTGTACAATGGCGATTATGTTCTGTACCTTTAAC
      G  G  K  P  M  P  L  L  A  C  T  M  A  I  M  F  C  T  F  N  131
394  GGCTATTTGCAAAGCAGATACTTGAGCCATTGGGCAGTGTATGCTGATGACTGGGTAACA
      G  Y  L  Q  S  R  Y  L  S  H  W  A  V  Y  A  D  D  W  V  T  151
454  GATCCCCGTTTTCTAATAGGTTTTGGCTTGTGGTTAGCCGGTATGTTGATAAACATCCAT
      D  P  R  F  L  I  G  F  G  L  W  L  A  G  M  L  I  N  I  H  171
514  TCAGATCATATCCTAAGGAATCTCAGAAAACCAGGAGACACTGGATACAAAATACCAAGG
      S  D  H  I  L  R  N  L  R  K  P  G  D  T  G  Y  K  I  P  R  191
574  GGAGGCTTATTTGAATACGTAACTGCAGCCAACTATTTTGGAGAAATCATGGAGTGGTGT
      G  G  L  F  E  Y  V  T  A  A  N  Y  F  G  E  I  M  E  W  C  211
634  GGCTATGCCCTGGCCAGCTGGTCTGTCCAAGGCGCAGCTTTTGCTTTCTTCACATTTTGT
      G  Y  A  L  A  S  W  S  V  Q  G  A  A  F  A  F  F  T  F  C  231
694  TTTTTATCTGGTAGAGCAAAAGAGCATCATCGGTGGTACCTCCAGAAATTTGAAGAGTAT
      F  L  S  G  R  A  K  E  H  H  R  W  Y  L  Q  K  F  E  E  Y  251
754  CCAAAGTTCAGAAAAATTCTAATTCCATTTTTGTTTTAAgtgcattttcaacgaaattat
      P  K  F  R  K  I  L  I  P  F  L  F  *                       263
814  cttcaagttgaagctttccaatggtgtttctctagggactttgtaaataagttatatctt
874  tgtaattttcctgctacttcatcattttcaagatgtcctctagaaatatttttttctagta
934  attttgcaagctacctaataagtacttaaataaactgaaatggaggttgaagtatcctac
994  tgtgtaacagatcagaatttcaaactccaggtaataactgctgacatttgttctaatttc
1054 gaatttacctcttttggctatgtcttgccaagtgtatctaagactagagtttacaactgt
1114 ctttgatggcattttcagaacaataaatgtcacaatcccttctatagccccctacagtga
1174 tcccttcaaggtcaacagcagtgttgctttcccccctgtagggctgggatatgtcttggag
1234 ccctctctcggaggccacagaggccgggggtagccattgtgcagtcatggcccaggggaa
1294 acttgccaaccttcattgtcaggtgctgtgtgtaagtggagaacttggggatagaggagg
1354 aagctcctggtggctcttccaaggcaggggcaaaggcatctggacttgttccagcccagc
1414 cccccaggtgacatcaccaggcagggaggggtgctggtggtggtttagacggagtaagtt
1474 gctttgcctgtgcaagtggctcccgggccctaaacaggcacctttaggccatgggtcact
1534 caccatgagccatcaatatgctctggtctgacatggtttctctttgtcctctagtctaga
1594 cctagtttttgttctgttctccacgtatggatatagtagagattattgtctgtgaaatt
1654 tctcctttgtggattttgagttttccgttgtagtgtaaagaatgattaccttctgtaaca
1714 ataaaaagaccacttttaagatttaaaaaaaaaaaaaaaaaaaaaaaa
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**Fig. 1. Nucleotide and deduced amino acid sequence of the monkey SR type 1 cDNA. Amino acids (represented by the one-lettered code) are indicated below their respective codons and numbered on the right beginning with the initiation methionine codon (M) and ending with a stop codon (asterisk). Numbers on the left indicate nucleotide positions. Underlined nucleotide sequences indicate canonical eukaryotic polyadenylation signals.**

human rat SR type 1 enzymes, respectively, the monkey SR type 1 protein shares significant amino acid identity with these proteins [Fig. 3(A)]. Overall, the monkey SR type 1 enzyme is highly homologous to its human protein counterpart, sharing about 93% amino acid sequence identity. Like the human protein, the monkey SR type 1 protein shares considerably lower sequence homology with the rat SR type 1 enzyme, having approx. 61% amino acid sequence identity between the two homologues.

In contrast to the type 1 enzymes, alignments of the predicted monkey SR type 2 amino acid sequences with its human and rat counterparts show that the three SR type 2 proteins are all the same length. Also, the type 2 isoenzymes share an even greater degree of protein sequence identity than do the SR type 1 proteins [Fig. 3(B)]. For example, the human and rat proteins share 95 and 76% amino acid sequence identity with the monkey type 2 enzyme, respectively. Of the 21 amino acid positions where point mutations have been identified in human patients with SR type 2 deficiency [27], all are conserved between the primate SR type 2

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-13   ggcacgaggcgcgATGCAGGTTCAGTGCCAGCAGAGCCCAGTGCTGGCAGGCAGCGCC
                    M  Q  V  Q  C  Q  Q  S  P  V  L  A  G  S  A    15
 47 ACTTTGGTCGCCCTTGGGGCACTGGTCTTGTACGTCGCGAAGCCCTCCGGCTACGGGAAG
     T  L  V  A  L  G  A  L  V  L  Y  V  A  K  P  S  G  Y  G  K    35
107 CACACGGAGAGCCTGAAGCCCGCGGCTACCCGCCTGCCAGCCCGCGCAGCCTGGTTCCTG
     H  T  E  S  L  K  P  A  A  T  R  L  P  A  R  A  A  W  F  L    55
167 CAGGAGCTGCCCTCCTTCGCGGTGCCCGCGGGGATCCTCGCTCGGCAGCCCCTCTCCCTC
     Q  E  L  P  S  F  A  V  P  A  G  I  L  A  R  Q  P  L  S  L    75
227 TTCGGGCCACCTGGGACGGTGCTTCTGGGCCTCTTCTGCGTACATTACTTCCACAGGACA
     F  G  P  P  G  T  V  L  L  G  L  F  C  V  H  Y  F  H  R  T    95
287 TTTGTGTACTCACTGCTCAATCGAGGGAGGCCTTATCCAGCTGTACTCATTTTCCGAGGC
     F  V  Y  S  L  L  N  R  G  R  P  Y  P  A  V  L  I  F  R  G   115
347 ATTGCCTTCTGCGCTGGAAATGGATTCCTTCAAAGCTACTATCTGATTTACTGTGCTGAA
     I  A  F  C  A  G  N  G  F  L  Q  S  Y  Y  L  I  Y  C  A  E   135
407 TACCCTGATGGGTGGTACACAGACATACGGTTTTGCTTGGGTGTCTTCTTATTTATTTTG
     Y  P  D  G  W  Y  T  D  I  R  F  C  L  G  V  F  L  F  I  L   155
467 GGAATGGGAGTCAACATCCATAGTGACTATATATTGCGCCAGCTCAGGAAGCCTGGAGAA
     G  M  G  V  N  I  H  S  D  Y  I  L  R  Q  L  R  K  P  G  E   175
527 ATCACCTACAGGATTCCAAAAGGTGGCTTGTTTACGTATGTTTCTGGAGCCAATTTCCTT
     I  T  Y  R  I  P  K  G  G  L  F  T  Y  V  S  G  A  N  F  L   195
587 GGTGAGATCATTGAATGGATCGGCTATGCGCTGGCCACTTGGTCCCTCCCAGCACTTGCA
     G  E  I  I  E  W  I  G  Y  A  L  A  T  W  S  L  P  A  L  A   215
647 TTTGCATTTTTCTCAGTTTGTTTCCTTGGGCTGCGAGCTTTTCACCACCATAGGTTCTAC
     F  A  F  F  S  V  C  F  L  G  L  R  A  F  H  H  H  R  F  Y   235
707 CTCAAGATGTTTGAGGACTACCCCAAATCTCGGAAAGCCCTTATTCCATTCATCTTTTAA
     L  K  M  F  E  D  Y  P  K  S  R  K  A  L  I  P  F  I  F  *   254
767 agaaaccaaattaaaaaggagcaaagctcccacaacgctgatgtaaactgtcaagctgct
827 gaaactgcaatttccatgatataatagtcctccatatatatatatatgtatgtgcatata
887 taatagtaggtctcctggcgttctgccagctggcctggggattctgagtggtgcctgctt
947 agagtttacttctacccttccagggacccctatcctgatctccaattgaagcttcaaaaa
1007 gccacttttccaaatggcgacggttgcttcttaggtattgctctgagaaagtacgaactt
1067 ctcctatttctttcactgggcaatccaagtacgcgtggcttcatacccactcccctgtca
1127 atgtaggacaactctgtaatcaagaatttttggcttgaaggcagtgcttatagacctta
1187 ttaaaggtatacattttgtacgcatacagagtagcagagatttaaactctgaagccacac
1247 agacccagagcaaacccactcccaaatgaaaaccccagtcacgtcttccttttccttggt
1307 taattaggaaagatgagaaattattagatagaccttgaatacaggagccctctcctcata
1367 gtgctgaaaagatactgatgcattgacctcatttccaaatgtatgcagtgtccttagttg
1427 atgagtgcctctgttttccagaagatttcacaatccccggaaaactggtatggctattct
1487 tgaaggccaggttttaataaccacaaagaaaaaggcatgaccctggatggctgctgggag
1547 agtagagaacagcatgatcctggatggctactaagaggatagagaacagttttacaatag
1607 acattgcaaactttcatgttcttggaaactggtggcaatagcccaaaaaaaaaaaaaaaa
1667 aa
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Fig. 2. Nucleotide and deduced amino acid sequence of the monkey SR type 2 cDNA. Amino acids (represented by the one-lettered code) are indicated below their respective codons and numbered on the right beginning with the initiation methionine codon (M) and ending with a stop codon (asterisk). Numberes on the left indicate nucleotide positions.

enzymes, and all but two (Gly-115 and His-230) are identically conserved in the proteins from all three species, thus reinforcing the concept of their importance for enzyme function.

A notable difference between the two primate type 1 proteins occurs at amino acid position 32 of the monkey enzyme. Residue 32 of the monkey type 1 SR is a leucine rather than the phenylalanine found at the corresponding position (amino acid 28) of the human enzyme. This Phe residue is included within a tetrapeptide (Ala-Val-Phe-Ala) proposed to be part of the human enzyme's steroid binding domain that influ-

Fig. 3. (*opposite*) Amino acid sequence comparison of all SR isozymes. This figure compares all of the SR enzymes published to date to either the monkey SR type 1 (A) or monkey SR type 2 (B) proteins. The monkey sequences are numbered beginning with the initiating methionine residue. Dashes indicate amino acid identity, whereas amino acid differences are shown in their corresponding position. Periods indicate sequence gaps introduced to maximize sequence homology. Brackets above the sequence denote the tetrapeptide sequence implicated in finasteride binding. Asterisks above sequence denote amino acid positions of naturally occurring mutants in SR type 2 deficient patients.

**A**

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                1                                                   50
Monkey          MATAVAEELL LAEERMLAAL AYLQCAVGCA VLARNRETNL AYGRHASPSF
Human           ----TG.... V----L---- ---------- -F----Q--S V-----L--H
Rat             ........ME -D-LCL-DM- V--EGFMAFV SIVGL-SVGS P---YSPQWP

                                                                   100
Monkey          RVRVPARAAW VVQELPSLAL PLYQYASESA PRLRSAPNCI LLAMFLVHYG
Human           -L-------- ---------- ---------- ---------- ----------
Rat             GI-----P-- FI-----MAW ---E-IRPA- A--GNL--RV ------I--V

                                                                   150
Monkey          HRCLIYPFLM RGGKPMPLLA CTMAIMFCTF NGYLQSRYLS HWAVYADDWV
Human           ---------- ---------- ---------C ---------- -C--------
Rat             Q-T-VF-V-I -----TLLVT FVL-FL---- ---V------ QF----E---

                                                                   200
Monkey          TDPRFLIGFG LWLAGMLINI HSDHILRNLR KPGDTGYKIP RGGLFEYVTA
Human           ---------- ---T------ ---------- ---------- ----------
Rat             -HPC--T--A ---V--V--- ---------- ---E------ --------S-

                                                                   250
Monkey          ANYFGEIMEW CGYALASWSV QGAAFAFFTF CFLSGRAKEH HRWYLQKFEE
Human           ---------- ---------- ---------- ---------- -E---R----
Rat             ------LV-- --F------L --VV--L--L ST-LT---Q- -Q--HE---D

                     263
Monkey          YPKFRKILIP FLF
Human           -------I-- ---
Rat             ---SR----- -VL
```

**B**

```
                1                                 *                 50
Monkey          MQVQCQQSPV LAGSATLVAL GALVLYVAKP SGYGKHTESL KPAATRLPAR
Human           ---------- ---------- ---A------ ---------- ----------
Rat             --IV-H-V-- -------ATM -T-I-CLG-- AS-------V SSGVPF----

                    **  *                                  *       100
Monkey          AAWFLQELPS FAVPAGILAR QPLSLFGPPG TVLLGLFCVH YFHRTFVYSL
Human           ---------- ---------- ---------- ---------- ----------
Rat             I--------- -V-SV-M--W --R------- N---A--SA- ------I---

                                *           *                     *  150
Monkey          LNRGRPYPAV LIFRGIAFCA GNGFLQSYYL IYCAEYPDGW YTDIRFCLGV
Human           ---------I --L--T---T ---V--G--- ---------- ------S---
Rat             -T----F--- -FL-AT---I ---L--A--- V------EE- ---V--SF--

                                *           *           * *           *  **200
Monkey          FLFILGMGVN IHSDYILRQL RKPGEITYRI PKGGLFTYVS GANFLGEIIE
Human           --------I- ---------- ------S--- -Q-------- ----------
Rat             --------I- -----T---- -----V---- -R-------- ----------

                      *     *             *     * *                 * 250
Monkey          WIGYALATWS LPALAFAFFS VCFLGLRAFH HHRFYLKMFE DYPKSRKALI
Human           ---------- ---------- L--------- ---------- ----------
Rat             ---------- V--F-----T L----MQ--Y ---------K ----------

                 254
Monkey          PFIF
Human           ----
Rat             ----
```

**Fig. 3**—*legend opposite.*

ences differential inhibitor response vs the rat type 1 SR [28]. Interestingly, the corresponding contiguous four amino acid sequences in the type 2 SRs are also not conserved across these three species [Fig. 3(B)]. With recognition that sequence variation within this specific tetrapeptide region can influence steroid and inhibitor affinities [28], comparative biochemical characterization of the monkey, human and rat SR isoenzymes was initiated. The results of these studies are presented below.

*RNA analysis in monkey prostate tissue*

To investigate and compare the mRNA transcript sizes of the monkey SR type 1 and type 2 genes, monkey prostate RNA was examined by Northern blot analysis using the monkey SR type 1 and type 2 cDNAs as hybridization probes. As shown in Fig. 4, one major band was detectable for each SR gene mRNA transcript in prostate RNA (the same size transcripts were also detected in monkey liver RNA-data not shown). The SR type 1 mRNA was approx. 1.9 kb, a size that is consistent with that of the cloned cDNA (1852 bp); accordingly, the cloned cDNA likely represents the entire mRNA transcript. The SR type 2 cDNA probe revealed a single mRNA of approx. 2.6 kb. Considering the size of the SR type 2 gene transcript, the cloned cDNA lacks at least 900 bp of sequence, perhaps missing nucleotides within both 5′ and 3′ untranslated regions.

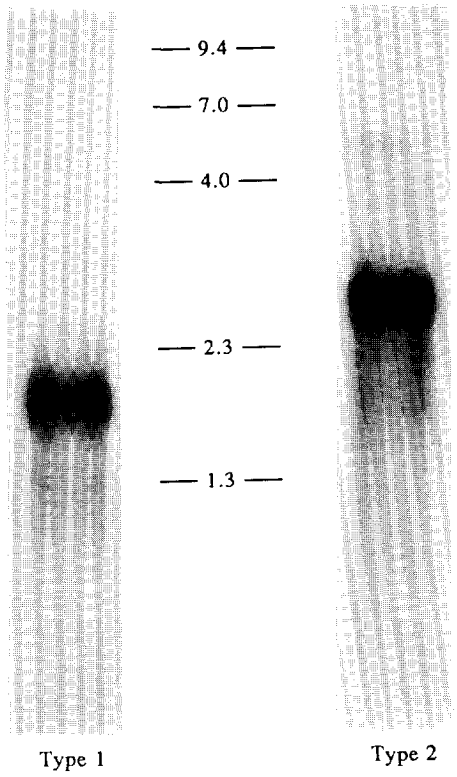


**Fig. 4. Northern blot analyses of *Cynomolgus* prostate mRNA. Northern blot analysis was used to examine the size of the monkey SR type 1 and type 2 mRNA transcripts in monkey prostate tissue. Ten microgram of poly(A)$^+$ RNA isolated from prostate was analyzed with the monkey SR type 1 cDNA as a hybridization probe. After the blot was exposed to X-ray film, the probe was removed and the blot was re-probed with a radiolabeled monkey SR type 2 cDNA probe and again exposed to X-ray film. Molecular weight markers are indicated in kb.**

*Kinetic characterization of monkey SRs type 1 and type 2*

To characterize the biochemical properties of the monkey SR proteins, both enzymes were individually expressed in a mammalian (CHO) cell line. As with previous reports of expression [8, 26], both enzyme activities were found localized to membrane fractions of cellular extracts. One of the original characteristics leading to recognition of two different SRs in the rat, and subsequently in man, was distinguishing profiles of enzyme activity vs pH [8]. Using conditions that typically involved micromolar concentrations of testosterone, the type 1 enzymes of both species showed highest activity above pH 7.0, while maximal catalysis by the second isoenzymes (type 2) occurred in a narrow acidic pH range of 4.5–5.5 [8]. Our results with the recombinant monkey isoenzymes expressed in CHO cells demonstrated pH activity dependence qualitatively identical to these previous observations, indicating that the type 1 and type 2 monkey SRs were most active at neutral and low pH respectively (Fig. 5).

In order to better appreciate the relative interspecies kinetic characteristics of the two human and monkey isoenzymes, several $\Delta^{4(5)}$-steroids were evaluated as potential substrates. The relative affinity of this selection of substrates was found to be qualitatively the same for both the type 1 (20α-hydroxyprogesterone ⩾ progesterone > androstenedione ⩾ testosterone > corticosterone) and type 2 (20α-hydroxyprogesterone ⩾ progesterone > testosterone > androstenedione > corticosterone) SRs. In addition, relative values for the efficiency of the enzymatic reactions, as indicated by the calculated second order rate constant $V_{max}/K_m$, are similar for the comparable isoenzymes between the two species (Table 1). These data suggest that the type 1 and type 2 enzymes from human and monkey have similar substrate selectivity.

More careful kinetic analysis with human SR type 2 has demonstrated that the activity associated with this enzyme is not exclusive to an acidic environment as originally characterized. The profiles of $V_{max}$ and $V_{max}/K_m$ as functions of pH with the human type 2 SR are depicted in Fig. 6. In this figure using testosterone as substrate, it is shown that the profile of maximal velocity is centered at low pH near 5.0 as had been originally observed. The $V_{max}/K_m$ profile for testosterone, however, shows maximal effect in the neutral to basic pH range. This dramatic and unusual effect on $V_{max}/K_m$ vs that for $V_{max}$ results from a significant decrease in the values for $K_m$ of testosterone upon increasing pH (e.g. 700 nM at pH 5.0 to 10 nM at pH 7.0), an observation recently described by other investigators [29]. Since $V_{max}/K_m$ is the calculated

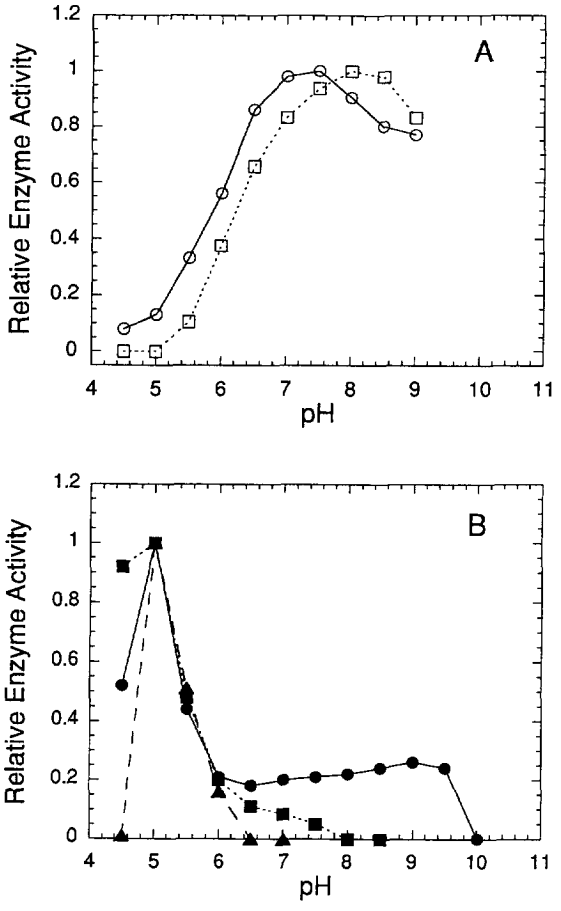


Fig. 5. Activities of recombinant primate type 1 and type 2 SRs. Type 1 (panel A) and type 2 (panel B) activities were determined over the indicated pH range as described under the Methods in the presence of 1–1.5 $\mu$M [$^{14}$C]testosterone. The activities have been normalized to the highest value in each data set and consequently are represented as relative activities. The activity values to which each curve was normalized were (Panel A) recombinant monkey type 1 (□, 113 pmol·min$^{-1}$·mg$^{-1}$) and recombinant human type 1 (○, 50 pmol·min$^{-1}$·mg$^{-1}$) and (Panel B) recombinant monkey type 2 (■, 65 pmol·min$^{-1}$·mg$^{-1}$), recombinant human type 2 (●, 15 pmol·min$^{-1}$·mg$^{-1}$), and monkey prostatic microsomes (▲, 12 pmol·min$^{-1}$·mg$^{-1}$).

second order rate constant for the reaction, the catalytic efficiency of the type 2 enzyme is actually greater at neutral than at low pH. It is estimated from these two sets of data that a single enzyme-associated protonation/deprotonation event influences the pH dependence of both $V_{max}$ (pK = 5.8 ± 0.2) and $V_{max}/K_m$ (pK = 5.7 ± 0.1) according to the WAVL and HABELL models described by equations 4 and 1, respectively [24].

Analysis of the monkey type 2 SR as a function of pH shows a similar effect on catalytic efficiency. Although not evaluated at the same level as that depicted in Fig. 6 for human isoenzyme 2, catalytic efficiency ($V_{max}/K_m$) was greater at pH 7.5 (870 ± 35 pmol·min$^{-1}$·mg$^{-1}$ $\mu$M$^{-1}$) than pH 5.0 (666 ± 105 pmol·min$^{-1}$ × mg$^{-1}$·$\mu$M$^{-1}$) resulting primarily from a significant decrease in the effective value of the Michaelis constant at neutral pH ($K_m$ = 2.5 ± 0.5 nM) vs that measured at the more acidic pH ($K_m$ = 700 ± 100 nM).

*Response of monkey SR isoenzymes to inhibitors*

One of the most discriminating characteristics between the human and rat type 1 and 2 SRs has been the differential isoenzyme response to known SR inhibitors. 4MA (1) has been shown to be equally potent on both isoenzymes from both species [28]. In contrast, inhibition by finasteride (2) is essentially equipotent on both rat enzymes, while the type 1 human SR is 50–100-fold more refractory to inhibition than human isoenzyme 2. Representative data from our laboratory confirming these observations are included in Table 2. It also has been shown that the steroidal acrylate epristeride (3), an uncompetitive inhibitor vs steroid substrate, is a dual inhibitor of the rat SRs, but shows selective inhibition of human type 2 vs type 1 SR [14].

Using chimeric enzymes, it has been suggested that the differential effects of inhibitors 1 and 2 result from steric interactions of the compounds' 17$\beta$-substituents with different amino acid side chains within the steroid binding domains of each isoenzyme [28]. The corresponding amino acid sequences proposed to be involved in these interactions (see Fig. 3) are Val-Ser-Ile-Val and Gly-Thr-Leu-Ile in the rat type 1 and type 2 SR and Ala-Val-Phe-Ala and Gly-Ala-Leu-Ala from human isoenzymes 1 and 2, respectively [28]. Interestingly, the corresponding amino acids of monkey type 1 (Ala-Val-Leu-Ala) and type 2 (Gly-Ala-Leu-Val) SRs are similar, but not identical, to the sequences found in the human enzymes, introducing the possibility that the isoenzymes between monkey and human may too display differential response to inhibitors.

Comparative results of the two recombinant monkey SRs with recombinant human enzymes and tissue-isolated rat enzymes with six selected compounds are included in Table 2. The inhibitory profiles of the monkey and human type 1 SRs closely parallel. Each of the compounds 1–5 are excellent inhibitors of type 2 enzyme from all three species. Both monkey and human type 1 SRs are refractory to the more potent inhibition of the rat type 1 isoenzyme by compounds 2 (finasteride), 3 (epristeride), 4 and 5, while all species' type 1 SR are exquisitely sensitive to inhibition by 4MA (1). Compound 6, which represents a class of nonsteroidal inhibitors [30], is selective for both type 1 primate SRs (Table 2).

A more comprehensive set of comparisons has been conducted with more than 30 compounds representing structurally diverse 3-oxo-4-aza steroidal [31], 3-carboxy steroidal [32, 33], and nonsteroidal [30] SR inhibitors. The empirical relationships between the apparent inhibition constants for type 1 and type 2 monkey and human SRs are presented in panels A and B, respectively, of Fig. 7. An excellent rank order correlation with both the types 1 and 2 isoenzymes of

*Table* 1(A). *Substrate preference of recombinant human steroid 5α-reductase isoforms**

| Steroid substrate | Isozyme 1† $K_m$ ($\mu$M) | Isozyme 1† $V_{max}$ (pmol/(min·mg)) | Isozyme 1† $V_{max}/K_m$ (pmol/(min·mg·$\mu$M)) | Isozyme 2† $K_m$ ($\mu$M) | Isozyme 2† $V_{max}$ (pmol/(min·mg)) | Isozyme 2† $V_{max}/K_m$ (pmol/(min·mg·$\mu$M)) |
|---|---|---|---|---|---|---|
| Testosterone | 3.5–5.2 | 220 | 40–60 | 0.7 | 88 | 115 |
| Androstenedione | 1.0 | 395 | 390 | 0.8 | 90 | 110 |
| Progesterone | 0.3 | 370 | 1230 | 0.3 | 80 | 260 |
| 20α-Hydroxyprogesterone | 0.2 | 570 | 2850 | 0.4 | 85 | 240 |
| Corticosterone | 18 | 210 | 12 | 4 | 42 | 11 |

*Table* 1(B). *Substrate preference of recombinant monkey steroid 5α-reductase isoforms**

| Steroid substrate | Isozyme 1‡ $K_m$ ($\mu$M) | Isozyme 1‡ $V_{max}$ (pmol/(min·mg)) | Isozyme 1‡ $V_{max}/K_m$ (pmol/(min·mg·$\mu$M)) | Isozyme 2‡ $K_m$ ($\mu$M) | Isozyme 2‡ $V_{max}$ (pmol/(min·mg)) | Isozyme 2‡ $V_{max}/K_m$ (pmol/(min·mg·$\mu$M)) |
|---|---|---|---|---|---|---|
| Testosterone | 2 | 245 | 120 | 0.2 | 86 | 660 |
| Androstenedione | 0.6 | 290 | 480 | 0.4 | 190 | 510 |
| Progesterone | 0.3 | 200 | 670 | 0.2 | 150 | 730 |
| 20α-Hydroxyprogesterone | 0.5 | 240 | 480 | ND | ND | ND |
| Corticosterone | 8 | 197 | 25 | 2 | 190 | 95 |

*Type 1 and type 2 SR activities were determined at pH 7.5 and pH 5.0, respectively.

†Experiments with human SRs were conducted using recombinant type 1 (0.05–0.10 mg protein per assay) and type 2 (0.02–0.15 mg protein per assay) enzymes expressed in CHO cells. Standard errors for all values were less than 20% of the indicated entries.

‡Experiments with monkey SRs were conducted using recombinant type 1 (0.06 mg protein per assay) and type 2 (0.030–0.150 mg protein per assay) enzymes expressed in CHO cells. Standard errors for all values were less than 20% of the indicated entries.

monkey and human origin can be observed over ranges of inhibitory potencies covering four-orders of magnitude. No corresponding relationship is observed upon similar analysis between the human or monkey and the rodent isoenzymes (not shown) as anticipated from the subset of data presented in Table 2, or from cross correlations between the type 1 and type 2 SRs within a given species. These comparisons of the species isoenzymes' response to three different classes of inhibitors indicate that both SRs from the *Cynomolgus* monkey are functionally indistinguishable from their human counterparts.

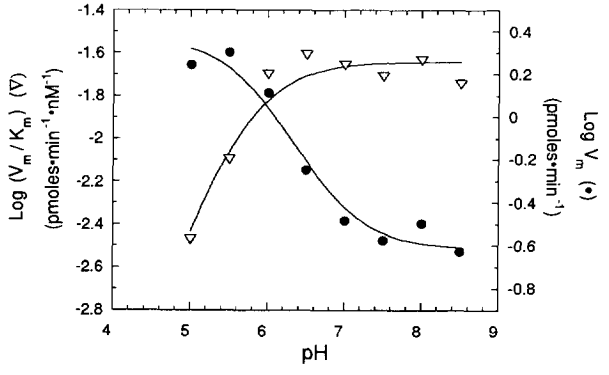


**Fig. 6. Dependence of kinetic parameters of human type 2 SR upon pH. Kinetic constants of the recombinant human type 2 SR were evaluated over the indicated pH range using testosterone as the steroid substrate as described under the Methods. Data for $V_m/K_m$ (▽) were fit to equation 1 yielding a value for $pK_a = 5.7 \pm 0.1$; the theoretical curve for the $V_{max}$ (●) data is represented by equation 4 with $pK = 5.8 \pm 0.3$.**

*Inhibition of primate type 2 SR as a function of pH*

Following the observations with human recombinant type 2 enzyme of the pH dependence upon $V_m$ and $V_{max}/K_m$, inhibition studies as a function of pH (Fig. 8) were conducted with a representative uncompetitive inhibitor, **4**, and a competitive inhibitor, **2** (finasteride, MK906). For the dienyl acid **4**, maximal inhibitory potency was observed over the neutral pH (6–8) range. The inhibitory profile of **4** was best evaluated by the BELL function, giving two inflection points: deproto-

*Table 2. Inhibition of SR isozymes by selected compounds*

| Enzyme* | Inhibitor 1 ($K_{i,app}$, nM) | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| *Human* | | | | | | |
| Type 1 | 3 | 100–120 | 410 | 290 | 1600 | 17 |
| Type 2 | 4 | 1–3 | 0.3–2 | 0.6 | 0.4 | >500 |
| *Monkey* | | | | | | |
| Type 1 | 3 | 220 | 500 | 250 | 1000 | 7 |
| Type 2 | 2 | 5 | 0.5 | 2.5 | 0.3 | NI@1250‡ |
| *Rat* | | | | | | |
| Type 1 | 8–10 | 6 | 20 | 10–30 | 150–250 | ND§ |
| Type 2 | 3 | 4 | 4 | 2 | 4 | ND |

*Source of types 1 and 2 human and monkey SRs were recombinant enzymes expressed in CHO cells. Rat types 1 and 2 activities were isolated from prostate and epididymus, respectively.

†Standard error for all $K_{i,app}$ values were less than 20%. Ranges indicate the results of multiple determinations.

‡No inhibition (NI) was observed at a concentration of 1250 nM compound **6**.

§Not determined (ND).

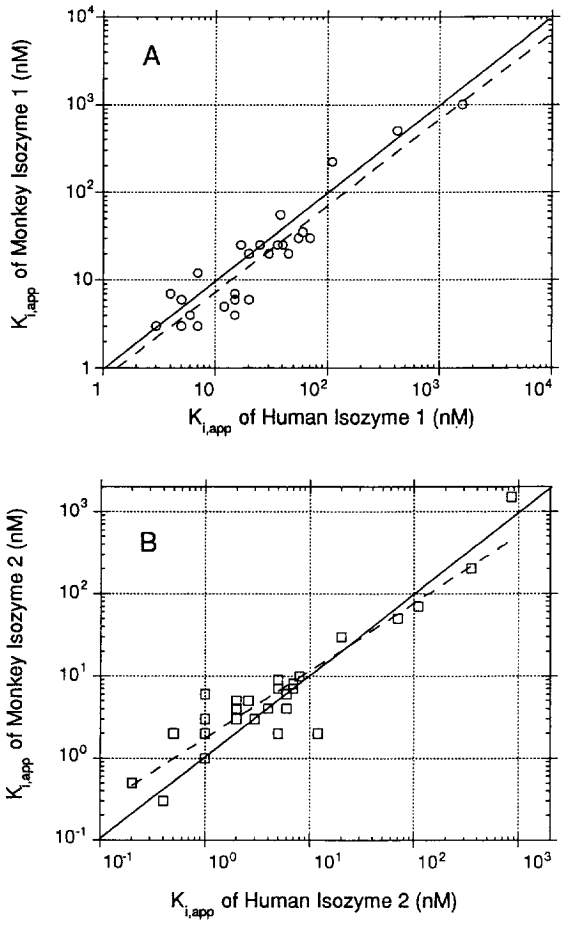


**Fig. 7. Comparisons of potencies of SR inhibitors on human and monkey enzymes. The inhibitory potencies ($K_{a,pp}$) of compounds representative of different structural classes of SR inhibitors (see text) were evaluated for the recombinant type 1 (panel A) and type 2 (panel B) human and monkey isoenzymes. The correlation of these determinations for each isozyme subtype from human and monkey are represented. The correlation coefficients ($R$ values) between the two type 1 and type 2 isoenzymes are 0.968 (panel A) and 0.940 (panel B), respectively.**

nation of one functionality ($pK_a = 5.0 \pm 0.4$) and protonation of a second functionality ($pK_b = 8.6 \pm 0.3$) are required for maximal inhibitory potency of the human type 2 enzyme activity. Inhibition by finasteride shows little dependence on pH as determined in this study. These profiles are qualitatively the same as those previously determined for the rat type 1 enzyme (from liver) with the same two inhibitors—a remarkable observation considering the low sequence homology (~40%) across species between the two enzymes [8]. Similarly, inhibition of the monkey type 2 SR by **4** at pH 7.5 ($K_{i,app} = 2.5 \pm 0.4$ nM) was more potent than that at pH 5.0 ($K_{i,app} = 4.2 \pm 0.7$ nM). These observations are consistent with the requirement for deprotonation of the C-3 acid functionality within the uncompetitive inhibitor **4** for optimal affinity with the type 2 SRs, as previously surmised using activity derived from rat liver microsomes [11, 34, 35] and now recognized to primarily consist of that species' type 1 enzyme.

## CONCLUSIONS

Previous results comparing the SR activity isolated from prostate of the *Cynomolgus* monkey have suggested this activity to be more similar to that found in the human tissue than is enzymatic activity from the same organ in the rat [8]. The cloning and expression of two proteins from the monkey, based on the prior work with the human and rat isoenzymes [8, 29], has enabled a more thorough set of comparative studies between the three species' SRs. These studies show that the type 1 and type 2 enzymes from monkey and human have primary sequence homologies of greater than 90% each. Yet with this high degree of sequence similarity, differences are observed within a tetrapeptide sequence that has previously been localized as a key structural determinant responsible for differential inhibitor response of rat and human type 1 SRs [28].

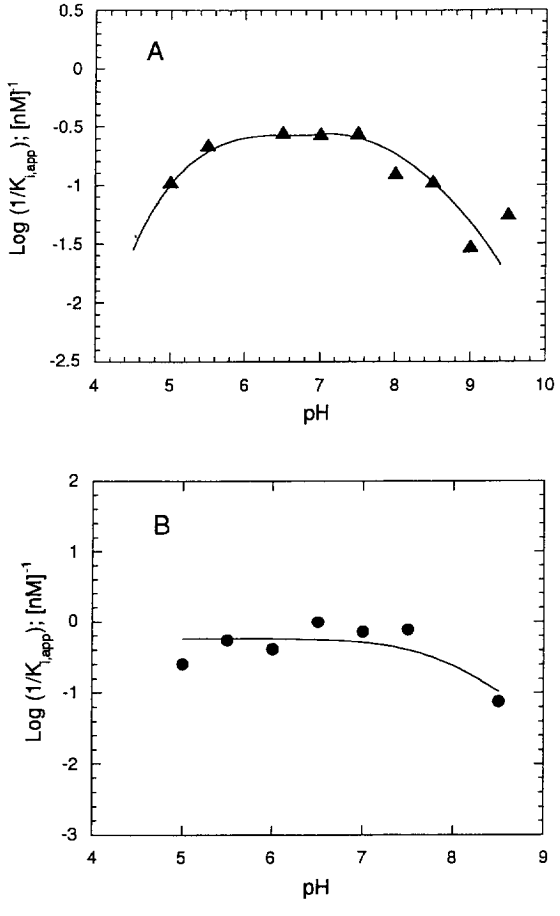


**Fig. 8. Inhibition of human type 2 SR as function of pH. Apparent inhibition constants ($K_{i,app}$) were determined over the indicated pH range as outlined under Methods using recombinant human type 2 SR with compounds 4 (panel A) and 2 (panel B). The data for compound 4 ($pK_a = 5.0 \pm 0.3$; $pK_b = 8.6 \pm 0.3$) was fitted to equation 3 (BELL); that for compound 2 ($pK_b = 7.9 \pm 0.4$) was fitted using equation 2 (HBBELL).**

Despite these sequence differences, however, the *in vitro* kinetic characteristics of the two primate's type 1 and type 2 SRs with a selection of steroid substrates and a structurally diverse set of recognized inhibitors appear functionally equivalent. By comparison, the poor correlations of inhibitor potencies on the rat and human type 1 and 2 SRs, along with these species differences in isoenzyme tissue expression [7, 36], introduce concern about the rodent's suitability as a model for comparative evaluation of new agents with potentially different profiles (e.g. compounds that are human isotype selective or dual isoenzyme inhibitors). Similarly, the mRNAs for types 1 and 2 SR are present in both human [10] and monkey prostate. However, like the enzymatic activity localized to the mature human prostate, the SR activity isolated from prostate of the monkey [11, Fig. 5(B)] is characteristic of type 2 isoenzyme.

Epristeride (3) and finasteride (2) represent two SR inhibitors that have been evaluated in man. With these compounds, suppression of plasma DHT concentrations in man has been used as a primary indicator of their effective biochemical pharmacology [37, 38]. Our experiments have shown that effects on plasma concentrations of DHT in the *Cynomolgus* monkey are comparable to those reported for man upon administration of compounds 2 or 3. These results suggest that the SR enzyme activities and response to type 2 selective inhibitors from both human and monkey are comparable. The monkey consequently would appear to represent an appropriate species for pharmacological assessment of novel inhibitors of the human type 1 and type 2 SRs.

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