THE IDENTIFICATION AND CHARACTERIZATION OF THE $C_{19}O_3$ STEROID METABOLITES OF 5α-ANDROSTANE-3β,17β-DIOL PRODUCED BY THE CANINE PROSTATE: 5α-ANDROSTANE-3β,6α,17β-TRIOL AND 5α-ANDROSTANE-3β,7α,17β-TRIOL

John T. Isaacs, Ian R. McDermott, and Donald S. Coffey

Department of Pharmacology and Experimental Therapeutics,
The James Buchanan Brady Urological Institute, Department of Urology
and The Oncology Center
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

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ABSTRACT

This study has identified the polar metabolites of 5α-androstane-3β,17β-diol(3β-diol) produced by the canine prostate. The major metabolite is 5α-androstane-3β,7α,17β-triol(7α-triol) accounting for approximately 80% of the total polar metabolites of 3β-diol. The remaining 20% is accounted for exclusively by another triol, 5α-androstane-3β,6α,17β-triol(6α-triol). This study has also characterized two enzymatic hydroxylases responsible for respective triol formation: 5α-androstane-3β,17β-diol 6α-hydroxylase(6α-hydroxylase) and 5α-androstane-3β,17β-diol 7α-hydroxylase(7α-hydroxylase). Both of these irreversible hydroxylases are located in the particulate fraction of the prostate and can utilize either NADH or NADPH as cofactor. Several in vitro steroid inhibitors of these hydroxylases were identified including cholesterol, estradiol and diethylstilbestrol. Neither of the hydroxylases were found to be decreased by castration (3 months) when expressed as activity/DNA. Using a variety of C_{19} androstane substrates, 6α- and 7α-triol were found to be major components of the total 3β-hydroxy-5α-androstane metabolites produced by the canine prostate.

INTRODUCTION

One of the major unanswered questions concerning the androgen dependent control of normal and abnormal growth of the prostate is the functional significance of the extensive metabolism of testosterone by this gland. Unlike estrogens which apparently undergo limited, if any, metabolism in target tissues, testosterone is extensively metabolized to a complex mixture of oxidized and reduced biotransformation products by androgen dependent tissues. The major reductive metabolites include 5α-dihydrotestosterone (DHT), 5α-androstane-3α,17β-diol(3α-diol), and

5α-androstane-3β,17β-diol(3β-diol). The importance of DHT as the major androgenic growth factor in the prostate has been established in several classical studies (1, 2). In contrast, the biological functions of the other metabolites have not been assigned.

In regard to the possible function of 3β-diol, Ofner et al. reported that this steroid can be hydroxylated by the canine and human hyperplastic prostate at position 6 or 7 to form $C_{19}O_3$ steroid triols (3). The predominant triol formed by these tissues was reported to be 5α-androstane-3β,7ξ,17β-triol. Morfin et al. confirmed and extended these observations to the normal as well as hyperplastic human prostate (4). These authors determined that the predominant triol formed by the human prostate is 7α-triol with small amounts of supposed 6β-triol also formed. Recent work from our laboratory has demonstrated that the rat prostate also actively hydroxylates 3β-diol to form triols. In contrast to the canine and human prostate, the rat prostate forms predominantly 5α-androstane-3β,6α,17β-triol(6α-triol) and small amounts of 5α-androstane-3β,7α,17β-triol(7α-triol) from 3β-diol (5). It was therefore of interest to rigorously assign the stereochemistry of the 3β-triols observed by Ofner et al. in the canine prostate and to compare the enzymatic characteristics of these canine hydroxylases with those of the rat prostate previously studied and reported (6).

MATERIALS AND METHODS

Steroids: All nonradioactive steroids were obtained as described previously (5). All 1,2[^{3}H]-labeled steroids were purchased from Amersham and had a specific activity of 40-60 Ci/mmole.

Animals: For the majority of work young mature male beagle dogs (mean age ± S.D., 2.3 ± 0.3 years; range, 1.6-3 years) were used. These were obtained from Laboratory Research Enterprises Ltd., Kalamazoo, Mi. and Buckshire Farms, Pa. The birthdate and pedigree of

each animal was available. Body weights ranged from 7.0 to 18.6 kg (11.3 ± 2.2 kg). In certain experiments (which are denoted in the appropriate cases), mongrel dogs obtained from the Department of Animal Services, Johns Hopkins Hospital were also used. Animals were anesthetized by intravenous pentabarbital and prostates quickly removed. The prostates were immediately cleaned of excess fat and adhesions and placed in ice-cold Medium 199. They were then sliced with a Stadie-Riggs tissue slicer to give relatively uniform prostatic slices (approx. 0.5 mm). In one experiment, beagle dogs were castrated via an abdominal incision and both the epididymides and testes were removed.

Thin-Layer Chromatography: TLC systems used were: 1) Chloroform: methanol (98:2.5) - 2 ascents on silica gel plates, 2) ethylacetate - 1 ascent on silica gel plates, 3) benzene:ethanol (9:1) - 2 ascents on silica gel plates, 4) chloroform:methanol (9:1) - 1 ascent on silica gel plates, and 5) benzene:ethanol (96:4) - 2 ascents on aluminum oxide plates. The dessicated silica gel plates used were Quantum Plates, type LK6D (Whatman Ltd.). The aluminum oxide plates used were always freshly heat-activated (140°C for 60 min) and were obtained from E. Merck (F_{254} neutral type E).

High Pressure Liquid Chromatography (HPLC): A Waters System 6000 high pressure liquid chromatograph was utilized to separate the polar metabolites of 3β-diol. The column utilized was a reversed-phase Waters C-18μ-Bondapak using the solvent system of methanol:H_2O (45:55) and a flow rate of 1 ml/min. Using this system 7β-triol (k' = 5.9), 6α-triol (k' = 7.2), 7α-triol (k' = 9.0), 6β-triol (k' = 12.2), 11β-triol (k ' = 23), 16α-triol (k ' = 17.7), and 3β-diol (k' = 62) were all easily separated from each other.

Slice Assay: Slice assays were performed as described unless otherwise stated. One hundred mg aliquots of canine prostatic slices were incubated in 2.0 ml of Medium 199 (Grand Island Biological Co.) containing either 1,2[^{3}H]-labeled testosterone, DHT, or 3β-diol at a final concentration of 50 nM. All the radioactive steroids were at a final specific activity of 0.5μCi/nmole). The incubations were at 37°C in a 95% O_2:5% CO_2 environment in a shaking water bath. At timed intervals, the incubations were terminated with 10 ml of chloroform: methanol (2:1). The chloroform:methanol layer was taken to dryness and processed as described previously (5). This entails separation and quantification of the steroid metabolites by TLC using several solvent systems. The results are expressed as pmoles of steroid/ 100 mg tissue.

Homogenate Assay for the 3β-Diol Hydroxylase Activities: Prostates were initially sliced with a Stadie-Riggs slicer and these slices were then minced with fine scissors. The minces were then homogenized in 1 mM dithiothreitol (DTT), 50 mM Tris, pH 7.4 buffer with an all glass conical homogenizer. Homogenates (corresponding to 40 mg of tissue) were incubated in 2.5 ml of 2 mM DTT, 50 mM Tris, pH 7.4 containing 5 x 10^{-4}M NADPH and 5μM ^{3}H-3β-diol (0.5μCi/nmole). Incubations were at 37°C in a shaking water bath for 15 minutes. The assay was terminated by the addition of 10 ml of chloroform:methanol (2:1). The chloroform:methanol layer was taken to dryness and processed as described previously (6). This involved routine separation and quantification of the triols as a group by silica gel TLC using

chloroform:methanol (9:1) as the solvent system followed by HPLC separation of the individual 6α- and 7α-triols.

β-Glucuronidase and Arylsulfatase Treatment of Polar Metabolites: One hundred mg of prostatic slices were incubated with 2 ml of Medium 199 containing 100 pmole of ^{3}H-3β-diol for 30 min at 37°C. Termination and extraction of the mixture was according to slice assay procedure. The polar metabolites of ^{3}H-3β-diol were isolated by means of silica gel TLC (chloroform:methanol (9:1)). An aliquot of polar metabolite was redissolved in 2 ml of 0.1 M acetate buffer, pH 5.5 containing 25μl of a 5U/ml solution of β-glucuronidase-arylsulfatase isolated from Helix pomatia (E.C.3.2.1.31-E.C.3.1.6.2) (obtained from Boehringer and Soehne, Germany). Incubation was at 37°C for 60 min followed by extraction in chloroform:methanol (2:1). The resulting extract was taken to dryness, redissolved and re-run on a silica gel plate with the chloroform:methanol (9:1) solvent system. An aliquot of the original polar metabolites (non-enzymatically treated) was also simultaneously run on this TLC plate. No difference in the distribution of radioactivity could be found between the enzymatic treated and non-treated polar metabolites.

Reversibility of the 3β-Diol Hydroxylase Activities: 1,2[^{3}H]-6α-triol and 1,2[^{3}H]-7α-triol were enzymatically produced from 1,2[^{3}H]-3β-diol as described previously (6). To individual 136 pmole aliquots of either ^{3}H-6α-triol or ^{3}H-7α-triol were added 12364 pmoles of unlabeled authentic 6α-triol or 7α-triol respectively. These mixtures (12,500 pmoles of 6α-triol or 7α-triol both having a specific activity of 0.5 μCi/nmole) were taken to dryness separately and each resuspended in 2.5 ml of 2 mM DTT - 50 mM Tris, pH 7.4 buffer containing either prostatic homogenates corresponding to 40 mg of tissue supplemented with 0.5 mM $NADP^{+}$ or 0.5 mM NAD^{+} or 100 mg of prostatic slices without added cofactor. Incubation was at 37°C for three hours. Termination, extraction, and separation of the steroids was according to methods described previously (6).

Subcellular Distribution of the 3β-Diol Hydroxylase Activities: An extremely simple differential centrifugation method was used (for reasons discussed in Results section) to obtain a particulate and cytosolic fraction of the canine prostate. Prostates were initially sliced with a Stadie-Riggs slicer and these slices were then minced with fine scissors. The resulting mince was then homogenized with an all glass conical homogenizer using as buffer 0.25 M sucrose, 1 mM $MgCl_2$, 2 mM DTT, 50 mM Tris, pH 7.4 (H-buffer). The homogenate was then filtered through bolting cloth and the filtrate (whole homogenate) was centrifuged at 100,000 G x 1 hr. The resulting pellet was resuspended with H-buffer to its original volume and represents the entire particulate fraction of the prostate. Aliquots of whole homogenate, particulate fraction, and cytosol (100,000 G supernatant) were assayed for the 3β-diol hydroxylase activities as described above under homogenate assay.

Nucleic Acid Determination: Tissue DNA was extracted and determined by the method of Coffey et al. (7).

RESULTS

5α-Androstane-3β,17β-Diol Metabolites: When 1,2[^{3}H]-5α-androstane-3β,17β-diol (1000 pmoles/20 ml of medium) was incubated with tissue slices from canine prostate (1 gram) for 30 minutes, only small amounts of DHT, epiandrosterone, 5α-androstane-3α,17β-diol and androstanedione are formed; the major transformation products are unidentified polar metabolites (Table 1).

Table 1

Metabolism of 5α-Androstane-3β,17β-Diol by Canine Prostatic Slices

Steroid Metabolites	Amount Formed (pmoles)	Relative %
Substrate Utilized		
5α-Androstane-3β,17β-diol	350 ± 31	
Metabolites		
Polar Metabolites		
Unconjugated	280 ± 23	80.0
Conjugated		
Glucuronide	<1	<.3
Sulfate	<1	<.3
Dihydrotestosterone	20 ± 5	5.7
Epiandrosterone	40 ± 6	11.4
Androstanedione	4 ± 2	1.1
Androstenedione	<1	<.3
Testosterone	<1	<.3
5α-androstane-3α,17β-diol	<1	<.3
Total Metabolites	344	98.2

Initial conditions: Substrate = 1000 pmoles of ^{3}H-3β-diol plus 1 gram of canine (beagle) prostatic slices in 20 ml of Medium 199 incubated at 37°C for 30 minutes. Separation of steroid metabolites was by TLC using the solvent system chloroform:methanol (98:2.5) with two ascents. This system does not resolve the 3α and 3β-diols from each other. This was achieved by aluminum oxide TLC with the benzene: ethanol (96:4) solvent system.

The polar metabolites of 5α-androstane-3β,17β-diol were determined not to be a conjugated form of the steroid (i.e., glucuronide or sulfate). This conclusion is based on the fact that incubation of the isolated polar metabolites with β-glucuronidase and arylsulfatase did not result in any change in the distribution of radioactivity as

determined by TLC between treated (β-glucuronidase-arylsulfatase) and non-treated polar metabolites. Ofner et al. had reported that the canine prostate hydroxylates 5α-androstane-3β,17β-diol at position 6 or 7 to form $C_{19}O_3$ steroid triols (3). 5α-Androstane-3β,6α,17β-triol (6α-triol) and 5α-androstane-3β,7α,17β-triol (7α-triol) were therefore tested for their abilities to co-migrate with the unidentified polar metabolite(s) of 5α-androstane-3β,17β-diol in several TLC systems. Four different silica gel TLC systems (systems 1-4, Methods section), were used to separate the [^{3}H]-3β-diol substrate from its polar metabolites produced by canine prostatic slices. (For the respective migrations obtained in each system, see (5)). In each system the major amount (approx. 95%) of the radioactivity corresponding to the polar metabolites was isopolar with both 6α- and 7α-triol. None of the systems used, however, were able to clearly resolve 6α- from 7α-triol. Actually other triols (i.e., 6β-, 7β-, 11β-, and 16β-triol) also migrated to this area. It was therefore necessary to further resolve the triols using the HPLC. Using HPLC, the polar metabolites of 5α-androstane-3β,17β-diol were resolved into two peaks of radioactivity corresponding to 6α- and 7α-triol (Figure 1). There was no radioactivity associated with either 5α-androstane-3β,6β,17β-triol (6β-triol) or 5α-androstane-3β,7β,17β-triol (7β-triol). Likewise, no radioactivity was associated with other $C_{19}O_3$ triols such as 5α-androstane-3β,11β,17β-triol (11β-triol) or 5α-androstane-3β,16α,17β-triol (16α-triol). Crystallization to constant specific activity was performed to definitively establish that the polar metabolites were indeed 6α- and 7α-triol, Table 2.

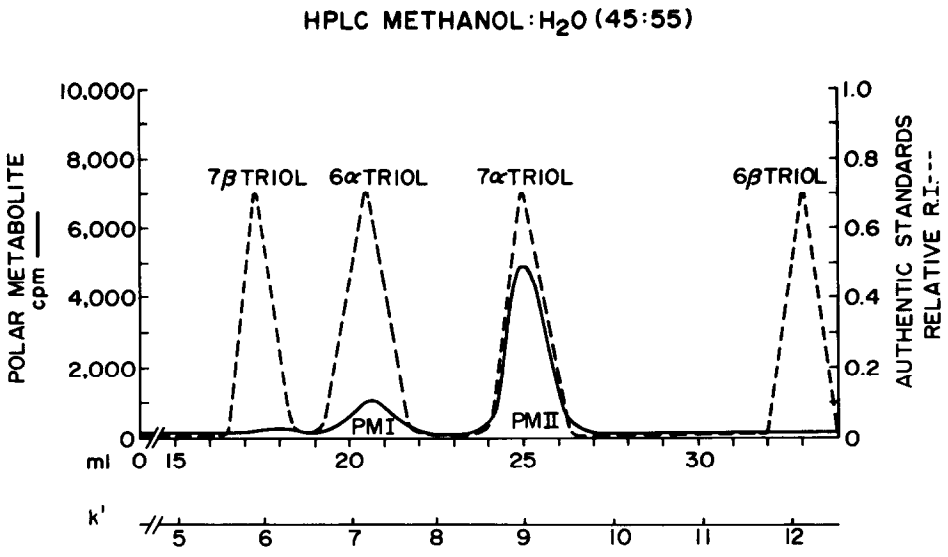

Figure 1.

High Pressure Liquid Chromatographic (HPLC) Resolution of the Polar Metabolites of ^{3}H-3β-Diol Produced by Canine Prostatic Slices

Canine (beagle) prostatic slices (100 mg) were incubated in 2.0 ml of Medium 199 containing 100 pmoles of ^{3}H-3β-diol (final [3β-diol] = 50 nM) for 1 hr at 37°C. After termination and extraction of the incubation mixture (as described in Methods section), the resulting residue was dissolved with 200 µl of chloroform:methanol (2:1) of which 25 µl was applied to a lane of a silica gel plate which was then developed in chloroform:methanol (9:1) (one ascent). The area corresponding to the polar metabolites was then removed and extracted with chloroform: methanol (2:1). This extract was taken to dryness and redissolved with 150 µl of carrier reference solution. This solution contained 7β-triol, 6α-triol, 7α-triol, 6β-triol, 16α-triol, and 11β-triol each at a concentration of 5 mg/ml of methanol. Twenty-five µl of this redissolved extract was then applied and run on HPLC which was equipped with a continuous recording differential refractometer. In this way, the elution profiles of the known reference triols could be followed by their relative R.I. (---). The solvent utilized was methanol:H_2O (45:55). Fractions from the HPLC were collected in 1 ml aliquots between 0-15 ml and 40-100 ml. Between these volumes 0.25 ml fractions were collected. All fractions were taken to dryness and redissolved with scintillation fluid and the radioactivity counted (——). The total radioactivity applied to the HPLC was separately determined on another 25 µl aliquot of the original material applied to the HPLC. The recovery of radioactivity in the fraction between 15-35 ml was 95% of the total cpm applied to the HPLC. PMI = polar metabolite I, PMII = polar metabolite II.

Table 2.

Crystallization to Constant Specific Activity of the Polar Metabolites of ^{3}H-5α-Androstane-3β,17β-Diol Produced by the Canine Prostate

#Consecutive Crystallization	Solvent	Specific Activity (cpm/mg) Crystals	Mother Liquor
A) Polar Metabolite I (PMI) + 6α-Triol (60 mg)			
Starting Specific Activity		864 ± 23	
1st	Acetone	849 ± 8	893 ± 7
2nd	Methanol/H_2O	847 ± 44	867 ± 11
3rd	Acetone/Hexane	838 ± 9	833 ± 14
B) Polar Metabolite II (PMII) + 7α-Triol (30 mg)			
Starting Specific Activity		8230 ± 13	
1st	Ethylacetate	8130 ± 74	8280 ± 45
2nd	Acetone/Hexane	8130 ± 88	8200 ± 89
3rd	Methanol/H_2O	8130 ± 93	8130 ±170

^{3}H-3β-diol was incubated with prostatic slices (beagle) and processed as described in Figure 1 except that 50 µl of the redissolved original extract was applied to the TLC plate and the resulting polar area was extracted, resuspended, and the entire volume was applied to the HPLC. Polar metabolite I (PMI) was collected (ml fraction 19-22) and taken to dryness. Authentic 6α-triol (60 mg) was added to the residue and the mixture was redissolved in methanol. This mixture was taken to dryness and an aliquot removed, weighed, and the radioactivity counted to give the starting specific activity (cpm/mg steroid). Consecutive crystallization was then performed from the indicated solvents. The second polar metabolite (PMII) was separately collected (ml fraction 24-27) and taken to dryness. Authentic 7α-triol (30 mg) was then added to the residue and processed as described above for 6α-triol. The mean of triplicate determination from a single crystallization is presented ± S.E.M.

The formation of the 6α- and 7α-triol was totally prevented if prostatic slices were heated in Medium 199 for 5 minutes at 90°C before the addition of ^{3}H-3β-diol. Also less than 5% of the normal rate of triol production was obtained if the incubation was performed at 4°C instead of 37°C. It was therefore concluded that hydroxylase enzymes metabolizing 5α-androstane-3β,17β-diol are present in the canine prostate.

In order to characterize the enzymes responsible for the hydroxylations of 3 β-diol, standard conditions for homogenate assays were defined. In preliminary work, it was found that when canine prostates were homogenized in 50 mM Tris pH 7.4 buffer immediately (less than 15 minutes) after removal from the animals and assayed immediately, reproduceable assays were obtained. However, if the time between removal and homogenization of the prostate exceeded 30 minutes, little homogenate hydroxylase activity could be found. Also if homogenates made rapidly (less than 15 minutes) after removal from the animal were allowed to stand at 0-4°C for any length of time, hydroxylase activity was lost. This loss of activity could be prevented by including 2 mM DTT in the original homogenization buffer. Therefore all reported homogenate data were determined on homogenates made within 15 minutes of removal of prostate with buffer containing 2 mM DTT, 50 mM Tris pH 7.4 and assayed immediately.

When 1,2[^{3}H]-3 β-diol at a concentration of 5 μM is incubated with canine prostatic homogenates corresponding to 10 mg of tissue, a linear rate of 6α- and 7α-triol formation occurs between 0-60 min, Figure 2A. Likewise, when ^{3}H-3β-diol is incubated with homogenates for 15 minutes, there is a linear relationship between the rate of triol formation and the amount of tissue (between 2.5-40 mg), Figure 2B. In both Figure 2A and B the rate of formation of 7α-triol is approximately four times the rate of formation of 6α-triol.

Both the homogenate 3β-diol 6α- and 7α-hydroxylase activities require cofactors, Table 3. The cofactor requirements are identical for both activities; both activities can use either NADH or NADPH.

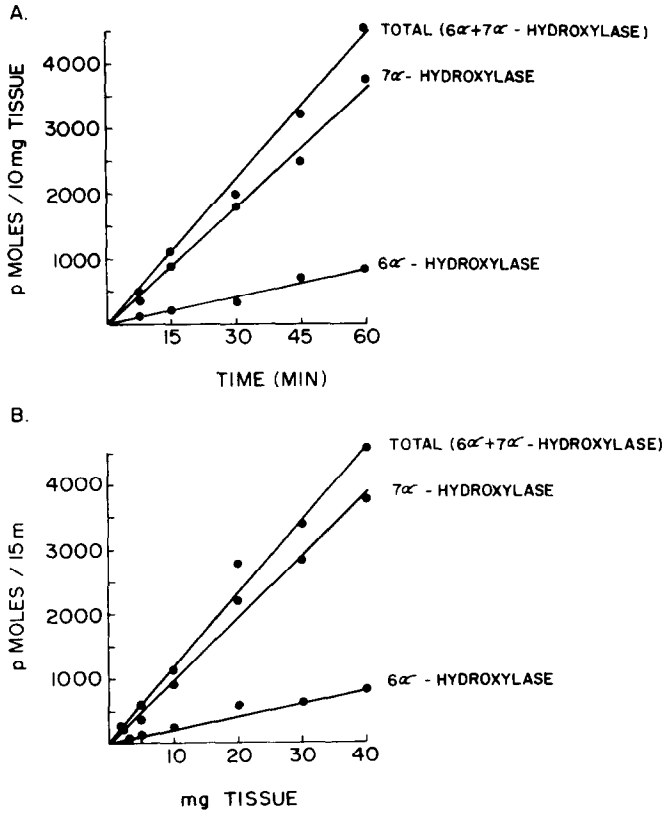

Figure 2.

Rate of 6α- and 7α-Triol Formation by NADPH Supplemented Canine Prostatic Homogenates versus time (A) and Tissue Amount (B).

A. Homogenates corresponding to 10 mg of tissue were incubated with 5 μM ^{3}H-3β-diol and 0.5 mM NADPH. At various times, aliquots were removed and metabolites quantified by TLC and HPLC as described in Methods section.

B. Homogenates corresponding to 2.5 - 40 mg of tissue were incubated for 15 minutes and metabolites quantified as in A.
Dog: Mongrels

Again the ratio of 7α/6α-triol production is approximately 4 regardless of which cofactor is added. The subcellular distribution of the 3β-diol hydroxylase activities was next examined, Table 4. When standard differential centrifugation techniques (5) were used to

Table 3

Cofactor Requirements for 3β-Diol Hydroxylase Activity of Canine* Prostatic Homogenates

Cofactors Added	3β-Diol Hydroxylase Activity (nmoles/hr/gram tissue)		
	Total (7α+ 6α) Hydroxylase	7α- Hydroxylase	6α- Hydroxylase
None	15 ± 2	12 ± 2	6 ± 1
NADPH	500 ± 100	402 ± 75	98 ± 20
NADH	495 ± 58	392 ± 51	103 ± 15
$NADP^+$	150 ± 20	120 ± 18	30 ± 9
NAD^+	100 ± 15	79 ± 12	21 ± 5

*Mongrels

Table 4

Subcellular Distribution of the 6α- and 7α-Hydroxylase Activity for 3β-Diol in Canine Prostate

Cellular Fraction	3β-Diol Hydroxylase Activity (nmoles of 3β-Diol Hydroxylated/ hr/gram tissue)		
	Total(6α+ 7α) Hydroxylase	6α- Hydroxylase	7α- Hydroxylase
Whole homogenate	495 ± 40	100 ± 10	396 ± 35
Particulate	395 ± 45	87 ± 15	308 ± 29
Cytosol	<1.0	<1.0	<1.0
Average Recovery	80 ± 15	87 ± 20	78 ± 12

separate nuclear, mitochondrial and microsomal fractions from the cytosol, the recovery of total hydroxylase activity was low (<30% of whole homogenate activity). Therefore the simple fractionation of prostatic homogenates into a total particulate (100,000 G pellet of the whole homogenate) and cytosolic fraction (100,000 G supernatant) was used. This simple fractionation scheme gave good reproduceable recoveries for both the 6α- and 7α-hydroxylases. Both activities were exclusively localized in the particulate fraction of prostate. Attempts to solubilize the respective enzymes from particulate fractions with 0.5% Triton X-100 were unsuccessful. No activity

could be recovered in either the Triton pellet or supernatant.

The direct effects of various steroids as potential inhibitors of the 3β-diol hydroxylase activities of the canine prostate were examined. Unlabeled test steroids at 5 μM concentration were separately coincubated with ^{3}H-3β-diol under standard homogenate assay conditions, Figure 3. Steroids possessing a 3β-hydroxyl group (cholesterol and epiandrosterone) were found to inhibit the formation of the triols. In addition, estradiol and diethylstilbestrol inhibited the total 3β-diol hydroxylase activities (6α- plus 7α-hydroxylase activity). Neither testosterone nor any of its metabolites, except epiandrosterone, were able to inhibit these activities.

Both the 3β-diol 6α- and 7α-hydroxylase activities were found to be irreversible. This was documented by separately incubating either ^{3}H-6α-triol or ^{3}H-7α-triol at a concentration of 5 μM with prostatic homogenates and slices. The homogenate assays were supplemented with either 0.5 mM $NADP^{+}$ or NAD^{+} while the slice assays contained no added cofactors. None of the incubations, whether homogenate or slice, produced any ^{3}H-3β-diol from either of the separate triols even if the incubations were continued for three hours at 37°C.

The androgen sensitivity of the 3β-diol 6α- and 7α-hydroxylase activities was also studied. These enzymes were assayed using the slice method with a ^{3}H-3β-diol concentration of 50 nM. Castration of the mature male dog results in a large decrease in prostatic wet weight, DNA, and the total glandular activities of the 6α- and 7α-hydroxylases. However, when these activities are expressed per unit

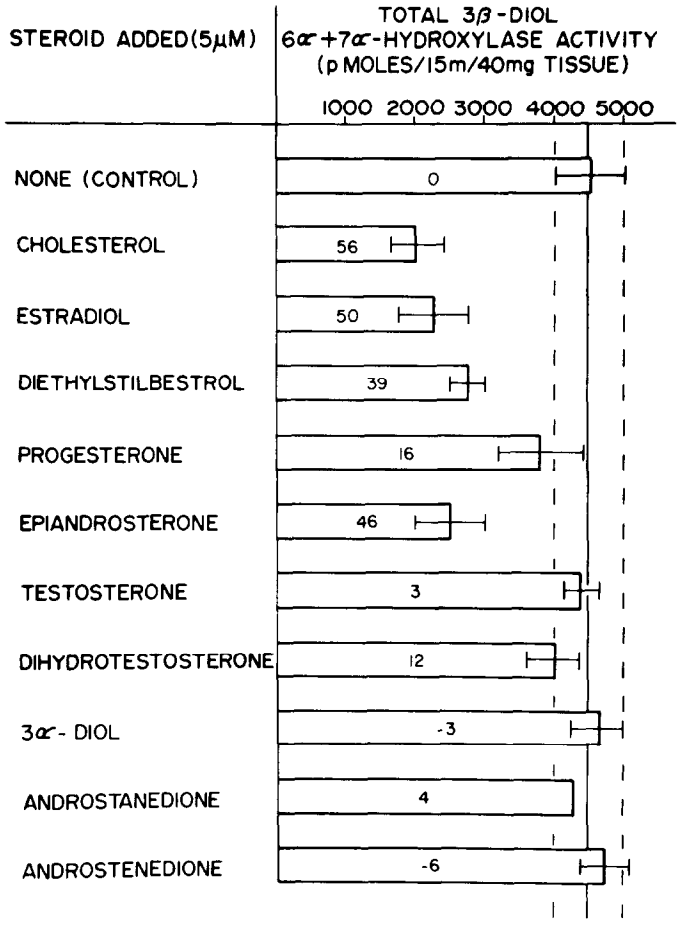

Figure 3.

The In Vitro Inhibitory Effects of Various Steroids on the Prostatic Total 3β-Diol Hydroxylase Activity

Assay contained 5 μM of ^{3}H-3β-diol combined with 5 μM of the indicated unlabeled steroid. The rate of formation of ^{3}H-6α-triol plus 7α-triol was determined as described in the Methods section. Number in column is % inhibition of the hydroxylase activity caused by test steroid and is based on the formula:

$$\% \text{ inhibition} = 100 - \frac{\text{activity is presence of test steroid}}{\text{activity of control}} \times 100$$

amount of DNA (per cell) there is no change in the specific activity of either enzyme following castration, Table 5.

Table 5.

Effects of Castration on the 3β-Diol 6α- and 7α-Hydroxylase Activities of the Mature Canine* Prostate

Treatment Group	Canine Prostatic 3β-Diol Hydroxylase Activity (pmoles/hr/mg DNA)		
	Total 6α + 7α-Hydroxylase	6α-Hydroxylase	7α-Hydroxylase
Mature Intact Control	144 ± 20	31 ± 8	112 ± 25
Three Month Castrate	169 ± 28	42 ± 11	126 ± 23

*Beagles. Assay: 100 mg of prostatic slices incubated with ^{3}H-3β-diol (50 nM) for 30 min at 37°C.

Testosterone and DHT were examined to see if these androgenic steroids could also be precursors for 6α- and 7α-triol formation, Figure 4. Both the triols are formed by prostatic slices (100 mg) starting with either 1,2[^{3}H]-labeled testosterone (Figure 4A) or DHT (Figure 4B); both steroids were at a concentration of 50 nM. The 3β-diol incubation (Figure 4C) was performed as a control and to show the linear rate of both triol formation and DHT production over one hour of incubation. The total triols formed (6α- plus 7α-triol), regardless of the starting steroid, account for the major proportion of the total 3β-hydroxy-5α-androstane metabolites in each case.

DISCUSSION

This study has identified the polar metabolites of 3β-diol and preliminarily characterized the enzyme responsible for their formation in the canine prostate. The predominant metabolite is 5α-androstane-3β,7α,17β-triol accounting for 75-80% of the total polar metabolites. The remaining 20-25% is exclusively accounted for by 5α-androstane-3β,

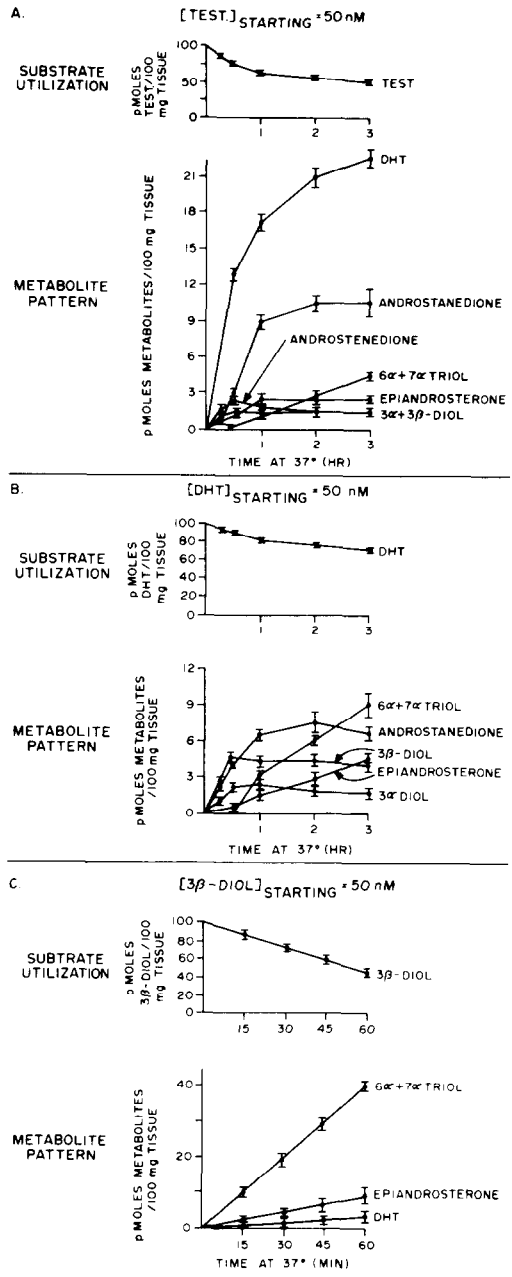
A.
[TEST.]STARTING = 50 nM
SUBSTRATE UTILIZATION
pMOLES TEST/100 mg TISSUE
TEST
METABOLITE PATTERN
pMOLES METABOLITES/100 mg TISSUE
DHT
ANDROSTANEDIONE
ANDROSTENEDIONE
6α+7α TRIOL
EPIANDROSTERONE
3α+3β-DIOL
TIME AT 37° (HR)
B.
[DHT]STARTING = 50 nM
SUBSTRATE UTILIZATION
p MOLES DHT/100 mg TISSUE
DHT
METABOLITE PATTERN
pMOLES METABOLITES /100 mg TISSUE
6α+7α TRIOL
ANDROSTANEDIONE
3β-DIOL
EPIANDROSTERONE
3α DIOL
TIME AT 37° (HR)
C
[3β-DIOL]STARTING = 50 nM
SUBTRATE UTILIZATION
p MOLES 3β-DIOL/100 mg TISSUE
3β-DIOL
METABOLITE PATTERN
pMOLES METABOLITES /100 mg TISSUE
6α+7α TRIOL
EPIANDROSTERONE
DHT
TIME AT 37° (MIN)

Figure 4.

6α,17β-triol. The formation of these triols has been shown to be irreversibly catalyzed by particulate enzymes which require NADPH or NADH as cofactors. Several in vitro steroid inhibitors of these 3β-diol 6α- and 7α-hydroxylases have been identified. These include cholesterol, estradiol, diethylstilbestrol and epiandrosterone. Neither of the hydroxylases were androgen-sensitive. Three months after castration, both the 6α- and 7α-hydroxylase activities were the same as in an intact control prostate when expressed as pmoles of triol formed/hr/mg DNA. These characteristics of canine prostatic 3β-diol metabolism are qualitatively different from those observed in the rat ventral prostate. The rat ventral prostate also actively metabolizes 3β-diol to polar metabolites (5, 6). The predominant metabolite of this tissue, however, is 6α-triol (75%) with the remaining metabolite exclusively being 7α-triol (25%). The enzymes responsible for these irreversible hydroxylations are also localized in the particulate fraction of the gland but specifically require NADPH. In addition, both activities are androgen-sensitive. The species differences between the rat and canine prostate in regard to triol formation is unclear.

In regard to the possible function of triol formation in the prostate, it is relevant that the canine prostate can form 6α- and 7α-triol not only starting from 3β-diol but also testosterone and DHT. The use of these latter two steroids, however, does not allow the unequivocal assignment of 3β-diol as the immediate precursor for the formation of the triols. While it is clear that the major pathway for triol formation is via 3β-diol directly, the possibility of other minor pathways for triol formation can not be excluded using these steroids.

Regardless of the starting steroid, however, 6α- and 7α-triol always accounted for the majority of the total 3β-hydroxy-5α-androstane metabolites formed by the canine prostate. This same observation was found for the rat ventral prostate (5). This might indicate that once 3β-diol is formed by the prostate it is rapidly metabolized irreversibly to the triols. While it is difficult to extrapolate the physiological significance of triol formation from the in vitro work performed in this study, this work may indicate that the contribution of the 3β-diol pathway (going ultimately to the triols) may account for significant amounts of DHT removal. The potential importance of this 3β-diol pathway can be appreciated when it is realized that only 3β-diol out of all the mixtures of oxidized and reduced metabolites of DHT can be further metabolized to products, 6α- and 7α-triol, which can not be converted back to DHT (due to irreversibility of the 6α- and 7α-hydroxylases). Furthermore, neither 6α- nor 7α-triol have any androgenic abilities when tested in a series of bioassays in the rat (5). It is therefore possible that the 3β-triols function as a common final pathway for the termination of androgenic abilities of the C_{19}-androstane steroids in the canine prostate. This possibility is strengthened by the observation of Ofner and Vena that when canine prostatic explants were cultured for 21 hr in the presence of ^{3}H-testosterone, 5α-reduced 7-hydroxylated products were found to be the major radiometabolite (8).

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Trivial names not identified elsewhere in the text: 5α-dihydrotestosterone (DHT): 17β-hydroxy-5α-androstan-3-one; Epiandrosterone: 3β-hydroxy-5α-androstan-17-one; Androstanedione: 5α-androstane-3,17-dione; Androstenedione: 4-androstene-3,17-dione; Dehydroepiandrosterone (DHEA): 3β-hydroxy-5-androsten-17-one; estradiol: estradiol-17β.