STUDIES ON THE EFFECT OF 5α-ANDROSTANE-3α,17α-DIOL IN THE CANINE PROSTATE IN VIVO

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ABSTRACT

Adult beagle dogs, castrated one month previously, were treated with 5α-androstane-3α,17α-diol (total dose 300 mg) over a period of one month. Examination of the prostate after treatment showed no significant change in size, weight or histological appearance from the castrate dog prostate. Subsequent administration of 5α-androstane-3α,17β-diol (300 mg) over the same period of time resulted in restoration of the prostate size, weight and histological appearance to that of the normal intact dog prostate. It is concluded that exogenously administered 5α-androstane-3α,17α-diol does not promote prostatic growth in the castrate beagle dog.

INTRODUCTION

There is considerable evidence that androgenic effects in sex accessory organs are mediated by testosterone or its intracellular metabolites (1). 17β-Hydroxy-5α-androstan-3-one(5α-dihydrotestosterone; DHT) was implicated in androgen action when it was found to be localized in the nuclei of rat ventral prostate (2,3,4). Studies on testosterone metabolism in prostatic tissue, both in vivo (2,5) and in vitro (6,7,8) have shown that, in addition to the formation of DHT, various 5α-androstanediols are produced.

In the canine prostate, 5α-androstane-3α,17α-diol has been implicated as the active intracellular androgen metabolite because of its ability in vitro to stimulate DNA (9) and RNA (10) polymerase activity and maintain the canine prostatic epithelium (11). There are conflicting reports concerning the binding of 5α-androstane-3α,17α-diol to androgen receptors in the canine prostate (12,13,14).

TABLE I

DOG PROSTATE : RESPONSE TO 5α-ANDROSTANE-3α,17α-DIOL VERSUS 5α-ANDROSTANE-3α,17β-DIOL

DOG	CASTRATE UNTREATED		CASTRATE 5α-ANDROSTANE-3α,17α-DIOL		CASTRATE 5α-ANDROSTANE-3α,17β-DIOL	
	ABSOLUTE (g)	RELATIVE (%)	ABSOLUTE (g)	RELATIVE*(%)	ABSOLUTE (g)	RELATIVE*(%)
A	2.7	100	1.2	43	10.0	371
B	6.6	100	6.1	91	13.3	201
C	5.5	100	4.0	72	8.2	149

MEAN PROSTATIC WEIGHT OF THE 3-NON-CASTRATE CONTROL DOGS = 14.2g

*RELATIVE TO UNTREATED CASTRATE VALUES

The initial identification of 5α-androstane-3α,17α-diol as a metabolite of testosterone in the canine prostate was also carried out in vitro (15). The present work was undertaken to evaluate the effect of 5α-androstane-3α,17α-diol in vivo on the dog prostate.

METHODS AND MATERIALS

MATERIALS (16)

5α-Androstane-3α,17α-Diol: 5α-Androstane-3α,17α-diol was synthesized in this laboratory (17) and had m.p. 225-226°C (from acetone/ water), undepressed on admixture with authentic material with the same m.p., (Lit (18) 228°C). The compound was homogeneous and was indistinguishable from authentic material by thin-layer chromatography (R_f 0.13, Silica gel GF_{254}, 9:1 chloroform: acetone) and by high pressure liquid chromatography (HPLC) (k'=10, Bondapak C18/CORASIL, 7:3 MeOH:H_2O). IR (KBr) 3300, 1430, 1360, 1053, 990, 950 cm^{-1}; NMR(d_5-pyridine) 5.23(2H, br, -OH), 4.28(1H, m, 3β-H), 3.97(1H, d, 17β-H) 0.86(3H, s, 19-Me), 0.71(3H, s, 18-Me); MS 292(M^+-CH3), 274 (M^+-H_2O). The MS and IR spectra were identical with those of authentic material.
$[\alpha]_D$ (EtOH) -10.3°

5α-Androstane-3α,17β-Diol: 5α-Androstane-3α,17β-diol was prepared by reduction of 3α-hydroxy-5α-androstan-17-one with sodium borohydride in dioxan-water (5:1) at 25° for 24 hours, and was purified by repeated crystallisation from methanol-water, m.p. 222-224°C, Lit. (19) 223°C. The steroid was confirmed to be homogeneous by t.l.c. (R_f 0.24, Silica gel GF_{254}, 9:1 chloroform/acetone) and by HPLC (k'=5, Bondapak C18/ CORASIL, 7:3 MeOH/H_2O) IR (KBr) 3400, 1360, 1270, 1048, 1004 cm^{-1}; NMR($d_5$5-pyridine) 4.27 (1H, m, 3β-H), 3.88(1H, t, 17α-H), 0.98(3H, s, 18-Me), 0.85(3H, s, 19-Me); MS 292(M^+), 274(M^+-H_2O), 259(M^+-H_2O-CH_3) 241(M^+-CH_3-H_2O-H_2O)
$[\alpha]_D$ (EtOH) +16°

For administration, a suspension of the steroid in propylene glycol was prepared such that the final concentration was 50 mg/ml. During a course of treatment the dogs received intramuscular injections (0.5 ml) three times per week over a period of four weeks. In both cases (i.e. 5α-androstane-3α,17α-diol, and 5α-androstane-3α,17β-diol), the total dosage received over this period was 300 mg.

ANIMALS

Six adult male beagles (age 2.5-4.0 years), average weight 17 kg., were used in these experiments. Three of these dogs were anesthetized with phenobarbital (Abbott, dose 25 mg/kg) and castrated. One month after castration, each of the three dogs underwent laparotomy for measurement of prostatic size. The dorso-ventral, caudo-cranial and width diameters of the prostate were measured with calipers. Prostatic weight was then estimated from a nomogram following the procedure of

Walsh and Wilson (20). After a ten-day recovery period, 5α-androstane-3α,17α-diol was administered to the dogs for one month. Laparotomies were then performed for re-measurement of prostatic size. After a twenty-day recovery period, the dogs were then treated with 5α-androstane-3α,17β-diol for one month. At the end of this time prostate sizes were again measured. Laparotomies were also performed on the three untreated dogs, and prostate sizes were determined for comparison purposes.

HISTOPATHOLOGY

Biopsy specimens, obtained from the ventral aspect of the prostate prior to and following each treatment, were fixed in 10% neutral buffered formalin and 5μ sections were stained with hematoxylin and eosin. The same procedure was adopted for the biopsy specimens obtained from the prostates of the untreated animals. Light microscopic, morphometric determinations were done as described by Weibel (21). Six sections per prostate and ten fields per section were studied. The relative volume of each tissue component was calculated from the following formula:

$$\text{Relative component volume} = V_M \times P_c$$

where V_M = prostatic volume calculated from caliper measurements (see text)

P_c = Relative percentage of component obtained by morphometric analysis

RESULTS

From Table 1 it can be seen that no significant change in prostatic weight and size was observed after treatment of three castrate beagle dogs with 5α-androstane-3α,17α-diol. Subsequent administration of 5α-androstane-3α,17β-diol in the same dosage to the same dogs produced a dramatic increase in prostate size and weight in all three cases (Table 1).

Histological and morphometric determinations, performed on the prostate biopsies before and after each course of treatment, correlate with the above observations. The normal adult dog prostate has a tubo-alveolar structure packed into lobules separated by stromal septa (Fig. 1). Following castration the prostatic epithelium involuted to a low cuboidal non-secretory epithelium (Fig. 2). After 5α-androstane-3α,17α-

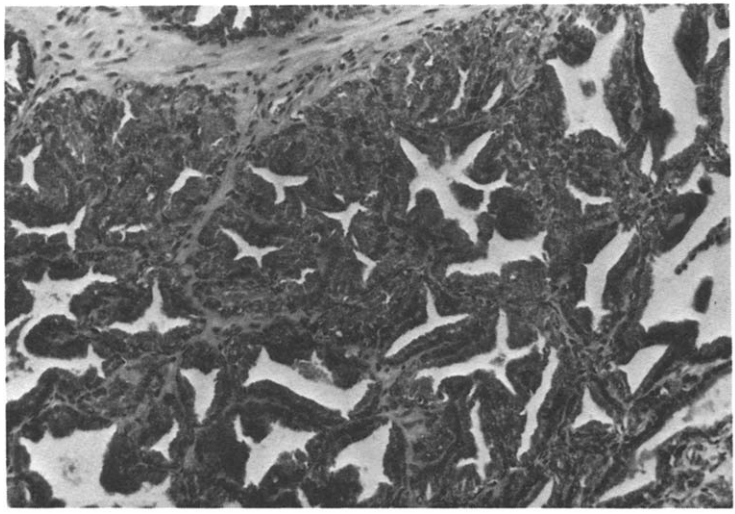


Fig. 1 (250X)

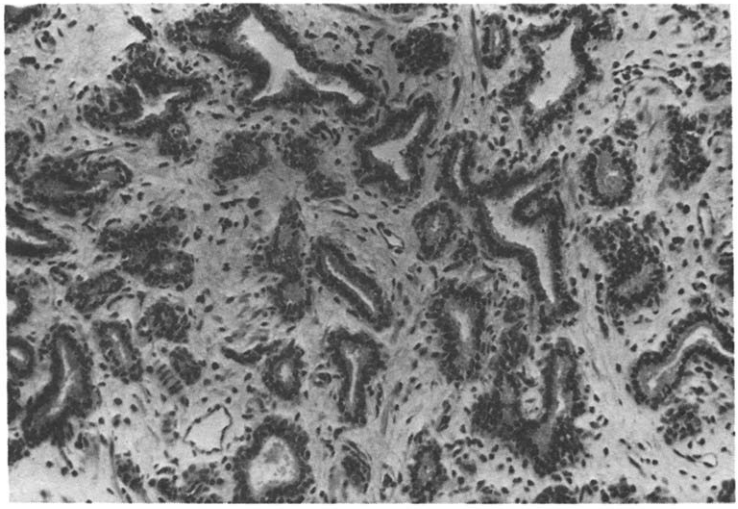


Fig. 2 (250X)

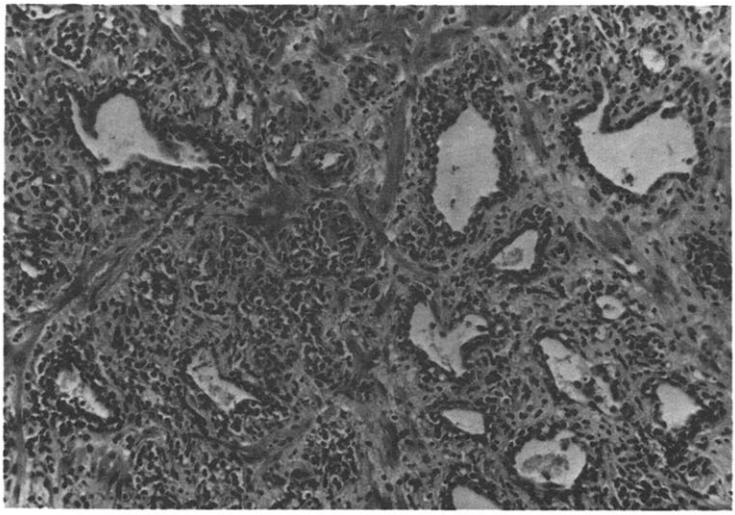


Fig. 3 (250X)

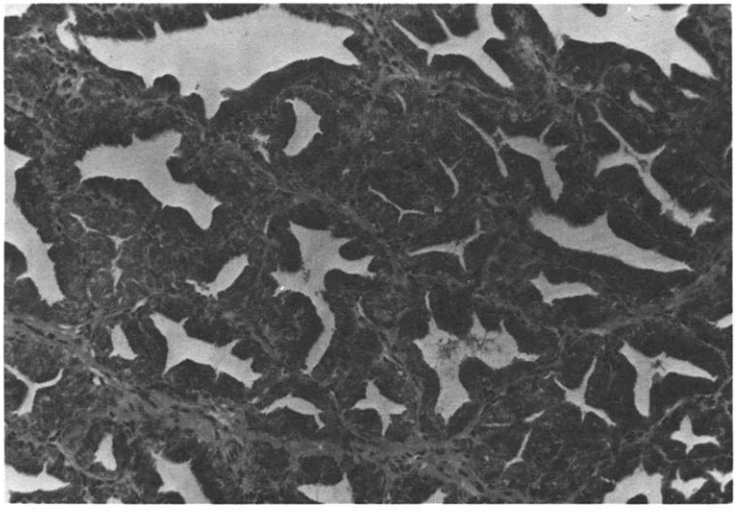


Fig. 4 (250X)

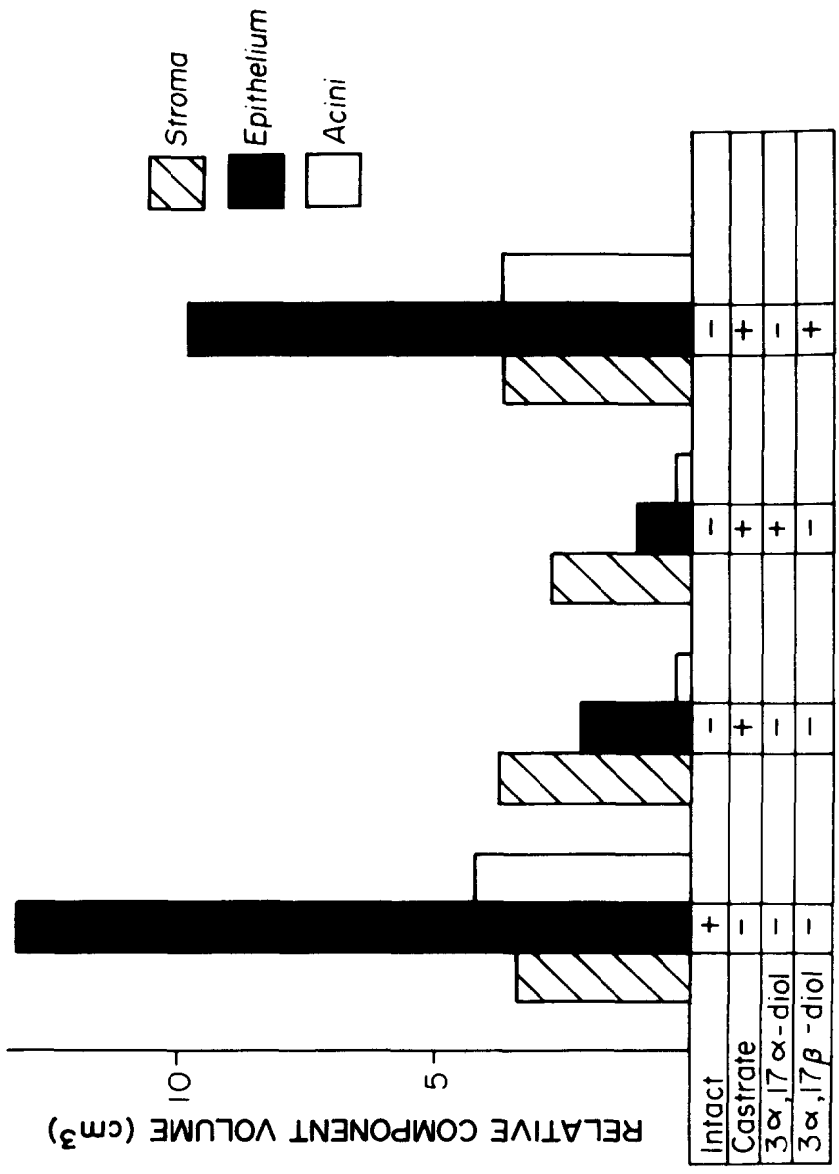
diol treatment, no stimulation of the epithelium was noted. On the contrary, further involution had occurred, consistent with the longer period of castration (Fig. 3). Administration of 5α-androstane-3α,17β-diol restored the prostate to the normal adult configuration (Fig. 4).

Morphometric determination of the percentage epithelium and stroma confirmed the inability of 5α-androstane-3α,17α-diol to initiate epithelial proliferation (Fig. 5). The untreated beagle dog prostates in this experiment comprised 63% epithelium and 16% stroma by volume. Following castration and involution for one month, the epithelial volume decreased to 16% of that of the intact control, but the stroma was unchanged. After the course of treatment with 5α-androstane-3α,17α-diol, the total epithelial volume decreased further to 7% of intact epithelial volume, while the stroma decreased to 79% of the value observed in intact control. Subsequent treatment with 5α-androstane-3α,17β-diol was found to restore the stromal volume to that of the intact control and the epithelium to 75% of the intact control animal volume.

DISCUSSION

Dihydrotestosterone is well accepted to be a major prostatic intracellular metabolite of testosterone and can stimulate prostatic growth *in vivo* (22,23,24) and *in vitro* (6,25). This, however, does not exclude the possibility that other metabolites of testosterone have important roles in the maintenance and stimulation of prostatic growth. Specifically, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol are thought to have roles in prostatic proliferation and cellular differentiation respectively (26). It is by no means established whether these steroids are themselves active and/or are further metabolised to other active compounds.

Fig. 5


Stroma
Epithelium
Acini
RELATIVE COMPONENT VOLUME (cm³)
10
5
Intact
Castrate
3α,17α-diol
3α,17β-diol
+
-
-
+
-
+
-
+
-
+
-
+
-
+
-
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The dog is the only readily available experimental animal to develop a condition similar to human prostatic hypertrophy. Therefore new aspects of testosterone metabolism in the dog are of definite interest. Pierrepoint et al. (9-13,15) have accumulated in vitro data implicating 5α-androstane-3α,17α-diol as an androgen in the dog, but no reports on in vivo experiments have appeared in the literature. However, Ruzicka and Kagi reported (18) many years ago that administration of 5α-androstane-3α,17α-diol to castrate rats in vivo failed to induce prostatic growth.

The protocol adopted for the in vivo testing of 5α-androstane-3α,17α-diol in dogs was closely similar to that of Walsh and Wilson (20). These authors have shown (20) that 5α-androstane-3α,17β-diol, when administered approximately one month (27) after castration, restored the involuted canine prostate.

In the present study, biopsies, taken one month after castration, confirmed the involution of all three prostates in the absence of exogenously administered steroid. Large doses of 5α-androstane-3α,17α-diol were then administered for a one-month period but no indication of prostatic growth was observed. In fact, histological and morphometric analysis of the prostate suggested that further involution had occurred (Fig. 3). The process of prostatic involution in dogs has been shown to continue for at least three months after castration (28,29).

In order to demonstrate that the involuted prostates after the 5α-androstane-3α,17α-diol treatment were indeed androgen-responsive, the known active androgen, 5α-androstane-3α,17β-diol (20), was then administered at the same dosage over the same period of time. Thus the dogs were used as their own controls (30). Prostatic growth was stimulated

as evidenced by size, weight and histology (Fig. 4).

Androgens are commonly defined as compounds which stimulate prostatic growth after exogenous administration to castrate animals. According to this definition, 5α-androstane-3α,17α-diol does not qualify as an active androgen in the dog. However, the present experiment does not exclude the possibility that exogenously administered 5α-androstane-3α,17α-diol does not reach the target organ due to prior metabolism or lack of transport.

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