BIOCHEMICAL AND HISTOLOGICAL STUDIES ON PROSTATES IN CASTRATED DOGS AFTER TREATMENT WITH ANDROSTANEDIOL, OESTRADIOL AND CYPROTERONE ACETATE

By

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ABSTRACT

The effect of cyproterone acetate (CA) on experimentally induced benign prostatic hyperplasia (BPH) in the castrated dog was investigated. BPH was induced by 6 months' treatment with 3α-androstanediol (3α-diol) alone and in combination with 17β-oestradiol (Oestradiol). RNA, DNA and zinc content of the glands were determined in addition to histological examination and measurement of the prostates. Two different types of prostatic enlargement were observed. First, 3α-diol induced typical diffuse canine hyperplasia with replacement of functional activity. DNA, RNA and the zinc content of total glands were increased compared with intact controls. Second, 3α-diol plus Oestradiol produced on the one hand a more striking increase of prostatic weights, but on the other a loss of typical morphological structure and function. Histologically, transformation of simple glandular epithelium into stratified squamous metaplasia occurred in addition to stimulation of fibromuscular tissue. Biochemically, a relative decrease of DNA per mg tissue was measured with a fall in the RNA to DNA ratio and zinc to the values of castrates. Administration of CA resulted in an abolition of the 3α-diol effect. Biochemical determinations and histological examinations revealed an effect similar to castration after treatment with 3α-diol plus CA. After treatment with 3α-diol plus Oestradiol plus CA fibromuscular stimulation as an oestrogen effect predominated in addition to glandular atrophy and metaplastic changes, especially in prostatic ducts. Epithelial hyperplasia is an effect of 3α-diol, whereas metaplastic proliferation only occurs in oestrogenized and androgenized dogs. In both types of prostatic enlargement CA prevents development of hyperplastic prostate.
Walsh & Wilson (1976) succeeded in inducing benign prostatic hyperplasia (BPH) in the castrated dog by treatment with steroid hormones. Six months' administration of 3α-androstanediol (3α-diol) resulted in a significant increase in prostatic weights compared to the prostates of intact control animals. A more striking effect was produced when 3α-diol was given in combination with 17β-oestriadiol (Oe2). In contrast to these findings testosterone and dihydrotestosterone (DHT) had failed to induce BPH under similar conditions (Gloyna et al. 1970; Wilson et al. 1975), although DHT was found to be the major steroid bound to nuclear chromatin in the prostate (Anderson & Liao 1968; Bruchovsky & Wilson 1968). Furthermore, the concentrations of DHT in hyperplastic prostates in dog and man were significantly greater than in normal glands (Geller et al. 1976; Gloyna et al. 1970; Siiteri & Wilson 1970). These results suggest that the accumulation of DHT alone seems not to be the causal mechanism for prostatic growth and that the reduced DHT-metabolite 3α-diol may possibly be involved in the development of BPH.

The present study was undertaken to examine the effect of the antiandrogen cyproterone acetate (CA) in the experimental BPH-model and to determine biochemical events associated with BPH induced by 3α-diol plus Oe2 in the castrated dog.

MATERIALS AND METHODS

Twenty-two dogs aged 1½ years were used. Before treatment the prostatic size of every dog was estimated by threedimensional caliper measurement (length, width, and dorsoventral depth) after retroperitoneal exposition of the gland by a midline incision. All animals were castrated except three dogs, which were kept as intact controls (group I). The other dogs were divided into four groups and received the following treatments over a period of 6 months: 3-androstanediol (3α-diol) (group II), 3α-diol plus oestradiol (Oe2) (group III), 3α-diol plus cyproterone acetate (CA) (group IV), 3α-diol plus Oe2 plus CA (group V). Total weekly dosage in every dog was 75 mg for 3α-diol, 0.75 mg for Oe2 and 600 mg for CA. The injections were given three times weekly, and the steroids were dissolved in 1 ml benzylbenzoate-castor oil 1:10 (v/v). The intact controls received the solvent alone.

At the termination of the experiment all dogs were killed and the prostates rapidly removed, measured and weighed. The concentrations of DNA and RNA were determined by the method of Webb & Levy (1955) and Webb (1956). The zinc concentration of the prostate was measured by atomic absorption spectrophotometry according to the method of Welz (1972) and Ketz et al. (1972). The organs were also examined histo-

Abbreviations used in the paper:
3α-androstanediol (3α-diol): 3α,17β-diol-5α-androstane;
dihydrotestosterone (DHT): 17β-hydroxy-5α-androstan-3-one;
oestradiol (Oe2): 3,17β-diol-1,3,5(10)-oestratriene;
cyproterone acetate (CA): 6-chloro-17-acetoxyl-1α,2α-methylene-pregna-4,6-diene-3,20-dione.
Relation between calculated volume and weight of the prostate in the dog

![Chart](image_url)

**Prostate weight g**

\[
Y = \text{prostatic weight (w)} \\
x = \text{prostatic volume (v)} \\
Y = mx + b \\
m = 1.285 = \tan \alpha \\
b = +0.635 \\
w = 1.285 \cdot v + 0.63 \\
n = 22
\]

**Fig. 1.**
Relation between calculated volume and prostatic weight in the dog.

logically after staining with haematoxylin-eosin and azan. The initial prostatic weights were determined from the constructed nomogram (Fig. 1). The linear function between prostatic weights and calculated volumes is well demonstrated by the correlation coefficient of 0.997. This nomogram makes it possible to determine prostatic weights from the calculated volume after *in situ* threedimensional measurement of the gland.

**RESULTS**

1. **Prostatic weights**

The average prostatic weights of castrated dogs before and after treatment in the differently treated groups are shown in Fig. 2. A comparison of the initial and final weights of the prostates shows a slight gain with an average of 2.5 g in the intact control (group I). The administration of 3a-diol in the castrated dog in group II causes a significant increase in prostatic weight with
an average gain of 12.8 g. A more striking effect in prostatic growth was found in group III after treatment with 3α-diol plus Oe₂. In this group the average weight increase was 22.1 g. The differences of prostatic weights between the controls and the treated animals were statistically significant ($P < 0.05$), but the difference between group II and group III was insignificant ($P > 0.10$). The administration of 3α-diol and CA (group IV) resulted in a marked reduction in final prostatic weight with an average decrease of 2.9 g as compared to the initial weight. After simultaneous treatment with 3α-diol plus Oe₂ plus CA (group V) there were no marked changes between initial and final weights in prostate glands. The reduction of prostatic weight in group IV was statistically significant ($P < 0.05$) compared to control group I, whereas there was no significant difference ($P > 0.10$) between group V and I.

The differences between the final prostatic weights in the different groups demonstrate that 3α-diol stimulates prostatic growth (group II). This effect is significantly ($P < 0.01$) abolished by CA (group IV). The more striking stimulation in prostatic growth after treatment with 3α-diol in combination with Oe₂ (group III) is significantly ($P < 0.02$) reduced after additional administration of CA (group V). Comparing the final prostatic weights in group IV (treatment with 3α-diol plus CA) and group V (treatment with 3α-diol plus Oe₂ plus CA) there is a significant difference of 3.4 g ($P < 0.01$).

![Effect of 5α-androstane-3α, 17β-diol, estradiol (E₂) and cyproterone acetate (CA) on prostate weight in the castrated dog](image)

Fig. 2.
Effect of 3α-diol, Oe₂ and CA on prostate weight in the castrated dog.
2. Histology

Representative histological sections of prostates removed from dogs of group I to V are shown in Fig. 3. In panel a of Fig. 3 the control gland (group I) exhibits characteristic acini lined with simple columnar epithelium surrounded by thin septae of connective tissue. 3α-diol administration in castrated dogs (group II) results in a similar histological picture with papillary infoldings of the tall columnar simple epithelia and enlarged lumina. 3α-diol plus Oe₂ induces an alteration of the morphological structure of the gland (group III). Histologically, epithelial proliferation is seen with stratified squamous metaplasia and complete obstruction of dilated acinar lumina filled with keratinized epithelial detritus. Furthermore, activation of the fibromuscular interacinar tissue is obvious (Fig. 3 c). As shown in panel d of Fig. 3 treatment with 3α-diol plus CA (group IV) results in complete atrophy of glandular epithelium and relative increase of the unchanged connective tissue. After treatment with 3α-diol plus Oe₂ plus CA (group V) atrophy of the glandular epithelium occurs, too (Fig. 3 c). In addition, squamous metaplasia in great ducts and activation of fibromuscular stroma including smooth muscle cells is revealed by histological examination.

3. DNA and RNA content

Hormonal manipulations in castrated dogs resulted in changes of the content of nucleic acids in the prostates. Fig. 4 demonstrates the effect of 3α-diol, Oe₂ and CA on total DNA and RNA of the glands.

The average total DNA (milligrams per gland) was 37 in the control group and increased significantly ($P < 0.05$) to 80 after 3α-diol treatment (group II) and to 87 after treatment with 3α-diol plus Oe₂ (group III). Total DNA decreased significantly ($P < 0.01$) to 14 after simultaneous treatment with CA and 3α-diol and to 17 after treatment with 3α-diol plus Oe₂ plus CA (group V). The highest total RNA content (milligrams per gland) found was 55 in 3α-diol treated castrated dogs (group II). A value of 42 was found in group III versus 28 in the intact control. A statistically significant reduction ($P < 0.01$) occurred after additional treatment with CA. The total RNA (milligrams per gland) was 4.5 in group IV and 6.8 in group V and significantly different from total RNA of group I, II and III ($P < 0.01$). Fig. 4 illustrates the values for RNA expressed per unit of DNA. This RNA : DNA ratio yields the highest values in the control group I with 0.75 and in the 3α-diol treated group II with 0.67, which is not significantly different ($P > 0.10$) from group I. In contrast, the ratio is significantly ($P < 0.01$) decreased to 0.48 after treatment with 3α-diol and Oe₂ (group III). Furthermore, a reduction of the ratio occurs after CA treatment. The values are 0.30 in group IV and 0.41 in group V. The RNA : DNA values of group III, IV and V are significantly different ($P < 0.01$) from values of group I and II.
Fig. 3.
Histological sections of prostates from dogs treated with hormones

a. untreated intact control (group I).
b. castrated dog after treatment with 3α-diol (group II).
c. castrated dog after treatment with 3α-diol plus Oe₂ (group III).
d. castrated dog after treatment with 3α-diol plus CA (group IV).
e. castrated dog after treatment with 3α-diol plus Oe₂ plus CA (group V).

Magnification: 400 x.
4. Zinc content

The average values of the zinc content in the prostates of the different groups are shown in Fig. 5. The results after measurement by atomic absorption spectrophotometry are expressed in percentage zinc concentration per dry weight. The highest zinc concentrations were found in the 3α-diol treated castrated dogs and in the intact controls \((1.67 \times 10^{-1}) \text{ (II)}\) and \(1.13 \times 10^{1}\) \text{ (I).} \text{ respectively). The}
difference between group I and II was statistically significant \((P < 0.05)\). The administration of 3α-diol plus \(\text{Oe}_2\) resulted in a statistically significant \((P < 0.02)\) decrease to about one-twentieth of the group II values and to one-fifteenth of the control values. A similar significant \((P < 0.01)\) degree of zinc reduction was found in group V after simultaneous treatment with 3α-diol plus \(\text{Oe}_2\) plus CA. Zinc concentrations fall significantly \((P < 0.01)\) to about one-fifteenth of the group II, and to one-tenth of the control values after treatment with CA plus 3α-diol. The differences of zinc concentrations between group III, IV and V were statistically insignificant \((P > 0.10)\).

![Figure 4](https://example.com/figure4.png)

**Fig. 4.**

Effect of 3α-diol, \(\text{Oe}_2\) and CA on nucleic acids of prostates in the castrated dog.
DISCUSSION

Several conclusions can be drawn from these studies, which were undertaken to examine the effect of CA on hormone-induced BPH in the castrated dog using biochemical analyses of the glands in addition to measurements and histological examinations: 3α-diol induces prostatic growth, thus confirming the findings of Walsh & Wilson (1976). After 6 months' treatment we found nearly the same mean increase of 12.8 g in prostatic weight compared with 10.1 g observed by Walsh & Wilson (1976). The biochemical determinations of the total DNA and RNA content and histological examinations of the glands proved that this weight gain was caused by epithelial hyperplasia according to the definition of Coffey (1974) and de Klerk et al. (1975). The presented results support numerous observations that androgens stimulate RNA and DNA synthesis in sex accessory tissue (Liao & Fang 1969; Sufrin & Coffey 1973; Lerner 1964; Williams-Ashman & Reddi 1972; Geller et al. 1976). Furthermore, the increase of prostatic zinc content compared with intact controls indicates that hyperplasia is associated with stimulated epithelial function, since the zinc content reflects the degree of glandular activity (Byar 1974). It was also demonstrated previously that testosterone increases the uptake of 65 zinc in intact dogs (Johnston et al. 1966) and baboons (Schoones et al. 1969), whereas a marked decrease was observed in intact and hypophysectomized and castrated

![Fig. 5](image)

Effect of 3α-diol, Oe₂ and CA on the zinc content of prostates in the castrated dog.
rats (Gunn & Gould 1956, 1957). Histologically, the increase of RNA, DNA and zinc in prostates stimulated by 3α-diol is associated with characteristic diffuse prostatic hyperplasia. No cellular hypertrophy was demonstrable on measuring the RNA to DNA ratio compared with the intact control. Gloyna et al. (1970) observed cellular hypertrophy after long term administration of DHT in castrated dogs, as measured by RNA to DNA ratio, whereas they found no prostatic hyperplasia but only restoration to normal weights (Gloyna et al. 1970). In castrated rats the RNA to DNA ratio returned to normal values after a stimulation period of 10 days with 3α-diol, while epithelial cells were restored to 65 % of normal total numbers (de Klerk et al. 1975). The 3α-diol effect on the acinar parenchyma of the prostate is completely abolished by CA, as monitored by prostatic weight, biochemical analyses and microscopic examination. The biochemical determinations of DNA, RNA and zinc content revealed significantly decreased values comparable to those found in castrated dogs (Gloyna et al. 1970; Mackenzie et al. 1962). These biochemical events are apparently conditioned by glandular atrophy as shown histologically. Similar microscopic findings were observed in intact dogs with spontaneous BPH after treatment with CA (Neri et al. 1968) and non-steroidal antiandrogens (Neri & Monahan 1972) as well as after castration (Huggins 1945; Neri et al. 1968). Atrophy of glandular epithelium was called a histological marker of gonadectomy in canine prostate (Leav et al. 1971), and the CA effect is like this. Prostatic growth produced by treatment with 3α-diol plus Oe2 is quite different from 3α-diol induced hyperplasia. In support of the findings of Walsh & Wilson (1976), we observed a more striking increase of prostatic size when 3α-diol was administered in combination with Oe2. The total DNA content nearly corresponds with that of the 3α-diol stimulated prostates in spite of the two-fold greater weight increase. Compared with the intact controls there was cellular proliferation accompanied by a decrease in cellular size as monitored by the increased DNA and decreased RNA to DNA ratio. Furthermore, the significantly reduced zinc content reflected the loss of normal epithelial function. These biochemical changes reflect a morphological alteration of the enlarged gland. The dominant histological statement is the stratified squamous metaplasia of the acinar epithelia with obstruction of dilated acinar lumina by epithelial detritus. The direct oestrogen/androgen antagonism with regard to prostatic secretion, first demonstrated by Huggins & Clark (1940) in the fistula dog, is to be explained by the loss of secretory activity of metaplastic epithelia. Furthermore, stimulation of fibromuscular tissue is seen after 3α-diol plus Oe2 treatment. Stimulation of fibromuscular tissue is a well known effect of oestrogens on organs that originate embryologically from the entodermal epithelium of the urogenital sinus (Neumann et al. 1975) and was also observed in ductus deferentes of oestrogenized castrated dogs (Senge et al., in press).
These two prostatic alterations, stratified squamous metaplasia and activation of fibromuscular tissue, occur spontaneously in dogs with oestrogen producing Sertoli cell tumours (Brody & Martin 1958; Huggins & Moulder 1945). The prostatic morphology of these dogs compares best with oestrogenized intact dogs or exogenously androgenized plus oestrogenized castrated dogs (Berg 1958).

After inhibition of 3α-diol by CA the changes in prostates which received 3α-diol plus Oe₂ plus CA presumably reflect the oestrogen effect. With regard to the prostatic size the atrophy of glandular epithelium is compensated by fibromuscular proliferation yielding nearly the same final weight as at the start of the experiment. The biochemical determination of total DNA, RNA and zinc indicates only minor differences in comparison to those of 3α-diol and CA treated dogs. The difference in DNA reflects the increased cell numbers in fibromuscular tissue. Metaplastic changes of persistent epithelium are also evident in Oe₂ plus 3α-diol plus CA treated castrated dogs, especially in prostatic ducts. This present result leads to the tentative conclusion that the metaplastic alterations in dogs are a primary response of sensitive tissue to oestrogen administration. In this context the possibility of an additional effect of prolactin should be discussed, since the hormonally treated dogs were not hypophysectomized. It has been demonstrated that prolactin influences the prostate (Farnsworth 1975) and increases under oestrogen treatment (Clemens & Meites 1974). Furthermore, the development of metaplasia of human adenoma tissue after heterotransplantation into the femoral muscles of newborn rats (Senge et al. 1973) or heterotransplantation into “nude” mice (Okada et al. 1975) was presumed to be a result of nutritional disturbances (Neumann et al. 1975). Proliferation of metaplasia only occurs in dogs as a common effect of oestrogen plus androgen. Metaplastic transformation of acinar epithelium involves loss of functional activities similar to that observed in human prostatic carcinoma (Kirchheim et al. 1974).

On the basis of the results presented in this report it may be concluded that three different pathophysio logic events occur in 3α-diol and Oe₂ induced BPH in the castrated dog: first, epithelial proliferation as a result of the 3α-diol effect; second, proliferation of metaplasia as a result of a common 3α-diol and Oe₂ effect; and third, fibromuscular proliferation of connective tissue as a result of the Oe₂ effect. Epithelial and fibromuscular proliferation is also involved in human BPH. The selective observation of different hormone dependent effects in this convincing experimental model of BPH and the possibility of abolishing definite effects may help to elucidate a number of unsolved problems in the development of BPH.
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