Short Communications

The Effect of Prostatic Metabolites of Testosterone and other Substances on the Isolated Deoxyribonucleic Acid Polymerase of the Canine Prostate

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The activity of DNA polymerase (DNA nucleotidyltransferase, EC 2.7.7.7) in androgen target-organs has not been widely studied. Earlier investigations in this laboratory (Fahmy, Griffiths, Mahler & Williams, 1967; Fahmy & Griffiths, 1968) showed that the isolated DNA polymerase from calf thymus tissue was responsive to various steroid hormones and suggested that further studies concerned with the hormone-responsiveness of a similar enzyme system from prostate tissue could be particularly germane to the understanding of the mechanism of action of these hormones. In this respect it was of note that testosterone and 5α -dihydrotestosterone (17β -hydroxy- 5α -androstane-3-one) stimulated DNA polymerase isolated from human hypertrophic prostatic tissue (Harper, Fahmy, Pierrepoint & Griffiths, 1970a) and, if this observation truly reflects the state *in vivo*, it would seem that this system of investigation could be of value in screening substances with regard to their clinical effectiveness in controlling prostatic dysfunction. This communication now describes further experiments on canine prostatic DNA polymerase.

Clinically normal prostate glands were removed from two 2-year-old healthy dogs and one 16-year-old dog. A further grossly enlarged and cystic gland was taken from an animal showing signs of bowel obstruction. The glands weighed 8.4, 12.3, 11.3 and 72.0 g respectively. Tissues were prepared as described by Shepherd & Keir (1966) and the semi-purified F_2 fraction was isolated. Enzyme preparations were stored in sealed vials at -20°C before use. The enzyme (100–250 μg of protein) was incubated at 37°C for 2 h with 50 nmol of [^3H]-thymidine triphosphate (specific radioactivity 10×10^6 c.p.m./ μmol) in 255 μl of medium containing 5 μmol of tris-HCl buffer, pH 7.5, 15 μmol of KCl, 100 nmol of EDTA, 1 μmol of MgCl_2 , 2 μmol of 2-mercaptoethanol and 50 nmol each of deoxy-GTP, deoxy-CTP and deoxy-ATP together with 50 μg of thermally denatured DNA. Either 5 μl of ethanol, or a compound in this volume of ethanol, was added to the reaction mixture. Protein content of

the enzyme was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). The results of this study are shown in Table 1.

A great deal of literature has accumulated on steroid metabolism by the human prostate (reviewed by Ofner, 1969), but a recent study (Harper, Pierrepoint, Fahmy & Griffiths, 1970b) was concerned primarily with the canine prostate. The 'prostatic metabolites' listed in Table 1 are those shown to be formed by these same canine prostate glands when incubated with [7α - ^3H]testosterone. Only three steroids stimulated the DNA polymerase activity, namely testosterone, 5α -androstane- $3\alpha,17\beta$ -diol and 5α -androstane- $3\alpha,17\alpha$ -diol. Noteworthy was the lack of effect of 5α -dihydrotestosterone on the enzyme system, in the light of experiments showing this steroid to be preferentially retained by rat prostatic nuclei either after testosterone administration *in vivo* (Bruchovsky & Wilson, 1968) or after incubation of the tissue with labelled testosterone (Anderson & Liao, 1968). Similar experiments with canine prostate tissue (Harper *et al.* 1970b) indicated a selective accumulation of 5α -androstane- $3\alpha,17\alpha$ -diol in the nucleus, and this, in association with the observed effect of the diol on the canine DNA polymerase, might suggest this steroid is an androgenic metabolite of testosterone in the dog. Whether it plays a more effective androgenic role in the canine prostate than does 5α -dihydrotestosterone, or testosterone itself, can be decided by further study. Relevant, however, are the experiments of Baulieu, Lasnitzki & Robel (1968a,b), showing that certain 5α -androstane diols as well as 5α -dihydrotestosterone stimulate cell division and induce epithelial hyperplasia in explants of rat prostate maintained in tissue culture.

The DNA polymerase isolated from the prostate of the old dog showed little response to steroids, although it was inhibited by the stilboestrol derivatives. It should be emphasized that dihydrodiethylstilboestrol generally shows a capacity equal to diethylstilboestrol in inhibiting isolated DNA polymerase enzyme systems (Harper *et al.* 1970a)

Table 1. *Effect of various compounds on canine prostatic DNA polymerase*

(S), Stimulation; (I), Inhibition. 0, No effect; —, not investigated.

	DNA polymerase activity (c.p.m./mg of protein)					
	11037	12173	3117	7310		
	% change in DNA polymerase activity					
	Hypertrophied prostate					
Compounds added to incubation (final concn. 40 μ M)	First young prostate	Second young prostate	Old prostate	Prep. 1	Prep. 2	Prep. 3
Testosterone	33.6 (S)	23.6 (S)	3.0 (S)	0	0	0
Prostatic metabolites						
Epitestosterone	0	0	—	—	—	—
5 α -Androstane-3,17-dione	—	—	0	0	0	—
5 α -Dihydrotestosterone	0	0	0	0	0	0
5 α -Dihydroepitestosterone	0	0	0	—	—	—
5 α -Androstane-3 α ,17 α -diol	12.8 (S)	10.6 (S)	0	15.1 (S)	10.5 (S)	11.6 (S)
5 α -Androstane-3 α ,17 β -diol	3 (I)	0	0	2.0 (S)	1.5 (S)	0
5 α -Androstane-3 β ,17 α -diol	0	0	0	0	0	0
5 α -Androstane-3 β ,17 β -diol	0	0	0	0	0	0
Androstenedione	0	0	—	0	0	0
Testosterone sulphate	0	—	—	—	—	—
Androsterone	0	0	—	—	—	—
Epiandrosterone	0	0	—	—	—	—
6 β -Hydroxyandrostenedione	0	0	—	—	—	—
Other compounds tested						
Androst-4-ene-3 β ,17 β -diol	0	0	—	—	—	—
5 β -Dihydrotestosterone	0	0	—	—	—	—
Oestradiol-17 β	1.3 (I)	1.9 (I)	0	—	1.2 (I)	0
Diethylstilboestrol	28 (I)	25 (I)	12.1 (I)	—	—	—
Hexoestrol	25 (I)	—	—	—	—	—
Dibutylstilboestrol	11 (I)	—	—	—	—	—
Dihydrodibutylstilboestrol	26 (I)	—	15.1 (I)	—	—	—
Cyproterone acetate	0	0	—	—	—	—
DPB	0	0	—	—	—	0
Chlormadinone acetate	—	—	—	—	—	0
17 α -Ethynyloestrone-3 β ,17 β -diol	0	0	—	—	—	—
17 α -Ethynyloestradiol-17 β	0	0	—	—	—	—
17 α -Vinyl-17 β -hydroxyoestrone	0	0	—	—	—	—

and, with its decreased oestrogenicity, might be of value in the treatment of prostatic hypertrophy and neoplasia.

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