Hormonal Effects *in vitro* on Prostatic Ribonucleic Acid Polymerase

By P. DAVIES,* A. R. FAHMY,*† C. G. PIERREPOINT‡ and K. GRIFFITHS‡

*Velindre Memorial Hospital and ‡Tenovus Institute for Cancer Research, Heath, Cardiff CF4 4XX, U.K.

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In recent years considerable interest has been directed towards the mechanism by which androgenic steroids control the growth and development of the prostate gland. In particular, the relationship between testosterone (17 β -hydroxyandrost-4-en-3-one) and its reduced metabolite 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androst-3-one) within the prostate has been the subject of intensive research. 5 α -Dihydrotestosterone was implicated in androgen action when it was found to be localized in the nuclei of the rat ventral prostate (Bruchovsky & Wilson, 1968*a,b*; Anderson & Liao, 1968). Further, Baulieu *et al.* (1968*a,b*) indicated that 5 α -dihydrotestosterone had a stimulatory effect on cell division and secretion in rat prostatic explants maintained in culture. Since it is well established that enhancement of RNA polymerase activity is one of the earlier androgenic effects in the prostate gland, it is noteworthy that Alfheim & Unhjem (1971) failed to observe a direct effect of 5 α -dihydrotestosterone on the activity of this enzyme system in homogenates, slices or nuclear preparations of rat ventral prostate *in vitro*.

Further studies on the effects of testosterone and its metabolites on the DNA-dependent RNA polymerase (nucleotide triphosphate–RNA nucleotidyl-transferase, EC 2.7.7.6) of nuclei prepared from rat and dog prostatic tissue are now reported. Observations on the inhibitory effect of various stilboestrol derivatives on this enzyme system are also described.

Materials and methods

Prostatic tissue from 12 Sprague–Dawley rats, aged 8–12 weeks, was pooled for each experiment and the nuclear preparation obtained by the method of Mainwaring (1969). In all preparative media, however, MgCl₂ was used instead of CaCl₂. Nuclei from the prostates of young dogs were prepared as described by Groom *et al.* (1971). The procedure of Mangan *et al.* (1967) was used to determine the activity of the DNA-dependent RNA polymerase in the nuclear preparations.

The enzyme preparation (equivalent to 60–100 μ g of DNA) in 0.25 M-sucrose–1.0 mM-MgCl₂ was incubated at 37°C for 15 min with 125 pmol of [8-¹⁴C]ATP (specific radioactivity 40 Ci/mol; The Radiochemical Centre, Amersham, Bucks., U.K.) in 500 μ l of a medium containing the following con-

stituents: 60 μ mol of tris–HCl buffer, pH 8.1, 3 μ mol of NaF, 2.5 μ mol of 2-mercaptoethanol, 15 μ mol of KCl, 2.5 μ mol of MgCl₂ and 300 nmol each of CTP, UTP and GTP. Either 50 μ l of ethanol, or one of the steroids or stilboestrol derivatives under investigation in this volume of ethanol to give a final concentration of 40 μ M, was added to the reaction mixture. DNA of the enzyme preparation was determined by the method of Burton (1956) as modified by Giles & Myers (1965).

Incubations were terminated by the addition of 5 ml of 10% (w/v) trichloroacetic acid. Precipitates were washed three times with cold 5% (w/v) trichloroacetic acid, once with cold ethanol and once more with cold 5% trichloroacetic acid. Acid-insoluble material was dissolved in 4 drops of Hyamine 10X (British Drug Houses Ltd., Poole, Dorset, U.K.) and kept overnight at 37°C, and 10 ml of scintillation liquid (Bray, 1960) was added. Radioactivity was measured in a Nuclear–Chicago model 6860 liquid-scintillation spectrometer. The incorporation of [¹⁴C]ATP into RNA was determined in terms of d.p.m./15 min per mg of DNA.

Results and discussion

The effects of the various compounds on the activity of the DNA-dependent RNA polymerase are shown in Table 1. Stimulation of the enzyme system from both rat and dog prostatic tissue was observed on addition of 5 α -dihydrotestosterone and to a smaller degree with testosterone. The effect of testosterone could, however, be due to its transformation by the nuclear 5 α -reductase enzyme system. The stimulatory effect of 5 α -dihydrotestosterone is of interest, again suggesting that this steroid plays a major role in controlling the growth and function of the prostate. The results also support the findings obtained by Bashirelahi *et al.* (1969) on the effects of testosterone and 5 α -dihydrotestosterone *in vitro* on the RNA polymerase enzyme system and contrast with the reported inhibitory effect of 5-dihydrotestosterone on the RNA polymerase of lysed thymus nuclei (Abraham & Sekeris, 1971).

Also noteworthy was the observed stimulation of the rat prostate RNA polymerase by both 5 α -androstane-3 α ,17 β -diol and also 5 α -androstane-3 β ,17 β -diol. These diols are metabolites of testosterone in the rat prostate (Griffiths *et al.*, 1970), and

† Deceased 16 May 1972.

Table 1. *Effect of incubation of rat and dog prostatic nuclei with various steroids and stilboesterol derivatives on the nuclear RNA polymerase activity*

Nuclear preparations were incubated for 15 min at 37°C in an assay medium containing these substances at a final concentration of 40 µM. Duplicate assays were carried out in each case. The incorporation of [8-¹⁴C]ATP into acid-insoluble material was determined and expressed as d.p.m./15 min per mg of DNA. Increases or decreases in incorporation are expressed as percentages of the value obtained in the absence of added steroid or stilboesterol derivative. Values followed by an asterisk (*) indicate inhibition, those without indicate stimulation. Dashes show that the compound was not studied in that particular experiment; the zeros indicate that there was no effect on the enzyme.

Compound (40 µM) added to enzyme assay system	Activation or inhibition of RNA polymerase activity (%)							
	Rat prostatic nuclear prep.					Canine prostatic nuclear prep.		
	(a)	(b)	(c)	(d)	(e)	(a)	(b)	(c)
Testosterone	3	3.7	4.3	3.5	3.1	5.8	14.6	7.2
5α-Dihydrotestosterone	41	40	43.5	45.4	36.3	21.8	26.9	18.7
Epitestosterone (17α-hydroxyandrost-4-en-3-one)	0	0	0	0	0	0	0	0
5α-Dihydroepitestosterone	0	0	0	0	0	0	0	0
5α-Androstane-3α,17α-diol	0	0	0	0	0	6.1	10.7	4.8
5α-Androstane-3α,17β-diol	27	32	33.6	39.0	23.9	0	0	0
5α-Androstane-3β,17α-diol	0	0	0	0	0	0	0	0
5α-Androstane-3β,17β-diol	31	31	35.1	38.1	28.5	2.6	2.5	1.0
Androst-5-ene-3β,17β-diol	0	0	0	0	0	0	0	0
Androst-4-ene-3,17-dione	0	0	0	0	0	0	0	0
3α-Hydroxy-5α-androstan-17-one	0	0	0	0	0	0	0	0
Dehydroepiandrosterone	0	0	0	0	0	0	0	0
Oestradiol-17β	—	—	31*	5*	9*	—	32*	19*
Diethylstilboesterol	—	—	37*	11*	8*	—	37*	28*
Hexoestrol	—	—	33*	11*	18*	—	12*	23*
Dibutylstilboesterol	—	—	42*	13*	15*	—	17*	26*
Dibutylidihydrostilboesterol	—	—	—	16*	23*	—	26*	31*

it is reported that the 3β,17β-diol stimulated the growth and activity of rat ventral prostatic tissue in culture (Baulieu *et al.*, 1968*a,b*). It is noteworthy that, in contrast with the rat prostate enzyme system, the 3α,17β-diol had no effect on the activity of the dog RNA polymerase. However, the 5α-androstane-3α,17α-diol stimulated the dog RNA polymerase and not the rat enzyme, corresponding to the results of previous investigations (Harper *et al.*, 1970), which showed that of the four 5α-androstanediol epimers only the 3α,17α-diol stimulated the DNA polymerase enzyme system isolated from the canine prostate. Together with experiments of Farnsworth (1970), indicating that the 3α,17β-diol controls the activity of adenosine triphosphatase in the prostate gland, these studies suggest that a number of the metabolites of testosterone may be concerned in eliciting the androgenic response in the prostate, and control of this pattern of metabolites may be necessary to promote normal growth and function. Imbalance of this

pattern might lead to prostatic neoplasia, and high concentrations of 5α-dihydrotestosterone have been reported in benign hypertrophic prostates (Siiteri & Wilson, 1970).

It should be noted that, in contrast with the studies carried out by Bashirelahi *et al.* (1969), no stimulation of the RNA polymerase was obtained with steroid concentrations below 40 µM. However, at steroid concentrations of 4 µM 5α-dihydrotestosterone and the 3α,17β- and 3β,17β-diols stimulated the enzyme system of rat prostatic nuclei when cytosol (remaining after nuclear sedimentation) was also added (Table 2). In similar experiments with the canine nuclear preparation, 5α-dihydrotestosterone and the 3α,17α- and 3β,17β-diols stimulated at the 4 µM concentration in the presence of cytosol. No stimulation was achieved with the 3β,17α-diol or any of the other steroids tested on rat or dog nuclear preparations, with or without cytosol, at concentrations of steroid up to 40 mM. The possibility that cytoplasmic

Table 2. *Effect of incubation of rat and dog prostatic nuclei with various steroids on the nuclear RNA polymerase activity in the presence and in the absence of a cytosol preparation*

Nuclear preparations were incubated for 15 min at 37°C in an assay medium containing steroid (4 µM) in the presence and in the absence of 250 µl of cytosol. Cytosol was obtained by centrifugation (at 100000g for 60 min) of the supernatant after removal of the nuclear pellet for RNA polymerase preparation. Incorporation of [8-¹⁴C]-ATP into acid-insoluble material was determined as d.p.m./15 min per mg of DNA. Accordingly 250 d.p.m. is equivalent to the incorporation of 1 pmol of ATP into RNA.

Steroid (4 µM) added to enzyme assay system	Incorporation of [¹⁴ C]AMP (d.p.m.)			
	Rat prostatic nuclei		Canine prostatic nuclei	
	-Cytosol	+Cytosol	-Cytosol	+Cytosol
None	8930	8893	10572	10284
	8856	8860	10532	10461
Testosterone	9142	9123	9687	10451
	9104	9090	10270	10070
5α-Dihydrotestosterone	9044	10816	10490	16155
	8963	11135	10016	14490
5α-Androstane-3α,17α-diol	8748	8804	10732	14604
	8813	8632	9188	14768
5α-Androstane-3β,17α-diol	9232	8884	10865	10716
	8535	8851	11401	10502
5α-Androstane-3α,17β-diol	8768	9936	10253	10336
	8690	10908	10626	10467
5α-Androstane-3β,17β-diol	8886	16317	10855	15249
	8767	16050	11272	16735

proteins concerned with steroid binding are involved in the observed stimulatory effects of the steroid on the RNA polymerase activity is worthy of further study.

Experiments carried out by Harper *et al.* (1970) indicated that diethylstilboestrol inhibited the activity of DNA polymerase isolated from human hyperplastic and neoplastic prostate tissue. At the same time the newly synthesized dibutylidihydrostilboestrol was shown to produce the same inhibitory effect. It is of interest therefore that both diethylstilboestrol and dibutylidihydrostilboestrol were shown to inhibit the RNA polymerase enzyme from rat and dog prostate tissue (Table 1), together with oestradiol-17β and other stilboestrol derivatives.

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