The Effect of Oestrogen on the Prostatic Metabolism of Testosterone in Tissue Culture

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During the last few years a great deal of information has accumulated with regard to the action of androgens on target tissues, especially that of testosterone (17 β -hydroxyandrost-4-en-3-one) on the prostate gland. Testosterone metabolism by the prostate both *in vivo* and *in vitro* (Farnsworth & Brown, 1963; Ofner, 1968; Gloyna & Wilson, 1969; Kowarski, Shalf & Migeon, 1969; Pike, Peeling, Harper, Pierrepoint & Griffiths, 1970) has been studied, and one of the most interesting observations has been that metabolites of testosterone, steroids such as 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androst-3-one) and the 5 α -androstanediols appear to have specific roles to play in eliciting the androgenic response (Baulieu, Lasnitzki & Robel, 1968*a,b*). Such investigations on the action of androgens must be relevant to the study of the aetiology and treatment of prostatic cancer, since an imbalance in the metabolism of testosterone by the prostate could induce the neoplastic state. Although the androgen-dependent nature of prostatic cancer and its treatment with oestrogens has been known for many years (Huggins, Scott & Hodges, 1941), little is known about the effect of oestrogen on prostatic metabolism. Oestrogens can influence the prostate indirectly via their action on the hypothalamus and testis, with a consequent decrease in testosterone secretion, but a direct action on the prostate is also probable (Farnsworth, 1969). Tissue-culture studies provide a means of investigating the action of compounds on tissue without the added complications of interference from the general metabolism. A study of the metabolism of testosterone by rat and canine prostatic explants, cultured in the presence and in the absence of natural and synthetic oestrogens, is reported in this paper.

Prostate glands from adult dogs and ventral prostates of 8-12-week-old Sprague-Dawley rats were dissected under sterile conditions from any surrounding fat and connective tissue. The tissue was then cut into 1 mm-cube explants and incubated in organ-culture dishes on lens-paper rafts supported by stainless-steel grids. Each culture dish contained 1 ml of Eagle's minimal essential medium fortified with 10% (v/v) of calf serum, 2 mM-glut-

amine, 200 units of penicillin and 100 μ g of streptomycin. [7 α -³H]Testosterone (20 μ Ci; specific radioactivity 1.51 Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) was added to each culture, and to some oestradiol-17 β , diethylstilboestrol or dibutylidihydrostilboestrol to give a final concentration of 17 μ M. Cultures were incubated in an atmosphere of air+CO₂ (95:5) at 37°C for 24 h. After incubation, explants from identical incubations were pooled, washed in phosphate-buffered saline (137 mM-NaCl-2.68 mM-KCl-8.09 mM-Na₂HPO₄-1.47 mM-KH₂PO₄), blotted dry and weighed before homogenization. In whole-tissue experiments the homogenate was analysed after the addition of 500 μ g of various carrier steroids. The individual steroids were purified by extensive t.l.c. and formation of derivatives, and measured either by absorption of u.v. light or by g.l.c. Radioactivity was determined by using a Nuclear-Chicago scintillation spectrometer as detailed by Harper, Pierrepoint, Fahmy & Griffiths (1971). Samples were taken for the determination of DNA (Burton, 1956) and protein (Lowry, Rosebrough, Farr & Randall, 1951); for the latter crystalline bovine serum albumin was used as the standard. In experiments where the nuclear fraction was examined, more cultures were pooled to obtain enough tissue for fractionation. The tissue was homogenized in 0.25 M-sucrose containing 3 mM-magnesium chloride and centrifuged at 700 *g* for 10 min to obtain a crude nuclear pellet. This was resuspended in 0.88 M-sucrose and layered over a gradient of 1.8-2.2 M-sucrose containing 3 mM-magnesium chloride and spun at 33000 *g*_{av.} for 45 min. The nuclear pellet was then examined for radioactive steroids in the same manner as the homogenate, and DNA was determined to estimate nuclear recovery. Table 1 shows the results of incubating [7 α -³H]testosterone with canine and rat prostatic explants in both the control and test cultures. The ratios of testosterone to 5 α -dihydrotestosterone, the most abundant metabolite, are given. The means of the specific radioactivities of the parent steroid and two derivatives are given in Table 1, and have been adjusted to 25 mg of tissue. The specific radioactivities showed significant

Table 1. *Incubation of rat and canine prostate explants with [³H]testosterone in the presence and in the absence of various oestrogens (17 µM)*

Species examined and tissue fraction analysed	Steroids investigated	Specific radioactivities of steroids investigated (d.p.m./nmol per 25 mg of tissue cultured)			
		Control	Oestradiol	Diethylstilboestrol	Dibutylidihydrostilboestrol
Rat: whole tissue	Testosterone	253.0	277.9	227.2	226.3
	5α-Dihydrotestosterone	1334.3	742.4	869.3	296.3
Rat: nuclear fraction	Testosterone	248.4	78.5	24.8	—
	5α-Dihydrotestosterone	3075.7	376.1	198.0	—
Dog: whole tissue	Testosterone	424.5	503.7	584.9	650.0
	5α-Dihydrotestosterone	144.7	121.2	54.2	68.4
Dog: whole tissue	Testosterone	741.0	708.0	661.0	773.0
	5α-Dihydrotestosterone	174.7	79.7	51.3	76.4

changes, depending on the co-incubated test compounds. The ratio of 5α-dihydrotestosterone to testosterone decreased in all cases, suggesting that 5α-dihydrotestosterone formation within the prostatic tissue had been directly suppressed by the oestrogens. Noteworthy was the apparent lower conversion of testosterone into 5α-dihydrotestosterone in the canine prostatic tissue, consistent with earlier studies (Harper *et al.* 1971). In experiments where the nuclei were examined the uptake of radioactive steroid into the nucleus was decreased in the presence of the oestrogens. However, whether this represents an effect of oestrogens on the binding of 5α-dihydrotestosterone to protein receptor (Fang & Liao, 1969) or the result of changes in the cytoplasmic metabolism of steroids induced by the compounds is not shown by these experiments. These compounds have no effect on the conversion of testosterone into 5α-dihydrotestosterone by rat nuclear preparations (Griffiths *et al.* 1970). Other metabolites of testosterone isolated from the rat prostate cultures were also examined. 5α-Androstane-diols were formed by the whole tissue, but the 17β-diols were formed in approx. 4 times the quantity of the 17α-diols. Mean specific radioactivities in the whole tissue were 43 and 41 d.p.m./nmol for the 3α,17β-diol and 3β,17β-diol respectively, and 3 and 15 d.p.m./nmol for the 3α,17α-diol and 3β,17α-diol respectively.

Baulieu *et al.* (1968a,b) showed in tissue-culture experiments with rat prostate explants that, although 5α-dihydrotestosterone maintained the cells as well as testosterone, it also caused some hyperplasia, whereas the metabolite 5α-androstane-3β,17β-diol produced hypertrophy of the gland without hyperplasia. 5α-Dihydrotestosterone has been implicated in the aetiology of prostatic hyperplasia in both men and dogs (Siiteri & Wilson, 1970; Gloyna, Siiteri & Wilson, 1970). Of interest are the results with dibutylidihydrostilboestrol, previously

shown to affect prostatic DNA polymerase (Harper, Fahmy, Pierrepont & Griffiths, 1970), which seems as effective as diethylstilboestrol in interfering with prostatic metabolism of testosterone and yet possesses little oestrogenic activity. This compound could possibly be of value in the treatment of prostatic tumours.

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