Androgen Dynamics *in vitro* in the Human Prostate Gland

EFFECT OF CYPROTERONE AND CYPROTERONE ACETATE

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Hyperplastic and adenocarcinomatous human prostatic tissue was superfused *in vitro* with radioactively labelled androst-4-ene-3,17-dione, testosterone and 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androst-3-one), with and without addition of the anti-androgens cyproterone and cyproterone acetate. Cyproterone competitively inhibited the entry of the androgens into the majority of the tissues, whereas cyproterone acetate increased this entry. These findings indicated that transport of androstenedione, testosterone and 5 α -dihydrotestosterone into prostatic tissue is performed by a specific mechanism, possibly involving a carrier situated in the cell membrane. The extent of metabolism of the three androgens was also modified: formation of 5 α -dihydrotestosterone from testosterone, and of the latter from androstenedione, was decreased by cyproterone and increased by the acetate. Acetate was more effective than cyproterone in decreasing the 'uptake' of the perfused androgens by the tissue; at the same time, it increased the androgen clearance from the tissue. As cyproterone acetate is the more potent of the two anti-androgens, the possibility that these findings *in vitro* are related to the different anti-androgenic potency exhibited by the two compounds *in vivo* is discussed. 'Uptake' of the two anti-androgens and the response to their action on androgen dynamics were similar in adenocarcinomatous and hyperplastic glands.

Cyproterone (6-chloro-17-hydroxy-1,2 α -methylene-pregna-4,6-diene-3,20-dione) and cyproterone acetate (17-acetoxy-6-chloro-1,2 α -methylene-pregna-4,6-diene-3,20-dione) are powerful anti-androgens, which exert multiple actions in many species. Cyproterone acetate has three times the anti-androgenic potency of cyproterone, and also has some progestational properties (for review, see Neumann *et al.*, 1970). Administration of these two compounds to male dogs and rats *in vivo* leads to a decrease in the size and function of the accessory sexual organs, particularly of the prostate gland (Neri *et al.*, 1968; Belham *et al.*, 1969; Fang & Liao, 1969; Geller *et al.*, 1969); some response has also been observed in the adenocarcinomatous prostate of man (Geller *et al.*, 1968). Further, cyproterone and cyproterone acetate diminish the retention of testosterone by the rat ventral prostate *in vivo* (Belham *et al.*, 1969; Fang & Liao, 1969; Geller *et al.*, 1969). Experiments with homogenates of rat ventral prostate and seminal vesicles have shown that the diminished retention is due to competition of the two anti-androgens with testosterone and 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androst-3-one) for binding sites on specific 'receptors' or, alternatively, to interference with the stability of the androgen-receptor complex (Belham *et al.*, 1969; Fang & Liao, 1969; Fang *et al.*, 1969; Stern & Eisenfeld, 1969). Cyproterone and cyproterone acetate may also decrease the stability of enzyme-substrate complexes (Breuer & Hoffmann, 1967).

We have previously reported investigations of androgen-tissue interaction *in vitro* in human and canine prostate, by means of a new experimental design. This design permits the separate measurement of the fraction of perfused steroids that is transported into the tissue (i.e. 'entry'), the extent of retention in the tissue, or 'uptake', and the removal of the steroid from the tissue, either by release or metabolism (Giorgi *et al.*, 1971, 1972a,b). Evidence has been obtained that the mechanism of androgen transport has some specificity. It has also been shown that hyperplastic human prostates are characterized by an excessive binding of the androgens, probably of a non-specific nature, which causes increased and protracted retention of the steroids in the tissue. The present paper describes the effect of cyproterone and cyproterone acetate on the dynamics of 'uptake' and metabolism of androgens in human hyperplastic and adenocarcinomatous prostates *in vitro*. The 'uptake' of the two anti-androgens *in vitro* was also measured in two hyperplastic and two adenocarcinomatous prostates.

Materials and Methods

Tissues studied

Details of the patients and the prostatic tissues investigated are summarized in Table 1. The prostates were removed by retropubic operation. Those from

Table 1. *Details of patients*

Expt. no.	Patient	Age (years)	Wt. of prostatic tissue removed at operation (g)	Histology
1, 2	J. R.	60	55	Nodular hyperplasia of the glandular and fibromuscular tissue
3	F. C.	68	48	Simple hyperplasia of the glandular and fibromuscular tissue
4	Ja. B.	84	30	Many areas of poorly differentiated carcinoma of prostatic origin
5, 15	H. I.	70	90	Most of the tissue consists of poorly differentiated carcinoma of prostatic origin
6	Th. S.	56	19	Simple hyperplasia of the glandular tissue
7	J. D.	61	42	Nodular hyperplasia of the glandular and fibromuscular tissue
8	R. P.	59	40	Nodular hyperplasia of the glandular and fibromuscular tissue
9	D. McA.	66	40	Nodular hyperplasia of the glandular and fibromuscular tissue
10	H. McC.	64	22	Simple hyperplasia of the glandular and fibromuscular tissue with areas of acute and chronic inflammation
11	R. S.	80	40	Simple hyperplasia of the glandular and muscular tissue
12	R. R.	65	80	Nodular hyperplasia of the glandular and fibromuscular tissue
13	G. A.	86	33	Nodular hyperplasia of the glandular and fibromuscular tissue
14	R. J.	66	54	Nodular hyperplasia of the glandular and fibromuscular tissue
16	R. C.	69	40	Nodular hyperplasia of the glandular and fibromuscular tissue; well-marked cystic change
17	J. F.	74	40	Nodular hyperplasia of the glandular and fibromuscular tissue; well-marked cystic change

patients Ja. B., H. I. and D. McA. showed areas of poorly differentiated carcinoma of prostatic origin; the others had the typical features of simple or nodular hyperplasia, with prevalence of fibromuscular over glandular tissue.

Radioactive steroids

[4-¹⁴C]Testosterone (specific radioactivity 58.2 mCi/mmol), [7 α -³H]androstenedione ([7 α -³H]-androst-4-ene-3,17-dione) (specific radioactivity 3.23 Ci/mmol) and 5 α -dihydro[1,2-³H]testosterone (specific radioactivity 33.3 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. [1,2 α -methylene-¹⁴C]Cyproterone (specific radioactivity 2.3 mCi/mmol) and [1,2 α -methylene-¹⁴C]cyproterone acetate (specific radioactivity 21.82 mCi/mmol) were gifts from Schering Chemicals Ltd., Burgess Hill, Sussex, U.K. Carrier steroids were purchased from Steraloids Ltd., Croydon,

Surrey, U.K. The radioactive and carrier steroids were purified before use, as described by Giorgi *et al.* (1971).

Experimental design

The experimental design was essentially that proposed by Gurpide & Welch (1969). A solution containing [³H]androstenedione, [¹⁴C]testosterone and 5 α -dihydro[³H]testosterone is superfused over tissue slices at a constant flow of 0.33 ml/min for 90 min, at which time a steady state is attained. The radioactive steroids are then measured in the tissue, perfusates and perfusing medium.

Details of the superfusion technique, purification of radioactive steroids in the samples and calculations have been reported previously (Giorgi *et al.*, 1971). [¹⁴C]Cyproterone or [¹⁴C]cyproterone acetate were superfused at a concentration of 9000 d.p.m./ml (2.63 and 0.37 μ mol of cyproterone and cyproterone

acetate/litre respectively); suitable amounts of unlabelled compounds were added to some of the media to obtain the concentrations shown in Table 2.

Results

In all the experiments reported, steroid concentration and isotope ratio were measured in the two perfusates collected 31–60min and 61–90min from the start of superfusion. There was no significant difference between the steroid concentration and isotope ratio in the two perfusates ($P>0.045$), and therefore it was concluded that in all the experiments, and for all the steroids, steady state had been attained (Giorgi *et al.*, 1971).

‘Uptake’ of [¹⁴C]cyproterone and [¹⁴C]cyproterone acetate by human prostate slices

The ‘uptake’ of the two anti-androgens was measured in two hyperplastic and two adenocarcinomatous glands. Samples of tissue slices of similar weight from the two hyperplastic glands were superfused with medium containing increasing amounts of cyproterone (Expt. 1) or cyproterone acetate (Expts. 2 and 3). The results are reported in Table 2. There was no variation in the ‘uptake’ of cyproterone within the concentration range in the medium from 2.63 to 17.49 μmol/litre. With cyproterone acetate, there were noticeable variations at the middle concentration of 15.74 μmol/litre. Although the significance of these variations was not clear, it was evident that the ‘uptake’ of both compounds increased almost proportionally with the concentration in the perfusing medium. Table 2 also shows that the ‘uptake’ of the two anti-androgens by the adenomatous tissues

(Expts. 4 and 5) was not significantly different from that observed in the hyperplastic glands.

Effect of addition of cyproterone on the entry, ‘uptake’, release and metabolism of androstenedione, testosterone and 5α-dihydrotestosterone by human prostate slices

To assess the effect of cyproterone on the dynamics of androgen ‘uptake’ and metabolism by prostate tissue, paired superfusions with two samples of slices from the same gland were performed. The two perfusing media contained the same concentration of [³H]androstenedione, [¹⁴C]testosterone and 5α-dihydro[³H]testosterone; one medium contained in addition unlabelled cyproterone (see Tables 3 and 4). In Expts. 8 and 11, three superfusions were performed, two with different concentrations of cyproterone in the medium. The gland used in Expt. 9 was adenocarcinomatous; the other five glands were hyperplastic. The results are reported in Tables 3 and 4 and in Fig. 1.

As observed previously (Giorgi *et al.*, 1971, 1972a), a similar portion of the three androgens entered the tissue and was irreversibly metabolized, and the ‘uptake’ of 5α-dihydrotestosterone was much greater than that of either androstenedione or testosterone (Table 3). Although these two steroids were never released unchanged into the medium, 5α-dihydrotestosterone was released in sizeable amounts in every experiment. Table 3 also shows that cyproterone at a concentration 24 times that of the androgens (Expts. 6–9) decreased the entry of androstenedione and testosterone in three experiments and the entry of 5α-dihydrotestosterone in all four. When the concentration of cyproterone and of the androgens in the

Table 2. ‘Uptake’ of [¹⁴C]cyproterone and [¹⁴C]cyproterone acetate by human prostate slices *in vitro*
The weight of the tissues was 0.456±0.040g. ‘Uptake’ is the ratio of concentration of the perfused compound in the tissue and in the perfusing medium.

Expt. no.	Compound superfused and concn. (μmol/litre)	‘Uptake’ of cyproterone	‘Uptake’ of cyproterone acetate
1 (Hyperplastic tissue)	Cyproterone 2.63	4.792	
	Cyproterone 8.74	4.589	
	Cyproterone 17.49	4.685	
2 (Hyperplastic tissue)	Cyproterone acetate 2.36		4.645
	Cyproterone acetate 15.74		9.763
	Cyproterone acetate 23.61		6.745
3 (Hyperplastic tissue)	Cyproterone acetate 2.36		9.602
	Cyproterone acetate 15.74		5.425
	Cyproterone acetate 23.61		8.184
4 (Adenocarcinoma)	Cyproterone 2.65	3.897	
	Cyproterone acetate 2.38		4.429
5 (Adenocarcinoma)	Cyproterone acetate 2.38		3.367

Table 3. *Effect of cyproterone on the entry, 'uptake', release and irreversible metabolism of [³H]androstenedione, [¹⁴C]testosterone and 5 α -dihydro-[³H]testosterone by human prostate slices in vitro*

The weight of the tissues was 0.511 ± 0.029 g. Value in *italics* indicate significant differences ($P < 0.0027$) between results with and without cyproterone. α , Fraction of perfused steroid entering the tissue; 'uptake', ratio of the concentration of the perfused steroid in the tissue and in the perfusing medium; β , fraction of the perfused steroid released unchanged from the tissue; *im*, fraction of the perfused steroid irreversibly metabolized. The subscripts A, T and DHT indicate androstenedione, testosterone and 5 α -dihydrotestosterone respectively.

Expt. no.	Concn. (μ mol/litre)		[³ H]Androstenedione				[¹⁴ C]Testosterone				5 α -Dihydro[³ H]testosterone			
	Androgen	Cyproterone	α_A	'Uptake' _A	β_A	<i>im</i> _A	α_T	'Uptake' _T	β_T	<i>im</i> _T	α_{DHT}	'Uptake' _{DHT}	β_{DHT}	<i>im</i> _{DHT}
6 (Hyperplastic tissue)	0.11	0	0.209	0.429	0	0.209	0.188	0.887	0	0.188	0.293	6.634	0.090	0.203
7 (Hyperplastic tissue)	0.11	2.63	<i>0.164</i>	0.481	0	<i>0.164</i>	<i>0.088</i>	0.878	0	0.088	<i>0.150</i>	6.687	<i>0.058</i>	<i>0.108</i>
	0.11	0	0.152	0.324	0	0.152	0.151	0.406	0	0.151	0.231	1.963	0.044	0.187
8a } (Hyperplastic tissue) {	0.11	2.63	0.158	<i>0.157</i>	0	0.158	0.172	<i>0.182</i>	0	0.172	<i>0.084</i>	2.240	0.058	<i>0.025</i>
	0.11	0	0.215	0.770	0	0.215	0.170	0.935	0	0.170	0.315	5.004	0.148	0.167
8b } (Hyperplastic tissue) {	0.11	0.09	0.233	1.399	0	0.233	0.191	1.221	0	0.191	0.314	4.224	0.141	0.173
8c } (Hyperplastic tissue) {	0.11	2.63	<i>0.147</i>	0.781	0	<i>0.147</i>	0.157	1.234	0	0.157	<i>0.212</i>	4.328	0.105	<i>0.106</i>
9 (Adenocarcinoma)	0.11	0	0.213	0.593	0	0.213	0.257	0.583	0	0.257	0.327	2.974	0.110	0.218
	0.11	2.63	<i>0.103</i>	0.491	0	<i>0.103</i>	<i>0.121</i>	0.584	0	<i>0.121</i>	<i>0.111</i>	2.890	<i>0.054</i>	<i>0.057</i>
10 (Hyperplastic tissue)	1.10	0	0.145	1.024	0	0.145	0.161	1.511	0	0.161	0.156	3.372	0.050	0.106
	1.10	17.49	0.141	<i>0.715</i>	0	0.141	0.144	<i>1.021</i>	0	0.144	0.178	<i>5.022</i>	0.043	<i>0.135</i>
11a } (Hyperplastic tissue) {	1.10	0	0.182	0.784	0	0.182	0.192	0.955	0	0.192	0.460	2.794	0.052	0.408
	1.10	8.74	0.179	1.219	0	0.179	0.219	1.581	0	0.219	0.547	4.273	0.055	0.492
11c } (Hyperplastic tissue) {	1.10	17.49	0.172	0.340	0	0.172	<i>0.128</i>	<i>0.527</i>	0	<i>0.128</i>	0.268	<i>1.539</i>	<i>0.021</i>	<i>0.247</i>

medium was raised to 17.49 and 1.10 $\mu\text{mol/litre}$ respectively (a ratio of 16:1) entry of androstenedione (α_A) was not affected, whereas that of testosterone (α_T) and dihydrotestosterone (α_{DHT}) were decreased in one experiment (Expt. 11c). The 'uptake' of androstenedione and testosterone was lower in the presence of cyproterone in one experiment at the lower androgen concentration (Expt. 7) and in the two experiments at the higher concentration (Expts. 10 and 11c). Apart from Expt. 11c, the 'uptake' of 5 α -dihydrotestosterone was not modified significantly by the anti-androgen. 5 α -Dihydrotestosterone was released in smaller amounts from the tissue in the presence of cyproterone in four cases (Expts. 6, 8c, 9 and 11c). As α_{DHT} was decreased proportionally more than the release of this steroid from the tissue (β_{DHT}), a greater portion of the steroid entering the tissue was returned unchanged to the medium. The irreversible metabolism of the three androgens was significantly diminished by cyproterone in most cases, and there were also qualitative changes in metabolism (Table 4).

It is noteworthy that cyproterone at a concentration similar to those of the androgens (Expt. 8b), or approximately seven times that of the androgens (Expt. 11b), had little or no effect on any of the parameters measured. In the same tissues, a greater concentration of cyproterone changed entry, 'uptake' and metabolism as described above (Expts. 8c and 11c). Thus the effect of cyproterone appeared to be

dependent on its concentration relative to that of the androgens. No difference in the action of the anti-androgen on the adenocarcinomatous tissue (Expt. 9) with respect to the hyperplastic tissues was evident.

Table 4 reports the conversion factor of androstenedione into testosterone (ρ_{AT}), of testosterone into androstenedione (ρ_{TA}) and of testosterone into 5 α -dihydrotestosterone (ρ_{TDHT}), measured in Expts. 6–11. The rate of intracellular formation (ng/min) of 5 α -dihydrotestosterone from testosterone (ν_{TDHT}) is also shown in Table 4. In all the tissues, ρ_{TDHT} was much greater than ρ_{AT} or ρ_{TA} , both in the presence and in the absence of cyproterone. There were variations in the interconversion of testosterone and androstenedione due to cyproterone, but they were not constant. In two cases (Expts. 6 and 9) the equilibrium was altered from a value favouring testosterone formation to an equilibrium favouring androstenedione formation. The conversion factor ρ_{TDHT} was significantly decreased by cyproterone in three cases (Expts. 7, 8c and 9). Also, as mentioned above, cyproterone decreased testosterone entry in most experiments. In consequence, the rate of formation of 5 α -dihydrotestosterone was decreased in superfusions with the anti-androgen (Expts. 6, 7, 8c, 9, 10 and 11b,c), irrespective of the variations of the conversion factor ρ_{TDHT} . Similarly, the rates of formation of testosterone and androstenedione were diminished in most experiments.

Table 4. *Effect of cyproterone on the interconversion in vitro by human prostate slices of labelled testosterone and androstenedione, and on the formation of labelled 5 α -dihydrotestosterone from testosterone*

The weight of the tissue was 0.511 ± 0.029 g. Values in *italics* indicate significant differences ($P < 0.0027$) between results with and without cyproterone. ρ_{AT} , Conversion factor of androstenedione into testosterone; ρ_{TA} , conversion factor of testosterone into androstenedione; ρ_{TDHT} , conversion factor of testosterone into 5 α -dihydrotestosterone; ν_{TDHT} , rate of formation of 5 α -dihydrotestosterone from testosterone.

Expt. no.	Concn. ($\mu\text{mol/litre}$)		Conversion factors			ν_{TDHT} (ng/min)
	Androgen	Cyproterone	ρ_{AT}	ρ_{TA}	ρ_{TDHT}	
6 (Hyperplastic tissue)	0.11	0	0.095	0.053	1.145	2.37
	0.11	2.63	0.077	0.152	1.109	1.26
7 (Hyperplastic tissue)	0.11	0	0.097	0.062	0.919	1.52
	0.11	2.63	0.111	0.047	0.306	0.58
8a } 8b } (Hyperplastic tissue) { 8c }	0.11	0	0.011	0.047	1.046	1.95
	0.11	0.09	0.011	0.017	0.961	2.12
	0.11	2.63	0.014	0.011	0.698	1.20
	0.11	0	0.111	0.048	0.875	2.47
9 (Adenocarcinoma)	0.11	0	0.032	0.183	0.599	0.80
	0.11	2.63	0.034	0.077	0.684	12.08
10 (Hyperplastic tissue)	1.10	0	0.001	0.047	0.872	13.79
	1.10	17.49	0.022	0.038	0.764	16.10
11a } 11b } (Hyperplastic tissue) { 11c }	1.10	0	0.014	0.021	0.565	13.50
	1.10	8.74	0.011	0.023	0.839	11.78
	1.10	17.49

Fig. 1. Intracellular clearance (g/min) of (a) [^3H]androstenedione, (b) [^{14}C]testosterone and (c) 5α -dihydro[^3H]testosterone with and without addition of cyproterone in human prostate slices

□, Intracellular clearances of androstenedione (IC_A), testosterone (IC_T) and 5α -dihydrotestosterone (IC_DHT) in the absence of cyproterone; ■, intracellular clearances in the presence of cyproterone. The number of each experiment is shown at the foot of the figure. HYPER, Hyperplastic tissue; CA, adenomatous carcinoma. In Expts. 6–9, the concentration of the androgens was $0.11\text{ }\mu\text{mol/litre}$ and that of cyproterone was $2.63\text{ }\mu\text{mol/litre}$; in Expts. 10 and 11, the concentration of androgens and cyproterone was 1.10 and $17.49\text{ }\mu\text{mol/litre}$ respectively.

The intracellular clearance of androstenedione (IC_A), testosterone (IC_T) and 5α -dihydrotestosterone (IC_DHT), calculated from the data in Tables 3 and 4, are illustrated in Fig. 1: IC_A and IC_T were always much greater than IC_DHT in the same tissue. The addition of cyproterone to the perfusing medium led to variations in the clearance of the three androgens. The intracellular clearance expresses the relationship between the amount of compound entering and being formed in the tissue, and the amount retained. As cyproterone diminished the entry and intracellular formation of the steroids in most instances, without decreasing substantially the retention, the clearances either became lower or remained

unchanged. There was an increase in IC_A or IC_T only in two experiments (Expts. 7 and 11c).

Effect of addition of cyproterone acetate on the entry, 'uptake', release and metabolism of androstenedione, testosterone and 5α -dihydrotestosterone by human prostate slices

Experiments involving paired superfusions of samples of slices from the same gland were also performed with medium containing [^3H]androstenedione, [^{14}C]testosterone and 5α -dihydro[^3H]testosterone, with or without addition of unlabelled cyproterone acetate. In Expts. 16 and 17, an additional

Table 5. Effect of cyproterone acetate on the entry, 'uptake', release and irreversible metabolism of [³H]androstenedione, [¹⁴C]testosterone and 5α-dihydro[³H]testosterone by human prostate slices *in vitro*

The weight of the tissues was 0.439±0.045 g. Values in *italics* indicate significant differences (*P*<0.0027) between results with and without cyproterone acetate. α, Fraction of perfused steroid entering the tissue; 'uptake', ratio of the concentration of the perfused steroid in the tissue and in the perfusing medium; β, fraction of the perfused steroid released from the tissue; *im*, fraction of the perfused steroid irreversibly metabolized. The subscripts A, T and DHT indicate androstenedione, testosterone and 5α-dihydrotestosterone respectively.

Expt. no.	Concn. (μmol/litre)		[³ H]Androstenedione				[¹⁴ C]Testosterone				5α-Dihydro[³ H]testosterone			
	Androgen	Cyproterone acetate	α _A	'Uptake' _A	β _A	<i>im</i> _A	α _T	'Uptake' _T	β _T	<i>im</i> _T	α _{DHT}	'Uptake' _{DHT}	β _{DHT}	<i>im</i> _{DHT}
12 (Hyperplastic tissue)	0.11	0	0.077	0.437	0	0.077	0.126	0.570	0	0.126	0.110	2.657	0.022	0.088
13 (Hyperplastic tissue)	0.11	2.36	0.123	0.203	0	0.123	0.074	0.231	0	0.074	0.116	2.034	0.020	0.096
	0.11	0	0.216	0.130	0	0.216	0.176	0.154	0	0.176	0.156	1.289	0.038	0.118
14 (Hyperplastic tissue)	0.11	2.36	0.232	0.106	0	0.232	0.254	0.148	0	0.254	0.293	3.212	0.126	0.168
	0.11	0	0.152	0.405	0	0.152	0.097	0.653	0	0.097	0.162	4.175	0.063	0.099
15 (Adenocarcinoma)	0.11	2.36	0.126	0.212	0	0.126	0.161	0.250	0	0.161	0.169	2.027	0.039	0.130
	0.11	0	0.064	1.719	0	0.064	0.064	1.719	0	0.064	2.461	3.583		
16a	0.11	2.36	0.085	2.247	0	0.085	0.085	2.247	0	0.085	0.147	3.102	0.040	0.106
16b } (Hyperplastic tissue)	1.10	0	0.095	1.079	0	0.095	0.067	0.735	0	0.067	0.227	2.310	0.033	0.194
16c } (Hyperplastic tissue)	1.10	15.74	0.154	0.845	0	0.154	0.177	0.756	0	0.177	0.208	3.098	0.040	0.168
17a } (Hyperplastic tissue)	1.10	23.61	0.129	0.639	0	0.129	0.163	0.732	0	0.163	0.289	2.974	0.034	0.255
	1.10	0	0.064	0.668	0	0.064	0.067	1.365	0	0.067	0.256	3.385	0.054	0.202
17b } (Hyperplastic tissue)	1.10	15.74	0.113	1.109	0	0.113	0.053	1.711	0	0.053	0.400	4.861	0.125	0.275
17c } (Hyperplastic tissue)	1.10	23.61	0.108	1.552	0	0.108	0.149	2.195	0	0.149				

Table 6. *Effect of cyproterone acetate on the interconversion in vitro by human prostate slices of labelled testosterone and androstenedione, and on the formation of labelled 5 α -dihydrotestosterone from testosterone*

The weight of the tissues was 0.439 ± 0.045 g. Values in *italics* indicate significant differences ($P < 0.0027$) between results with and without cyproterone acetate. ρ_{AT} , Conversion factor of androstenedione into testosterone; ρ_{TA} , conversion factor of testosterone into androstenedione; ρ_{TDHT} , conversion factor of testosterone into 5 α -dihydrotestosterone; ν_{TDHT} , rate of formation of 5 α -dihydrotestosterone from testosterone.

Expt. no.	Concn. ($\mu\text{mol/litre}$)		Conversion factors			ν_{TDHT} (ng/min)
	Androgen	Cyproterone acetate	ρ_{AT}	ρ_{TA}	ρ_{TDHT}	
12 (Hyperplastic tissue)	0.11	0	0.027	0.015	0.511	0.71
	0.11	2.36	0.041	0.018	1.057	0.87
13 (Hyperplastic tissue)	0.11	0	0.020	0.075	0.603	1.16
	0.11	2.36	0.039	0.010	0.924	2.58
14 (Hyperplastic tissue)	0.11	0	0.046	0.209	1.083	1.15
	0.11	2.36	0.195	0.023	0.755	1.33
16a	1.10	0	0.046	0.094	1.098	7.37
16b	1.10	15.74	0.173	0.094	1.111	18.67
16c	1.10	23.61	0.195	0.080	0.705	12.64
17a	1.10	0	0	0.071	1.100	8.09
17b	1.10	15.74	0.021	0.076	1.100	6.41
17c	1.10	23.61	0.040	0.019	0.575	9.41

superfusion was performed with a third sample of slices. The concentration of the three androgens and of cyproterone acetate in each perfusing medium is shown in Tables 5 and 6.

From Table 5 it appears that the effect of cyproterone acetate on the entry of the three androgens was completely different from that of cyproterone. At both concentrations of androgen in the medium, 0.11 and $1.10 \mu\text{mol/litre}$, α_A was either increased (Expts. 12, 16b,c and 17b,c) or remained unchanged; α_T was increased in all but one superfusion, and α_{DHT} was increased in Expts. 13, 16c and 17b,c. 'Uptake' of androstenedione tended to be decreased by cyproterone acetate in all cases with the exception of Expt. 17b,c; 'uptake' of testosterone was decreased in two experiments (Expts. 12 and 14), whereas 'uptake' of 5 α -dihydrotestosterone was decreased in one experiment only (Expt. 14). In Expt. 17, the 'uptake' of the three androgens was increased by cyproterone acetate added in two different concentrations; 'uptake' of 5 α -dihydrotestosterone was also higher in Expt. 13. Significantly more 5 α -dihydrotestosterone was released from the tissue in these two experiments in the presence of the anti-androgen. The metabolism of the three androgens varied in parallel with their entry.

As shown in the last two experiments reported in Table 5, cyproterone acetate had the same effect on entry, 'uptake' and metabolism of the labelled steroids, at concentrations 14 and 20 times greater than that of the androgens. In one adenocarcino-

matous tissue (Expt. 15), the anti-androgen did not modify the 'uptake' of testosterone and 5 α -dihydrotestosterone and increased α_T , as in the other tissues.

Table 6 illustrates the qualitative changes induced by cyproterone acetate in the metabolism of the androgens. These changes were much more consistent than those observed with cyproterone. The conversion factor of androstenedione into testosterone (ρ_{AT}) became greater in all the experiments, whereas the conversion factor of testosterone into androstenedione (ρ_{TA}) decreased, especially in Expts. 13, 14 and 17c. The conversion factor of testosterone into 5 α -dihydrotestosterone (ρ_{TDHT}) was increased in two experiments at the lower androgen concentration in the medium (Expts. 12 and 13); in another one of these experiments (Expt. 14) and in the two experiments at the higher androgen concentration (Expts. 16c and 17c) ρ_{TDHT} was decreased. In the same tissues, a lower concentration of cyproterone acetate had no effect (Expts. 16b and 17b). However, owing to the increase in testosterone entry induced by the anti-androgen, the rate of formation of 5 α -dihydrotestosterone from testosterone (ν_{TDHT}) was always augmented. Also the rate of formation of testosterone from androstenedione increased in all the experiments.

The intracellular clearance of the three androgens in the presence and absence of cyproterone acetate is shown in Fig. 2. The anti-androgen increased IC_A and IC_T in four experiments (Expts. 12, 13, 14 and 16c) and IC_{DHT} in three. In the other cases, the

Fig. 2. Intracellular clearance (g/min) of (a) [^3H]androstenedione, (b) [^{14}C]testosterone and (c) 5α -dihydro[^3H]testosterone with and without addition of cyproterone acetate in human prostate slices

□, Intracellular clearances of androstenedione (IC_A), testosterone (IC_T) and 5α -dihydrotestosterone (IC_DHT) in the absence of cyproterone acetate; ■, intracellular clearances in the presence of cyproterone acetate. The number of each experiment is shown at the foot of the figure. HYPER, Hyperplastic tissue. In Expts. 12–14, the concentration of the androgens was $0.11\text{ }\mu\text{mol/litre}$ and that of cyproterone acetate was $2.36\text{ }\mu\text{mol/litre}$; in Expts. 16 and 17, the concentration of androgens and cyproterone acetate was 1.10 and $23.6\text{ }\mu\text{mol/litre}$ respectively.

clearances were practically unchanged, with the exception of IC_A in Expt. 17c. These findings showed that the changes in retention and removal of the androgens caused by cyproterone acetate were proportionally greater than the simultaneous increase of their entry and rate of intracellular formation.

Discussion

We have reported that oestradiol- 17β and androstenedione can interfere with the entry of testo-

sterone and 5α -dihydrotestosterone into human and canine prostate tissue *in vitro* (Giorgi *et al.*, 1972a,b). The present finding that cyproterone and cyproterone acetate also influence the entry of androgens into prostate slices supports the view that a specific mechanism is responsible for the transport of steroids into prostate tissue. Caution must be exercised in extending our observations *in vitro* to the situation *in vivo*. Because of the low specific radioactivity of the ^{14}C -labelled androgens, we had to use $0.11\text{ }\mu\text{mol}$ of androgen/litre in the superfusing medium. The

concentration of unbound testosterone in male plasma is about $0.1 \times 10^{-3} \mu\text{mol/litre}$ (Vermeulen *et al.*, 1971). If it is assumed that the concentration in the fluid bathing the cells *in vivo* is of the same order of magnitude as that of unbound steroid in plasma, the cells in our experiments were exposed to much higher concentrations of steroids than they normally are. As discussed by Stein (1967), when solutes are transported into cells by specific systems, such as for instance facilitated diffusion, results obtained at high concentrations might not be representative of those obtained at lower concentrations.

We find that cyproterone and cyproterone acetate have completely opposite effects on the transport of androgens into the prostate slices. Cyproterone inhibited entry of the androgens only when its concentration in the medium was much higher than that of the androgens. This might indicate competition between the two types of compounds for some membrane component. On the other hand, the greater entry of the androgens in the presence of cyproterone acetate could be explained as co-transport. Competition and co-transport entail the existence of a specific carrier in the cellular membrane (Stein, 1967). The existence of such a carrier would be compatible with the finding, reported by Hubbell *et al.* (1970), that in the erythrocyte membrane steroids are bound both to the protein and the lipid component.

We observe in contrast with others working with the rat (Belham *et al.*, 1969; Fang *et al.*, 1969; Geller *et al.*, 1969) that cyproterone and cyproterone acetate have no constant effect on the 'uptake' of androstenedione, testosterone and 5α -dihydrotestosterone by hyperplastic human prostate slices. It might be that the two anti-androgens are effective only on 'specific' binding of the three steroids. This effect could be masked in our experiments, in which, owing to the high concentrations of androgens used, 'non-specific' binding was probably predominant. The discrepancy between our results and those reported for the rat ventral prostate might also depend on the type of tissue studied. However, the acetate increased androgen metabolism, as well as release, in the tissue slices, and androgen clearances became greater. On the contrary, the alcohol decreased the metabolism and either left the androgen clearances unchanged or decreased them. From this it could be speculated that the greater anti-androgenic effect of cyproterone acetate, as compared with cyproterone, observed *in vivo*, resides in its ability to accelerate removal of androgens from target tissues.

Cyproterone seemed to decrease the activity of 17α -hydroxysteroid dehydrogenase and of 5α -steroid reductase in human prostate *in vitro*, as it does in testes and liver of rats (Breuer & Hoffmann, 1967; Hoffmann & Breuer, 1968; Denef *et al.*, 1968).

Cyproterone acetate did not seem to have any direct effect on the activity of these two enzymes.

Finally, there was no evident difference between adenocarcinomatous and hyperplastic glands with regard to the 'uptake' of the two anti-androgens. The effect of these two compounds on entry, 'uptake' and metabolism of androstenedione, testosterone and 5α -dihydrotestosterone was also similar in the two types of tissue.

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