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IN VITRO [7-^{3}H]-TESTOSTERONE METABOLISM BY THE RAT VENTRAL PROSTATE—EFFECT OF SOME EXOGENOUS COMPOUNDS*

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SUMMARY

Fragments of rat ventral prostate were incubated *in vitro* with [7-^{3}H]-testosterone and the effect of some compounds on testosterone metabolism in this system was studied. Progesterone, 17-hydroxyprogesterone and ethynylestradiol inhibited the formation of dihydrotesterone (DHT), 3α-androstanediol, 5α-androstanedione and androsterone. There was a concomitant apparent stimulation of 17β-hydroxysteroid dehydrogenase activity. Medroxyprogesterone acetate (MPA) had no effect on formation of DHT but inhibited formation of 3α-androstanediol. This inhibition of 3α-hydroxysteroid dehydrogenase activity was confirmed when [4-^{14}C]-DHT was incubated with the prostatic tissue in the presence of MPA. Prostaglandin E_2 or the presumed absence of prostaglandins had no effect on 5α-reduction of testosterone in this system.

INTRODUCTION

It has recently been recognized that testosterone is metabolized in its target tissues. Thus, in the ventral prostate, dihydrotestosterone (DHT), 3β and 3α androstanediols, androstenedione, androsterone and 5α-androstanedione have been identified as testosterone metabolites [1]. Of these, DHT and 3α-androstanediol (DIOL) which are formed by 5α-reduction of testosterone are the predominant ones while the rest which are formed *via* the 17-ketonic pathway are relatively minor ones. In other target organs like the human hair follicles metabolites formed *via* the 17-ketonic pathway are the principal testosterone metabolites [2]. Some of the testosterone metabolites may by themselves be biologically active and DHT is increasingly being considered as an active androgenic hormone at the tissue level in some target organs [1]. DIOL also has shown androgenic activity [3, 4]. Therefore, compounds that may modify testosterone metabolism in target organs have therapeutic implications and will also be useful in further elucidation of the mechanism of testosterone action at the tissue level. Some steroidal compounds like progesterone and its derivatives have already been shown to significantly inhibit the formation of DHT and DIOL from exogenous labelled testosterone in the target organs [5, 6]. However, the effect of such inhibitors of testosterone 5α-reductase on the other testosterone metabolites that are formed *via* the 17-ketonic pathway in target organs is not known.

Apart from compounds which inhibit the formation of all the 5α-reduced metabolites of testosterone it would be of interest to seek inhibitors that would selectively affect one of the 5α-reduced metabolites. Recently, Saenger *et al.*[7] have demonstrated that after incubating cultured human skin fibroblasts with [4-^{14}C]-testosterone in the presence of medroxyprogesterone acetate (MPA) there was a marked reduction in the formation of DIOL with a concomitant increase in DHT as compared to control incubations. Such selective inhibition in the formation of DIOL from testosterone by MPA has, however, not yet been demonstrated in the prostatic tissue. Although Massa and Martini[8] using fragments of rat ventral prostate have observed inhibition in the formation of 5α-reduced metabolites from testosterone by MPA their results are expressed as a sum of DHT and DIOL and they have not noted any selective inhibition in the formation of the latter. On the other hand, Albin *et al.*[9] observed that administration of MPA to rats did not result in changes in the 5α-reductase activity of the ventral prostate or in the pattern of metabolites formed when prostate homogenates from the MPA treated animals were incubated *in vitro* with [^{3}H]-testosterone. Thus, there was some need to further study the effect of MPA on testosterone metabolism in the ventral prostate.

In view of these considerations, using fragments of rat ventral prostate, we have studied *in vitro*: (1) the effect of MPA on [7-^{3}H]-testosterone metabolism and on the formation of DIOL from [4-^{14}C]-DHT; (2) the effect of progesterone and 17-hydroxyprogesterone (17

* The abbreviations and trivial names used are: 3α-androstanediol (DIOL): 5α-androstane-3α,17β-diol; 5α-androstanedione: 5α-androstane-3,17-dione; androstenedione: 4-androstene-3,17-dione; androsterone: 3α-hydroxy-5α-androstan-17-one; 5α-dihydrotestosterone (DHT): 17β-hydroxy-5α-androstan-3-one; ethynylestradiol: 17-ethynyl-1,3,5(10)-estratriene-3,17β-diol; 17-hydroxyprogesterone (17 OHP): 17-hydroxy-4-pregnene-3,20-dione; medroxyprogesterone acetate (MPA): 6α-methyl-17-hydroxy-4-pregnene-3,20-dione acetate; progesterone: 4-pregnene-3,20-dione; testosterone: 17β-hydroxy-4-androsten-3-one.

OHP) on [7-^{3}H]-testosterone metabolism by isolating metabolites of the 17β-hydroxy and the 17-ketonic pathways; and (3) the effect of presence or presumed absence of prostaglandins on 5α-reduction of [7-^{3}H]-testosterone. We wish to report here the results of these experiments.

MATERIALS AND METHODS

Steroids

[7-^{3}H]-Testosterone (S.A. 25 Ci/mmol), [4-^{14}C]-DHT (S.A. 50·6 mCi/mmol) and [1,2-^{3}HN]-DIOL (S.A. 44 Ci/mmol) were obtained from New England Nuclear, Boston, U.S.A., and purified before use by paper chromatography. Nonradioactive steroids were obtained from Sigma Chemical Co., St. Louis, U.S.A., and from Ikapharm, Ramat-Gan, Israel.

Detection and counting of radioactivity. Radioactivity on paper chromatograms and thin-layer plates was detected with the aid of a radiochromatogram scanner (Model 7290, Packard Instrument Company, Downer's Grove, Ill.). Counting of ^{3}H and ^{14}C content of samples was done on a 3-channel liquid scintillation spectrometer (Tri-Carb, Model 3375, Packard).

Incubations. Male Wistar rats (225 g) were sacrificed by decapitation and the ventral prostate was removed. The gland was cut into small pieces before incubation.

In testing the effect of a compound on metabolism of [7-^{3}H]-testosterone roughly equal portions of the same gland were randomly selected, weighed and distributed in two beakers. Each beaker contained approximately 4·4 × 10^6 d.p.m. of [7-^{3}H]-testosterone dissolved in 0·2 ml of propylene glycol, 5 ml of Krebs–Ringer bicarbonate buffer, pH 7·4, and 10 mg glucose. One beaker served as control where no exogenous steroid other than the substrate was added while in the other beaker a particular concentration of an exogenous steroid had been added. The beakers were then incubated in a Dubnoff metabolic shaking incubator at 37°C with air as the gas phase for 3 h. The incubations were terminated by addition of acetone and the beakers were kept in deep freeze until processed. In some experiments [4-^{14}C]-DHT, 5·5 × 10^5 d.p.m. was used as substrate, other conditions being the same as described above.

Various such incubations were done, ventral prostate gland from one animal being used for studying the effect of a compound at any one concentration. From the testosterone incubates, DHT, DIOL, androsterone, androstenedione and 5α-androstanedione were isolated. However, in some incubates (see Table 1) only DHT and DIOL were looked for. In experiments using [4-^{14}C]-DHT as substrate DIOL was isolated. The experimental procedure followed for the isolation of these compounds is described below.

Before extraction, 100 μg of nonradioactive steroids (that were to be looked for) were added to the incubate to obtain a measure of losses incurred during the isolation procedure. In some experiments this was done by adding appropriate amounts of radioactive steroids bearing isotope label different than the one in the substrate. Four volumes of ethanol acetone (1:1 v/v) mixture were then added to the incubate. After filtration of the supernatant through paper (Whatman no. 1) the paper and the beaker were washed with additional amounts of the solvent mixture. The tissue was then extracted with an identical mixture of ethanol–acetone in a glass homogenizer and the extract after filtering through the filter paper was combined with the first supernatant. Ethanol and acetone were removed in a rotary flash evaporator and the aqueous residue was extracted with methylene chloride (3 × 30 ml). The separation and isolation of metabolites from the methylene chloride extract was done by serial paper partition (PPC) and silica gel thin-layer chromatography (t.l.c., for systems A and C, precoated silica gel sheets with inorganic fluorescent indicator Type K 301 R, Eastman, and for system B, precoated silica gel sheets of 0·25 mm thickness, Merck). The following PPC systems were used: (I) Bush A, (II) hexane–propylene glycol, and (III) hexane–benzene (1:1 v/v)/propylene glycol. The t.l.c. systems used were: (A) chloroform–acetone (98:2 v/v), (B) chloroform–methanol (98·25:1·75 v/v) and (C) cyclohexane–ethyl acetate (60:40 v/v).

The methylene chloride extract was chromatographed in PPC system I and three areas corresponding to (1) DIOL + testosterone, (2) DHT + androstenedione + androsterone and (3) 5α-androstanedione were eluted.

Area 1. DIOL and testosterone were separated by PPC in system III for 7 h. Both the compounds were further purified by t.l.c. A.

Area 2. Androstenedione was separated from DHT and androsterone by t.l.c. B. It was then chromatographed in PPC II for 16 h. DHT and androsterone were isolated as separate fractions following rechromatography in PPC I.

Area 3. 5α-Androstanedione in this area was isolated by t.l.c. C.

Determination of recoveries. At the end of the isolation procedures, the recovery of each of the added carrier steroid and the radioactivity isolated along with it were determined. For the determination of mass of different steroids gas–liquid chromatography was used. The stationary phase was XE-60, other conditions were as described by Lehoux *et al.*[10]. The recovery of added steroids although variable was usually more than 40%.

Identification of isolated fractions. To the isolated fractions from each incubate additional amounts of the respective nonradioactive steroid were added and the fractions were crystallized to constant S.A. Radiochemical purity of a fraction was admitted when the specific activity of three consecutive crystal crops did not differ from their mean by more than 5%. Fractions containing a labelled steroid added after incubation were identified on the basis of constancy of isotope ratios on crystallization.

Calculation of the effect of an exogenous steroid on the formation of testosterone metabolites. The purity of an isolated metabolite fraction was calculated by comparison of the specific activity of that material before

Table 1. Effect of exogenous compounds on the *in vitro* metabolism of [7-^{3}H]-testosterone (approximately 4·4 × 10^{6} d.p.m., S.A. 25 Ci/mmol) by rat ventral prostate. Each value is expressed as a percentage of the corresponding control*

Expt. no.	Compound added in 5 ml incubation medium	Metabolite				
		DHT	DIOL	Androsterone	Androstenedione	5α-Androstanedione
	None	100·0	100·0	100·0	100·0	100·0
1	Progesterone (5 μg)	38·2	20·9	46·1	256·0	30·4
2	Progesterone (10 μg)	5·9	4·9	8·0	381·0	14·9
3	17-Hydroxyprogesterone (5 μg)	46·3	29·0	104·7	104·5	110·6
4	17-Hydroxyprogesterone (10 μg)	37·2	18·7	28·0	315·0	46·7
5	17-Hydroxyprogesterone (15 μg)	20·6	6·2	8·4	513·0	50·2
6	MPA (5 μg)	95·8	33·8	38·9	121·0	79·6
7	MPA (10 μg)	91·0	26·1	26·0	103·0	81·3
8	MPA (15 μg)	90·1	20·6	28·0	61·6	63·8
9	Ethynylestradiol (15 μg)	22·8	51·5	44·2	254·0	44·9
10	Cortisol (15 μg)	95·7	103·2	102·9	103·2	108·5
11	Prostaglandin E_2 (25 μg)	101·3	100·6	—	—	—
12	Indomethacin (100 μg)	96·2	99·5	—	—	—

* Percentage of conversion of the substrate to metabolites in control samples in the different experiments was variable and was of the following order: DHT, 20–30%; DIOL, 4–8%; androsterone, androstenedione and 5α-androstanedione, each 1–3%.

(pool) and after crystallization (mean of crystals). This, together with appropriate correction for losses estimated to have occurred during the experimental procedure, gave the actual amount (in terms of radioactivity) of the compound present at the end of the incubation. The d.p.m. value thus arrived at was then expressed per 100 mg of tissue. As mentioned earlier, in a set of two incubation beakers that were utilized per gland one served as control. The d.p.m./100 mg value in each metabolite fraction of the control beaker was considered as 100 and the d.p.m./100 mg value in the corresponding fractions of the experimental beaker was then expressed as per cent of this control value.

RESULTS AND DISCUSSION

Results of a series of 12 experiments in which the effect of exogenous compounds on the *in vitro* [7-^{3}H]-testosterone metabolism by rat ventral prostate was studied are shown in Table 1. Each value in the table is the result of a single experiment and is in comparison of its individual control. Steroids were tested at concentrations of 5–15 μg/5 ml of the incubation medium and prostaglandin E_2 and indomethacin at concentrations known to elicit their effects *in vitro*. In view of the experimental work involved in isolating multiple testosterone metabolites from both the control and experimental samples in each incubation, experiments were not done in duplicate. However, when a compound showed a significant effect on testosterone metabolism it was tested at different concentrations in further experiments. This not only provided confirmation of the effect of that compound but also showed that the changes from control were related to the concentration of that compound in the incubation medium (Table 1). Crystallization data for metabolites isolated in one experiment (Experiment 2, 10 μg progesterone) are given in Table 3. Isolated metabolites in other experiments were of similar radiochemical purity. Of the isolated [7-^{3}H]-testosterone metabolites, in control incubates DHT was the predominant one and accounted for 20–30% of the substrate. About 4–8% of testosterone was converted to DIOL. Androstenedione, 5α-androstanedione and androsterone were formed in lesser amounts (each about 1–3% of the substrate). Unutilized substrate was found in all of the incubates. 3β-Androstanediol was looked for but could not be isolated in any significant amounts.

As seen in Table 1, addition of MPA to the incubates did not result in any significant changes in the formation of DHT from [^{3}H]-testosterone. However, further

Table 2. Effect of exogenous compounds on the formation of 3α-androstanediol by rat ventral prostate after *in vitro* incubation with [4-^{14}C]-dihydrotestosterone (5·5 × 10^{5} d.p.m., S.A. 50·6 mCi/mmol). Each value is expressed as a percentage of corresponding control*

Expt. no.	Compound added in 5 ml incubation medium	3α-Androstanediol formed
	None	100·0
13	Medroxyprogesterone acetate (10 μg)	31·0
14	Medroxyprogesterone acetate (10 μg)	28·5
15	Medroxyprogesterone acetate (15 μg)	23·2
16	Medroxyprogesterone acetate (15 μg)	21·0
17	Ethynylestradiol (15 μg)	97·6

* Percentage of conversion of the substrate to DIOL in control samples in the different experiments was variable and was of the order of 10–12%.

Table 3. Crystallization data for radioactive metabolites isolated after incubation of rat ventral prostate with [7-^{3}H]-testosterone (appropriate amounts of carrier steroids were added before crystallization)

Compound identified	Beaker no.*	Pool S.A. [^{3}H]-d.p.m./mg	Crystals S.A. [^{3}H]-d.p.m./mg		
			C-3	C-4	C-5
Dihydrotestosterone	1	48,148	46,373	45,301	45,458
	2	3288	2843	2821	2816
3α-Androstanediol	1	17,375	13,508	14,064	13,843
	2	2327	1192	1247	1235
Androsterone	1	1755	1260	1277	1232
	2	123	80	84	81
Androstenedione	1	1220	804	828	824
	2	3114	2061	2134	1992
5α-Androstanedione	1	4950	4053	3856	4034
	2	540	420	439	428

* Beaker no. 1—control; beaker no. 2—containing 10 μg progesterone.

metabolism of DHT to DIOL was inhibited by MPA. There was also an inhibition in the formation of androsterone as compared to that of 5α-androstanedione through which it is derived. Thus, the inhibition in the formation of DIOL and androsterone shows that MPA inhibited testosterone metabolism at the 3α-hydroxysteroid dehydrogenase step without showing significant effect at the 5α-reduction step especially in the formation of DHT. There was, however, a slight inhibition in the formation of 5α-androstanedione. This inhibition of 3α-hydroxysteroid dehydrogenase by MPA was confirmed by incubating the prostate tissue with [4-^{14}C]-DHT in the absence and presence of added MPA (Table 2). Thus our observations and those of Saenger *et al.*[7] with cultured human skin fibroblasts show that in *in vitro* systems in the presence of adequate amounts of MPA there is a change from normal in the pattern of testosterone metabolism in target organs. Such selective inhibition of certain testosterone metabolites could be exploited in studying the mechanism of androgen action in *in vitro* systems. We may add that in the experiments of Albin *et al.*[9], referred to earlier, where no change in the pattern of prostatic testosterone metabolism was observed after administration of MPA to rats it is possible that the compound did not reach the prostate in amounts sufficient to elicit such changes.

From the studies of Briggs and Briggs[11] it appeared that another compound which may alter the pattern of testosterone metabolism in the prostate could be ethynylestradiol. They observed that infusion of this compound together with [^{3}H]-testosterone into human prostate gland reduced the amount of DHT formed but resulted in increased DIOL formation. In our experiment, however, addition of ethynylestradiol to prostate tissue incubates resulted in reduced conversion of [^{3}H]-testosterone to both DHT and DIOL (Table 1). Similarly after incubating [4-^{14}C]-DHT in the presence of ethynylestradiol there was no change in the formation of DIOL as compared to the control (Table 2). The reasons for our not observing stimulation of DIOL formation by ethynylestradiol are not clear. However, it may be that we tested the compound in *in vitro* conditions while Briggs and Briggs[11] had infused it into human prostate along with [^{3}H]-testosterone before excising the tissue.

As observed by earlier investigators [5, 6] addition of progesterone and 17 OHP to the incubates inhibited 5α-reduction of [^{3}H]-testosterone by the prostate as indicated by reduced formation of DHT and DIOL (Table 1). Similarly formation of 5α-androstanedione and androsterone was also inhibited in these samples. There was, however, an apparent stimulation of 17β-hydroxysteroid dehydrogenase activity in these incubates and androstenedione was found in larger amounts as compared to control incubates. This stimulation was also observed when a different type of compound, ethynylestradiol, inhibited 5α-reduction of testosterone (Table 1). Thus it appears that under conditions where 5α-reduction of testosterone is inhibited there would be a concomitant stimulation of 17β-hydroxysteroid dehydrogenase. This may be of more significance in target organs such as human hair follicles where androstenedione is the major testosterone metabolite [2]. Although this stimulation in androstenedione formation could be partly explained on the basis of inhibition in its further metabolism by 5α-reduction (Table 1) it is difficult to determine from the present experiments the exact mechanism of this stimulation. This is because the experiments were done with fragments of whole tissue, without addition of "cofactors" and in the presence of high concentrations of inhibitors.

The lack of effect of cortisol on the formation of any of the testosterone metabolites indicates that the experimental system used in our studies can be selective.

In view of the ubiquitous presence of prostaglandins we tested the effect of addition of one of the prostaglandins, PGE_2, on the *in vitro* metabolism of testosterone by rat ventral prostate. Essentially no change from control was detected in the 5α-reduction of [^{3}H]-testosterone (Table 1). Similarly, indomethacin in concentrations known to inhibit prostaglandin biosynthesis *in vitro* [12] had no effect. In another experiment, indomethacin (twice, 20 mg/kg [13]) was administered to two male rats. The ventral prostate tis-

sues from these animals were incubated *in vitro* with [^{3}H]-testosterone and the incubates were extracted as described before. Scanning of the initial paper chromatograms after PPC in system I did not reveal any changes from the control samples and hence the experiment was not followed further. It may be added that in all the cases reported in Table 1 where any significant changes in [^{3}H]-testosterone metabolism were observed scanning of chromatograms at the stage of the above-mentioned PPC reflected these changes.

Thus, using whole rat ventral prostate tissue we have demonstrated that MPA changed the normal pattern of *in vitro* testosterone metabolism in that tissue. Further, it was observed that steroidal inhibitors of testosterone 5α-reductase caused an apparent stimulation of 17β-hydroxysteroid dehydrogenase. Prostaglandin E_2 or the presumed absence of prostaglandins did not have any effect on 5α-reduction of testosterone in this system.

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