*Journal of Steroid Biochemistry*, 1977, Vol. 8, pp. 1109–1115. Pergamon Press. Printed in Great Britain

# METABOLISM *IN VITRO* OF DIHYDROTESTOSTERONE, 5α-ANDROSTANE 3α, 17β-DIOL AND ITS 3β-EPIMER, THREE METABOLITES OF TESTOSTERONE, BY THREE OF ITS TARGET TISSUES, THE ANTERIOR PITUITARY, THE MEDIAL BASAL HYPOTHALAMUS AND THE SEMINIFEROUS TUBULES*

LIDIA WEI LIU KAO†, ANIBAL PEREZ LLORET‡ and JUDITH WEISZ

Division of Reproductive Biology, Department of Obstetrics and Gynecology, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033, U.S.A.

(*Received* 7 *February* 1977)

**SUMMARY**

To examine the metabolism of three 5α-reduced derivatives of testosterone (T) by seminiferous tubules (ST), anterior pituitary (AP) and medial basal hypothalamus (MBH) of adult rats, these tissues were incubated with either tritiated dihydrotestosterone (DHT), 5α-androstan-3α, 17β diol (3α-adiol) or its 3β-epimer (3β-adiol). The products and residual precursors were quantified using $^{14}C$ labeled tracers for recovery. Both DHT and 3β-adiol were extensively metabolized. The % DHT metabolized ranged from 60% (MBH) to 93% (ST) with 3α-adiol forming the major product (ST = 69%, AP = 54%, MBH = 38%). The % 3β-adiol metabolized ranged from 68% (ST) to 90% (AP). The metabolism of 3β-adiol by ST differed from that by the other tissues; reversibility of 3β-oxidoreduction, with substantial accumulation of DHT (13%) and 3α-adiol (36%) occurred only with ST. With AP and MBH these metabolites were identifiable only with increased substrate concentrations. In all incubations with AP and MBH with 3β-adiol the bulk of the radioactivity was associated with some polar metabolites (AP = 76%, MBH = 62%). An inverse relationship between polar metabolite formation and 3β-oxidation was suggested by the much smaller amounts of such metabolites being formed by ST.

In all tissues the bulk of 3α-adiol remained unmetabolized. The % conversion of 3α-adiol to DHT was relatively high for AP and MBH (av = 27%) but low for ST (av = 7%).

## INTRODUCTION

The involvement of dihydrotestosterone (DHT), formed *in situ* from testosterone (T), in mediating the action of T on some of its target organs is now well established[1]. In addition, a limited amount of data are available suggesting that the 3α and 3β reduced metabolites of DHT can exert certain effects in some target tissues, and thus need also to be considered as possible mediators of some of the diverse androgenic effects of T[2, 3]. This proposition is based principally on the observation that the nature of the effects of the 3α and 3β reduced metabolites may differ from those of T or DHT. Thus, Robel *et al.*[2] have reported that in organotypic cultures of prostatic tissue, DHT promotes cell division while 5α-androstane-3α, 17β-diol (3β-adiol) increases the secretory capacity of the cells. In our own studies[3], we found that the release of LH and FSH by hypothalamic extract, from anterior pituitaries (AP) of male rats, *in vitro*, could be modified not only by T or DHT but also by the 5α-androstane-3α, 17β-diol (3α-adiol) or its 3β-epimer. Using a continuous flow incubation (perifusion) system to monitor trophic hormone release from the AP during successive ten-min periods over the course of several h, it was possible to demonstrate not only that all four steroids could alter pituitary responsiveness but also that there were certain reproducible differences between their effects on the sequence of changes in the amounts and ratios of FSH and LH released.

For the interpretation of the data from the *in vitro* experiments, such as those carried out by Robel *et al.* on the prostate[2], or by us on the AP[3], it is important to know the extent to which the metabolites presented to the isolated target tissues may undergo further metabolism and to characterize the nature of the products formed. Robel *et al.* cited as evidence for the independent action of 3β-adiol on

* Supported by Grant MH24279, The National Institutes of Health and by The Rockefeller Foundation.

† Postdoctoral Fellow, The Population Council.

‡ Postdoctoral Fellow, The Ford Foundation.

Present Address: Centro de Investigaciones en Reproducción, Facultad de Medicina, Paraguay 2155-Pisolo, Buenos Aires, Argentina.

The following trivial names are used in this paper: dihydrotestosterone (17β-hydroxy-5α-androstan-3-one,); testosterone, (17β-hydroxy-androsten-3-one,); androsterone, (3α-hydroxy-5α-androstan-17-one,); epiandrosterone, (3α-hydroxy-5α-androstan-17-one,); androstanedione, (5α-androstane-3, 17-dione); androstenedione, (4-androstene-3, 17-dione); estradiol, (1,3,5(10)-estratrien-3,17β-diol); estrone, (1,3,5(10)-estratrien-3-ol, 17-one).

the prostate not only the qualitative differences in the effects observed but also that $3\beta$-adiol is not reconverted to DHT by that tissue to any significant extent. This is in contrast to $3\alpha$-adiol which has been shown to be readily converted to DHT both *in vivo*[4–6] and by target tissues *in vitro*[3–6]. A systematic investigation of the potential of the isolated AP to metabolize the three $5\alpha$-reduced derivatives *in vitro* has not yet been reported and was the initial purpose of this study.

Two target tissues of androgens, the medial basal hypothalamus (MBH) and the seminiferous tubules (ST), in addition to the AP were studied for their potential to metabolize DHT, $3\alpha$ and $3\beta$-adiol. The MBH of male rats was investigated in anticipation of future studies on the effects that various $5\alpha$-reduced forms of T may have on LH-RH release *in vitro*. The studies of the ST represented an extension of our previous investigation of the potential of this tissue to metabolize T and DHT *in vitro*[7].

In preliminary experiments we found that the metabolism of $3\beta$-adiol by ST differed from that by the other tissues. Only in incubations of ST was conversion of $3\beta$-adiol to DHT and $3\alpha$-adiol readily demonstrable. Consequently, a comparison of the reversibility of $3\beta$-oxido-reduction in incubations with the different target tissues and the basis for differences between their ability to form DHT from $3\beta$-adiol became a focal point of this study.

## EXPERIMENTAL

Tissues were obtained from 50–55 or 70–90 day old male Sprague–Dawley rats (Charles River, C. D.). The animals were housed in air-conditioned quarters (lights on 7 a.m.–7 p.m.) and had free access to Purina Chow and water. They were killed by decapitation. The dissection of ST and interstitial tissue (IT) has been described previously[7]. The AP was bisected at the isthmus. The medial basal hypothalamic fragments (MBH) (av. weight = 3–4 mg/fragment) were dissected as described for the deep basal hypothalamic specimens by Kavanagh and Weisz[8]. They included all of the arcuate nucleus, median eminence, the caudal end of the ventromedial nucleus and most of the posterior periventricular nucleus. The ventral prostate (P), cleaned of fat and minced with scissors, was used in some incubations primarily to permit comparison with findings from other laboratories.

*Chemicals and radioactive steroids.* All solvents were of reagent grade. Toluene and methanol were further purified by distillation. Glucose-6-phosphate (G6P) and NADP were purchased from Sigma Corp., the nonlabeled steroids from Steraloids, Inc. and [$7\alpha$-$^{3}$H]-testosterone (5 Ci/mmol) from Amersham Searle Corp. The other tritiated precursors, [1, 2-$^{3}$H]-dihydrotestosterone (44 Ci/mmol) [1, 2-$^{3}$H]-$5\alpha$-androstane-$3\alpha$, $17\beta$-diol (44 Ci/mmol) and the $^{14}$C labeled steroids tracers ([4-$^{14}$C]-testosterone [4-$^{14}$C]-androstenedione [4-$^{14}$C]-dihydrotestosterone [4-$^{14}$C]-estrone and [4-$^{14}$C]-estradiol-$17\beta$) were purchased from New England Nuclear Corp. Tritiated $3\beta$-adiol precursor as well as the $^{14}$C labeled $3\alpha$ and $3\beta$ adiols used as tracers were prepared by sodium borohydride reduction of $^{3}$H and $^{14}$C labeled DHT, without addition of carrier, as described previously[7]. The steroids formed were purified by paper chromatography developed in 85% methanol in water–cyclohexane or 90% acetic acid in water–cyclohexane followed by the sequence of thin layer and paper chromatographic systems used for isolating the products of incubations, as described below.

*Incubations.* The amounts of tissue incubated were: 100 mg for the ST, AP and the prostate and 50 mg for the MBH. The clumps of IT incubated averaged 2–4 mg. The medium in each flask had the following composition: 2.7 ml TC Hanks medium (Difco), 1 mg NADP, 1 mg glucose-6-phosphate and 0.28 ml of 1.3% $NaHCO_3$. The sodium bicarbonate was added to the medium to raise its pH to 7.3–7.4 at 37° when gased with 95% $O_2$ and 5% $CO_2$. The steroid precursors were added in 20–50 $\mu$l of ethanol. The incubations were carried out at 37° except those of testicular tissues which were incubated at 34°. They were terminated after one h by addition of 5 ml ethyl acetate and freezing. The samples were stored at − 20° till extracted.

The stability of each of the labeled substrates was tested by incubating it in the medium not containing tissue.

*Isolation and quantification of the steroids.* The methods for extraction of metabolites, their separation and identification have been described in detail previously[7]. The amounts of labeled T, DHT, $\Delta^4$A, $3\alpha$ and $3\beta$-adiol in the incubation were quantified using $^{14}$C labeled tracers[7]. Radiochemical homogeneity was considered to have been achieved when in three successive crystallizations the ratios of $^{3}$H/$^{14}$C in the crystals and the mother liquors did not deviate from the mean more than ± 5% (Table 1). For three steroids, epiandrosterone, $5\alpha$-androstane-dione ($5\alpha$ A) and androsterone, $^{14}$C labeled tracers were not available. The amounts of these steroids formed were estimated on the basis of the recoveries of DHT-$^{14}$C trace on the same thin layer or paper chromatography from which the steroid was isolated. The radioactive homogeneity of these steroids was not established.

Radioactivity was counted in a Packard Liquid Scintillation Counter as described previously[9]. Sufficient counts were accumulated to give a counting accuracy of ± 2%.

## RESULTS

*Metabolism of DHT—substrate concentration = 0.033 $\mu M$ (Table 2)*

*Dihydrotestosterone* was actively metabolized by all tissues, in particular by those derived from the testis. In incubations of ST only an average of 6.5% of the

Table 1. Representative $^{3}H/^{14}C$ ratios of successive crystallizations

| | Testosterone acetate | | Androstenedione | | Dihydro testosterone Acetate | | 5α-Androstane-3α, 17β diacetate | | 5α-Androstane-3β, 17β diacetate | | Estradiol diacetate | | Estrone acetate | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CR | ML | CR | ML | CR | ML | CR | ML | CR | ML | CR | ML | CR | ML |
| 1 | 231.70 | 233.53 | 6.21 | 6.59 | 10.01 | 10.44 | 89.71 | 88.38 | 1.78 | 7.56 | 0 | 0.137 | 0 | 0.025 |
| 2 | 226.73 | 233.22 | 6.16 | 6.38 | 9.78 | 10.05 | 89.81 | 93.57 | 1.52 | 2.15 | 0.003 | 0.045 | 0 | 0 |
| A* 3 | 234.24 | 232.40 | 6.32 | 6.26 | 9.74 | 10.04 | 88.89 | 86.71 | 1.52 | 1.73 | 0 | 0 | | |
| 4 | 230.80 | 233.62 | 6.32 | 6.46 | 10.23 | 10.12 | 88.65 | 86.92 | 1.47 | 1.51 | | | | |
| 5 | | | | | | | | | 1.46 | 1.47 | | | | |
| 6 | | | | | | | | | 1.45 | 1.46 | | | | |
| 7 | | | | | | | | | 1.49 | 1.49 | | | | |
| 1 | | | | | 1.42 | 1.92 | 3.95 | 6.82 | 116.44 | 127.00 | | | | |
| 2 | | | | | 1.37 | 1.44 | 3.71 | 4.24 | 116.45 | 116.25 | | | | |
| B* 3 | | | | | 1.36 | 1.31 | 3.31 | 3.43 | 112.64 | 115.66 | | | | |
| 4 | | | | | 1.35 | 1.36 | 3.22 | 3.29 | 109.10 | 112.83 | | | | |
| 5 | | | | | | | 3.30 | 3.33 | | | | | | |

All crystallizations were carried out from ether: hexane or *iso*-octane: aceteone except those of 5α-androstane-3β, 17β diacetate for which methanol and water were used. CR = Crystals; ML = Mother liquor. A* = an example of anterior pituitary incubated with testosterone. B = an example of anterior pituitary incubated with 5α-androstane-3β, 17β-diol.

substrate remained unconverted. The corresponding figure for the IT incubations was 12.5%. The residual DHT in the MBH incubations was consistently higher, (30–45%). The range of values obtained for AP was wider, 11–33%.

The wider range of values for residual DHT in the AP incubations can be accounted for by the differences in values obtained in two sets of incubations in which animals of somewhat different ages were used; the amount of residual DHT was lower in the two incu-

Table 2. Metabolism of dihydrotestosterone (DHT), 5α-androstan-3α, 17β diol (3α-adiol) and 5α-androstan-3β, 17β diol (3β-adiol) by rat anterior pituitary (AP), medial basal hypothalamus (MBH), seminiferous tubule (ST), interstitial tissue (IT) and prostatic tissue (P) *in vitro*.

| | % Substrate converted—mean and (range) | | | | |
|---|---|---|---|---|---|
| | AP | MBH | ST | IT | P |
| DHT Incubations—No. | 4 | 3 | 2 | 2 | |
| DHT (Residual) | 21.7 | 40.0 | 6.5 | 12.5 | — |
| | (11.2–32.8) | (30.5–45.1) | (6.2–6.7) | (8.3–16.7) | |
| 3α-Adiol | 54.1 | 37.7 | 68.5 | 25.8 | — |
| | (46.8–59.0) | (34.6–43.7) | (68.4–68.5) | (18.5–33.1) | |
| 3β-Adiol | 0.61 | 0.24 | 1.5 | 8.7 | — |
| | (0.50–0.71) | (0.10–0.32) | (1.5–1.6) | (6.5–10.8) | |
| Polar metabolites | 6.0 | 2.0 | N.A.* | N.A. | — |
| | (4.4–7.3) | (1.9–2.0) | — | — | — |
| 3α-Adiol incubations—No. | 2 | 2 | 4 | 2 | 2 |
| 3α-Adiol (Residual) | 60.3 | 64.6 | 75.1 | 38.4 | 33.1 |
| | (59.1–61.5) | (63.7–65.5) | (71.8–78.1) | (28.7–48.1) | (28.3–37.9) |
| DHT | 26.6 | 27.0 | 6.7 | 13.5 | 48.9 |
| | (23.7–29.4) | (26.2–27.9) | (3.2–9.9) | (12.4–18.9) | (44.8–53.0) |
| 3β-Adiol | 1.5 | 0.17 | 0.76 | 7.7 | 0.27 |
| | (1.4–1.6) | (0.16–0.18) | (0.51–1.11) | (7.1–8.3) | (0.26–0.28) |
| Polar metabolites | N.A. | 0.80 | N.A. | N.A. | N.A. |
| | | (0.69–0.91) | — | — | — |
| 3β-Adiol incubations—No. | 2 | 2 | 4 | 2 | 2 |
| 3β-Adiol (Residual) | 9.5 | 27.4 | 31.9 | 21.0 | 5.3 |
| | (7.7–11.1) | (24.1–30.7) | (26.8–34.4) | (19.8–22.1) | (4.7–5.9) |
| DHT | < 0.09 | 0 | 12.9 | 23.7 | < 0.40 |
| | — | — | (7.8–18.5) | (19.6–27.4) | — |
| 3α-Adiol | < 0.09 | 0.45 | 36.0 | 23.7 | < 0.72 |
| | — | (0.41–0.48) | (32.0–43.0) | (20.0–27.4) | — |
| Polar metabolites | 75.6 | 62.4 | N.A. | N.A. | 77.5 |
| | (64.4–86.8) | (62.1–62.6) | — | — | (72.5–77.5) |

Age of rats = 70–90 days except for two incubations of AP and of MBH with DHT for which 50–55 day old donors were used. Substrate concentration = 0.033 $\mu$M.

* Not analyzed.

bations with the AP from the 70–90 day animals than in the two in which tissues from younger, 50–55 day old, rats were used (11.2 and 12.7% vs. 30.7 and 32.8%).

The principal metabolite identifed in all incubations with DHT was *3α-adiol*. It accounted on the average of 69% of the radioactivity in incubations of the ST and 54% in those of the AP. The figures were somewhat lower for the MBH (38%) and for the IT (26%). In contrast, the amounts of *3β-adiol* that accumulated were small; a mean of 0.6% of the substrate for AP, 0.2% for MBH, 1.5% for ST and 8.7% for IT.

The two androstanediols and the residual DHT, the three steroids that were fully identified and quantified, accounted for between 76–77% of the radioactivity in incubations with AP, MBH or ST but for only 47% in those with the IT. *Androsterone*, a metabolite less rigorously identified and quantified, accounted for an additional 13.8–15.1% of the radioactivity in incubations with ST and 7.6–13% in those with IT. In incubations with AP and MBH only small amounts of this metabolite were found (AP = up to 3.7%, MBH = 0.1–2.3%). The amounts of 5α A and of *epiandrosterone* isolated from incubations with the four tissues were also small. An additional small amount of radioactivity was found localized close to the origin of the thin layer chromatograms in extracts from incubation with AP and MBH. We assume this to be due to some *polar metabolite* or metabolites (*vide infra*).

Thus, the per cent of unknown metabolites was small for all tissues except for the IT.

*Metabolism of 3α-adiol—substrate concentration = 0.033 μM (Table 2)*

The *residual* precursor, *3α-adiol*, accounted for more than half the radioactivity in incubations of AP, MBH and ST (mean = 60–75%) and for about 1/3 with the IT and the P. Conversion of 3α-adiol to *DHT* took place in incubations with all tissues studied. The P had the highest potential to form *DHT* (48.9% of the substrate) and the ST the lowest (6.7%). The values for the other tissues were intermediate (AP = 26.6%, MBH = 27% and IT = 13.5%).

Only small amounts of *3β-adiol* were found, less than 2%, in incubations with all the tissues except with IT in which 7.7% of the substrate was recovered as this metabolite.

Thus, the residual substrate, 3α-adiol and its two metabolites, DHT and 3β-adiol, accounted for between 80–90% of the total radioactivity in incubations with all the tissues except in those with IT. Unidentified metabolites in incubations with IT reached levels as high as 40%.

*Metabolism of 3β-adiol—substrate concentration = 0.033 μM (Table 2)*

More than 90% of the *3β-adiol* was metabolized in incubations with AP and P and between 80–90% with the other tissues. At this low substrate concentration, *DHT* was not formed in identifiable amounts in incubations with either AP, MBH or P. In contrast, 12.9 and 23.7% of the radioactivity was recovered as DHT from incubations with ST and IT, respectively. In incubations with these two tissues, *3α-adiol*, formed presumably via DHT, also accumulated in substantial amounts (ST = 36.0%, IT = 23.7%). Small amounts of 3α-adiol, between 0.41–0.48%, were also isolated from incubation of MBH but not from those of AP or P. In incubations with the three tissues in which DHT did not accumulate, i.e. AP, MBH and P, the bulk of the radioactivity appeared to be associated with some *polar metabolite* or metabolites that remained close to the origin on the thin layer chromatogram (AP = 75.6%; MBH = 62.4%; and P = 77.5%).

Thus, in incubations with ST and IT, the bulk of the radioactivity, 80% and 64% respectively, could be accounted for by the unmetabolized substrate, 3β-adiol and its two metabolites, DHT and 3α-adiol. In contrast, in incubations of AP, MBH and P between 70–95% of the substate was converted to *unknown metabolites*. To localize these metabolites on the thin layer chromatograms, the silica gel was eluted from the plates in 13.5 $cm^2$ areas. Between 62% and 87% of the total radioactivity remained close to the origin and was presumably associated with some *polar metabolite or metabolites*. This finding led us to consider the possibility that the failure of DHT formation from 3β-adiol by the AP, MBH and P, under the conditions of our incubations, was not necessarily due to irreversibility of 3β-reduction of DHT but to a rapid, possibly irreversible, conversion of the relatively small amount of 3β-adiol substrate to polar metabolites. The more ready formation of DHT and 3α-adiol from 3β-adiol by tissues derived from the testis could then be the result of a lesser tendency for 3β-adiol to be converted to the polar metabolites and not because of any fundamental difference in 3β-reductase. To test this hypothesis we incubated fixed amounts of AP, MBH and ST, the three tissues of primary interest to us, with increasing amounts of substrate and quantified the amounts of DHT, 3α-adiol and polar metabolites accumulated.

*Metabolism of 3β-adiol—effect of increasing substrate concentration (Table 3)*

The potential of the AP and MBH to form *DHT from 3β-adiol* became evident when the substrate concentration was raised to 0.33 μM (Table 3). The amounts of DHT that accumulated were, however, small in comparison with those formed by the ST; in the incubation with AP they averaged 1/70th and in those with MBH, 1/24th of those found in the ST incubations. *Polar metabolite* formation was inversely related to the amounts of DHT formed; it was 100-fold greater in the AP incubation and 75-fold greater in the MBH incubation than in that with ST.

Table 3. Metabolism of 5α-androstane-3β, 17β diol (3β-adiol) by anterior pituitary (AP), medial basal hypothalamus (MBH) and seminiferous tubules (ST) of rats *in vitro*; effect of increasing substrate concentration on amounts of dihydrotestosterone (DHT), 5α-androsterone-3α, 17β diol (3α-adiol) and of unidentified polar metabolite(s) formed

| Tissue | mass | Substrate Conc. μM | Substrate Mass/3 ml* nmol | 3β-Adiol (residual) | nmol of Steroid formed DHT | nmol of Steroid formed 3α-Adiol | Polar metabolites |
|---|---|---|---|---|---|---|---|
| AP | 100 mg | 0.033 | 0.1 | 0.009 | <0.00009 | <0.00009 | 0.076 |
| | | 0.33 | 1.0 | 0.140 | 0.001 | 0.002 | 0.802 |
| | | 3.33 | 10.0 | 2.274 | 0.019 | 0.031 | 7.502 |
| | | 16.67 | 50.0 | 20.105 | 0.070 | 0.290 | 29.285 |
| MBH | 50 mg | 0.033 | 0.1 | 0.027 | <0.0002 | 0.0005 | 0.062 |
| | | 0.33 | 1.0 | 0.451 | 0.003 | 0.005 | 0.527 |
| | | 3.33 | 10.0 | 3.952 | 0.031 | 0.071 | 5.783 |
| | | 16.67 | 50.0 | 27.215 | 0.115 | 0.250 | 21.915 |
| ST | 100 mg | 0.033 | 0.1 | 0.032 | 0.013 | 0.036 | N.A. |
| | | 0.33 | 1.0 | 0.504 | 0.072 | 0.244 | 0.007 |
| | | 3.33 | 10.0 | 2.730 | 1.038 | 3.576 | 0.220 |
| | | 16.67 | 50.0 | 32.855 | 3.070 | 7.670 | 0.445 |

The incubations with 0.33, 3.33 and 16.67 μM concentration of substrate were carried out on aliquots from a single pool of homogenized tissue. Those with 0.033 μM on minced tissues were from different pools of animals. *The tissues were incubated in 3 ml medium for one h.

With all three tissues the amounts of *DHT* that accumulated increased in a linear fashion with increasing substrate concentration. They reached 70 and 116 pmol in AP and MBH incubations respectively with the highest substrate concentration used (16.6 μM) amounting to about 1/40th and 1/27th of that in the corresponding ST incubations. The radioactivity associated with the *polar metabolites* also increased progressively with the substrate concentration while *3α-adiol*, not detectable in incubations of AP with 0.033 μM 3β-adiol, accumulated in identifiable amounts when the substrate concentration was raised ten-fold. In incubations with all three tissues there was a progressive linear increase also in the amount of this metabolite with increasing substrate concentration. The ratio of 3α-adiol to DHT was, in all instances, in favor of 3α-adiol (Table 3).

*Metabolism of testosterone—substrate concentration = 0.033 μM (Table 4)*

There were no major differences in the pattern of metabolites formed by the different tissues from T with the following exceptions: the presence of *3β-adiol*, albeit in small amounts, could be confirmed only in incubations of AP and MBH. Formation of $\Delta^4$A was conspicuous only in incubations with ST and IT in which this metabolite accounted for 11.2 and 20.7% of the total radioactive material present.

*Control incubations*

Testosterone was the only precursor which, when incubated in the absence of tissue, formed one of the metabolites being studied; 0.1–0.3% of the T was recovered as $\Delta^4$A. Recovery of all precursors from control incubations was essentially 100%.

Table 4. The amounts of dihydrotestosterone (DHT), 5α androstan-3α, 17β-diol (3α-adiol), 5α androstan-3β, 17β-diol (3β-adiol) and androstenedione ($\Delta^4$A) by rat anterior pituitary (AP), medial basal hypothalamus (MBH) seminiferous tubules (ST) and interstitial tissue (IT) incubated with tritiated testosterone (T). The substrate concentration was 0.033 μM.

| | %-Substrate converted—mean and (range) AP | MBH | ST | IT |
|---|---|---|---|---|
| T (Residual) | 74.7 | 76.1 | 82.4 | 66.1 |
| | (70.6–79.8) | (74.7–77.6) | (82.2–82.6) | (59.0–69.9) |
| DHT | 2.4 | 1.2 | 1.5 | 0.21 |
| | (1.4–3.5) | (1.0–1.3) | (1.5–1.5) | (0.14–0.35) |
| 3α-Adiol | 10.6 | 1.6 | 3.9 | 0.14 |
| | (5.1–14.8) | (1.2–2.1) | (3.7–4.1) | (0.10–0.16) |
| 3β-Adiol | 0.13 | 0.007 | <0.21 | <0.06 |
| | (0.03–0.25) | (0.005–0.01) | — | — |
| $\Delta^4$A | 0.91 | 0.50 | 11.2 | 20.7 |
| | (0.30–1.52) | (0.31–0.68) | (11.0–11.3) | (17.9–22.2) |
| No. of incubations | 4 | 2 | 2 | 2 |

Age of rats = 70–90 days except for two incubations of AP and of MBH for which 50–55 day old donors were used.

## DISCUSSION

The three tissues primarily under investigation, the AP, the MBH and the ST of the rat were shown to have the potential to metabolize further DHT, 3α as well as 3β-adiol. The most extensively metabolized precursors were DHT and 3β-adiol. In confirmation of previous reports, the major metabolite in incubations of all three tissues with DHT was 3α-adiol[10–12]. 3β-Reduction of DHT also occurred in the incubations with these tissues but to a much smaller extent. The 3β-reductive pathway has been previously identified in the ST[12, 13] and P[2]. Some 3β-reduction has been stated to occur in incubations of MBH and AP without, however, documentation of the experimental findings[14].

Identification of the 3β-reductive pathway presents certain problems. Not only are the amounts of 3β-adiol that accumulate small, but judging from the incubations with 3β-adiol as the precursor, most of the 3β-adiol formed may be metabolized further. This phenomenon was much more conspicuous in AP, MBH and P incubations than in those with tissues derived from the testis. Thus, to determine if 3β-reduction takes place in a tissue, it is necessary to quantify not only the amount of 3β-adiol but also the amounts of its metabolites formed.

The major products of the metabolism of 3β-adiol appear to be highly polar. This extensive conversion of 3β-adiol to polar metabolites appears to hinder the formation of DHT from 3β-adiol. Thus, in incubations of AP, MBH or P with 3β-adiol, a precursor of which 60–70% is converted by these tissues to polar metabolites, no DHT could be identified when low substrate concentrations were used. Reversibility of 3β-reduction of DHT in these tissues became evident only when higher substrate concentrations were used. In contrast, in incubations of ST with 3β-adiol, a tissue in which polar metabolite formation occurs only to minimal extent, substantial amounts of DHT were identified even at the lowest substrate concentration. Assuming that 3α-adiol is formed via DHT, one may deduce that nearly 50% of the 3β-adiol incubated with ST must have undergone 3β-oxidation. The conversion of 3β-adiol to DHT *in vivo* has been demonstrated[12]. However, the sites of 3β-oxidation cannot be deduced from these experiments.

While we did not analyze for polar metabolites in the incubations of IT with 3β-adiol, we assume that polar metabolite formation by this tissue, as with ST, is insignificant since substantial DHT and 3α-adiol formation occurred.

The nature of the polar metabolites formed remains to be determined. Judging from our findings with further paper and column chromatography of the radioactivity eluted from close to the origin from the t.l.c. there are at least two such metabolites (V. Gay, L. Kao and J. Weisz, unpublished data). The possibility that needs to be considered is that one or both of these may be responsible for the effects of 3β-adiol on gonadotrophin regulation[3] or on prostatic cells in culture[2] rather than, or in addition to, the 3β-adiol presented to the tissue.

The pattern of metabolite formation appeared to be similar for the different tissues. There were quantitative differences in the actual amounts of the various metabolites formed. The data clearly cannot serve as a basis for precise quantitative comparisons between the metabolic potentials of the different tissues. One fundamental problem is the difficulty, at this time, of working with controlled and comparable tissue: substrate ratios. This is due to a lack of homogeneity of each of the tissues studied. It is reasonable to assume that in the AP, the MBH and the ST, only a portion of the total cell population serves as the target for the T or is involved in the metabolism of the parent steroid and its metabolites. Differences in the steroid metabolizing potential of different cell types within a target organ have already been demonstrated for the ST and the AP[15, 16] but it is not known what proportion of the total cell population are actual target cells. Nevertheless, some of the differences between the tissues were of sufficient magnitude and occurred so consistently that we think they may reflect true difference between tissues rather than artifacts of incubation conditions and as such deserve additional study. Among these are the ready functional reversibility of the 3β-oxido-reductase system in the tissues derived from the testes versus the others. This may in turn be linked to differences in the extent of polar metabolite formation. The accumulation of relatively large amounts of androstenedione from T appears also to be a feature characteristic of testicular tissues as was the low yield of DHT from 3α-adiol, especially with the ST. Since DHT in the circulation of the rat is rapidly converted to 3α-adiol[17, 18], the efficiency with which 3α-adiol can be reconverted to DHT by a particular tissue may have a bearing on the levels of DHT that can be achieved within that tissue following administration of DHT. Thus, the more ready reversibility of 3α-adiol to DHT in the prostate than in the ST needs to be considered when evaluating the much greater effectiveness of DHT in maintaining and restoring prostatic weight in castrate rats than in its ability to maintain or restore spermatogenesis[19].

More incubations with AP from animals of different ages would be needed to determine whether the differences in metabolism of DHT found in the present study was a function of the age of the animals or to random differences between different groups of animals. Age dependent changes in 5α and 3α reductase activity in the AP have been demonstrated by Denef *et al.*[20, 21].

The extent to which the quantitative aspect of the findings *in vitro* are applicable to the *in vivo* situation or to *in vitro* experimental conditions that differ from those used in our studies can be debated. However, the demonstration that these tissues have the potential to metabolize further the various 5α-reduced

metabolites of T needs to be considered in experiments designed to test the function of these steroids.

In the perifusion system used by us to test the effects of the three 5α-reduced metabolites of T on the responsiveness of the AP to HE[3], it is evident that no matter which metabolite was infused, a variable amount of the other two was probably formed. Specifically, at the concentration of 3β-adiol used (0.33–33 μM), small amounts of DHT were likely to have been produced. In their studies of the prostatic tissue cultures, Robel *et al.* applied 3β-adiol at concentrations ranging from 3.5–70 μM[2] but checked for reconversion of 3β-adiol to DHT only with 0.3 μM 3β-adiol. After 22 h of incubation, no labeled DHT was identified. However, judging from our findings, it will be necessary to check DHT formation from 3β-adiol using the higher substrate concentrations before discounting reversibility of 3β-reduction.

It is questionable whether any definitive conclusions can be drawn about the action of a metabolite from experiments in which the individual steroids are applied and a biological effect quantified. The proposition that DHT, 3α and 3β-adiol may have distinct actions on tissues such as the AP or prostate must rest, at this time, on differences in the characteristics or nature rather than simply the magnitude of the response they elicit. Since interconversion between various metabolites seems to occur with such regularity, it is impossible to define the exact role of each metabolite or the extent to which one can act in the absence of the other(s). To determine these will require the development of new tools such as specific blockers of metabolizing enzymes.

*Acknowledgements*—The authors are grateful to Ms. Margaret Shamonsky for her technical help and to Ms. Doris Sauder for her patience during the preparation of this manuscript.

## REFERENCES


1. King R. J. B. and Mainwaring W. I. P: *Steroid–Cell Interactions.* University Park Press, Baltimore (1974) pp. 1–440.
2. Robel R., Lasnitzki I. and Baulieu E.-E.: *Biochimie* **53** (1971) 81–96.
3. Kao L. W. L. and Weisz J.: *Endocrinology* **96** (1975) 253–260.
4. Bruchovsky N. *Endocrinology* **89** (1971) 1212–1222.
5. Horst H.-J., Dennis M., Kaufman J. and Voigt K. D.: *Acta endocr., Copenh.* **79** (1975) 394–402.
6. Krieg M., Horst H.-J. and Sterba M. L.: *J. Endocr.* **64** (1975) 529–538.
7. Perex Lloret A. and Weisz J.: *Endocrinology* **95** (1974) 1306–1316.
8. Kavanagh A. and Weisz J.: *Neuroendocrinology* **13** (1973) 201–211.
9. Weisz J. and Gibbs C.: *Endocrinology* **94** (1974) 616–620.
10. Steinberger E. and Ficher M.: *Endocrinology* **89** (1971) 679–684.
11. Kasai H., Mijutari S. and Matsumoto K.: *Acta endocr., Copenh.* **74** (1973) 177–185.
12. Sowell J. G., Folman Y. and Eik-Nes K. B.: *Endocrinilogy* **94** (1974) 346–354.
13. Folman Y., Ahmad N., Sowell J. G. and Eik-Nes K. B.: *Endocrinology* **92** (1973) 41–47.
14. Massa R. and Martini L.: *Gynec. Invest.* **2** (1971) 253–270.
15. Dorrington J. H. and Fritz I. B.: *Endocrinology* **96** (1975) 879–889.
16. Lloyd R. V. and Karavolas H. J.: *Endocrinology* **97** (1975) 517–526.
17. Gay V. L.: *Fed. Proc.* **34** (1975) 303.
18. Gay V. L.: *Program of Ninth Annual Meeting of the Society for the Study of Reproduction*, Philadelphia, (August 1976).
19. Bartke A., Harris M. E., Weisz J. and Watson D.: *Fertil. Steril.* (in press).
20. Denef C., Magnus C. and McEwen B. S.: *J. Endocr.* **59** (1973) 605–621.
21. Denef C., Magnus C. and McEwen B. S.: *Endocrinology* **94** (1974) 1265–1274.
22. Wilson J. D. and Gloyna R. E.: *Recent Prog. Horm. Res.* **26** (1970) 309–336.
23. Baulieu E. E., Jung I., Blondeau J. P. and Robel P.: *Adv. Biosci.* **7** (1971) 179–189.
24. Robel P., Blodeau J.-P. and Baulieu E.-E.: *Biochim. biophys. Acta* **373** (1974) 1–9.