UPTAKE AND BINDING IN VIVO OF $^3$H LABELLED ANDROGEN IN THE RAT EPIDIDYMIS AND DUCTUS DEFERENS

By

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ABSTRACT

Following the administration of $[^3]$H-testosterone and $[^3]$H-5α-dihydrotestosterone to adult castrated male rats, a selective uptake of androgen was found in the epididymis and ductus deferens, in contrast to the much lower uptake by skeletal muscle. Simultaneous administration of non-labelled androgen reduced the uptake in the epididymis and ductus deferens by about 60–70 per cent. One day after castration, the uptake in the epididymis was about 54 per cent higher than in the intact animals.

The 105 000 × g supernatant fraction of homogenates obtained from both the ductus deferens and the epididymis, contained androphilic macromolecules excluded from Sephadex G-100 gel.

The 600 × g crude nuclear fraction of homogenized epididymal tissue contained salt extractable androgen binding macromolecules.

The accessory sex glands of male animals are androgen dependent organs. There is now a large amount of evidence showing that both the prostate gland and the seminal vesicles are able to accumulate and retain androgen (Tveter & Attramadal 1968; Fang et al. 1969; Tveter 1970). This retention of androgen is probably due to the presence of specific binding sites for androgen both in the cytoplasm (Unhjem et al. 1969; Tveter & Unhjem 1969; Fang et al. 1969; Mainwaring 1969b) and in the nuclei (Bruchovsky & Wilson 1968b; Mainwaring 1969a; Unhjem 1970).

The epididymis and ductus deferens are also target organs for androgens. As for the prostate and the seminal vesicles, the height and histochemistry of the epithelial cells, as well as the secretory activity of these organs are strongly contingent upon the presence of appropriate amounts of circulating androgen.
Likewise, withdrawal of androgenic hormones rapidly causes atrophy and regressive changes (Cavazos 1958; Allen & Slater 1957, 1958; Mann 1964). This androgen dependence of the epididymis and ductus deferens might indicate an uptake and retention of androgen in these organs, similar to that in the prostate and the seminal vesicles.

The aims of the present study were to examine the epididymis and ductus deferens with regard to 1) the kinetics of the uptake of androgen under various experimental conditions, after the injection in vivo of both [³H]testosterone and [³H]5α-dihydrotestosterone and 2) the binding of androgen to cytoplasmic and nuclear macromolecules.

MATERIALS AND METHODS

Animals
Adult male rats of a local strain, weighing about 250 g, were used. The animals were castrated through an abdominal incision 24-48 hours before the start of the experiments. The animals were fed on a standard laboratory diet.

Steroids
[1,2-³H]Testosterone (51 Ci/mMole) and [1,2-³H]5α-dihydrotestosterone (5α-androstan-17β ol-3-one; 44 Ci/mMole) were obtained from the New England Nuclear Corporation. The purity was guaranteed by the manufacturers to be higher than 98 per cent. The solvents, benzene and ethanol, were evaporated and the labelled compounds re-dissolved in saline containing 10 per cent ethanol. Non-labelled 5α-dihydrotestosterone, obtained from the Sigma Chemical Co., was dissolved in propyleneglycol/ethanol in the ratio 3:1 (v/v) to give a final concentration of 250 µg/ml.

Uptake studies
a) The kinetics of the uptake were studied in forty animals. The rats were injected with either [³H]testosterone or [³H]5α-dihydrotestosterone in a dose of 15 µCi/100 g body weight. The labelled androgens were given intravenously into the tail vein. The animals were killed at different time intervals (7½, 15, 30, 60 and 120 min), and specimens were taken from the epididymis, ductus deferens and from the rectus abdominis muscle. The tissue specimens were carefully dissected free from surrounding fat and connective tissue and were gently blotted to remove any surface moisture before weighing.

b) Effect of non-labelled androgen. In order to investigate the influence of non-labelled androgen on the uptake of [³H]5α-dihydrotestosterone, four animals were given 250 µg 5α-dihydrotestosterone intraperitoneally 15 min before the administration of the labelled compound. The animals were killed after 2 h and the radioactivity was determined in the epididymis, ductus deferens, muscle and liver. Four control animals were given the vehicle of the non-labelled compound only.

c) Effect of castration. To investigate the influence of castration on the uptake, six animals castrated 24 h previously and six intact, non-castrated animals were injected with 11.4 µCi/100 g body weight of [³H]testosterone (42.3 Ci/mMole). The animals were killed after 1 h and the epididymis and specimens from the rectus abdominis muscle were rapidly removed. The tissues were homogenized in 0.1 M Tris-HCl buffer,
pH 7.4, and aliquots were taken for the determination of radioactivity and for measurement of protein according to the method of Lowry et al. (1951).

**Gel filtration studies**

In these experiments, adult castrated animals were given $[^3]$H]testosterone or $[^3]$H]5α-dihydrotestosterone and killed 1 h later. Pooled tissues from two animals were homogenized with a Potter Elvehjem all glass homogenizer in 0.1 M Tris-HCl buffer, pH 7.4 at 0°C. The homogenates were centrifuged at 105,000 × g for 60 min at 3°C in a Beckman Ultracentrifuge (Modell Spinco L/L2, rotor L-50 at 37,000 r.p.m.), and the supernatant fractions were subjected to gel filtration as described elsewhere (Hansson, Tveter & Attramadal, in press).

In order to obtain a crude nuclear fraction, pooled epididymal tissue from two animals was homogenized with a Potter Elvehjem homogenizer in 8 ml 0.32 M sucrose medium, pH 7.4, containing 1 mM CaCl$_2$. The homogenates were filtered through two layers of gauze and centrifuged at 600 × g for 10 min in a Sorvall refrigerated centrifuge at 3°C (Modell RC-2, rotor SS-34, 2300 r.p.m.). This nuclear pellet was washed twice and dissolved in 4 ml 0.1 M Tris-HCl buffer, pH 7.4 containing 1 M NaCl. The nuclear suspension was agitated for 30 min at 0°C, and then centrifuged at 20,000 × g for 15 min at 3°C (Sorvall RC-2, rotor SS-34, 12,800 r.p.m.). The supernatant fractions were chromatographed on a column of Sephadex G-100 as described elsewhere (Hansson, Tveter & Attramadal, in press).

**Determination of radioactivity**

The radioactivity was determined by liquid scintillation counting in a Nuclear Chicago Marks I apparatus (Hansson, Tveter & Attramadal, in press). All measurements were performed in duplicate samples and at least 4 × 10$^4$ counts were recorded. Aliquots were taken from each fraction after gel filtration for the determination of radioactivity. The radioactivity was calculated as cpm in 0.5 ml of each fraction in some experiments, and as cpm/fraction in others.

**RESULTS**

The uptake of radioactivity in the epididymis and ductus deferens was significantly higher than in the muscle tissue, both after the administration of $[^3]$H]testosterone and $[^3]$H]5α-dihydrotestosterone *in vivo*. Almost identical uptake patterns were found in both experiments (Fig. 1, right and left). The uptake in the epididymis tended to be somewhat higher than in the ductus deferens. The radioactivity in both the epididymis and the ductus deferens was retained for a prolonged period of time, while the radioactivity in the muscle specimens showed a continuous decrease after the administration. In the epididymis and ductus deferens, the highest uptake was found 15–30 min after the iv administration of the labelled compounds, almost the same values being retained after 2 h. After the administration, the ratio between the uptake in the accessory genital organs and that in muscle increased. When $[^3]$H]testosterone was given, the maximum ratio was obtained after 1 h, while after the administration of
Uptake of radioactivity by the epididymis, the ductus deferens and skeletal muscle following the administration in vivo of 15 μCi [3H]testosterone or [3H]5α-dihydrotestosterone per 100 g body weight (ca. 0.2 μg/animal). The rats were injected intravenously into the tail vein, and at the time indicated, the animals were killed by decapitation. The radioactivity was determined by liquid scintillation, after digestion for about 6 h in Soluene TM-100. Each point on the curve is the mean value of 4 animals (8 specimens), and the results were calculated as DPM/mg wet weight ± SEM. Left: testosterone; right: 5α-dihydrotestosterone.

[3H]5α-dihydrotestosterone the maximum ratio seemed to appear somewhat later (Fig. 2).

The injection of non-labelled 5α-dihydrotestosterone shortly before the administration of the labelled androgen, reduced the uptake in the epididymis from 390 DPM/mg to 140 DPM/mg, and from 385 DPM/mg to 110 DPM/mg in the ductus deferens. On the other hand, the uptake in the muscle and liver was apparently not influenced by the administration of the non-labelled compound (Fig. 3).

In the castrated animals, the uptake in the epididymis was about 54 per cent higher than in the intact non-castrated rats (Table 1). The uptake of radioactivity by the muscle specimens was, however, not influenced by orchidectomy.

Gel filtration of the 105,000 × g supernatant fractions from the epididymis and ductus deferens, revealed two main peaks as determined by the optical density at 280 nm. The major part of the radioactivity was eluted corresponding to the macromolecules excluded from the gel bed, and a smaller portion was associated with material of lower molecular weight. The binding of radioactivity to soluble macromolecules excluded from the G-100 gel was found in both the epididymis and ductus deferens. The distribution of radioactivity was essentially
The ratio between the uptake of radioactivity in the epididymis and ductus deferens and that in muscle at different intervals after the administration of [3H]testosterone (left) and [3H]5α-dihydrotestosterone (right).

The effect of non-labelled androgen on androgen uptake by different organs of castrated male rats. The animals were injected with 250 µg 5α-dihydrotestosterone intraperitoneally 15 min before the intramuscular injection of 30 µCi [3H]5α-dihydrotestosterone per 100 g body weight. The animals were killed after 2 h and the radioactivity was determined as DPM/mg wet weight ± SEM (vertical bars). Open columns: control animals; Hatched columns: non-labelled androgen injected.
Table 1.
The effect of castration on the uptake of radioactivity in the epididymis and muscle after the injection of \([\text{\textsuperscript{3}H}]\)testosterone \textit{in vivo} (11.4 \(\mu\text{Ci}/100\) g body weight). The animals were castrated 24 h before sacrifice, and were killed 1 h after the administration of the labelled androgen. The results are given as CPM/mg protein \(\pm\) standard error of the mean (SEM).

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Epididymis</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact animals</td>
<td>6</td>
<td>152.3 (\pm) 10</td>
</tr>
<tr>
<td>Castrated animals</td>
<td>6</td>
<td>223.6 (\pm) 21.8</td>
</tr>
</tbody>
</table>

the same both after the administration of \([\text{\textsuperscript{3}H}]\)testosterone and \([\text{\textsuperscript{3}H}]\)5\(\alpha\)-dihydrotestosterone (Figs. 4, 5 and 6).

Gel filtration chromatography of the NaCl extracts from the epididymal nuclear 600 \(\times\) g pellet, revealed a sharp and distinct macromolecular peak. Almost all the radioactivity was bound to the macromolecules excluded from the gel, in both the experiments using \([\text{\textsuperscript{3}H}]\)testosterone (Fig. 7), and \([\text{\textsuperscript{3}H}]\)5\(\alpha\)-dihydrotestosterone (Fig. 8).

![Graph](image-url)

\textit{Fig. 4.}
Dotted line shows the elution pattern obtained when subjecting the 105 000 \(\times\) g supernatant of the epididymis to gel filtration on Sephadex G-100 (1.9 \(\times\) 29 cm), 3 ml fractions were collected. Solid line represents radioactivity 1 h after the im injection of 60 \(\mu\text{Ci}\) \([\text{\textsuperscript{3}H}]\)testosterone per animal.

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Dotted line shows the elution pattern obtained when subjecting the 105,000 × g supernatant fraction of the epididymal homogenate to gel filtration (Sephadex G-100, 2.5 × 30 cm). 4 ml fractions were collected. Solid line represents the radioactivity 1 h after the im injection of 60 μCi [3H]5α-dihydrotestosterone per animal.

DISCUSSION

The present study indicates that both the epididymis and the ductus deferens are able to accumulate and retain androgen. Androgen is concentrated in these organs, as compared with the lower uptake in skeletal muscle. Moreover, the uptake of androgen in the accessory genital organs increases simultaneously with decreasing values in muscle. Thus, there seems to be a fundamental similarity with regard to the uptake of androgen by the prostate (Tveter & Attramadal 1968; Fang et al. 1969) the seminal vesicles (Tveter & Unhjem 1969), the epididymis and the ductus deferens of castrated male rats. The reduced uptake in the epididymis and ductus deferens after the administration of non-labelled androgen, as well as the increased uptake in the epididymis following castration, suggests that these organs contain specific androgenic »receptors« with a limited capacity for binding androgen. Non-labelled androgen will thus compete with the labelled molecules for the binding sites, while castration unmasks »receptors« by reducing the endogenous circulating androgen in the blood.

Appelgren (1969) has recently studied the distribution of radioactivity in various organs after infusion of 14C-labelled testosterone by whole body autoradio-
**Fig. 6.**
Dotted line shows the elution pattern obtained when subjecting the 105,000 × g supernatant fraction of the ductus deferens homogenate to gel filtration. Solid line represents the radioactivity 1 h after the im injection of 60 μCi [3H]testosterone per animal. (Sephadex G-100, 2.5 × 30 cm).

**Fig. 7.**
Dotted line shows elution pattern obtained when subjecting the NaCl extract of the 600 × g crude nuclear fraction of epididymal homogenate to gel filtration. Solid line represents radioactivity 1 h after the im injection of 50 μCi [3H]testosterone per animal. (Sephadex G-100, 2.5 × 30 cm).
Fig. 8.
Dotted line shows the elution pattern obtained when subjecting the NaCl extract of the 600 × g crude nuclear pellet of epididymal homogenate to gel filtration. Solid line represents the radioactivity 1 h after the im injection of 50 μCi [³H]5α-dihydrotestosterone per animal.

He found a very high concentration of radioactivity in the liver and kidney, thus indicating the main routes of excretion of testosterone. In the male accessory sex organs, however, the amount of radioactivity was very low. This might be due to the use of ³H-labelled hormone with a low specific activity. Tveter & Attramadal (1968) have demonstrated the need for using minute amounts of testosterone with a very high specific activity in order to demonstrate the selective uptake of androgen in the prostate. The very high concentration of radioactivity in the ductus deferens found by Appelgren (1969) might be due to the secretion of testosterone into the testicular fluid (Vogelmayr et al. 1966). Skinner & Rowson (1967) have shown that the passage of testosterone along the ductus deferens is necessary for the normal development of the ampullary glands.

The present findings also indicate that the epididymis and ductus deferens contain androphilic macromolecules in the 105 000 × g supernatant fraction of homogenized tissue. This corresponds to previous observations on the prostate (Unhjem & Tveter 1969; Unhjem et al. 1969; Mainwaring 1969b; Fang et al. 1969; Baulieu & Jung 1970), and the seminal vesicles (Tveter & Unhjem 1969). The physiological significance of this cytosol «receptor» is not quite clear. Recent investigations seem to favour the assumption that the binding of androgen to cytoplasmic macromolecules is an obligatory step, which precedes the firm binding and retention of androgen by the nuclei (Fang et al. 1969).
The NaCl extracts of the 600 × g nuclear pellet from the epididymis contained macromolecules that bound radioactivity as previously shown in the prostate (Bruchovsky & Wilson 1968b; Anderson & Liao 1968; Mainwaring 1969a; Unhjem 1970). These nuclear androgen-binding macromolecules are probably acidic proteins (Bruchovsky & Wilson 1968b; Mainwaring 1969a). The nuclear binding of androgen may have some effect on gene transcription, a possibility which may explain the increased RNA- and protein synthesis after androgen stimulation (Wilson & Loeb 1965; Williams-Ashman 1965; Liao 1968; Liao & Stumpf 1968).

Recently it has been demonstrated that cell-free homogenates from the epididymis metabolize several steroid hormones, and that testosterone is reduced to 5α-dihydrotestosterone and 5α-androstane-3,17-diol (Inano et al. 1969). The same conversion of testosterone has also been shown after incubation of tissue slices from the epididymis in vitro (Gloyna & Wilson 1969). The transformation of testosterone to 5α-dihydrotestosterone and 5α-androstane-3,17-diol has turned out to be specific for the androgen target organs that bind and accumulate androgen (Farnsworth & Brown 1963; Shimazaki et al. 1965; Bruchovsky & Wilson 1968a; Anderson & Liao 1968; Tveter & Aakvaag 1969; Unhjem et al. 1969). The fact that [3H]dihydrotestosterone is taken up and retained to almost the same extent as [3H]testosterone, suggests that 5α-dihydrotestosterone may be the main metabolite bound to the cytoplasmatic and nuclear binding sites as previously shown in the prostate.

The epididymis contains large amounts of semen, which may be able to metabolize androgen (Seamark & White 1964; Scott et al. 1963). The significance of this for epididymal function is unknown. The extent to which the uptake and binding of androgen in the rat epididymis might be due to the sperms or to the epithelial and fibro-muscular components of the epididymis is not known. Further studies, including autoradiography, are necessary to solve this problem.

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REFERENCES

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