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5α-Reductase Activity in Rat Adipose Tissue[1] (42592)

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Abstract. We measured the 5α-reductase activity in isolated cell preparations of rat adipose tissue using the formation of [^{3}H]dihydrotestosterone from [^{3}H]testosterone as an endpoint. Stromal cells were prepared from the epididymal fat pad, perinephric fat, and subcutaneous fat of male rats and from perinephric fat of female rats. Adipocytes were prepared from the epididymal fat pad and perinephric fat of male rats. Stromal cells from the epididymal fat pad and perinephric fat contained greater 5α-reductase activity than did the adipocytes from these depots. Stromal cells from the epididymal fat pad contained greater activity than those from perinephric and subcutaneous depots. Perinephric stromal cells from female rats were slightly more active than those from male rats. Estradiol (10^{-8} *M*), when added to the medium, caused a 90% decrease in 5α-reductase activity. Aromatase activity was minimal, several orders of magnitude less than 5α-reductase activity in each tissue studied.

The formation of dihydrotestosterone (DHT) from testosterone (T) requires the activity of the enzyme 5α-reductase (1, 2). The tissues of the reproductive tract of the human male contain relatively high levels of 5α-reductase activity (2), and activity has also been described in other tissues (3). We reported that 5α-reductase activity was present in fat tissue of the human forearm as a result of studies *in vivo* (4). More recently, Perel *et al.* have reported on the levels of 5α-reductase activity in the fat tissue of human female mammary glands (5).

Although enzyme activities in tissues may vary between species, 5α-reductase activity has been described in various rat tissues (2, 6). We, therefore, used rat fat tissue in order to determine the extent of 5α-reductase activity in this tissue and the cells responsible for the activity. Our findings form the basis for this report.

Materials and Methods. Sprague–Dawley rats (150–200 g) were obtained from Charles River Laboratories (Wilmington, MA) and fed standard rat chow, *ad libitum*. They were sacrificed by cervical dislocation and fat tissue was removed and treated as described below.

Collagenase suitable for isolation of adipocytes was obtained from Sigma Chemical Co. (St. Louis, MO). All solvents were HPLC grade (J. T. Baker Co., Phillipsburg, NJ) and were used without further purification. [7-^{3}H]Testosterone ([^{3}H]T; sp act 30 Ci/mmole), [4-^{14}C]dihydrotestosterone ([^{14}C]DHT; sp act 58 mCi/mmole), and [4-^{14}C]estradiol ([^{14}C]E_2; sp act 58 mCi/mmole) were obtained from New England Nuclear Corp. (Boston, MA). The [^{3}H]T was chromatographed on Whatman No. 1 paper in a Bush A system (methanol:water:heptane; 8:2:10, v/v/v) prior to use. The purities of the [^{14}C]DHT and [^{14}C]E_2 were checked using thin-layer chromatography and, being >98% pure, they were used without further purification. T, DHT, E_2, and estrone (E_1) were obtained from Steraloids, Inc. (Wilton, NH) and recrystallized from methanol prior to use.

The adipose tissue from three to six rats was removed and stromal cells and adipocytes were isolated separately from each rat essentially as described (7–9). The tissue, 4–8 g, was minced well with scissors and weighed. The minced tissue was placed into a plastic vial and Krebs buffer, pH 7.4, containing 1 mg/ml collagenase and 1% bovine serum albumin was added to give a concentration of 1 g of tissue per 5 ml buffer. The mixture was gassed with oxygen for 1 min and incubated in a Dubnoff incubator at 37°C for 20 min. At the end of the incubation, the mixture was sieved through silk into plastic centrifuge tubes and centrifuged briefly at 1000 rpm. The lower layer

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containing the stromal cells was carefully removed and saved. The supernatant containing the adipocytes was brought up to the original volume with Krebs buffer and centrifuged three times at 1000 rpm. The lower layer was discarded each time. After the final centrifugation, the adipocytes were resuspended in buffer. Aliquots, 0.25 ml, of the cell preparations were used to determine the DNA content (10).

Individual flasks containing 2 ml of cell suspension, either stromal cells or adipocytes, 1 μCi [^{3}H]T, and in most cases 50 μg of DHT were incubated for 180 min at 37°C in an atmosphere of 95% O_2:5% CO_2. Following the incubation 200 dpm of [^{14}C]DHT was added to the incubation media which were extracted twice with 10 ml of ethyl acetate:cyclohexane (2:1, v/v). The pooled extracts were washed once with 1 ml of $NaHCO_3$ and once with 1 ml of H_2O. The solvents were removed under vacuum and the residues were partitioned between carbon tetrachloride:chloroform (CCl_4:$CHCl_3$; 5:1, v/v) and 1 *N* NaOH (11). The CCl_4:$CHCl_3$ phase containing the DHT and other androgens was separated and the solvents were removed under vacuum. The DHT was purified by multiple chromatographic and derivitization steps (4, 11), which included an initial separation on paper chromatography in the Bush A system. The DHT–androstenedione area was eluted and chromatographed using silica gel thin-layer chromatography in the system $CHCl_3$:MeOH (98:2, v/v), with parallel channels containing DHT standard. The DHT area was identified by spraying the parallel channels with polymolybdic acid and the DHT area in the sample channels was eluted and acetylated. The DHT–acetate was chromatographed using silica gel thin-layer chromatography in the system benzene:ethyl acetate (90:10, v/v). The DHT–acetate area was identified as above and eluted and the acetate was hydrolyzed using 0.5 ml of 0.15 *N* NaOH in 80% MeOH. The resultant DHT was chromatographed using silica gel in the system $CHCl_3$:MeOH (98:2, v/v). Following the last purification step, the DHT was taken up in Optifluor (Packard Instrument Co., Downers Grove, IL) and the radioactivity was measured in a liquid scintillation spectrometer (11). The tritium counts in [^{3}H]DHT were corrected for procedural losses with the ^{14}C counts.

In certain experiments, [^{14}C]E_2 was added to the incubation just prior to the extraction and both phases of the partition step were processed. The alkali containing the estrogens was acidified and the estrogens were extracted and purified using several thin-layer chromatographic steps before and after acetylation (12). The final chromatographic step involved HPLC. The radioactivity in estradiol was measured in a liquid scintillation spectrometer and the net [^{3}H]E_2 was calculated using the [^{14}C]E_2 to correct for losses through the procedure. In several experiments, we measured the ^{3}H in the purified estrone fractions but did not correct for losses through the procedure. In all experiments looking for aromatization, we did not add 50 mg DHT to the flasks.

In all incubations, either boiled tissue or no-tissue controls were run in parallel with the other flasks. The results of the controls were subtracted to obtain the net picomole steroids formed per hour of incubation per flask.

Statistical analyses were done using Student's *t* test or the Student–Newman–Keuls test where appropriate.

Results. As shown in Table I, 5α-reductase activity was present in stromal cells from both the epididymal fat pad and from perinephric fat. The activity in the stromal cells was significantly greater than the activity in the adipocytes from the respective areas. Although the activity in the stromal cells from the epididymal fat pad was fourfold greater than in the stromal cells from perinephric fat, the difference in activity in the adipocytes from the two areas was minimal.

In initial experiments we were unable to find significant amounts of radioactivity above background as 5α-androstenediols and did not look further for these metabolites of DHT. As noted, in most experiments we added 50 μg DHT to the incubation flask, but in one experiment, stromal cells from epididymal fat pads were incubated with and without 50 μg DHT and no differences could be detected between the amounts of DHT formed.

Using the stromal cells from the epididymal fat pad, we calculated a K_m of 1.4 μ*M* and a V_{max} of 4.6 n*M* for the 5α-reductase enzyme.

In order to compare the 5α-reductase activity in fat tissue from male and female rats, we measured DHT production in stromal cells from the perinephric fat of male and female

TABLE I. FORMATION OF DIHYDROTESTOSTERONE (DHT) BY STROMAL CELLS AND ADIPOCYTES FROM THE EPIDIDYMAL FAT PAD

Tissue source	Cell type	DHT formed (pmole/3 hr/mg DNA)	P value
Epididymal fat pad	Stromal	0.357 ± 0.040[a]	<0.01
	Adipocyte	0.024 ± 0.001	
Perinephric fat	Stromal	0.081 ± 0.001	<0.01
	Adipocyte	0.017 ± 0.001	

Note. Stromal cells and adipocytes from epididymal fat pads and perinephric fat of three male rats were incubated separately in 2 ml Krebs buffer with 1 μCi [7-^{3}H]testosterone and 50 μg DHT for 3 hr at 37°C. The [^{3}H]DHT formed was isolated and purified and the radioactivity was measured.

[a] Mean ± SE.

rats. As noted in Table II, the stromal cells from female rats contained greater activity than those obtained from the perinephric fat of male rats ($P < 0.01$). However, the stromal cells from the epididymal fat pads contained greater activity ($P < 0.01$) than stromal cells from the perinephric fat of either sex. In another experiment the stromal cells from the epididymal fat pads contained greater activity than stromal cells from periovarian fat (data not shown).

The epididymal fat pad stromal cells contained greater activity ($P < 0.01$) than stromal cells prepared from perinephric or subcutaneous fat (Table III) and stromal cells from perinephric fat were more active than those from subcutaneous fat ($P < 0.01$). However, the aromatase activity was similarly low in cells from each tissue and far less than the 5α-reductase activity.

When estradiol (10^{-8} *M*) was added to the stromal cells from the epididymal fat pad the 5α-reductase activity was significantly reduced ($P < 0.01$) (Table IV).

TABLE II. 5α-REDUCTASE ACTIVITY OF STROMAL CELLS FROM PERINEPHRIC FAT FROM MALE AND FEMALE RATS AND FROM THE EPIDIDYMAL FAT PAD

Tissue source	Sex	DHT formed (pmole/3 hr/mg DNA)
Perinephric	F	0.207 ± 0.020[a,b]
Perinephric	M	0.125 ± 0.048
Epididymal fat pad	M	0.511 ± 0.004[b,c]

Note. Stromal cells were isolated from the perinephric fat from three female rats. Stromal cells were isolated from the perinephric fat and epididymal fat pad from six male rats. The isolated cells from each rat were incubated separately in 2 ml Krebs buffer with 1 μCi [7-^{3}H]testosterone and 50 μg DHT for 3 hr at 37°C. The [^{3}H]DHT formed was isolated and purified and the radioactivity was measured.

[a] Mean ± SE.

[b] Significantly different from perinephric fat from male rats; $P < 0.01$.

[c] Significantly different from perinephric fat from male and female rats; $P < 0.01$.

Discussion. The ability of adipose tissue from humans to convert T to DHT has been shown by Longcope and Fineberg (4) *in vivo* and Perel *et al.* (5) *in vitro.* We have now demonstrated that this activity is present in the adipose tissue of both male and female rats. While adipocytes possess enzymatic activities (7, 8) certain steroid metabolic enzymes have been noted to occur primarily in the stromal cells (5, 13). Stromal cell preparations consist of vascular endothelium, mast cells, macrophages, and other connective tissue elements (8) and are support elements for the adipocytes. Although the prostate epithelial cells possess higher levels of 5α-reductase activity than prostate stromal cells, the latter have been shown to convert T to DHT (6). Our finding that stromal cells from fat from various sites had 5α-reductase activity would suggest that this activity might be widespread in vascular tissue. However, it is of interest that the stromal cells from the epididymal fat pad were much higher in 5α-reductase activity than stromal cells from other sites. This may indicate that there is outgrowth of vascular tissue from the 5α-reductase-rich epididymis into the fat pad. This outgrowth may be a mechanism to ensure sufficient DHT for growth and function of the adjacent tissue. The lower levels of activity in the fat of female reproductive tissues

TABLE III. 5α-REDUCTASE AND AROMATASE ACTIVITIES IN EPIDIDYMAL FAT PAD, PERINEPHRIC FAT, AND SUBCUTANEOUS FAT STROMAL CELLS

	DHT formed (pmole/3 hr/mg DNA)	E_2 formed (pmole/3 hr/mg DNA)
Epididymal fat pad	$0.331 \pm 0.031^{a,b,c}$	0.0013 ± 0.0003
Perinephric fat	0.141 ± 0.021^{c}	0.0013 ± 0.0003
Subcutaneous fat	0.025 ± 0.008	0.0010 ± 0.0001

Note. Stromal cells from five rats were prepared and incubated separately as in Table I.
[a] Mean ± SE.
[b] Significantly different from the other two groups; $P < 0.01$.
[c] Significantly different from subcutaneous fat; $P < 0.01$.

may, therefore, reflect the fact that DHT is not necessary in these tissues.

It should be noted that there were differences in 5α-reductase activity among different groups of rats. Thus, the activity in the 5α-reductase activity of the epididymal fat pads differed between individual experiments. The reasons for these differences are uncertain but should not reflect on the experiments, all of which involved comparisons of cells from rats within individual shipments.

We found a difference in 5α-reductase activity between stromal cells from various sites, but Perel *et al.* (5) did not report such a difference for human adipose stromal cells. However, differences between adipose tissue depots for other enzymatic activities have been reported (14). Perel *et al.* (5) measured 5α-reductase activity in adipose tissue from women whereas we used rats, so species differences may play a role in the differing findings.

The K_m of the 5α-reductase for T in the epididymal fat pad is not too different from that reported in homogenates of prostate tissue (0.83 μM) by Frederiksen and Wilson (15). Bruchovsky and Wilson (1) were able to find conversion of T to DHT only in the accessory sex tissues of the male rat. However, after injecting [^{3}H]T into rats, they looked for [^{3}H]DHT only in certain tissues and it is unclear whether they specifically examined adipose tissue. Our data and those of Perel *et al.* (5) would indicate that adipose tissue does contain 5α-reductase activity.

TABLE IV. INHIBITION OF 5α-REDUCTASE ACTIVITY IN EPIDIDYMAL FAT PAD STROMAL CELLS BY ESTRADIOL

	DHT formed (pmol/3 hr/mg DNA)
Control	0.297 ± 0.041^{a}
Estradiol (10^{-8} M)	0.022 ± 0.014

Note. Stromal cells from four rats were prepared and incubated separately as described in Table I except that estradiol (10^{-8} M) was added to duplicate flasks of stromal cells from each rat.
[a] Mean ± SE.

As has been reported by others (16, 17), the addition of estradiol proved to be an effective inhibitor of 5α-reductase activity. When estradiol (10^{-8} M) was added to the epididymal fat pad stromal cells, we found an inhibition of 90%. To see whether the 5α-reductase activity might be modulated by the *in situ* production of estrogen by stromal cells, we measured the [^{3}H]estrogen formed from the [^{3}H]T added to the cells. However, we found little or no aromatase activity in our incubations which were done under conditions similar to those used when we found aromatase activity in the rat Leydig cells (18). It is unlikely that our failure to find aromatase activity in the adipose stromal cells was due to technical reasons. It appears, therefore, that stromal cells from rat adipose tissue possess little or no aromatase activity. This is in contradistinction to the findings of several groups using human fat stromal cells (13, 19).

Thus 5α-reductase activity is present in stromal cells prepared from fat depots of male and female rats. There is a variation in the amounts of 5α-reductase measured depending on the area from which the fat was obtained. Stromal cells from the epididymal fat pad contain the highest concentration of 5α-reductase activity relative to DNA content.

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