TESTOSTERONE METABOLISM IN TARGET TISSUES: Hypothalamic and Pituitary Tissues of the Adult Rat and Human Fetus, and the Immature Rat Epiphysis (1)

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ABSTRACT

Rat pituitary, hypothalamic and cerebral cortical minces are demonstrated to effect the conversion of testosterone (T) to 5α-androstan-17β-ol-3-one (DHT) and Δ^4-androstene-3,17-dione (Δ^4A). The pituitary effected the greatest conversion of T to both DHT and Δ^4A. The hypothalamus converted more T to DHT than did the cerebral cortex; but the conversion of T to Δ^4A in these two tissues occured to approximately the same extent. Analysis of the time course of the conversion of T to DHT in these tissues demonstrated the conversion to increase in a linear fashion over 180 min., while the maximum conversions in the hypothalamus and cerebral cortex were reached by 120 min.

The conversion of T to DHT and Δ^4A was also demonstrated in the human fetal hypothalamus and pituitary, and in the distal femoral epiphysis of the growing rat. In the femoral epiphysis, 5α-androstane-3α,17β-diol was also identified.

Recently, the conversion of testosterone (T) to 5α-androstan-17β-ol-3-one, (dihydrotesterone,DHT), a compound with marked androgenic activity (2), has been demonstrated in two target tissues of androgen action, the prostate of the rat and human (3-7), and in human skin (8-10). To assess whether this reaction might occur in other potential sites of androgen action, radio-labeled T was incubated

with minced preparations of mature rat hypothalami, pituitaries and cerebral cortices; human fetal hypothalamic and pituitary tissues; and the epiphyseal end of the femur of an immature rat.

MATERIALS AND METHODS

Materials. Testosterone-7α-^{3}H, specific activity (SA) 1.9 Ci/mM (batch 4), obtained from Nuclear Chicago Co., was purified by paper chromatography (PC) in systems I and II (Table 1). Recrystallization of an aliquot of this material with authentic crystalline T demonstrated radiochemical homogeneity.

All organic solvents were redistilled prior to use. Crystalline steroid standards were recrystallized prior to use and melting points were determined. Chromatographic behavior was also assessed.

Incubation Procedure. For the study of T metabolism in adult rat neural tissues, comparable weight pools of six pituitaries, hypothalami, and cerebral cortices were prepared from 59 day old male Holtzman rats and incubated within 30 minutes of sacrifice. The human fetal hypothalamic and pituitary tissues were obtained from a female fetus of 10 weeks gestational age. In all cases, incubations were performed within twenty minutes of removing the tissue.

Tissue minces of rat pituitary, hypothalamus and cerebral cortex were individually prepared and incubated with 7.76μCi of T-7α-^{3}H in a calcium free Krebs-Ringer bicarbonate buffer, ph 7.4, without added co-factors, at 37C for 0, 60, 120, and 180 minutes. Tissue-less control incubations were simultaneously performed for each time interval. The immature rat femoral epiphysis and human fetal pituitary and hypothalamic preparations were incubated for 90 min. under the same conditions. Incubations were performed in a Dubnoff metabolic incubator with 95% oxygen and 5% carbon dioxide as the gas phase. The average total weight of the rat pituitary pools used was 44 mg. (range 40-48 mg.), that of the hypothalamic pools was 122 mg. (range 109-138 mg.), and

that of the cerebral cortices was 130 mg. (range 120-143 mg.). The human fetal hypothalamic and pituitary tissues were not weighed. The reactions were terminated by the addition of 80% ethanol.

Extraction and Purification. Following the addition of 300 µg of DHT and Δ^4-androstene-3,17-dione (Δ^4A) to the pools, three extractions with 80% ethanol were carried out followed by two extractions with absolute ethanol. In addition, the femoral epiphyseal preparation was precipitated with 70% methanol at -17C for 48 hrs. A dichloromethane-water partition was then performed in a countercurrent fashion using 3 separatory funnels with 6 lower phase transfers.

Chromatography. PC was performed on Whatman No. 1 paper. All chromatography was carried out at 24 ± 2C. The PC systems are listed in Table 1, and will be referred to by number. Chromatography on silica gel impregnated glass fiber sheets was performed as described by Payne and Mason (11).

TABLE 1. Chromatographic systems employed

No.	Solvents	(Ref.)	Ratios	Type of chromatography
I	Heptane, benzene, methanol, H_2O	(12)	3.3:1.7:4:1	PC[a]
II	Cyclohexane, benzene, formamide	(13)		PC
III	Ligroine, propylene glycol	(14)		PC
IV	Heptane, methanol, H_2O	(12)	5:4:1	PC
V	Isooctane, t-butanol, 1M NH_4OH	(15)	3:5:5	CPC[b]
VI	Chloroform	(11)		ITLC[c]

[a] PC = paper chromatography
[b] CPC = column partition chromatography
[c] ITLC = glass fiber chromatography

Estimation of Steroids. T and Δ^4A were detected on

paper chromatograms by ultraviolet absorption. DHT was detected with alkaline m-dinitrobenze reagent (16). Spectrophotometric quantitation of Δ^4A was performed in ethanol at 240 mμ. DHT acetate was quantitated using the Zimmermann reaction for 17 ketosteroids except that the color was developed in the dark for 10 min. and samples measured at 540 mμ. Corrections were performed as described by Allen (17). Radioactive samples were counted in an automated 3-channel Packard Tri-Carb Scintillation Spectrometer (Model 3375). Each sample was counted in 10 ml of toluene containing 3.0 g/l PPO (2, 5-diphenyl-oxazole) and 100 mg/l dimethyl POPOP (1, 4-bis-2- (4 methyl 5-phenyl-oxazolyl) benzene) for sufficient time to assure a counting error no greater than 3%. Appropriate quench corrections were performed using an internal standard technique.

Establishment of radiochemical homogeneity. Varying amounts of crystalline authentic steroids were added to each of the radioactive steroids isolated. They were successively recrystallized until constant SA was achieved.

RESULTS

Greater than 97% of the ^{3}H-labeled material incubated was recovered following ethanol extraction. Following partition between dichloromethane and water, only 0.23 - 1.6% of the starting radioactive material was found in the aqueous fraction. Following Celite column partition chromatography in system V, the bulk of this radioactive material in the aqueous fraction was found in the first holdback volume, in which unconjugated ("free") steroids are found, and was not analyzed further.

All of the extracts from the dichloromethane

fractions were submitted to the same identification procedures. These fractions were submitted to PC in system I, and three peaks of radioactivity were noted with Rf values of 0.37, 0.55 and 0.66.

The peak with an Rf of 0.37 corresponded to authentic T. The radioactive material in this peak from each tissue was subjected to PC in system II. A single peak of radioactivity (Rf = 0.54), corresponding to the mobility of authentic T, was observed. This radioactive material from each tissue was then separately acetylated (acetic anhydride: anhydrous pyridine, 1:2) and submitted to PC in system III. Again, a single peak (Rf = 0.43) with a mobility corresponding to authentic carrier T acetate was found in all tissues. Following these chromatographic procedures, aliquots of the T acetate-like radioactive material were taken for recrystallization. The SA of the first crystals and mother liquors were within 4% of the SA of the starting material.

Following the initial PC of the radioactive material from the femoral epiphysis in system I, a "shoulder" in the testosterone-like radioactive material was observed in the chromatogram scan which was slightly more polar than T. This material was acetylated and subjected to

PC in system III where it ran with an Rf of 0.92. This material was then saponified with 2.5% Na_2CO_3 and developed on silica gel impregnated glass fiber sheets in system VI. Two peaks of radioactivity were noted with Rf values of 0.71 and 0.77. The radioactive material with the Rf value of 0.77 had a mobility identical to carrier 5α-androstane-3α-17β-diol. It was mixed with additional carrier and recrystallized to constant SA (Table 2b). The identity of the radioactive material with an Rf of 0.71 could not be established.

The radioactive material with Rf's of 0.55 and 0.66 following the initial PC in system I corresponded to the mobilities of DHT and Δ^4A respectively. The radioactive material from these two peaks was pooled and subjected to PC in system III for 18 hrs. Two peaks with mobilities identical to carrier DHT (Rt = 0.47) and Δ^4A (Rt = 1.01) were found. The radioactive material eluted from the DHT-like peak was acetylated, again placed in PC in system III, and the chromatogram developed until the solvent had reached the front. In this system, authentic DHT acetate ran in an identical fashion to the radioactive material, with an Rf of 0.78. Additional carrier DHT acetate was added to this material and recrystallization to constant SA was performed (Tables 2a

and 2b). The radioactive material corresponding to Δ^4A was submitted to PC in system IV, and developed until the solvent had reached the front. Again, radioactivity and carrier Δ^4A traveled in an identical fashion. Additional authentic crystalline Δ^4A was added and recrystallization to constant SA effected (Tables 2a and 2b).

As can be seen in Fig 1, of the rat neural tissues studied, the pituitary effected the conversion of T to DHT to the greatest extent (26.2×10^{-7} μmoles/mg). This conversion increased in a linear fashion over the time range studied. The hypothalamus was also capable of effecting this conversion, but to a lesser extent than the pituitary (9.01×10^{-7} μmoles/mg). In the hypothalamus, the reaction appeared to reach a maximum by 120 minutes, and then fall slightly. Cerebral cortical tissue also possessed 5α-reducing capacity, but as can be seen in Fig 1, to a lesser extent than in either of the two other tissues (3.54×10^{-7} μmoles/mg). No DHT was found in the tissue-less control incubations.

In addition to DHT, as can be seen in Fig 2, Δ^4A was also found in all three neural tissues. Again, the pituitary had the greatest 17β dehydrogenase enzymatic capacity (3.82×10^{-7} μmoles/mg). However, there was

no appreciable difference between hypothalamic and cortical activity in regard to this enzymatic function (1.47×10^{-7} and 1.43×10^{-7} μmoles/mg respectively). Small quantities of Δ^4A (0.10 - 0.17%) were found in the control flasks. These values have been subtracted from the corresponding tissue incubation values.

As can be seen in Table 2b, both DHT and Δ^4A were also formed in the human fetal pituitary and hypothalamus, and in the distal femoral epiphysis of the growing rat. In the human fetal hypothalamus, DHT and Δ^4A were formed in approximately equal amounts. In the fetal pituitary, more Δ^4A than DHT was formed. In the femoral epiphysis, approximately equal amounts of DHT and Δ^4A were found (DHT, 309 dpm/mg; Δ^4A, 368 dpm/mg).

Discussion

The data presented herein demonstrate that T can be converted to DHT and Δ^4A in the mature rat pituitary, hypothalamus and cerebral cortex, as well as in the hypothalamus and pituitary of the human fetus and the distal femoral epiphysis of the growing rat. The use of viable mince preparations of these tissues rather than homogenates obviated the need for co-factors.

In the rat brain tissues studied, the greatest

conversion both to DHT and Δ^4A, per mg of tissue, occured in the pituitary gland. DHT was formed to a greater extent in the hypothalamus than in the cerebral cortex, while Δ^4A was formed in approximately the same amounts in both of the latter tissues. A linear increase in the formation of DHT was observed in the pituitary tissues during the 180 minutes studied. It is possible that these conversions are related to the degree of T uptake by these tissues (18).

Uptake of T in both pituitary and hypothalamus following infusion of this compound to the adult rat as observed recently by Whalen et al. (19) and Samperez et al. (18) plus the present finding that these tissues can convert T to DHT, suggests the possibility that DHT may play a role, either by itself or in conjunction with T, in the regulation of pituitary luteinizing hormone. To evaluate this postulate, the comparative effects of T and DHT on the suppression of radioimmunoassayable LH in the rat (20) are being evaluated currently.

The finding of DHT and Δ^4A demonstrates the presence of both 5α reductase and 17β dehydrogenase enzymes in these tissues. In other studies on steroid hormone metabolism in brain tissue, Sholiton et al. (21) found that the rat brain was capable of oxidizing or reducing

cortisol and cortisone. Grosser and Axelrod (22) found that tissue from various areas of the brains of fetal, newborn and adult baboons also converted cortisol to cortisone. They also found cortisol and cortisone acetate in the fetal and newborn brain. Although steroid acetates were sought in the presently described experiments, none was found. Knapstein et al. (23) demonstrated the sulfurylation of dehydroepiandrosterone in the human fetal brain. In the present experiments, steroid sulfates, including T sulfate, were sought but not found. The binding and retention of DHT in rat prostate have been demonstrated to occur in the nuclei (3,7). It remains to be ascertained whether, in neural tissues, the reaction is also intranuclear.

Upon intravenous administration of tritiated testosterone to normal rats, DHT was identified in prostate, seminal vesicle and preputial gland, all presumed targets of androgen action. In contrast, no DHT was found in liver, heart, lungs, testes, or levator ani muscle, although, with the exception of the levator ani, there was 3 to 9 times as much radioactivity in the latter tissues as in those in which DHT was found (3).

The finding of the conversion of T to DHT in

human fetal hypothalamic and pituitary tissues is of particular interest since we (25) and others (26,27) have demonstrated the capacity of the human fetal testes to biosynthesize T and because of the recent finding of gonadotropins in human fetal pituitaries (28).

Since androgens are also known to exert action on the epiphyseal ends of long bones in growing mammals, the conversion of T to DHT and 5α-androstane-3α,17β-diol (which also has significant androgenic activity (2)) in the femoral epiphysis is of interest. Our findings of 17β dehydrogenase and 5α reductase activity in the growing rat are in contrast to those of Murota and Tamaoki, who studied both progesterone and T metabolism in the epiphyses of femora and tibiae from 13 day old chick embryos and found 3α-hydroxy, 5β reduced metabolites (29). It is possible that the type of reduction varies with the species involved.

Whether the *in vitro* conversions demonstrated herein also occur *in vivo* remains to be ascertained. Current investigations in this laboratory are being directed toward this question, as well as the subcellular site of the reactions.

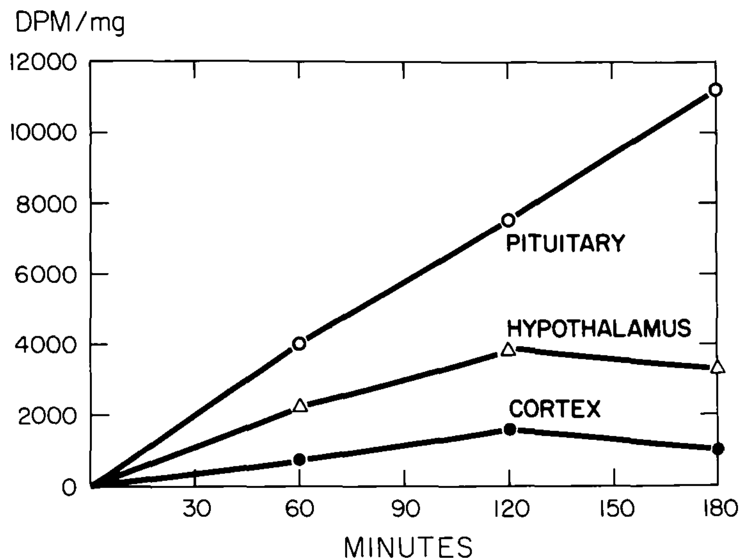

Fig. 1. Conversion of testosterone to androstan-17B-ol-3-one by adult rat brain tissues. Incubations at each time interval were performed on a pool of tissue obtained from six animals.

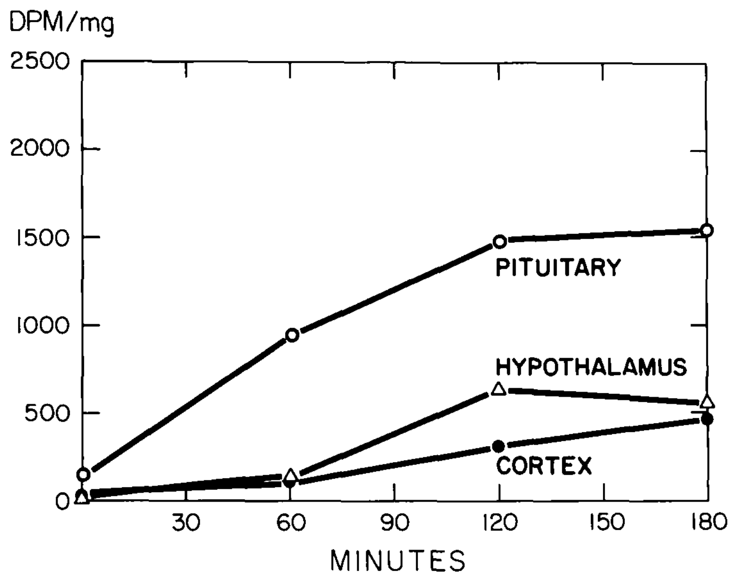

Fig. 2. Conversion of testosterone to Δ^4 androstene-3, 17-dione by adult rat brain tissues.

Table 2a. Recrystallization of 5α-androstan-17β-ol-3-one and Δ^4 androstene-3,17-dione from adult rat tissues incubated with testosterone 7α-^{3}H

Tissue	5α-androstan-17β-ol-3-one (as acetate)		Δ^4 androstene-3,17-dione	
		dpm/mg		dpm/mg
Rat Pituitary	SM[a]	2027	SM	1618
	C_1[b]	1985	C_1	1553
	C_2	1979	C_2	1583
	C_3	2033	C_3	1534
	ML_3[c]	2104		
Rat Hypothalamus	SM	3910	SM	1304
	C_1	3844	C_1	1332
	C_2	3819	C_2	1338
	C_3	3749	C_3	1323
	ML_3	3889	ML_3	1370
Rat Cerebral Cortex	SM	1117	SM	1487
	C_1	1061	C_1	1517
	C_2	1072	C_2	1558
	C_3	1117		
	ML_3	1166	ML_2	1625

a) SM = starting material
b) C = crystal
c) ML = mother liquor

Table 2b. Recrystallization of radioactive metabolites derived from tissues incubated with testosterone-7α-^{3}H

Tissue	5α-androstan-17β-ol-3-one (as acetate)		Δ^4 androstene-3,17-dione		5α-androstane-3α,17β-diol	
		dpm/mg		dpm/mg		dpm/mg
Human Fetal Pituitary	SM	387	SM	1046		
	C_1	257	C_1	1181		
	C_2	216	C_2	1103		
	C_3	220	ML_2	1071		
	ML_3	245				
Human Fetal Hypothalamus	SM	1481	SM	2735		
	C_1	1457	C_1	2789		
	C_2	1415	C_2	2749		
	ML_2	1418	ML_2	2557		
Immature Rat Epiphysis	SM	1990	SM	4184	SM	1972
	C_1	1918	C_1	4198	C_1	2041
	C_2	1792	C_2	4192	C_2	2144
	C_3	1884	ML_2	4113	C_3	2232
	ML_3	1854			C_4	1927
					ML_4	1893

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