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STEROID METABOLISM IN FOETAL TISSUES—IV CONVERSION OF TESTOSTERONE TO 5α-DIHYDROTESTOSTERONE IN HUMAN FOETAL BRAIN

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SUMMARY

This study reports the measurement of the conversion of testosterone to 5α-dihydrotestosterone by a double isotope method *in vitro* in hypothalamic and cerebral tissue of 23 human foetuses age 12–26 weeks of gestation. The following percentages of conversion by 100 mg of tissue were found: male hypothalamus 4·36% ± 0·77 (SEM) $n = 13$, male cerebrum 1·36% ± 0·27 (SEM) $n = 13$; female hypothalamus 3·73% ± 0·82 (SEM) $n = 10$, female cerebrum 1·90% ± 0·48 (SEM) $n = 10$. These data indicate that 5α-dihydrotestosterone formation in the brain of the human foetus is regionally different with higher conversion in the hypothalamus than in the cerebrum. These differences are statistically significant. A correlation between 5α-reductase activity in the human foetal male hypothalamus and cerebrum was found. The data are discussed in relation to the development of the neuroendocrine functions in the human foetal brain.

INTRODUCTION

Recent findings have indicated that androgen-dependent tissues (prostate, seminal vesicles, etc.) metabolize testosterone (T) to 5α-dihydrotestosterone (DHT) [1, 2]. This raises the question as to whether central nervous structures in the developing human foetus involved in endocrine regulation also possess this enzyme activity. If so, some steroid metabolites might modify feedback mechanisms, synthesis and release of neurohormones and influence sexual behaviour [3–6]. Animal experiments have demonstrated the uptake of androgens in the brain [7–9] and specifically the accumulation of DHT in certain brain structures [10, 11]. This is in good agreement with recent findings of a DHT receptor in the hypothalamus [12]. In previous studies a distinct difference in aromatization of steroids in cerebrum and hypothalamus in animals and the human foetus has been reported [13–15] and there are reports that such differences might also exist for 5α-reductase [20, 21].

Since the formation of DHT seems to be a decisive step for androgen action [1–4] and only two human foetuses have been studied so far [21], the metabolism of T to DHT in cerebrum and hypothalamus of 23 human foetuses has been examined.

EXPERIMENTAL

Tissue from 23 foetuses obtained from spontaneous and therapeutic abortions was examined. The weeks of gestation ranged from 12 to 26 and the crown-heel length from 7 to 34 cm. The foetal brain tissues were used for the incubation experiments within 15 min after delivery, hypothalamic and cerebral cortex tissues were dissected and immediately prepared for incubation.

All solvents were of reagent grade and purchased from Merck (Darmstadt, Germany) except for isooctane (Fluka, Buchs, Switzerland) and ligroin (Riedel, Hannover, Germany). Cofactors and enzyme preparations were obtained from Boehringer (Mannheim, Germany).

Crystalline and radioactive steroids. Unlabelled DHT (17β-hydroxy-5α-androstan-3-one) and its acetate were purchased from Steraloids (Pawling, U.S.A.). The labelled steroids were obtained from the Radiochemical Centre (Amersham, England) and New England Nuclear (Dreieichenheim, Germany).

[4-^{14}C]-Testosterone (59·0 mCi/mmol) was purified by celite column chromatography with system C-1 prior to use (Table 1); [7α-^{3}H]-17β-hydroxy-5α-androstan-3-one (44·0 Ci/mmol) was used as purchased.

Chromatography and measurement of radioactivity. Celite column chromatography was carried out with glass columns 65 cm long and an i.d. of 1 cm. They were packed first with 2 g celite mixed with 1 ml of ethylene glycol followed by 20 g celite mixed with 15 ml ethylene glycol. The sample was dissolved in 1·5 ml ethylene glycol, mixed with 2 g celite and added on the column. Elution of the steroids was carried out first with 100 ml isooctane followed by a gradient consisting of 200 ml isooctane and 200 ml of a mixture isooctane–ethyl acetate 70:30 v/v. Thin-layer chromatography (t.l.c.) was performed on glass plates (20 × 20 cm.) coated with silica gel G (0·25 mm thick) and developed in the solvent systems listed in Table

Table 1. Solvent systems for chromatography

Chromatography system						
Celite column C - 1	Ethylene glycol : Isooctane : Isooctane / Ethyl acetate 70/30					
Thin layer (Silica gel G)			Rf T	Rf T acetate	Rf DHT	Rf DHT acetate
T 1	Chloroform : Ethyl acetate	13 : 1 x 2	0.42	-	0.62	-
T 2	Methylene chloride:Dioxane	19 : 1	0.39	-	0.71	-
T 3	Chloroform	- x 2	-	0.75	-	0.91
T 4	Isooctane : Ethyl acetate	13 : 7	-	0.45	-	0.79

l at room temperature. Further details are described elsewhere [16].

Radioactivity was determined in a Packard liquid scintillation spectrometer Model 3380 equipped with an automatic absolute activity analyzer Model 544. The radioactivity was located after column chromatography by counting 1/10 of every second fraction and after t.l.c. with a thin-layer scanner Berthold LB 2723.

Outline of the method. For each incubation 100 mg of tissue was homogenized in 10 ml 1/15 M phosphate buffer, pH 7·4 and incubated with measured amounts of [4-^{14}C]-T and [7α-^{3}H]-DHT, 100 mg glucose-6-phosphate, 50 mg NADP, 25 mg NADPH and 54 IU glucose-6-phosphate dehydrogenase for 4 h at 37°C in a shaking water bath under air. The incubations were stopped and extracted three times with ethyl acetate. After evaporation of the organic solvent the remainder of the extract was first chromatographed on a celite column with system C-1. The DHT fraction was further chromatographed on t.l.c. in system T 1 and T 2 after addition of 500 μg of unlabelled authentic DHT. The residue was then acetylated with 0·5 ml pyridin and 0·5 ml acetic acid anhydride over 18 h at room temperature. After evaporation of the solvents the residue was further chromatographed in system T 3 and T 4. After repeated crystallization to constant isotope ratio [15], calculation of the per cent conversion was carried out based on previous *in vivo* [18] and *in vitro* studies [17, 19]. The per cent conversion of T to DHT was calculated according to the following formula:

$$\%\ \text{conversion of T to DHT} = \frac{\text{isotope ratio of the isolated DHT}}{\text{isotope ratio of the incubated tracers}} \times 100.$$

For statistical analysis Students *t*-test was used. Results are expressed as means (SEM). Blanc values for the method were obtained by incubating the radioactive tracers, cofactors and enzyme preparation without viable foetal tissue. The blanc value found was 0·03% ± 0·01 ($n = 7$).

RESULTS

As shown in Table 2, a highly significant difference for the conversion of T to DHT in human foetal male and female hypothalamus and cerebral cortex was found. Comparison of the results from hypothalamic tissue of 13 male foetuses with hypothalamic tissue from 10 female foetuses revealed a higher conversion in male than in female hypothalamic tissue (4·36% ± 0·77 vs 3·73% ± 0·82). However, this difference was not statistically significant. The rate of conversion by cerebral tissue of 13 male foetuses compared with 10 female foetuses was not significantly different (1·36% ± 0·27 vs 1·90% ± 0·48). A correlation between the per cent conversion of T to DHT in human foetal male hypothalamic and cerebral tissue was found as illustrated in Fig. 1. According to the celite column chromatograms other metabolites including androstanediol are present, but due to the design of the method quantitation could not be done.

DISCUSSION

The specificity of this method is determined by the use of authentic labelled and unlabelled steroids, multiple chromatography, acetate formation and repeated crystallization to constant isotope ratio.

Previous studies have indicated a variation of 5α-reductase activity in different areas of the brain in animals [20, 34]. This concerns testosterone

Table 2. Per cent conversion T to DHT in brain tissues of 13 human male and 10 human female foetuses

Male foetuses		Female foetuses	
Hypothalamus	Cerebrum	Hypothalamus	Cerebrum
6.59	1.10	5.74	0.61
3.46	0.85	6.65	3.46
7.11	1.15	4.43	2.30
4.06	0.85	2.92	1.38
8.33	2.69	4.04	4.65
9.13	3.82	4.93	3.36
2.12	1.64	1.31	1.34
1.20	0.30	2.58	1.83
3.04	1.68	0.08	0.12
0.47	0.52	0.18	0.13
5.00	0.99		
4.82	1.80		
1.25	0.38		
Mean (SEM)			
4.36± 0.77	1.36± 0.27	3.73±0.82	1.90±0.48
$p < 0.001$		$p < 0.025$	

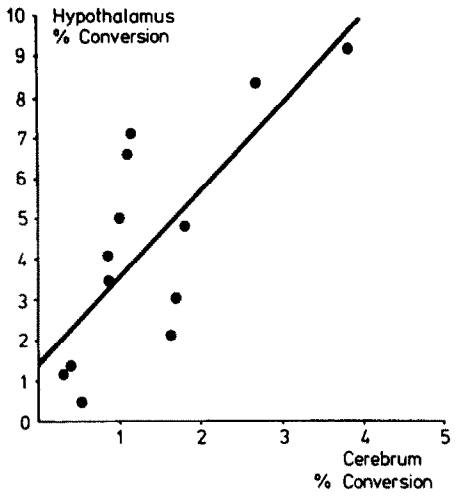

Fig. 1. Correlation of the percent conversion of T to DHT in hypothalamic and cerebral tissues of human male foetuses ($y = 1{\cdot}46 + 2{\cdot}124\ x$, $r = 0{\cdot}754$, P 0·01).

[3, 4, 20], progesterone [22, 23] and 20α-hydroxyprogesterone [24] as well. Accumulation of testosterone in hypothalamic tissue has been demonstrated by a variety of techniques [7–9]. Furthermore, differentiation of the neural mechanism of gonadotrophin secretion depends, at least in the rat, on steroids such as testicular androgens which suppress cyclicity and absence of gonadal activity leaves the hypothalamus in a cyclic state [25]. In the human, hypothalamic differentiation appears to occur early in foetal life [5]. Testosterone biosynthesis and secretion seems to parallel these events. This is substantiated by changes in testosterone concentration in cord blood [26, 27, 33], by the testosterone concentration in the foetal testicular tissue [28, 29, 33], by *in vitro* steroid metabolism and the histological-histochemical changes of the human foetal testis [17]. Therefore, the active metabolism of testosterone in human foetal hypothalamic tissue demonstrated in this study is considered to be of biological importance, particularly since differences have been found in both the hypothalamus and cerebrum (Table 2) similar to those observed in animal studies [20]. These data are in close agreement with the findings on aromatization of androgens by these tissues in which similar differences have been demonstrated in animals and the human foetus [13–15]. To illustrate this further the following comparison could be made in cerebral (c) and hypothalamic (h) tissue of a foetus of 15 weeks of gestation: (a) conversion of androstenedione to estrone [15] and (b) conversion of T to DHT were measured simultaneously demonstrating the difference of cerebral and hypothalamic tissue steroid metabolic activity: (a) 0·07% (c): 1·40% (h); (b) 1·15% (c): 7·11% (h). A small sex difference of 5α-reductase activity found in the hypothalami of rats [20] was also found for the human foetuses. T and other steroids have been shown to influence 5α-reductase activity under experimental conditions [3, 31, 32]. Whether differences in concentration of endogeneous testosterone or other steroids in the human foetus have modified the results presented in this paper cannot be ascertained. The correlation of 5α-reduction in male hypothalamic and cerebral tissue is noteworthy (Fig. 1) and underlines the possible significance of certain steroid metabolic events for human foetal neuroendocrine differentiation.

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