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TESTOSTERONE AND DIHYDROTESTOSTERONE LEVELS IN EPIDIDYMIS, VAS DEFERENS, SEMINAL VESICLE AND PREPUTIAL GLAND OF MICE AFTER hCG INJECTION

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Summary—Temporal changes of testosterone (T) and dihydrotestosterone (DHT) levels were measured by RIA in epididymis, vas deferens, seminal vesicle and preputial gland of adult male mice after a single injection of hCG. The response of circulating T to hCG stimulation was rapid and persisted over a period of 48 h. The temporal changes of androgen content of target organs paralleled the modifications of circulating T. In all organs the high androgen levels attained at 1 or 4 h plateaued until 24 h, decreased thereafter and returned to basal values at 72 h. The concentration of T by sex accessory organs was more accelerated by hCG injection than its conversion into DHT.

INTRODUCTION

The male gonad exerts an early inductive and morphogenetic influence on the development of the genital system [1] and the dependence of male sex accessory glands upon testicular androgens continues during adult life. It has been demonstrated that testosterone enters the cell of target organs and becomes transformed into active metabolites, particularly 5α-dihydrotestosterone (DHT) which, after binding to specific receptors, alter the transcription of specific genes leading to the appearance of new species of RNA and to protein synthesis [2, 3, 4]. Circulating androgens are obviously indispensable to normal target organ function but mechanisms for local testosterone (T) concentration and hormone metabolism play also a major role. The binding of hCG and subsequent stimulation of cyclic AMP formation and T production and secretion have been extensively studied [for reviews see 5, 6] but it is unknown if hCG stimulation affects the target organs steroid response.

The present study examined endogenous levels of T and DHT in accessory sex organs after hCG stimulation. Since a single injection of hCG induces a biphasic response in testicular androgen production in men [7, 8, 9] and since clear-cut differences between species have been reported [10], temporal changes in T and DHT levels were determined in accessory sex organs of adult mice.

EXPERIMENTAL

Animals

Adult male mice of the Swiss strain CD1 Charles River France (80–90 days) were housed 5 per cage. Laboratory animal food and water were available *ad libitum*. hCG (ISH Laboratories) was prepared in 0.9% saline solution and administered intraperitoneally. The dose used (8 IU) was chosen to elicit a maximal response [11]. Groups of 15 animals were injected with 8 IU of hCG and killed 1, 4, 12, 24, 48 and 72 h later. Males injected with saline solution alone ($N = 15$) 4 h before killing served as controls (0 h level). Blood was collected by decapitation, centrifuged and stored at −25°C until assayed. The organs were excised and immediately weighed and frozen at −25°C. The secretions of the accessory sex organs were not extruded before weighing.

Steroids measurements

T and DHT were measured by a radioimmunoassay method previously described [12]. The organs were homogenized in a 5 mM solution of *N*-ethyl-maleimide that inhibits the transformation of T into DHT. Plasma and organs extraction with ethyl acetate–isooctane after addition of tritiated T and DHT, was followed by a chromatography on a celite column which separates T from DHT. Recovery of added radioactive standard was 74 ± 10% (mean ± SD) for T and 60 ± 10% for DHT. The intra and interassay variations were 6 and 6.1% for T and 9 and 8.6% for DHT. The sensitivity of the method was 45 ± 10 pg for T and 55 ± 12 pg for DHT. RIA was performed using an antibody raised in rabbits against testosterone-3-*O*-carboxymethyl oxime bovine serum albumin at a final dilution of 1:45,000. The major steroids reacting in this system were T (100%) and DHT (74%). Among the 17 other steroids studied, the cross reactions of 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol were 5 and 3% respectively, for the others it was less than 2%.

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Table 1. Temporal changes in the weight (mg) of accessory sex organs of hCG treated mice

Time (h) after hCG injection	Accessory sex organs			
	Epididymides	Vasa deferentes	Seminal vesicles	Preputial glands
Pretreatment	92 ± 5	34 ± 2	215 ± 13	140 ± 11
1	89 ± 3	31 ± 3	231 ± 12*	144 ± 10
4	95 ± 3	33 ± 2	293 ± 14*	149 ± 12
12	96 ± 3	33 ± 1	300 ± 15*	157 ± 8
24	98 ± 4	35 ± 2	336 ± 14*	162 ± 7
48	98 ± 5	35 ± 2	315 ± 14*	158 ± 10
72	94 ± 4	35 ± 2	302 ± 15*	154 ± 7

Values are means ± SEM. ($N = 15$ in each group). *Significantly different from the 0 h control values.

Statistical evaluation was made by one-way analysis of variance (ANOVA) and Duncan's New Multiple Range Test when necessary. F-tests were calculated at the 0.01 level of significance. All data were expressed as the mean ± SEM.

RESULTS

The analysis of variance indicated that hCG treatment did not affect the weight of accessory sex organs except that of seminal vesicle which was increased from 4 to 72 h after injection ($F = 5.40$; $P < 0.001$) [Table 1]. DHT has not been measured in plasma since it was undetectable in adult male and only present at low levels after hCG stimulation (418 ± 80 pg/ml) [13]. Analysis of variance showed that circulating T concentrations were significantly affected by hCG injection ($F = 59$; $P < 0.001$). A significant increase was detected 1 h after injection and a 36–40 fold increase recorded at 1 h and 4 h (from 1500 ± 500 pg/ml to 59,000 ± 4000 pg/ml at 1 h). The level then decreased to 35,000 ± 8000 pg/ml at 12 h, plateaued at clearly elevated concentrations until 24 h and decreased gradually over a period of 48 h (Fig. 1).

One hour after hCG injection, T and DHT were not detected in quantities of spleen (a non-target organ) equivalent to those of the three accessory sex organs used ($N = 10$). Since the ratio of T to DHT differed with the organs, the levels of T and DHT were summed (total androgens Fig. 1) in order to compare the androgen content of the three organs. Analysis of variance showed that the androgen contents of the epididymis ($F = 59$), vas deferens ($F = 21$), preputial gland ($F = 19$) and seminal vesicle ($F = 49$) were significantly ($P < 0.001$) influenced by hCG treatment. Significant increases in the androgen content of all organs studied were detected 1 h after intraperitoneal injection of hCG. The immediate (1 h) androgen content increase in epididymis was about 10-fold (from 1200 ± 200 to 12,500 ± 1400 pg/2 epid.) and gradually decreased until 72 h (Fig. 1). The androgen content of vas deferens, seminal vesicle and preputial gland followed a similar pattern (Fig. 1). For all organs, maximal values were seen 1 or 4 h after hCG injection. If we examine separately T and DHT they parallel the changes recorded for total androgens and their levels return to pretreatment values at 72 h (Fig. 1). The concentrations of androgens in epididymis ($F = 31$), vas deferens ($F = 46$), preputial gland ($F = 21$) and seminal vesicle ($F = 45$) were significantly ($P < 0.001$) altered by hCG injection. For all organs, maximal concentrations were measured 1 or 4 h after hCG injection and they returned to basal values at 72 h (Table 2). In controls, the concentrations of androgens in accessory sex organs expressed as ng/g were higher than those of plasma expressed as ng/ml (Table 2). From 1 to 48 h after hCG administration, androgen concentrations in epididymis and vas deferens were higher than those measured in plasma, seminal vesicle and preputial gland. The lower T concentrating capacity of seminal vesicle may be due, in part, to increase of seminal fluid induced by hCG injection (Table 1). However, if the androgen concentrations in seminal vesicle were calculated according to control

Table 2. Temporal changes in the plasma T and in androgen (T + DHT) concentrations in accessory sex organs of hCG treated mice

Time (h) after hCG injection	Plasma ng/ml	Epididymis ng/g	Vas deferens ng/g	Seminal vesicle ng/g	Preputial gland ng/g
Pretreatment	1.5 ± 0.5	13 ± 2.5	19 ± 4	9 ± 2.0	3 ± 0.7
1	59 ± 4*	140 ± 13*	251 ± 19*	40 ± 3*	22 ± 1*
4	56 ± 5*	159 ± 17*	206 ± 15*	45 ± 3*	32 ± 3*
12	35 ± 8*	111 ± 10*	149 ± 15*	30 ± 3*	25 ± 3*
24	40 ± 9*	144 ± 10*	161 ± 11*	25 ± 1*	23 ± 3*
48	11 ± 2*	62 ± 6*	52 ± 19*	14 ± 2	11 ± 1*
72	2.4 ± 0.8	25 ± 3*	11 ± 2	7 ± 1	4 ± 0.6

Values are means ± SEM ($N = 15$ in each group). *Significantly different from the 0 h control levels.

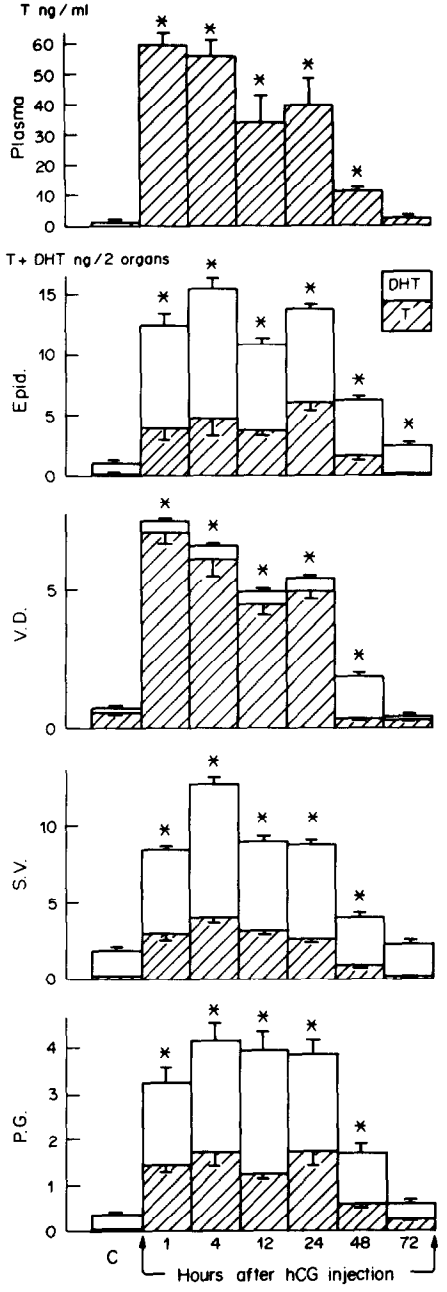

Fig. 1. Peripheral T concentrations and androgen (T + DHT) contents in epididymis (Epid.), vas deferens (V.D.), seminal vesicle (S.V.) and preputial gland (P.G.) after a single injection of 8 IUhCG in adult mice. Each bar represents the mean of androgens: DHT (open bar) + T (hatched bar). Each point is the mean ± SEM of 15 measurements. The asterisks indicate statistically significant differences from the 0 h control levels ($P < 0.001$).

seminal weight, they stayed lower than in plasma (39, 58, 41, 40, 19 and 10 ng/g at 1, 4, 12, 24, 48 and 72 h respectively.)

In controls, as in hCG treated males, DHT is the predominant androgen in epididymis, seminal vesicle and preputial gland, T in vas deferens. The temporal changes in T/DHT ratios indicate that hCG increases the concentration of T in all accessory sex organs but the conversion of T into DHT is not stimulated since the ratios are always higher or equal to those measured in controls (Table 3). The ability of the vas deferens to convert T into DHT is limited, as indicated by the maximal increase observed for DHT content (139 ± 30%) compared to those measured in epididymis (943 ± 107%), preputial gland (778 ± 94%) and seminal vesicle (455 ± 37%). Except for preputial gland, the T/DHT ratios returned to control values at 48 h.

DISCUSSION

The basal and maximal concentrations of plasma T reported herein were in good agreement with those previously measured in the laboratory [13] and by others [14]. The prolonged testicular response to hCG we observed paralleled with the uptake of hCG by testes which increased up to 24 h [15]. In rats [16, 17] and men [7, 8, 9], the circulating levels of T decrease from the acute peak (about 2 h) but remain significantly elevated at 24 h, rising to a second peak 48 to 72 h after initial injection. This biphasic pattern was not observed in mice: after the first increase, plasma T levels were maintained at a plateau for 24 h and declined gradually thereafter to basal levels [14, present results]. The discrepancy between results obtained in rats and mice may be due to differences between species or to the doses of hCG used since it has been shown that in rats a slight biphasic pattern response was observed only with the highest dose of hCG injected [15].

Although the steroidogenic changes after hCG injection have been well documented in plasma and testes, our study offers, for the first time, an extensive analysis of accessory sex organs steroid responses after hCG stimulation. Very few data are available concerning the quantification of androgens in accessory sex organs after hCG injection. In rats, intravenous administration of hCG for 1 h did not increase significantly T and DHT levels in caput epididymis [18]. Our results showed that testicular stimulation induced a rapid (1 h) and significant increase in the androgen content of all target organs studied. The temporal changes in androgen content of target organs paralleled those of circulating T: the levels attained at 1 h plateaued until 24 h, decreased thereafter and returned to the pretreatment values at 72 h. Considering the dramatic increases in serum T concentrations and the fact that each organ contains a few percentage of blood, a contribution of blood to the tissue androgen levels measured cannot be excluded. However, since androgens are not detectable in spleen of hCG treated males, the contribution of blood may be probably slight. The differences observed in the ability of accessory sex organs to concentrate circulating androgens may perhaps be related to their level of specific binding proteins since it has been shown that the androgen responsiveness of target organs can be explained by quantitative

Table 3. Temporal changes in the T/DHT ratio in accessory sex organs of normal and hCG treated mice

Time (h) after hCG injection	Accessory sex organs			
	Epididymis	Vas deferens	Seminal Vesicle	Preputial gland
Pretreatment	0.27 ± 0.09	3.31 ± 1.80	0.21 ± 0.08	0.20 ± 0.02
1	0.53 ± 0.15	19.10 ± 2.30	0.54 ± 0.08	0.96 ± 0.17
4	0.59 ± 0.10	12.80 ± 1.00	0.46 ± 0.04	0.69 ± 0.09
12	0.49 ± 0.05	11.00 ± 0.50	0.52 ± 0.05	0.46 ± 0.05
24	0.77 ± 0.15	10.60 ± 1.10	0.42 ± 0.02	0.86 ± 0.18
48	0.30 ± 0.04	4.40 ± 0.40	0.24 ± 0.03	0.59 ± 0.08
72	0.12 ± 0.02	2.20 ± 0.30	0.11 ± 0.02	0.65 ± 0.05

Values are means ± SEM (N = 15 in each group).

differences in their receptor levels and their 5α-reductase activity [19].

In agreement with earlier studies in rats [20, 21, 22], men [23] and mice [24] the present results showed that the accessory sex organs of controls and hCG treated animals contained both T and DHT, DHT being the predominant androgen in epididymis, seminal vesicle and preputial gland, T in vas deferens. The low or undetectable DHT levels measured in plasma of male mice [13] suggest that this hormone is essentially synthesized locally in the target organs, from circulating T. Because of the temporal changes in the T/DHT ratios, it is most likely that the concentration of T was more accelerated by hCG injection than its metabolism in target organs. Then, it seems that during the 72 h following hCG injection, the conversion of T into DHT by the accessory sex organs was not stimulated by increasing circulating levels of T and hCG.

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