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THE INFLUENCE OF GONADOTROPHIN (HCG) TREATMENT ON THE STEROIDOGENESIS BY INCUBATED RAT TESTES

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SUMMARY

The production of steroids and the metabolism of testosterone by the incubated testes from normal and HCG treated rats were studied. The production experiments show that HCG (3 UI/day for 10 days) induces an increase of testosterone and 5α-androstanediol production and a strong depression of 7α-hydroxytestosterone formation. The metabolism experiments indicate an increased transformation of testosterone to 5-reduced metabolites and a decreased conversion to 7α-hydroxytestosterone by the testes of HCG treated rats. It is concluded that HCG provokes a shift in the testicular metabolism pattern of testosterone from the 7α-hydroxylation to the 5α-reduction pathway.

INTRODUCTION

Studies made during the last decade [1–19] indicate that the metabolic transformation of testosterone by the testes of the rat is controlled mainly by four enzyme systems localized in the microsomial fraction: 5α-reductase, 17β-hydroxysteroid-dehydrogenase, 3β-hydroxysteroid - dehydrogenase + Δ^5 - Δ^4 - isomerase, and hydroxylation enzyme systems such as 7α-hydroxylase. Two of these enzyme systems hold a key position in the metabolization process of testosterone: 5α-reductase, which transforms testosterone to 5α-reduced compounds (mainly 5α-androstanediol), and 7α-hydroxylase, which converts the active hormone to 7α-hydroxytestosterone [6]. The activity of both enzymes is related to the age of the rats [20–26]. Indeed, the activity of 5α-reductase is high in the testes of young and low in those of adult rats; conversely, the activity of 7α-hydroxylase is very low in young rats and high in adult rats.

Inano *et al.*[19] have observed that the testicular microsomial fraction from mature rats injected daily for 14 days with high doses of human chorionic gonadotrophin (HCG) (68 IU) shows a decreased 7α-hydroxylase activity. In experiments in which the testes of adult rats were incubated, we observed that injections of smaller doses of HCG (10 IU daily for 10 days) provoke a considerable decrease of the production of 7α-hydroxytestosterone and an increase in 5α-androstanediol [25]. The present investigation was undertaken to study the influence of more physiological doses of HCG on the production and metabolism of steroids by incubated testes of the adult rat.

EXPERIMENTAL PROCEDURE

Male rats (150 days old) from the inbred laboratory strain were injected intraperitoneally for 10 consecutive days with 3 IU of HCG (Pregnyl®, Organon) or with saline (control rats). Twenty-four hours after the last injection the rats were sacrificed by decapitation, the testes were immediately excised and decapsulated and the testicular tissue was put in an incubation vessel containing 7 ml ice-cold Krebs–Ringer buffer solution enriched with NADP (1.8 mmol/l) and glucose-6-phosphate (5 mmol/l). All incubations were performed at 37°C under continuous shaking and under a constant flow of carbogen. The incubations were stopped by addition of 60 ml acetone–methanol (1:1 v/v) to the incubation mixture (buffer + testes).

Two kinds of incubation experiments were performed. In a first series of experiments (*metabolization experiments*) the metabolism of testosterone by the incubated testes was studied at different incubation times; in these experiments 25 μl ethanol containing 1000 ng [4-^{14}C]-testosterone were added to the incubation vessel and the incubations were stopped after 15, 30, 45, 60, 90 or 120 min. In other experiments the testes were preincubated for one h before the

The following trivial names are used in this paper:
testosterone: 17β-hydroxy-4-androsten-3-one
androstenedione: 4-androstene-3,17-dione
5α-androstanedione: 5α-androstan-3,17-dione
5α-androsterone: 3α-hydroxy-5α-androstan-17-one
epi-androsterone: 3β-hydroxy-5α-androstan-17-one
5α-androstanediol-3α: 5α-androstan-3α,17β-diol
5β-androstanediol-3α: 5β-androstan-3α,17β-diol
5α-androstanediol-3β: 5α-androstan-3β,17β-diol
5β-androstanediol-3β: 5β-androstan-3β,17β-diol
5α-dihydrotestosterone: 17β-hydroxy-5α-androstan-3-one
7α-hydroxytestosterone: 7α,17β-dihydroxy-4-androsten-3-one
7α-hydroxyandrostenedione: 7α-hydroxy-4-androstene-3,17-dione

addition of 25 μl ethanol containing 1 ng [1,2,6,7-^{3}H]-testosterone to the incubation vessel; 0.25 ml incubation liquid was taken 10 s (time zero) and 10, 20, 30, 40, 50 and 60 min after the addition of the tritiated testosterone and added to 2 ml acetone–methanol (1:1 v/v).

Finally, the metabolism of 7α-hydroxytestosterone and 5α-androstanediol by the incubated testes was studied; in these metabolism experiments, the testes were incubated for 15, 30, 60 or 120 min with 2000 ng of either [4-^{14}C]-7α-hydroxytestosterone or [1,2-^{3}H]-5α-androstanediol.

In a second series of experiments (*production experiments*), the amounts of testosterone, 5α-androstanediol (3α + 3β) and 7α-hydroxytestosterone produced by the incubated testes after different incubation times (15, 30, 45, 60, 90 and 120 min) were measured in the absence of exogenous steroid precursor.

The endogenous testosterone, 5α-androstanediol (3α + 3β) and 7α-hydroxytestosterone content of the testes at the time of sacrifice was estimated in decapsulated testes added immediately without incubation to a mixture of 7 ml water and 60 ml acetone–methanol (1:1 v/v).

Isolation and estimation of steroids

After being left overnight in the deep-freeze at −20°C, the acetone–methanol–water extract was filtered through a Whatman n° 1 filter and the filtrate was reduced to 3 ml by evaporation under a stream of nitrogen. The remaining water phase was extracted with 80 ml dichloromethane and the extract evaporated under a stream of nitrogen. The steroids were fractionated and estimated by liquid scintillation counting, fluorimetry or gas chromatography, as previously described [26].

The testosterone in the 0.25 ml incubation liquid of the experiments with preincubated testes was isolated by Sephadex LH 20 and paperchromatography, and estimated by radioimmunoassay using a highly specific antiserum prepared in rabbits with testosterone 3-carboxyoxime.

RESULTS

Testicular weight of the rats was not modified by the 10-day treatment with HCG. Mean weight of a pair of testes was 2.41 g ± 0.18 (S.D.) for the normal rats (n = 18) and 2.47 g ± 0.19 (S.D.) for the HCG-treated rats (n = 22). The weight of the prostate and seminal vesicles was increased by about 38% and 64% respectively.

1. *Metabolization experiments*

1a. *Metabolization of [4-^{14}C]-testosterone without preincubation.* The results concerning the metabolism of [4-^{14}C]-testosterone without a preincubation period are summarized in tables 1 and 2. They show that the testes from HCG-treated rats metabolize [4-^{14}C]-testosterone at a lower rate than the testes from normal rats. Much higher percentages of unmetabolized [4-^{14}C]-testosterone are found in the incubations of the HCG-treated rats than in those of controls; this is especially pronounced after 30 min of incubation.

Testicular tissue from both the HCG-treated and the normal rats transforms testosterone to a large number of metabolites, including androstenedione, 5α-androstanedione, 5α-dihydrotestosterone, 5α-androsterone, epiandrosterone, 5α-androstanediol (3α and 3β), 7α-hydroxytestosterone, 7α-hydroxyandrostenedione and a group of 11 unidentified metabolites, most of which are produced in amounts less than 0.2% of the incubated [4-^{14}C]-testosterone. Epiandrosterone, 5α-androsterone and 5α-dihydrotestosterone, as immediate precursors of 5α-androstanediol (3α or 3β), were estimated as one group, while one of the unidentified metabolites, X_1, was also measured. The percentage transformation of [4-^{14}C]-testosterone to these metabolites varies with the duration of incubation (Table 1). After 15 min approximately equal percentages of androstenedione are found in the incubates of the normal and the treated rats (±10%); from that time on, the percentages of that metabolite decrease more rapidly with incubation time for the normal than for the HCG-treated rats. Although only very small amounts of 5α-androstanedione are found at each incubation time, they are higher in the incubates of the HCG-treated than in those of the normal rats. Small amounts of 5α-androsterone, epiandrosterone and 5α-dihydrotestosterone are found in the incubates of the normal rats (from 1.2%–2.1%); in the incubates of the HCG-treated rats, the percentages of these metabolites are markedly higher and increase continuously with incubation time from 3.2%–6.6% (mean values). HCG treatment also results in an accumulation of androstanediol. While relatively low percentages (from 2.7–5.1%) androstanediol (5α + 5β) are found in the incubates of the normal rats, much higher percentages, which increase with incubation time (from 4.6%–20.5%), especially of both epimers of 5α-androstanediol, occur in the incubates of the HCG-treated rats. Finally, results of table 1 show that the transformation of [4-^{14}C]-testosterone to 7α-hydroxytestosterone, 7α-hydroxyandrostenedione and to the unidentified metabolite X_1, is strongly depressed by HCG. The percentages of these metabolites increase continuously with incubation time in both the incubates of the HCG-treated and the control rats; however, at each incubation time the amounts of 7α-hydroxytestosterone, 7α-hydroxyandrostenedione and of X_1 in the incubates of the normal rats are, respectively, about 5–10 times, 3–6 times and 2–5 times higher than in those of the HCG-treated animals.

In Table 2 the amounts of [^{14}C]-labelled metabolites found in the incubates were expressed for each incubation time as percentages of the amount of [4-^{14}C]-testosterone metabolized at that time. The

Table 1. Metabolization of [4-^{14}C]-testosterone by incubated testicular tissue of normal (N) and HCG (3 IU/day for 10 days) treated rats

Incubation time: min	Testosterone metabolites																					
	Unmetabolized testosterone		Androtenedione		5α-androstanedione		7α-hydroxytestosterone		7α-hydroxyandrostenedione*		Compound X_1		5α-dihydrotestosterone epiandrosterone 5α-androsterone		Androstanediol 5α, 3β		Androstanediol 5α, 3α		Androstanediol 5β, 3α or 3β		Androstanediol 5α, 3α or 5β, 3α	
	N	HCG	N	HCG	N	HCG	N	HCG	N	HCG	N	HCG	N	HCG	N	HCG	N	HCG	N	HCG	N	HCG
15	42.2	52.4	10.0	9.8	0.4	0.6	6.7	1.1	4.0	0.9	0.3	0.2	1.3	2.6	0.7	1.6	0.9	1.3	1.0	1.7	2.7	4.6
	50.1	55.2	12.3	10.3	0.5	0.8	8.1	1.4	5.0	1.0	0.4	0.3	1.5	3.8	1.1	1.9	0.9	1.7	1.1	2.2	3.0	5.6
30	33.9	49.8	5.9	9.0	0.4	1.2	11.8	1.7	4.8	1.7	1.0	0.3	1.6	2.8	1.4	2.3	1.8	2.7	1.1	2.3	4.3	7.3
	44.1	54.1	7.7	10.5	0.5	1.4	14.0	1.8	5.0	1.8	1.1	0.4	1.9	3.4	1.8	3.1	2.1	3.6	1.2	2.6	5.1	9.3
60	18.6	45.3	2.4	7.3	0.3	0.6	26.2	3.5	5.7	1.6	2.9	0.5	1.6	3.3	1.2	2.9	1.2	4.0	0.6	2.2	3.1	9.1
	21.8	50.6	3.4	8.9	0.3	0.7	31.6	4.7	6.4	1.9	3.0	0.5	2.1	3.9	1.3	3.9	1.6	4.9	0.7	2.7	2.7	11.5
90	8.2	42.5	1.3	6.5	0.2	0.7	40.0	2.2	7.9	1.5	3.9	0.7	1.1	4.7	1.5	4.7	1.3	7.2	0.9	1.6	3.7	13.5
	11.3	47.6	1.7	8.1	0.2	0.9	46.2	4.7	10.5	1.8	4.9	0.8	1.3	6.9	1.9	6.0	1.8	9.9	1.0	2.2	4.7	18.1
120	7.0	34.6	1.0	4.1	0.2	0.8	43.2	8.1	5.7	2.2	4.3	1.7	1.2	6.0	1.3	7.0	1.6	8.3	0.4	1.0	3.3	16.3
	8.1	40.8	1.0	5.4	0.2	0.8	48.6	10.9	7.7	2.5	6.1	2.2	1.4	7.6	2.0	8.1	1.7	11.0	0.6	1.4	4.4	20.5

* The detailed description of the identification of this substance will be published separately.

The data of two groups of experiments are given. Recovered unmetabolized testosterone and metabolites are expressed as a % of added [4-^{14}C]-testosterone.

Table 2. Metabolism of [4-^{14}C]-testosterone by incubated testicular tissue from normal (N) and HCG (3 IU/day for 10 days) treated rats.

Incubation time	15 min		30 min		60 min		90 min		120 min	
	N	HCG	N	HCG	N	HCG	N	HCG	N	HCG
Metabolized	57.8	47.6	66.1	50.2	81.4	54.7	91.8	57.5	93.0	65.4
testosterone	49.9	44.8	55.9	45.9	78.2	49.4	88.7	52.4	91.9	59.2
	17.3	20.5	8.9	17.9	2.9	13.3	1.4	11.3	1.0	6.3
androstenedione	24.6	22.9	13.7	22.8	4.3	18.0	1.9	15.4	1.1	9.1
	0.7	1.3	0.6	2.4	0.4	1.1	0.2	1.2	0.2	1.2
5α-androstanedione	1.0	1.8	0.9	3.1	0.4	1.4	0.2	1.7	0.2	1.3
5α-androsterone										
epi-androsterone	2.4	5.5	2.4	5.5	1.9	6.0	1.2	8.1	1.3	9.1
5α-dihydrotestosterone	3.0	8.4	3.4	7.4	2.7	7.9	1.5	13.1	1.5	12.8
Sum of										
5-reduced	7.6	16.4	9.5	22.5	6.1	23.8	5.4	32.9	5.0	27.1
metabolites	9.8	22.8	13.4	30.7	7.5	32.6	7.0	49.4	6.5	48.8
7α-OH-testosterone	11.6	2.3	17.8	3.4	32.2	6.4	43.6	3.8	46.4	12.4
	16.2	3.1	25.0	3.9	40.4	9.5	52.1	8.9	52.9	18.4
	6.9	1.9	7.3	3.4	7.0	2.9	8.6	2.6	6.1	3.4
7α-OH-androstenedione	10.0	2.3	8.9	3.9	8.2	3.8	11.8	3.4	8.4	4.2
	0.5	0.4	1.5	0.6	3.6	0.9	4.2	1.2	4.6	2.6
X_1	0.8	0.7	1.9	0.9	3.8	1.0	5.5	1.5	6.6	3.7

Metabolites are expressed as a % of the amount of metabolized [4-^{14}C]-testosterone. Data of two series of experiments are given.

calculated values demonstrate that at any incubation time 7α-hydroxytestosterone, 7α-hydroxyandrostenedione and the metabolite X_1 represent a much higher, and the 5(α + β)-reduced metabolites a much lower, percentage of the [4-^{14}C]-testosterone metabolites for the normal than for the HCG-treated rats.

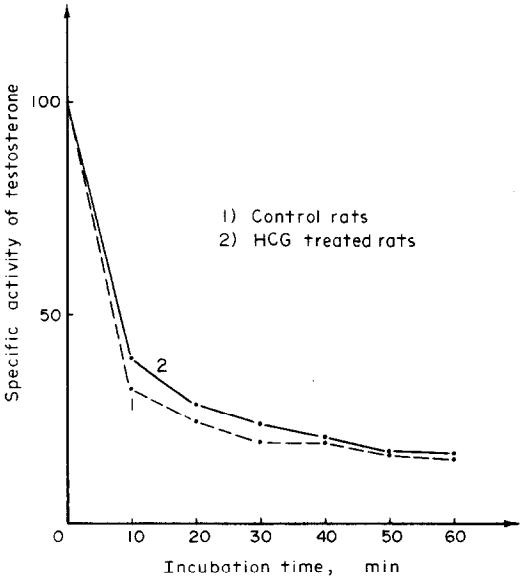

Fig. 1. Metabolization of testosterone by incubated testicular tissue of normal and HCG (3 IU/day for 10 days) treated rats. S.A. (c.p.m./ng) of testosterone isolated from the incubation medium after different incubation times; 1 ng [1,2,6,7-^{3}H]-testosterone was added after an incubation period of 1 h.

1b. *Metabolization of* [1,2,6,7-^3H]*-testosterone with preincubation.* The results of the experiments in which [1,2,6,7-^{3}H]-testosterone was added after a preincubation period of one h in order to reach a steady state in the testosterone levels of the incubates are presented in Fig. 1. From the curves, it is clear that the S.A. of testosterone measured in the incubation medium decreases in the same way as a function of time in the testicular incubates of both the normal and the HCG-treated rats.

1c. *Metabolization of* [1,2-^3H]*-5α-androstanediol and* [4-^{14}C]*-7α-hydroxytestosterone.* The results of the experiments concerning the metabolism of α-androstanediol and of 7α-hydroxytestosterone by the incubated testes are presented in Fig. 2. The curves indicate that the disappearance rate of both metabolites is not influenced by treatment with HCG. The curves also show that 7α-hydroxytestosterone is metabolized at a much lower rate than 5α-androstanediol; after 60 and 120 min, respectively, only 22% and 17% unmetabolized 5α-androstanediol are found, in contrast with 68% and 62% unmetabolized 7α-hydroxytestosterone.

2. *Production experiments*

The results of the endogenous production of testosterone, 7α-hydroxytestosterone and 5α-androstanediol, i.e. without addition of a precursor to the incubation vessel, are represented in Table 3. They clearly demonstrate that the content of testosterone in the incubates from normal or HCG-treated rats increases continuously with incubation time up to

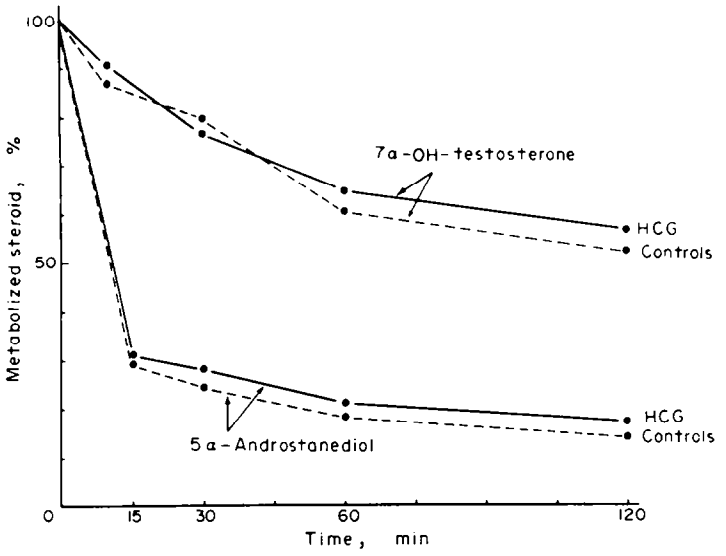

Fig. 2. Metabolization of [1,2-^{3}H]-5α-androstanediol (2 μg) and [4-^{14}C]-7α-hydroxytestosterone (2 μg) by incubated testicular tissue of normal and HCG (3 IU/day for 10 days) treated rats. HCG: testes from HCG rats. Controls: testes from normal rats.

about 60 min; from 60–120 min, constant testosterone levels are seen in the incubates. The incubates from HCG-treated rats contain about 4–5 times more testosterone than those from normal rats. The testosterone content of the testes of both groups of rats prior to the incubation shows about the same ratio (115 ng versus 30 ng). The 5α-androstanediol content of the testicular incubates from HCG-treated rats continuously increases with time of incubation and reaches a maximum value of about 260 ng (mean) after 120 min, whereas very small or undetectable amounts of that compound are found in the incubates of normal rats. The 5α-androstanediol content of the testes in both groups of rats prior to the incubation was under the limit of sensitivity of the method used.

The 7α-hydroxytestosterone content continuously increases with incubation time up to 120 min, both for the normal and the HCG-treated rats. The production of that metabolite is, however, very strongly depressed by the HCG treatment and much higher amounts of 7α-hydroxytestosterone are found in the incubates of the normal rats than in those of the HCG-treated animals. The 7α-hydroxytestosterone content of the testes from normal rats before the incubation is about 40 ng, while that from HCG-treated rats was under the limit of sensitivity of the method.

DISCUSSION

The metabolism of testosterone by testicular tissue of the rat has been studied extensively during the last decade. From the metabolites produced, it can be deduced that the testicular inactivation process is based mainly on both reduction and hydroxylation

Table 3. Production of testosterone, 7α-hydroxytestosterone and 5α-androstanediol by incubated testicular tissue of normal (N) and HCG (3 IU/day for 10 days) treated rats

Incubation time: min	*In vitro* production (ng/pair of testes) of: Testosterone N	HCG	7α-Hydroxytestosterone N	HCG	5α-Androstanediol N	HCG
15	67	260	178	40	<25	28
	93	312	242	62		35
30	96	415	320	56	<25	42
	158	605	268	94		66
45	125	615	368	118	<25	95
	175	695	445	135		128
60	155	875	515	125	<25	165
	225	890	585	155		186
90	145	870	578	137	<25	194
	205	878	710	175		230
120	165	860	640	128	<25	220
	225	940	810	172		290

The data of two groups of animals are given.

reactions; some of the metabolites, however, could not be identified up to now. Experiments in our laboratory (unpublished data) indicate that the testes of the rat are able to transform testosterone to at least 24 different metabolites *in vitro*. A great number of these compounds, however, are produced in very small quantities (less than 0.2%) and therefore were omitted from consideration in this study. The identity of the other metabolites (Table 1), with the exception of X_1, was ascertained by paper chromatography, counter current distribution and crystallization to constant S.A. The amounts of metabolite X_1 isolated from the incubation were not sufficient for identification studies; however, the fact that a single peak is obtained after paper chromatography in different systems and after counter current distribution suggests that this metabolite is a pure substance.

The metabolization experiments without a preincubation period do not allow one to conclude that there is a lower metabolization rate of testosterone in the HCG-treated testes, since the interference from endogenously-produced testosterone with the added [4-^{14}C]-testosterone is much higher in these incubates than those of the control animals.

The experiments concerning the metabolism of [^{3}H]-testosterone under steady-state conditions (constant testosterone levels are reached after one h of incubation both in the incubated testes and the incubation medium), show (Fig. 1) that the S.A. of testosterone in the incubates of HCG-treated rats decreases at the same rate as in those of normal rats; this indicates that the turnover rate of testosterone is not modified by the HCG treatment. The much higher testosterone levels in the incubations of the HCG-treated rats (Table 3) are hence caused by an increased production capacity of that hormone by the testicular tissue of these rats. Notwithstanding the high testosterone concentrations, a very limited part of the testosterone is converted through the hydroxylation pathway to 7α-hydroxytestosterone by the testes of HCG-treated rats. Since only minor differences are observed between the disappearance curves of 7α-hydroxytestosterone for normal and HCG-treated rats (Fig. 2), it can be concluded that the low 7α-hydroxytestosterone levels found in the incubates of HCG-treated rats result from a depressed formation through the 7α-hydroxylase enzyme system.

The results of the metabolization experiments also indicate that HCG treatment depresses the transformation to 7α-hydroxyandrostenedione and metabolite X_1, since at the different incubation times a much lower percentage of the metabolized testosterone has been converted to these compounds. Concomitant with the decreased production of 7α-hydroxytestosterone, 7α-hydroxyandrostenedione and of metabolite X_1, increased amounts of 5α-reduced compounds, mainly 5α-androstanediol, are found after the HCG treatment. This marked increase of the 5α-androstanediol levels is not due to a delayed metabolization of that metabolite (Fig. 2), but rather to increased conversion of testosterone through the 5α-reductase pathway. The fact that the percentage distribution of the metabolized [4-^{14}C]-testosterone over androstenedione, 5α-androstanedione and 5α-androsterone + epiandrosterone is considerably higher after HCG treatment at any incubation time implies a more exclusive oxidation of testosterone at C17 through the 17β-hydroxysteroid-dehydrogenase.

The results of our experiments show that the daily injection of HCG, for ten days to adult rats induces a shift in the testicular metabolism pattern of testosterone from the normally-predominating 7α-hydroxylase pathway to the 5α-reductase pathway. Inano *et al.*[19] also demonstrated that the microsomial fraction from testes of mature rats injected daily for 14 days with high doses of HCG (68 IU) showed a considerably reduced 7α-hydroxylase and 17β-hydroxysteroid dehydrogenase activity. Our studies indicate that more physiological doses of HCG (3 IU) also depress the hydroxylation reaction at position 7 (and possibly at other positions) of testosterone. The fact that a nearly 50% decrease in the cytochrome P-450 system levels (unpublished data) was found in the testicular microsomial fraction of our HCG-treated rats correlates well with these findings. In contrast to the decreased activity of the 17β-hydroxysteroid dehydrogenase reported by Inano *et al.*[19] after HCG treatment, our results indicate an increased activity of this enzyme system following HCG treatment. This difference may be due to the methods used, and particularly to the fact that the activity of the 17β-hydroxysteroid dehydrogenase was estimated by Inano *et al.* solely from the conversion of androstenedione to testosterone.

Our results do not elucidate the mechanisms by which HCG influences the testicular enzyme systems which regulate the metabolism of testosterone. The effect of HCG could be either direct, or mediated through the increased testosterone production, since a decrease of the testicular 7α-hydroxylase activity was observed by Inano *et al.*[19] after prolonged injections of high doses (1 mg/day for 14 days) of testosterone.

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