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17α-HYDROXYLASE AND TESTOSTERONE BIOSYNTHESIS IN RAT TESTES

P. KREMERS, CH. TIXHON and J. GIELEN
Laboratoire de Chimie Médicale, Institut de Pathologie,
Université de Liège au Sart-Tilman, Belgium

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SUMMARY

The biological properties of 17α-hydroxylase, the first enzyme in the conversion of C_{21} to C_{19} steroids, were studied in relation to the plasma level of testosterone in order to approach the problem of the regulation of the hormone's biosynthesis. The 17α-hydroxylase specific activity varies with the age of the animals and reaches a maximum 24 h after the plasma FSH peak, i.e. at the 30th day of life. The plasma testosterone concentration begins to rise during the following days to reach a plateau about 10 days after the FSH peak. A single administration of human chorionic gonadotropin (hCG) depressed the 17α-hydroxylase activity, but induced a significant rise in the plasma testosterone concentration. A more prolonged treatment with gonadotropins (200 IU/day/for 5 days) also enhanced the testicular 17α-hydroxylase activity. On the other hand, administration of testosterone, even in rats pretreated with hCG, reduced 17α-hydroxylase activity. This was not the case in hypophysectomized rats. Estrogen administration (10 μg/kg/day) also reduces the enzymatic activity. This effect is not mediated by the hypothalamo–hypophysial axis, but results from a direct action at the testicular level on enzyme biosynthesis. Our results seem to indicate that steroid-17α-hydroxylase is not an important regulatory step in testosterone biosynthesis.

INTRODUCTION

In testicular microsomes, the hydroxylation of progesterone or pregnenolone on the 17α position is the first step in the metabolic pathway leading from C-21 steroids to androgens. The 17α-hydroxylase, situated at a strategic position, is likely to play an important regulatory role. The production of the end product of the pathway, namely testosterone, has been extensively studied; however, much less is known about the activity of the various enzymes required for its biosynthesis [1,2]. Qualitatively and quantitatively, the production of androgens by the testes varies with the sexual maturation of the animals. In fact, testosterone is produced in large amounts only at the time of complete sexual maturity, i.e. after the fortieth day of life in the rat [3]. The administration of various hormones can also greatly modify the production of androgens. Pituitary hormones, for example, stimulate the biosynthesis of testosterone while androgens and estrogens depress the production of the hormone [4,5].

We have recently described a new and specific assay to measure the activity of progesterone and pregnenolone-17α-hydroxylase [6]. Thus, it was possible for us to investigate in detail the activity of this enzyme in relation to testosterone production and in particular under experimental conditions in which hormone production is modified. In this paper, we describe some biological properties of testicular 17α-hydroxylase. The neonatal development of the enzymatic activity was compared to plasma levels of FSH, LH and testosterone. The effect of hypophysectomy and hormone administration (hCG, testosterone and estradiol) on 17α-hydroxylase activity was compared to their effect on the testosterone level.

Reprint orders: P. Kremers, Laboratoire de Chimie Médicale, Institut de Pathologie, Université de Liège, B-4000 SART-TILMAN par Liège 1 Belgium

MATERIALS AND METHODS

Human chorionic gonadotropin (hCG) was purchased from Organon (Pregnyl), testosterone propionate and estradiol benzoate were obtained from Schering, S.G. The other chemicals and reagents were of analytical grade. The hormones were administered subcutaneously, solubilized in medicinal oil or in a saline solution.

Sprague–Dawley rats (Centre des Oncins, Lyon, France) were used throughout the experiment. For experiments requiring animals of a well defined age, rats of the same origin were bred in our own animal house. The animals were sacrificed by a blow on the head and carefully bled. The testes were removed and rapidly cooled in ice-cold isotonic KCl solution. All of the following manipulations were performed at 4°. The testes were first pressed through a metal disc perforated with 1.5 mm diameter holes. The resulting pulp was then diluted with 4 parts of the same solution and homogenized in a Potter–Elvehjem tube with a teflon pestle. The homogenate was centrifuged for 10 min at 9000 *g* in a refrigerated Sorvall apparatus. The 9000 *g* supernatant was used as the enzyme

source for our assays. They were stored at −20° for as long as 4 weeks without any significant loss of enzymatic activity.

17α-Hydroxylase activity was determined as described in detail elsewhere [6,7] using pregnenolone specifically tritiated at the 17α-position as the substrate. The limit of sensitivity was 0.01 nmol/h × mg protein.

Blood was collected in heparinized tubes after decapitation of the animal. These tubes were centrifuged for 5 min at 3000 *g* and the plasma was stored at −20°. Plasma LH and FSH levels were determined by radioimmunoassay according to Hendrick *et al.* FSH values were expressed in terms of NIAMD-FSH-RP-1, biological potency 2.1 and LH as NIAMD-LH-RP1, biological potency 0.03 × NIH-LH-S1 [8,9]. Testosterone was determined on the diluted plasma using a radioimmunoassay kit (TESTOK/G. 75 − R) from C.I.S. Belgium and was expressed in ng/ml.

RESULTS

1. *Steroid-17α-hydroxylase activity as a function of the age of the rats*

The activity of 17α-hydroxylase in the testes and the plasma concentrations of testosterone and FSH were measured in rats from the fifteenth day of life until adulthood (Fig. 1). The plasma FSH level increased with the age of the animals and reached a peak value on the 29th day of life. Thereafter, the concentration decreased and remained at a constant level after the 36th day. The plasma LH concentration did not vary significantly with the age and is not depicted on the figure. The testicular 17α-hydroxylase activity, when expressed in nmol/h × mg of protein, varied almost in parallel with the plasma FSH and reached a peak value on the 30th day. The hydroxylase S.A. decreased thereafter to reach the level characteristic of the adult animal around the 36th day. If the total enzymatic activity rather then the S.A. is presented (nmol/h × 2 testes) the result is quite different. The total 17α-hydroxylase activity increased continously from the 14th up to the 40th day of life where it finally reached a maximal and stable value. In fact, this pattern parallels the growth and the maturation of the testes. Similarly, plasma testosterone concentration increased rapidly after the 29th day, reaching a plateau around the 41st day.

2. *Steroid-17α-hydroxylase activity as a function of various hormonal treatments*

A. *Gonadotropins.* hCG (100 I.U.) Was administered daily to adult rats for 10 days. The hydroxylase activity was measured frequently following the first injection and at various times during the treatment (Fig. 2). The results of this experiment clearly indicated the existence of a biphasic response of the enzymatic activity to the hormonal treatment. Shortly after the first hCG injection, the hydroxylase activity decreased rapidly and was almost undetectable from the tenth until the twenty-fourth h. Thereafter, the enzymatic activity increased consistently up to the sixth day of the treatment and reached a plateau whose value corresponded to about twice that of the initial activity. A similar biphasic response of enzymatic activity to hCG administration has been observed by Purvis *et al.* [11]. On the other hand, the plasma testosterone level rose immediately and attained a maximal value 24 h after the hormonal treatment. It should be noted that the weight of the testes did not signficantly change during the first 10 days of treatment.

In hCG treated hypophysectomized rats, the 17α-hydroxylase activity did not drop initially as in the intact animals, but instead, decreased immediately

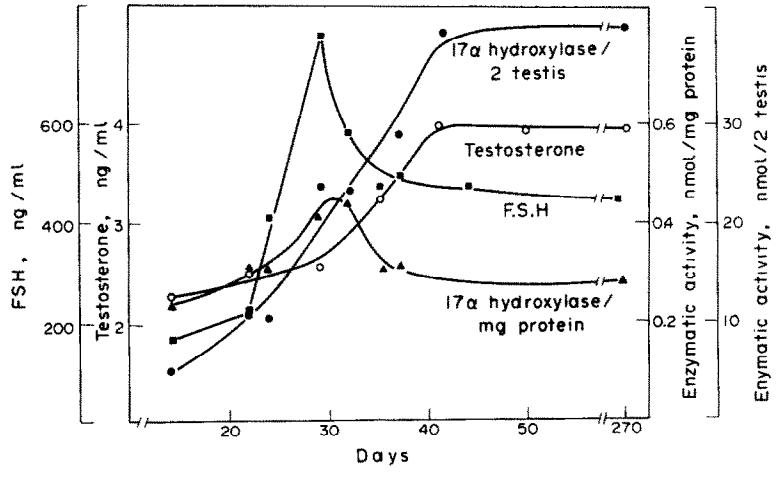

Fig. 1. Variation with the age of the animal of testicular 17α-hydroxylase activity and plasma FSH and testosterone concentrations. Each point represents the mean value for 5 animals. 17α-hydroxylase activity is expressed as nmol/mg post mitochondrial supernatant protein or as nmol/2 testis, FSH and testosterone concentrations in ng/ml of plasma.

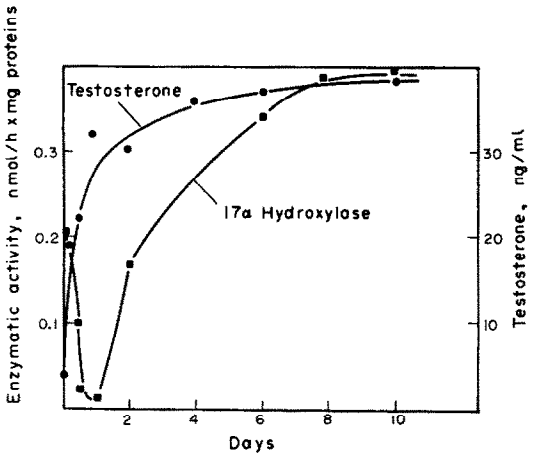

Fig. 2. Changes in testicular 17α-hydroxylase and plasma testosterone level after hCG treatment (500 IU/Kg of body weight). Rats were killed 6, 8, 12 and 24 h after the first hCG injection. The experiment was then continued with a daily hCG administration (at 3 P.M.), the rats being sacrificed at 9 A.M. Results are given as mean value ± S.D. (5 animals for each point).

after the hormone administration. After 3 days of treatment, the relative increase of 17α-hydroxylase and of plasma testosterone concentration were much higher in hypophysectomized rats than in intact animals (Table 1).

B. *Testosterone*

After a subcutaneous administration of testosterone (5 mg/day × kg), the testicular pregnenolone-17α-hydroxylase activity progressively decreased and became undectable after 6 days of treatment (Fig. 3). In hypophysectomized rats, the enzymatic activity, already depressed, was further decreased by this hormonal treatment. If testosterone is administered to rats which were pretreated for 7 days with hCG, an important difference is observed between the control and hypophysectomized animals (Table 2).

In control hCG pretreated animals, testosterone administration counterbalanced the effect of hCG. On the other hand, when testosterone was given to hypophysectomized hCG pretreated rats, the enzymatic activity remains at the level of the hCG treated animals.

C. *Estrogen*

The daily administration of estradiol benzoate (10 μg/kg) (Table 3) rapidly precipitated a decrease of the testicular-17α-hydroxylase activity. Twenty-four h after the first injection, the enzymatic activity had already reached a level corresponding to about 30% of the control. In hypophysectomized hCG treated rats, estradiol also decreases significantly the enzymatic activity. This is in contrast to the effect of testosterone which did not change the enzymatic activity in the same rats. It should be noted that *in vitro*, estradiol, testosterone and hCG have no effect on testicular-17α-hydroxylase activity [7].

D. *Half-life of testicular-17α-hydroxylase and of plasmatic testosterone*

The use of conventional methods to measure the half-life of an enzyme, such as the administration of a protein synthesis inhibitor like cycloheximide, was not possible in this case. In the hours following such an administration, no significant loss of hydroxylase activity could be noted in the testes.

For this reason, we approached the problem by another method. Control and hypophysectomized rats were treated for 10 days with hCG (100 IU/day).

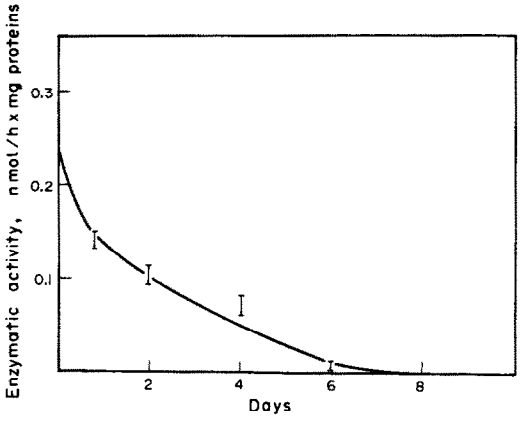

Fig. 3. Changes in testicular 17α-hydroxylase activity after a daily intraperitoneal administration of testosterone (5 mg/kg). Mean values of 5 rats are given. Vertical brackets indicate S.D.

Table 1. Effect of hCG treatment on testicular-17α-hydroxylase and on plasma testosterone

State of the animals	Treatment	Length of treatment (days)	17α-hydroxylase nmol/h/mg protein	Testosterone ng/ml
Normal	Saline	3	0.2 ± 0.01	3.7 ± 0.8
	hCG (500 IU/kg)	1	0.05 ± 0.01*	25 ± 2*
	hCG (500 IU/kg)	3	0.6 ± 0.02*	35 ± 1.5*
Hypophysectomized	Saline	3	0.06 ± 0.01	0.3 ± 0.1
	hCG (500 IU/kg)	1	0.20 ± 0.01*	5.5 ± 0.5*
	hCG (500 IU/kg)	3	0.40 ± 0.04*	6.5 ± 0.6*

The results are the mean and standard deviation for 5 animals (3 months old). * Statistically different from the corresponding controls (saline) at the 0.01 level of significance (Student's *t*-test).

Table 2. Effect of hCG and testosterone on testicular 17α-hydroxylase in control and hypophysectomized rats

Treatment	Normal rats	Hypophysectomized rats
NaCl	0.20 ± 0.02	0.02 ± 0.01
hCG	0.40 ± 0.05	0.65 ± 0.16
hCG-testosterone	0.23 ± 0.05*	0.75 ± 0.10

Enzymatic activity (mean ± S.D.; average of 5 animals) determined on 9000 *g* supernatant are given in nmol/h/mg protein. Control and hypophysectomized rats were treated for 10 days with physiological saline or hCG (500 IU/kg). The last group received hCG for 7 days and then testosterone and hCG simultaneously for the last 3 days.
* Statistically different from hCG treated group at 0.01 level of significance (Student's *t*-test).

Table 3. Effect of estrogen administration on testicular 17α-hydroxylase

Treatment	Length (days)	Normal rats	Hypophysectomized rats
Oil	3	0.20 ± 0.02	0.02 ± 0.01
Estradiol (100 μg/kg)	1	0.07 ± 0.03*	<0.01
Estradiol (100 μg/kg)	3	0.03 ± 0.02*	<0.01
hCG (500 IU/kg)	3	0.30 ± 0.03*	0.61 ± 0.25*
hCG + Estradiol	1	0.16 ± 0.05†	0.45 ± 0.16*

Testicular 17α-hydroxylase activity (nmol/h × mg protein) was determined after subcutaneous administration of estradiol benzoate (100 μg/kg). The first group received a single injection 24 h before sacrifice. The second group, one injection daily for three days. The third group received hCG for three days. The last group hCG for 3 days with one injection of estradiol on the third day. Sacrifice took place on the 4th day. Each value represents the mean ± S.D. for 5 rats. * Statistically different from controls (oil) at the 0.01 level of significance (Student's *t*-test). † Statistically different from control and from hCG treated animals.

Table 4. 17α-hydroxylase and testosterone half-lives

Rats	Testicular 17α-hydroxylase half-life (days)	Plasma testosterone
Normal	2.2 ± 0.3	1.7 ± 0.2
Hypophysectomized	2.5 ± 0.2	2.1 ± 0.4

The apparent half-lives were estimated from the physiological decay the enzymatic activity. For the purpose of comparison, the rate of decay of plasma testosterone levels was expressed as a half-life, i.e. the time necessary to observe a 50% decay of the hormonal level. The results are the mean and S.D. from 5 rats and are expressed in days. They were calculated from the semi logarithmic plot of enzymatic activity (or testosterone concentration) versus time.

The spontaneous decay of both 17α-hydroxylase and plasma testosterone concentration was estimated by measuring the two parameters during the 6 following days. It was then possible to calculate an apparent half-life for the hydroxylase activity as well as the rate of the plasma testosterone decay (Table 4). These two parameters were of the same order of magnitude, nevertheless, in both control and hypophysectomized rats, the plasma testosterone concentration decayed more rapidly compared to the testicular hydroxylase activity.

DISCUSSION

It is well known that testicular steroid biogenesis undergoes important changes during sexual maturation [12,13]. Our results are the first to correlate the testicular-17α-hydroxylase activity with plasma parameters (testosterone, FSH, LH). The 17α-hydroxylase S.A. rose with the age of the animal to reach a peak at the 30th day of life, immediately after the FSH peak; thereafter, FSH concentration and 17α-hydroxylase specific activity decreased to a new

level corresponding to that of the adult animal. The absolute 17α-hydroxylase activity (nmol/h/2 testes) rose from the 14th to the 40th day of life parallel to the increase in the plasma testosterone level and probably also in correlation with the maturation of the gland. Nevertheless, the increase in 17α-hydroxylase S.A. can be explained by either a specific enrichment in 17α-hydroxylase or by a preferential increase of the hormone producing tissue at this particular age.

After hCG treatment, testicular-17α-hydroxylase and the plasma level of testosterone did not vary in a parallel way. In normal rats, 17α-hydroxylase was first depressed 24 h following the injection of hCG. On the other hand, the plasma testosterone concentration rose immediately following hCG administration. This experiment indicates that 17α-hydroxylase activity is not likely to be rate limiting in the hCG induced production of testosterone. Indeed testosterone level rises in the plasma whereas 17α-hydroxylase activity decreases in the testes.

The decay of plasma testosterone is more rapid than testicular-17α-hydroxylase activity. This difference may be partially explained by the fact that plasma hormone levels result mainly from two antagonistic enzymatic processes: testicular biosynthesis and hepatic catabolism. Our results concerning the half-life of 17α-hydroxylase activity are similar to those found by Purvis and coworkers [5,10] for testicular cytochrome P-450, progesterone-17α-hydroxylase and C_{17}–C_{20} lyase. Thus, an overall regulation of the whole microsomal enzymatic complex seems to occur in the control of testosterone productions.

Testosterone and estradiol reduce the testicular-17α-hydroxylase in normal rats. However, in hypophysectomized rats pretreated with hCG, only the administration of estradiol was able to provoke a reduction of the enzymatic activity. As none of these hormones has a direct *in vitro* action on the enzyme [7], one must assume that testosterone acts via the hypothalamo-hypophysial axis, whereas estradiol acts directly at the testicular level. Since, we measure the enzyme concentration, it seems likely that estradiol is acting on the biosynthesis of a component of the 17α-hydroxylase multienzymatic complex.

Supporting our results, Tamaoki[14] has reported that treatment with hexestrol (a synthetic estrogen) also reduced the progesterone-17α-hydroxylase, provoking the appearance of an unusual metabolite in the testes, 3β-hydroxy-5α-pregnan-20-one.

The control of testosterone production by the hypothalamo–hypophysial axis could be explained by different hypotheses, such as: Another enzyme of the testosterone specific metabolic pathway is the rate limiting step, the microsomal enzymes involved in the biosynthesis of testosterone are regulated as a whole. This hypothesis is in agreement with the *in vitro* research of Mahajan and Samuels[16]. Another possibility is a control of the amount of substrate available for the hormone synthesis.

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