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STIMULATION *IN VIVO* OF TESTICULAR 7α-HYDROXYLASE ACTIVITY IN IMMATURE RATS BY LH AND hCG

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SUMMARY

Testicular C_{19}-steroid 7α-hydroxylase activity rises during puberty in the rat. Hormonal mechanisms responsible for this rise are unknown. The present study is to the best of our knowledge the first to report successful stimulation of testicular 7α-hydroxylase in immature rats.

Administration of crude and purified hCG every day for 3 or more days to immature rats in doses of 3 I.U. lead to significant stimulation of testicular 7α-hydroxylase activity. Purified preparations of subunits of hCG did not stimulate 7α-hydroxylase activity. Stimulation of testicular 7α-hydroxylase was, however, recorded when purified subunits of hCG were allowed to recombine and then administered to the animals.

Several preparations of LH failed to cause stimulation of 7α-hydroxylase when administered to immature rats. Treatment of immature rats with mixtures of LH and FSH, LH and prolactin or LH together with FSH and prolactin was also ineffective in this respect. Large doses of highly purified ovine-LH, rat LH and human LH were, however, able to stimulate testicular 7α-hydroxylase activity in immature rats. Preparations comprising hybrids of ovine-LH and hCG (oLHα + hCGβ, hCGα + oLHβ) stimulated testicular 7α-hydroxylase activity in immature rats. Administration of the same amounts of a hybride preparation of human and ovine-LH (oLHα + hLHβ) to immature rats was ineffective in this respect.

Administration of testosterone or estradiol to immature rats lead to supression of testicular 7α-hydroxylase activity. Combined treatment of such rats with hCG and testosterone or with estradiol and hCG augmented testicular 7α-hydroxylase activity to the same degree as that of animals treated with hCG only.

INTRODUCTION

The testis of mature rats produces large amounts of 7α,17β-dihydroxy-4-androsten-3-one (7α-hydroxy-testosterone) *in vitro*, and testicular production rate of this steroid equals that of testosterone [1–3]. 7α-hydroxylation of androgens curbs their androgenic potencies [4–6], but 7α-hydroxy-testosterone will inhibit testicular enzymes like 5-ene-3β-hydroxy steroid dehydrogenase [7], 5α-reductase and 3α-hydroxy steroid dehydrogenase [3] *in vitro*.

Testicular 7α-hydroxylase is low in immature rats compared to that of mature animals [2, 3, 8]. The testis of immature rats will in addition to 7α-hydroxy-testosterone also produce [8] small amounts of 7α-17β-dihydroxy-5α-androstan-3-one (7α-hydroxy-Dht), 5α-androstan-3α,7α,17β-triol (7α-hydroxy-3α-A'DIOL) and 5α-androstane-3β,7α,17β-triol (7α-hydroxy-3β-A'DIOL). Testicular 7α-hydroxylase activity starts to increase when the rats are 40 days old and at 60 days of age the adult level of activity is reached [2, 3, 8]. Little is, however, known about factors involved in the induction of 7α-hydroxylase in the immature rat testis. The purpose of this study was to investigate whether known hypophyseal or gonadal hormones could promote this enzymic activity in the testis of the immature rat.

EXPERIMENTAL

Materials

[1α,2α(*n*)-^{3}H]-Testosterone (SA 53 Ci/mmol) and [1α,2α(*n*)-^{3}H]-7α-hydroxy-testosterone (SA 36 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England. Scintillation fluid (Scint Hei-I) was obtained from F. Heidrenreich, Oslo, Norway, and was diluted with absolute ethanol (2:100 v/v). Unlabelled steroids were delivered by Steraloids Inc., U.S.A., except for 7α-hydroxy-testosterone which was kindly supplied by Dr K. Irmscher, E. Merck, Darmstadt, Germany. 7α,17β-dihydroxy-5-androstan-3-one, 5α-androstan-3α,7α,17β-triol and 5α-androstane-3β,7α,17β-triol were synthesized from 7α-hydroxy-testosterone. Synthesis and characterization of these steroids will be published elsewhere [6].

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Labelled steroids were purified by thin layer chromatography (TLC). TLC was performed on 20 × 20 cm silica gel-60 F254 plates purchased from E. Merck, Darmstadt.

From Sigma Chemicals Corp. were obtained NADP (Sigma-grade), glucose-6-phosphate, glucose-6-phosphate dehydrogenase type XV. All other chemicals and solvents were purchased from E. Merck, Darmstadt and were of *pro analysi* quality.

Hormone preparations

National Institute of Arthritis, Metabolism and Digestive Diseases and the National Pituitary Agency, University of Maryland School of Medicine, U.S.A., generously provided the following hormone preparations: NIH-FSH-S9, NIH-FSH-S12, NIAMDD-FSH-13(AFP-2846-C, potency 15 U/mg), NIH-LH-B10, NIH-LH-S17, NIH-LH-S19, NIAMDD-oLH-21 (AFP-2557-B, potency 2.5 U/mg), NIAMDD-rLH-I-4 and NIH-Prolactin-S10.

Purified preparations of hCG: CR-119 (11600 I.U./mg), CR-121 (13450 I.U./mg) and its subunits CR-119α (14.5 I.U./mg) and CR-119β-2 (12.7 I.U./mg) were gifts from the Center of Population Research, The National Institute of Child Health and Human Development of the National Institute of Health, U.S.A. Dr D. N. Ward and Dr W.-K. Lui Shum, Dept. of Biochemistry, M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, TX, U.S.A., generously provided the hybrid preparations: oLHα + hCGβ (WKS-16-37A), hCGα + oLHβ (WKS-16-38A) and oLHα + hLHβ (NSJ-4-37). Human growth hormone (hGH) and purified human-LH (hLH, 0303 1978, about 30,000 I.U./mg) were donated by Dr A. Aakvaag, Aker Hospital, Oslo, Norway. Crude-hCG (Pregnyl®, 3250 I.U./mg, lot nr. 91637) was a gift from Organon, Oss, The Netherlands. Crude-hCG (Physex-Leo® was also purchased from Leo Pharamaceutical Products, Ballerup, Denmark. TSH (Actyron) was obtained from Ferring Läkemedel, Sweden and ACTH (®Synacthen Depot) was obtained from CIBA. Professor Dr W. R. Butt, Dept. of Clinical Endocrinology, The Birmingham and Midland Hospital for Women, England, generously provided anti-hCG (F 96) and anti β-hCG (F 98). Anti-hCGβ-111-145 was a gift from Dr V. C. Stevens, Dept. of Obstetrics and Gynecology, Ohio State University, U.S.A.

Experiments in vivo

Immature Wistar male rats which were 20–23 days old at the start of experiments were used. The animals were separated from their mothers when the investigations started. Rat chow and tap water were provided *ad libitum*. The different preparations tested were administered subcutaneously in the neck region. Steroids were administered in sesam oil, other preparations were administered in 0.9% NaCl or in sesam oil containing 5% (weight) beeswax. Immature animals receiving vehicle only served as controls in all experiments.

Experiments in vitro

Twenty four hours after the last injection the rats were sacrified by decapitation. The testes were removed and testicular homogenates prepared as previously described [3]. Conditions of incubations and assay of testicular 7α-hydroxylase activity are discussed elsewhere [8]. Total 7α-hydroxylase activity was recorded as the sum of formation of the following isolated steroids: 7α-hydroxy-testosterone, 7α-hydroxy-Dht, 7α-hydroxy-3α-A'DIOL and 7α-hydroxy-3β-A'DIOL.

The protein content of the testicular homogenates was determined by the Bio-Rad protein assay (Bio-Rad laboratories Rich. CA, U.S.A.).

Purified preparations of human chorionic gonadotrophin subunits were recombined using the method of Morgan *et al.*[9].

RESULTS

Administration of 400 I.U. hCG (Physex Leo®, 3000 I.U./mg) every other day, or 200 I.U. every day, for 7 days to 23 day old rats, resulted in a four to five-fold increase in testicular 7α-hydroxylase activity compared to control rats receiving vehicle only (Table 1). Crude-hCG from Organon (Pregnyl, 3250 I.U./mg, lot no. 91637) administered for the same time and in the same doses, displayed similar effects on this enzymic activity in 23 day old rats (Table 1).

NIH-LH-S17 (100 μg/day for 7 days) administered to 23 day old rats did not, however, result in testicular 7α-hydroxylase activity above that of control animals (Table 1).

Mixtures of NIH-LH-S17 and NIH-prolactin-S10 (100 μg LH + 150 μg prolactin) or 75 μg LH + 220 μg prolactin + 100 μg NIH-FSH-S9 were given to 23 day old rats every day for 7 days. No stimulation of testicular 7α-hydroxylase activity was recorded compared with control rats receiving vehicle only (Table 1). Administration of prolactin (300 μg per day for 7 days), TSH (Actyron 1 I.U. per day for 7 days), ACTH (Synacthen-depo, 100 μg/100 g b.wt/day for 7 days) or hGH (100 μg/100 g b.wt/day for 10 days) to 23 day old rats did not result in any stimulation of testicular 7α-hydroxylase activity (Table 1).

Eight immature rats were injected subcutaneously with 200 I.U. of crude hCG (Pregnyl-Organon). A pair of these rats was sacrificed 1, 2, 3 and 5 days after the first injection. Each day the remaining rats were administered 200 I.U. hCG. The rats sacrificed the fifth day had then received a total of 5 injections of hCG. Control rats were subjected to the same treatment, but received vehicle only. The testes of these animals were removed and assayed for 7α-hydroxylase activity. The data obtained from this experiment are depicted in Fig. 1. Two days after the first injection, the hCG treated rats had significant higher tes-

Table 1. Effect of administering different hypophyseal hormones and hCG on testicular 7α-hydroxylase activity (pmol/min/mg protein) in immature rats

Hormone preparation administered	Dose	7α-Hydroxylase activity	
		Experimental group	Control group
hCG-(Physex-Leo®)	400 I.U.	450 ± 33	100 ± 17
hCG-(Pregnyl®-Organon)	400 I.U.	458 ± 92	100 ± 17
hCG-(Physex-Leo®)	200 I.U./day	583 ± 58	100 ± 17
hCG-(Pregnyl®-Organon)	200 I.U./day	379 ± 42	100 ± 25
NIH-LH-S17	100 μg/day	78 ± 7	100 ± 10
NIH-LH-S17	100 μg/day		
+NIH-P-S10	150 μg/day	104 ± 31	100 ± 10
NIH-LH-S17	75 μg/day		
+NIH-FSH-S9	100 μg/day		
+NIH-P-S10	220 μg/day	93 ± 28	100 ± 10
NIH-P-S10	300 μg/day	115 ± 18	100 ± 13
TSH (Actyron®)	1 I.U./day	100 ± 17	100 ± 17
ACTH (Synacthen-depo)	100 μg/100 g b.wt/day	37 ± 2	100 ± 9
hGH	100 μg/100 g b.wt/day	122 ± 42	100 ± 5

Hormones were dissolved in 0.9% NaCl and administered subcutaneously to 23 days old rats every day for 7 days, excpet hGH which was administered every day for 10 days. The high doses of hCG (400 I.U.) was administered every other day (total of four injections). Control groups received saline only. Mean data ± SD ($n = 4$) are expressed as % of control values (≈ 100%).

ticular 7α-hydroxylase level than the control animals (Fig. 1). Testicular 7α-hydroxylase activity continued to rise in a linear fashion in hCG treated rats in the 5 day period of the experiment (Fig. 1).

Different doses of crude hCG (Pregnyl-Organon) were administered to 23 day old rats every day for 3 days. Significant stimulation of testicular 7α-hydroxylase activity was observed with doses down to 3 I.U. (1 μg) per day (Fig. 2). Six I.U. (2 μg) of crude hCG given each day for 3 days resulted in full stimulation (Fig. 2). Two preparations of purified hCG, CR-119 (11 600 I.U./mg) and CR-121 (13 450 I.U./mg) were tested for ability to stimulate testicular 7α-hydroxylase in immature rats. Both preparations were potent stimulators of this enzymic activity when given in doses of 2 μg each day for 3 days to 22 day old rats (Table 2). Purified hCG-α subunit (CR-119, 14,5 I.U./mg) and purified hCG-β subunit (CR-119-2, 12,7 I.U./mg) were both ineffective in stimulating testicular 7α-hydroxylase activity in immature rats when given in doses of 2 μg/day for 3 days (Table 2). Purified hCG-α and hCG-β subunits were recombined as described by Morgan *et al.* [9]. The preparation of recombined hCG was administered to 22 day old rats for 3 days in a dose per day equivalent (on weight base) to 2 μg hCG-α + 2 μg hCG-β. This resulted in significant stimulation of testicular 7α-hydroxylase activity (Table 2).

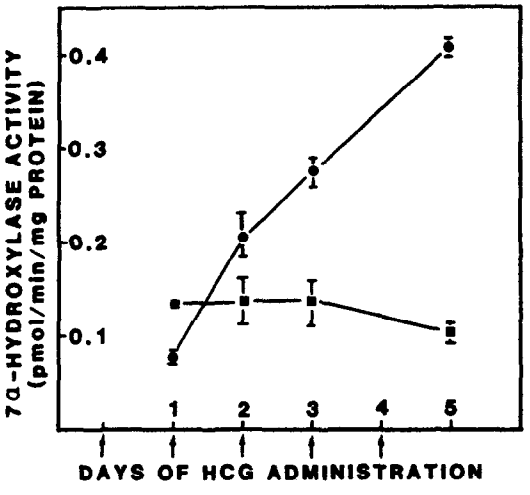

Fig. 1. Effect of administering hCG (Pregnyl-Organon) for different periods of time on testicular 7α-hydroxylase activity in 23 day old rats. Eight immature rats were injected with 200 I.U. hCG. A pair of these rats was sacrificed 1, 2, 3 and 5 days after their first injection. Each day the remaining rats were given 200 I.U. hCG (arrows). The rats sacrificed the fifth day had thus received a total of 5 injections (1000 I.U.) of hCG. Control rats were subjected to the same treatment but received vehicle only. The testes of these rats were removed and assayed for 7α-hydroxylase activity (pmol/min/mg protein). ●: HCG treated animals. ■: Saline treated animals (controls).

To saline solutions of crude hCG (®Pregnyl), different antisera preparations were added in amounts enough to neutralize the hCG-activity present. The antisera preparations used were: F-96-anti-hcG, F-98-anti-β-hCG and an antiserum direct towards the 111–145 peptide part of the β-hCG chain. The antiserum treated hCG solutions were administered to immature rats in a dose equivalent to 1 μg of hCG per day for 3 days. No stimulation of testicular 7α-hydroxylase activity was recorded following this treatment compared to control rats receiving antiserum diluted in saline only (Table 3).

Purified preparations of LH and FSH were tested

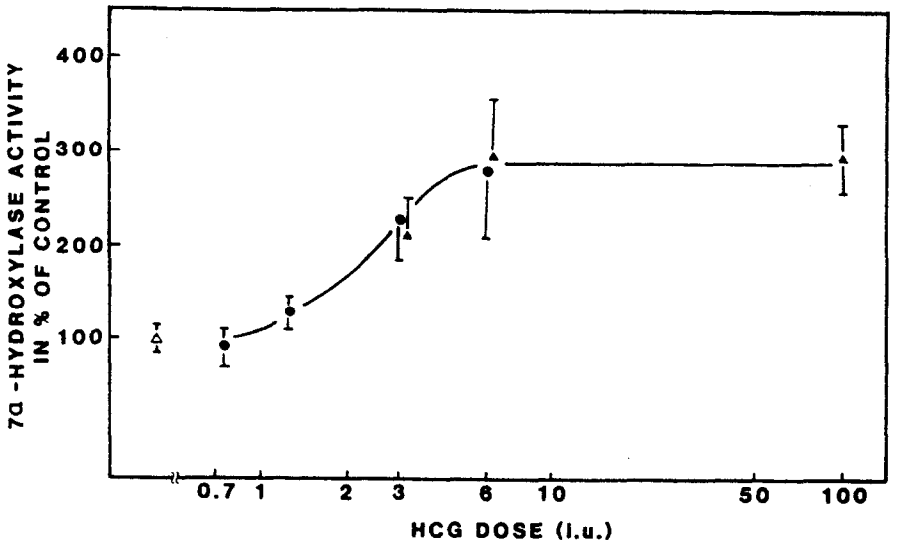

Fig. 2. Effect of administering different doses of hCG on testicular 7α-hydroxylase activity in immature rats. Groups of four rats were injected every day for 3 days with different doses of hCG (Pregnyl-Organon). Control rats received vehicle only. Mean values of testicular 7α-hydroxylase activity (pmol/min/mg protein) ± SD, are expressed as % of control values (=100%). Administered hCG dose is presented on logarithmic scale. Two independent experiments were done: ▲: values from experiment 1. ●: values from experiment 2. △: saline control value.

for their ability to stimulate testicular 7α-hydroxylase activity in immature rats. Administration of a large dose of purified LH (NIAMDD-oLH-21, 100 μg per day for 3 days) resulted in a significant increase in testicular 7α-hydroxylase activity in such rats (Table 4). When smaller doses (5 μg per day for 3 days) of this purified LH were administered to immature rats, no stimulation of testicular 7α-hydroxylase activity could be recorded (Table 4). Injections of 5 μg (150 I.U.) of highly purified human LH (hLH-0303 1978) each day for 3 days to immature rats gave significant increase in testicular 7α-hydroxylase activity compared to control rats (Table 4). A dose of 0.2 μg (6 I.U.) hLH injected every day for 3 days was, however, not able to stimulated testicular 7α-hydroxylase activity when administered to immature rats (Table 4). Purified rat LH (NIAMDD-rLH-I-4) administered to 23 day old rats in a dose of 6.5 μg per day for 3 days, resulted in a significant stimulation of testicular 7α-hydroxylase activity. Immature (23 day old) rats injected 3 days with a dose of 35 μg per day of purified FSH (NIAMDD-oFSH-13), did not have testicular 7α-hydroxylase activity significantly different from control rats injected with vehicle only (Table 4).

In some of the experiments the ventral prostate of the experimental rats was dissected and weighed. The groups of rats showing significant stimulation of testicular 7α-hydroxylase activity in response to hormone treatment, had significantly higher ventral prostate weight (mg/100 g b.wt) compared to rats receiving vehicle only (Table 4). Treatment of 23 day old rats with 5 μg per day of NIAMDD-oLH-21 for 3 days resulted in ventral prostate weights significantly above that of control rats. testicular 7α-hydroxylase

Table 2. Effect of administering different preparations of hCG, hCG-α-chain, hCG-β-chain and recombined hCG-α-chain and hCG-β-chain on testicular 7α-hydroxylase (pmol/min/mg protein) activity in immature rats

Preparation administered	Dose	7α-Hydroxylase activity	
		Experimental group	Control group
hCG (Pregnyl-Organon)	2 μg (6 I.U.)/day	224 ± 46	100 ± 21
hCG-CR-121 (purified-hCG)	2 μg (26 I.U.)/day	204 ± 34	100 ± 21
hCG-CR-119 (purified-hCG)	2 μg (24 I.U.)/day	218 ± 7	100 ± 23
hCG-CR-119α (purified-hCG-α-chain)	2 μg (0.03 I.U.)/day	93 ± 29	100 ± 23
hCG-CR-119β-2 (purified-hCG-β-chain)	2 μg (0.025 I.U.)/day	66 ± 22	100 ± 23
hCG-CR-119α (2 μg) + hCG-CR-119β (2 μg)	2 + 2 μg/day	210 ± 51	100 ± 21

The preparations were dissolved in 0.9% NaCl and given subcutaneously to 22 day old rats every day for 3 days. Purified hCG-α-chain and hCG-β-chain were recombined as described by Morgan *et al.*[9] and administered to immature rats in a dose equivalent to 2 μg hCG-α-chain and 2 μg hCG-β-chain. Control groups received vehicle only. Mean data ± SD (n = 4) are expressed as % of control values (=100%).

Table 3.

Preparation administered	Dose	7α-Hydroxylase activity: Experimental group	7α-Hydroxylase activity: Control group
hCG (Pregnyl-Organon)	1 μg (3 I.U.)/day	248 ± 67	100 ± 24
hCG (Pregnyl-Organon) + anti hCG (F-96)	1 μg (3 I.U.)/day	88 ± 33	100 ± 49
hCG (Pregnyl-Organon) + anti hCG β (F-98)	1 μg (3 I.U.)/day	135 ± 39	100 ± 11
hCG (Pregnyl-Organon) + anti hCG-β-111-145	1 μg (3 I.U.)/day	116 ± 12	100 ± 5

To saline solutions of crude hCG (Pregnyl-Organon) were added antisera directed towards hCG, hCG-β-chain or hCG-β-chain-111-145 peptide fragment, in suffificent quantities to neutralize the hCG-activity present. The ensuing mixtures were administered subcutaneously to 22 day old rats every day for 3 days. Control rats received saline solutions of antisera only. Mean values of testicular 7α-hydroxylase activity (pmol/min/mg protein), ±SD, (n = 4), are expressed as % of the control values (= 100%).

activity in these rats was, however, in the same range as the control animals (Table 4).

Different preparations comprising recombinations of different LH and hCG subunits, were tested for their potency to alter testicular 7α-hydroxylase activity. Hybrid preparations consisting of oLHα + hCGβ (WKS-16-37A) or hCGα + oLHβ (WKS-16-38A) were both able to stimulate testicular 7α-hydroxylase activity when given to immature rats each day for 3 days at a dose of 6.5 μg and 6.7 μg respectively (Table 5). Administration of a hybrid of oLHα + hLHβ at a dose of 6.7 μg per day for 3 days did not result in significant stimulation of testicular 7α-hydroxylase activity (Table 5).

The effect of changing the vehicle for administration of LH and hCG from 0.9% NaCl to sesam oil containing 5% (weight) beeswax was investigated. Stimulation of testicular 7α-hydroxylase activity in immature rats was recorded when 2 μg hCG (Pregnyl-Organon) was injected every day for 3 days in saline or in sesam oil–beeswax. NIAMDD-oLH-21 was ineffective in this respect when administered in saline or in sesam oil–beeswax at a dose of 5 μg per day for 3 days (Table 5).

Testosterone was administered to immature rats in different doses and in periods of 3 days or 7 days. Testicular 7α-hydroxylase activity in testosterone treated rats were significantly below that of control rats receiving vehicle only (Table 6). Administration of estradiol (100 μg/100 g b.wt per day) for 7 days to immature rats resulted in a depression of testicular 7α-hydroxylase activity (Table 6). Stimulation of testicular 7α-hydroxylase activity were recorded in immature rats treated concomitantly with testosterone and hCG or estradiol and hCG (Table 6).

DISCUSSION

Testicular 7α-hydroxylase activity in immature rats could reproducibly be stimulated by injections of different preparations of crude and purified hCG (Tables 1 and 3, Figs 1 and 2). LH preparations displayed in this respect less potency *in vivo* (Tables 1, 2 and 4). Testicular enzymic activities like alcohol dehydro-

Table 4. Effect of administering different preparations of LH and NIAMDD-oFSH-13 on testicular 7α-hydroxylase activity and prostate weight in immature rats

Hormone preparation administered	Dose	7α-Hydroxylase activity: Experimental group	7α-Hydroxylase activity: Control group	Ventral prostate weight: Experimental group	Ventral prostate weight: Control group
hCG (Pregnyl-Organon)	2 μg (6 I.U.)/day	287 ± 39	100 ± 28	162 ± 7	100 ± 17
NIAMDD-oLH-21	5 μg/day	87 ± 4	100 ± 28	131 ± 15	100 ± 17
NIAMDD-oLH-21	100 μg/day	188 ± 29	100 ± 28	167 ± 18	100 ± 17
hLH	0.2 μg (6 I.U.)/day	97 ± 22	100 ± 28	106 ± 17	100 ± 17
hLH	5 μg (150 I.U.)/day	190 ± 47	100 ± 38	141 ± 7	100 ± 10
NIAMDD-rLH-I-4	6.5 μg/day	156 ± 21	100 ± 21		
NIAMDD-oFSH-13	35 μg/day	135 ± 38	100 ± 8		

The hormone preparations were dissolved in 0.9% NaCl and administered to 23 day old rats every day for 3 days. 7α-Hydroxylase activity (pmol/min/mg protein) and ventral prostate weights (mg/100 g b.wt) are expressed as mean values ±SD (n = 4) in % of the control values (= 100%).

Table 5. Effect administering different preparations comprising hybrid molecules of oLH, hLH and hCG or oLH and hCG in sesam oil/beeswax on testicular 7α-hydroxylase activity in immature rats

Preparation administered	Dose	7α-Hydroxylase activity	
		Experimental group	Control group
oLHα + hCGβ hybrid (WKS-16-37A)	6.5 μg/day	171 ± 43	100 ± 24
hCGα + oLHβ hybrid (WKS-16-38A)	6.7 μg/day	139 ± 5	100 ± 8
oLHα + hLHβ hybrid (NSJ-4-37)	6.7 μg/day	128 ± 25	100 ± 8
NIAMDD-oLH-21	5 μg/day in 0.9% NaCl	91 ± 19	100 ± 22
NIAMDD-oLH-21	5 μg/day in sesam oil/beeswax	111 ± 22	100 ± 16
hCG (Pregnyl-Organon)	2 μg (6 I.U.)/day in 0.9% NaCl	246 ± 53	100 ± 22
hCG (Pregnyl-Organon)	2 μg (6 I.U.)/day in sesam oil/beeswax	179 ± 37	100 ± 16

The hybrid oLHα + hCGβ was given in a dose of 6.5 μg/day every day for 3 days while the hybrids hCGα + oLHβ and oLHα + hLHβ were given in a dose of 6.7 μg/day for 3 days. oLH and hCG in saline or in sesam oil/beeswax (5% beeswax by weight) were administered every day for 3 days. Control rats received vehicle only. Mean values of 7α-hydroxylase activity (pmol/min/mg protein) are expressed as % of control values (= 100%).

genase [10] nonspecific carboxyl esterase [11, 12], 5-ene, 5β- and 5α-3β-hydroxy-steroid dehydrogenases [12–16] and 5α-reductase [15] have previously been shown to be inducible in the testis of normal and hypophysectomized immature rats by administration of LH and hCG. The present study is to our knowledge the first to demonstrate successful stimulation of 7α-hydroxylase activity in immature rat testis *in vivo*. Lacroix *et al.*[17] have administered hCG to 16 day old rats for 10 days without observing stimulation of testicular 7α-hydroxylase activity. Most 7α-hydroxylase assays published depend on measurements of 7α-hydroxy-testosterone only [1–3, 17, 18], while our 7α-hydroxylase assay also includes measurements of 7α-hydroxy-Dht, 7α-hydroxy-3α-A'DIOL and 7α-hydroxy-3β-A'DIOL [8]. 5α-Reduced metabolites of testosterone dominate quantitatively in the immature rat testis [2, 3] and measurements of 7α-hydroxylated 5α-reduced androgens give a more sensitive 7α-hydroxylase assay in such testes.

The testis possesses prolactin and TSH receptors [19–21] and prolactin and growth hormone can prevent LH receptor loss following injections of large doses of LH or following hypophysectomy [22, 23]. However, administration of prolactin, hGH or TSH did not have any effect on testicular 7α-hydroxylase activity (Table 1). Injections of ACTH resulted in a significant decrease in testicular 7α-hydroxylase activity (Table 1). Treatment of immature rats with cortisol leads to reduced plasma testosterone levels and in reduced androgen secreting capacity of Leydig cells exposed to hCG *in vitro*[24, 25].

Our results suggests that induction of testicular 7α-hydroxylase following hCG administration to immature rats is due to the hCG molecule itself and not to contaminations in the hormone preparations employed (Table 2). HCG displays in addition to LH activity also some FSH activity which is probably an intrinsic property of the hCG molecule [26, 27]. Treatment with FSH alone or in combinations with

TABLE 6. Effect of administering steroids or steroids together with hCG on testicular 7α-hydroxylase activity in immature rats

Hormone administered	Dose	Days of treatment	7α-Hydroxylase activity	
			Experimental group	Control group
Testosterone	1 mg/100 g b.wt/day	3	68 ± 8	100 ± 18
Testosterone	3 mg/100 g b.wt/day	3	50 ± 23	100 ± 35
Testosterone	100 μg/100 g b.wt/day	7	52 ± 4	100 ± 13
Testosterone	3 mg/100 g b.wt/day	7	24 ± 8	100 ± 24
Estradiol	100 μg/100 g b.wt/day	3	50 ± 10	100 ± 17
Testosterone + hCG (Pregnyl-Organon)	3 mg/100 g b.wt/day 2 μg (6 I.U.)/day	3	379 ± 74	100 ± 35
Testosterone + hCG (Pregnyl-Organon)	3 mg/100 g b.wt/day 2 μg (6 I.U.)/day	7	296 ± 74	100 ± 24
Estradiol + hCG (Pregnyl-Organon)	100 μg/100 g b.wt/day 16 μg (200 I.U.)/day	7	240 ± 67	100 ± 17
HCG (Pregnyl-Organon)	16 μg (200 I.U.)/day	7	273 ± 27	100 ± 17

Rats were injected every day for 3 or 7 days as indicated in the Table. Mean testicular 7α-hydroxylase activity (pmol/min/mg protein) ± SD ($n = 4$) are expressed as % of control values (= 100%).

LH and prolactin did not stimulate testicular 7α-hydroxylase activity in the immature rat (Tables 1 and 4).

Administration of LH in sesam oil/beeswax has previously been shown to increase the biological potency of LH *in vivo*[14, 28], but this vehicle did not increase biopotency of NIAMDO-oLH-21 in our study (Table 5).

Androgen production increases in the testes of immature rats following administration of LH or hCG [29, 30]. This leads to an increase [29] in ventral prostate weight (Table 4). Androgen production, and thereby ventral prostate weight increase, seems to be stimulated with lower doses of LH than required for stimulation of 7α-hydroxylase activity (Table 4).

The 7α-hydroxylase seems not to be substrate induced, since administration of testosterone to immature rats gave a decrease in testicular 7α-hydroxylase activity (Table 6). Injections of estradiol had similar effects (Table 6). Treatment of immature rats with testosterone or estradiol together with hCG, augmented 7α-hydroxylase activity to the same degree as that of animals treated with hCG only (Table 6). This indicates that the effect of administering testosterone or estradiol on testicular 7α-hydroxylase activity is not at the level of the testis, but possibly due to suppression of circulating LH and FSH [5, 31, 32]. It has been shown previously that the Leydig cells possess an estrogen receptor [33] and that administration of estradiol or testosterone to intact or hypophysectomized rats will influence testicular properties like LH/hCG receptor affinity, C_{17}-C_{20}-lyase activity and 17α-hydroxylase activity [21, 31, 32, 34].

The biological role of 7α-hydroxylated androgens is still unknown. Such steroids display no androgenic or anabolic properties tested *in vivo*[4–6]. The activity of testicular 7α-hydroxylase increases during puberty [2, 3]. The present study has demonstrated that administration of LH or hCG to immature rats will lead to increased 7α-hydroxylation of androgens in the testis. The low potency displayed by LH compared to hCG in our study may be due to faster removal of LH and/or lower affinity for LH on the LH/hCG receptor in the testis of the immature animal [37]. HCG is more stable in the bloodstream than LH due to the higher carbohydrate content of hCG [38, 39]. Blood plasma levels of LH are similar in immature and mature rats [40–42] and testicular responsiveness in terms of androgen production following LH and hCG exposure, is not very different in mature and immature rats [43].

Our results indicate that LH is the natural stimulator of testicular 7α-hydroxylase during puberty. The mechanisms by which this occurs are, however, not clear and await further investigation.

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