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EFFECTS OF HCG AND PMS ON BIOCONVERSION OF PROGESTERONE, ANDROSTENEDIONE AND TESTOSTERONE IN IMMATURE RAT TESTES

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SUMMARY

The present study was conducted to determine whether exogenous gonadotropins will alter the 5α-reductase activity in immature rat testes. Newborn and 10-day-old rats were treated with either human chorionic gonadotropin (HCG, 50 IU/day) or pregnant mare serum (PMS, 50 IU/day). All the animals were sacrificed at 20 days of age. Testicular tissue was incubated with three different radiolabeled substrates: [^{3}H]-progesterone, [^{3}H]-androstenedione or [^{3}H]-testosterone.

The percentage of conversion of progesterone or androstenedione to testosterone was essentially unchanged; formation of 5α-reduced androgens was not prevented but greatly reduced. When testosterone was used as substrate, it was avidly metabolized by testicular tissue of 20-day-old rats of both groups, those treated with gonadotropins and the untreated controls. The data show that daily administration of gonadotropins to male rats, started either at day 1 or at day 10 from birth, significantly alter the steroid metabolism in the testes of 20-day-old rats. Specifically, the activities of 5α-reductase and 3α-hydroxysteroid dehydrogenase are drastically changed.

INTRODUCTION

The production of androgens by the testes is under control of pituitary gonadotropins [1–3]. In experiments with perfused testes an acute effect of gonadotropins on testosterone secretion has been clearly demonstrated [4], and Samuels and Helmreich were the first to show the stimulatory effect of pituitary gonadotropins upon the enzymes involved in androgen biosynthesis [5].

Our previous studies of *in vitro* progesterone metabolism by rat testicular tissue at different stages of development [6–8], revealed a biphasic pattern in the capacity of the tissue to convert progesterone to testosterone. These studies also demonstrated that the changing pattern of steroidogenesis was directly related to the age of the animals. At birth, metabolism of progesterone resulted in accumulation of testosterone, and as testes continued to develop, the major accumulating metabolites were 5α-reduced androgens (at approximately 20 days of age). In the adult testes, the metabolic pattern reverted to that observed at birth, testosterone again being the major metabolite.

It has been suggested [6] that secretory activity of the fetal Leydig cells is controlled by maternal chorionic gonadotropin, and that the cells remain stimulated for some time after birth. This would explain the high rate of testosterone production at birth. When testosterone production decreases (at approximately 20 days of age) 5α-reductase activity increases markedly. This increase in 5α-reductase activity prevents accumulation of testosterone [9, 10].

The present study was conducted to determine whether daily administration of gonadotropins commencing on day 1 or day 10 after birth will alter 5α-reductase activity in testes of 20-day-old rats and maintain the high degree of testosterone accumulation observed at birth.

5α-Androstanediol (Anl): 3α,17β-dihydroxy-5α-androstane; 5α-Androstanedione (And): 5α-androstan-3, 17-dione; Androstenedione (A): 4-androsten-3, 17-dione; 5α-Androsterone (An): 3α-hydroxy-5α-androstan-17-one; 20α-Dihydroprogesterone (20α-DP): 20α-hydroxy-4-pregnene-3-one; 17α-hydroxyprogesterone (17α-OH-P): 17α-hydroxy-4-pregnene-3,20-dione; Isoandrosterone (I-An): 3β-hydroxy-5α-androstan-17-one; Progesterone (P): 4-pregnene-3,20-dione; Testosterone (T): 17β-hydroxy-4-androsten-3-one; NADP,Na: nicotinamide adenine dinucleotide phosphate, sodium salt; G-6-P,Na_2: glucose-6-phosphate, disodium salt.

MATERIALS AND METHODS

A. *Animals.* Fifteen newborn male rats of the Long Evans strain, raised in our laboratories, were used. They were distributed into the following five groups: Group 1—control group, untreated rats ($n = 3$). Group 2—rats receiving, from day 1 of birth, 50 IU of HCG/rat/day, for 20 days ($n = 3$). Group 3—rats receiving, from day 10 of birth, 50 IU of HCG/rat/day, for 10 days ($n = 3$). Group 4—rats receiving, from day 1 of birth, 50 IU of PMS/rat/day, for 20 days ($n = 3$). Group 5—rats receiving, from day 10 of birth, 50 IU of PMS/rat/day, for 10 days ($n = 3$).

All animals were sacrificed at 20 days of age. Testicular tissue from each rat was incubated with three different radiolabeled steroids: [^{3}H]-progesterone, [^{3}H]-androstenedione, or [^{3}H]-testosterone.

B. *Incubation techniques.* Approximately 50 mg of testicular tissue was gently teased with fine scissors and placed into a flask containing 1.5 ml of incubation medium and the appropriate radiolabeled precursor. The tissue was incubated in a Dubnoff metabolic shaker for 3 h at 37°C under air. The incubation was terminated by the addition of 10 ml of ethyl acetate, and at the same time appropriate ^{14}C-tracers (approximately 40,000 d.p.m. each of [^{14}C]-androstenedione, [^{14}C]-estradiol, [^{14}C]-estrone, [^{14}C]-17α-hydroxyprogesterone, [^{14}C]-progesterone and [^{14}C]-testosterone) were added to the mixture in order to correct for losses during subsequent manipulations. The material was frozen until further processing.

Composition of incubation medium for total volume of 1.5 ml: 1.0 ml Hanks' solution, 25 μl ethanol, 175 μl NADP, Na solution (3.257 μmol/ml), 175 μl G-6-P, Na_2 solution (36.17 μmol/ml), 125 μl 1.3% $NaHCO_3$.

New York) were purified on silica gel column and recrystallized to correct melting point.

For paper chromatography, Whatman 1 chromatographic paper and the following chromatographic solvent systems were used: 1. Bush A, heptane–methanol–water (100:80:20, by vol.). 2. Bush 3, heptane–benzene–methanol–water (66:34:80:20, by vol.). For thin-layer chromatography, silica gel precoated aluminum sheets (E. Merck, Darmstadt, Germany) and chloroform–acetic acid (10:1, V/V) solvent system was used.

The human chorionic gonadotropin, HCG (Follutein, Squibb, New York) was obtained in vials containing 10,000 IU. The lyophilized material was dissolved in saline to yield 50 IU/0.1 ml. The pregnant mare serum, PMS (Equinex, Ayerst, New York), obtained in vials containing 50,000 IU, was dissolved in saline to yield 50 IU/0.1 ml.

E. *Computations.* (a) In cases of metabolites where ^{14}C-labeled tracers were utilized, the conversion of the substrate to the different metabolites (expressed in terms of radioactivity or weight) was calculated, after isotopic dilution and recrystallization to constant ^{3}H/^{14}C ratio, using the following formulas:

$$\%\,\text{conversion} = \frac{\text{ratio } ^3\text{H}/^{14}\text{C in last crystals} \times \text{d.p.m. } ^{14}\text{C-tracers}}{\text{d.p.m. } ^3\text{H-substrate}} \times 100$$

$$\%\,\text{conversion} \times \text{nmol substrate} \times 10 = \text{pmol metabolite in incubation}$$

$$\frac{\text{pmol metabolite in incubation} \times 1000}{\text{weight of tissue (mg) in incubation}} = \text{pmol metabolite/g tissue}$$

C. *Extraction, identification of steroids and measurement of radioactivity.* The procedures for extraction and identification of steroids (sequential chromatography, derivative formation, isotopic dilution, and recrystallization to constant ratio ^{3}H/^{14}C, or constant specific activity, d.p.m./μmol), and measurement of radioactivity have been described in detail elsewhere [11, 12].

D. *Chemicals.* The following radioactive substrates (New England Nuclear Corp., Boston, MA) were utilized: [7-^{3}H]-progesterone, supplied at a specific activity of 20 μCi/nmol, was diluted with unlabeled progesterone to yield 2.70 μCi/nmol; 19 × 10^6 d.p.m., 3.17 nmol were used per incubation flask. [1, 2-^{3}H]-androstenedione, supplied at a specific activity of 50 μCi/nmol was diluted with unlabeled androstenedione to yield 2.56 μCi/nmol; 21 × 10^6 d.p.m., 3.70 nmol were used per incubation flask [7-^{3}H]-testosterone, supplied at a specific activity of 10 μCi/nmol, was diluted with unlabeled testosterone to yield 2.65 μCi/nmol; 26 × 10^6 d.p.m., 4.42 nmol were used per incubation flask. The ^{14}C-tracers (New England Nuclear Corp), were diluted with ethanol in order to yield approximately 40,000 d.p.m./ml of solution. Analytical grade solvents were redistilled before use. Non-radioactive steroids (Steraloids, Inc., Pawling,

(b) In cases of some metabolites (e.g., androsterone) ^{14}C-labeled tracers were not available. When such metabolites migrated as a clean, well defined peak in the first chromatogram, and when in the course of subsequent manipulations (sequential chromatography and isotopic dilution and recrystallization to constant specific activity) the material appeared to be radiochemically pure, the percentage of conversion was calculated on the basis of the amount of radioactivity in the corresponding peak eluted from the first chromatogram (Bush A). (c) Testosterone, 5α-androstanediol and 17α-hydroxyprogesterone have the same R_F values in Bush A system. The material in the radioactive peak, migrating with the mobility of testosterone in Bush A system, was acetylated and rechromatographed in the same system. As a result of this procedure, three major radioactive peaks were obtained with chromatographic mobilities of testosterone acetate, 17α-hydroxyprogesterone and 5α-androstanediol diacetate, respectively. The percentage of conversion of progesterone to testosterone and 17α-hydroxyprogesterone was calculated from the ^{3}H/^{14}C ratios in the last crystals. The percentage of conversion to 5α-androstanediol was computed from the relative distribution of radioactivity in each of the three peaks, referring back to the total radioac-

Table 1. Body and organ weights of animals used in the experiment

	Group 1 (control) (n = 3)	Group 2 (HCG, 20 days) (n = 3)	Group 3 (HCG, 10 days) (n = 3)	Group 4 (PMS, 20 days) (n = 3)	Group 5 (PMS, 10 days) (n = 3)
Body weight (g)	31.0 ± 0.0*	34.3 ± 0.7	35.7 ± 0.3	29.0 ± 1.0	31.0 ± 1.2
Right testis (mg)	59.1 ± 1.3	248.2 ± 2.8	154.1 ± 29.1	355.1 ± 4.6	274.1 ± 19.7
Left testis (mg)	58.9 ± 0.9	227.7 ± 1.3	148.6 ± 15.6	361.9 ± 6.7	269.8 ± 17.7
Prostate (mg)	20.9 ± 4.5	42.1 ± 1.3	40.9 ± 1.6	43.4 ± 2.3	41.2 ± 2.4
Seminal vesicle (mg)	3.0 ± 0.8	13.8 ± 2.6	10.7 ± 2.3	27.2 ± 1.1	26.4 ± 1.1
Pituitary (mg)	1.6 ± 0.2	1.4 ± 0.1	1.7 ± 0.2	1.7 ± 0.1	1.8 ± 0.1

* Mean ± S.E.

tivity in zone I of the first Bush A chromatogram. This computation was considered to be justified because all three radioactive peaks were found to be essentially radiochemically pure.

It is recognized that procedures "b" and "c" permit only an estimation of the percentage of conversion and should not be considered to reflect precise figures of conversion of progesterone to these metabolites.

RESULTS

The body and organ weights are summarized in Table 1. A marked increase of testicular weight was observed in gonadotropin-treated animals.

Other observations drawn from Table 1 are:

1. treatment with gonadotropins (HCG or PMS) from day 1 after birth, resulted in a greater increase in testicular weight than treatment commenced on day 10 after birth.
2. treatment with PMS resulted in a greater increase in testicular weight than treatment with HCG; the same relationship held for the seminal vesicle.

Metabolism of [^3H]-progesterone

Table 2 summarizes the data of the metabolism of [^{3}H]-progesterone by testicular tissue from the five groups of rats. The testicular tissue from the gonadotropin-treated (both, HCG and PMS) rats utilized less progesterone than control tissue; however, the percentage of conversion of progesterone to testosterone was essentially unchanged. Formation of 5α-reduced androgens was not prevented but greatly reduced. A sharp decrease in the accumulation of 5α-androstanediol in all four experimental groups was observed; formation of 5α-androsterone, apparently, was not affected as much as that of 5α-androstanediol. Androstenedione accumulated in large amounts in incubates of testicular tissue from rats treated with PMS from the 10th day of age. The four experimental groups yielded also a large amount of polar substances, which were not identified. Treatment with HCG resulted in a sharp increase of 20α-dihydroprogesterone, particularly in incubates of testicular tissue from animals treated since birth.

Metabolism of [^3H]-androstenedione

Results of this study are summarized in Table 3. No significant differences in utilization of androstenedione were observed among the four experimental groups or in comparison to the control group. Accumulation of testosterone was not influenced by treatment with gonadotropins. Formation of 5α-androstanediol was sharply diminished, especially in incubates of tissue from rats treated with PMS, but 5α-androsterone and 5α-androstanedione remained unchanged. Here again, the four experimental groups showed a large conversion to polar compounds, which were not identified.

Metabolism of [^3H]-testosterone

Table 4 summarizes the data from this study. Testosterone was avidly metabolized by tissue from each group of animals. Accumulation of 5α-androstanediol was again less in incubates from gonadotropin-treated animals, but not as pronounced as in other experiments. Accumulation of 5α-androsterone and 5α-androstanedione was greater in the experimental groups treated with HCG (no difference with the control group) than in the groups treated with PMS. Accumulation of androstenedione remained unchanged, except in Group 5, where it was elevated. This finding is in accord with that obtained for this group using [^{3}H]-progesterone as substrate.

DISCUSSION

Results from the present study clearly reveal that exogenous gonadotropins (HCG or PMS), injected daily into male rats (either since birth or commencing at 10 days of age), drastically decrease the 5α-reductase activity and do not significantly alter, per weight unit of tissue, the low levels of testosterone accumulation in incubates of testicular tissue obtained from 20-day-old rats. This occurred in spite of the obvious stimulation of the gonad by those gonadotropins, as evidenced by a dramatic increase in weight, and hypertrophy and hyperplasia, of the Leydig cells.

Several investigators, Slaunwhite and Samuels[13], Nayfeh and Baggett[14], Nayfeh *et al.*[15], Inano and

Table 2. Metabolism of $[^3H]$-progesterone in incubates of testicular tissue

Metabolites	(%)*	Group 1 (control) (pmol/g tissue)	(%)	Group 2 (HCG, 20 days) (pmol/g tissue)	(%)	Group 3 (HCG, 10 days) (pmol/g tissue)	(%)	Group 4 (PMS, 20 days) (pmol/g tissue)	(%)	Group 5 (PMS, 10 days) (pmol/g tissue)
Polar zone (not identified)	17		16		21		43		46	
Testosterone	3	2000	4	2800	3	1650	2	1350	2	1060
17α-Hydroxyprogesterone	8	5200	2	1400	3	1650	1	670	2	1060
5α-Androstanediol†	22	14,400	1	700	1	550	5	3400	1	
20α-Dihydroprogesterone‡	3	2000	19	13,500	6	3300	2	1350	4	2100
Androstenedione	1	650	1	700	4	2200	2	1350	16	8400
5α-Androsterone‡	38	24,800	3	2100	13	7100	15	10,100	20	10,600
Progesterone (unconverted)	5		42		30		22		6	
Less polar than P (not identified)	—		6		18		8		2	

* Percentage of conversion of $[^3H]$-progesterone to the indicated metabolites.
† Percentage of conversion estimated on the basis of computations explained in paragraph "c" under "Computations".
‡ Percentage of conversion based on the radioactivity recovered after the first chromatography in Bush A system (paragraph "b" under "Computations").

Table 3. Metabolism of $[^3H]$-androstenedione in incubates of testicular tissue

Metabolites	(%)*	Group 1 (control) (pmol/g tissue)	(%)	Group 2 (HCG, 20 days) (pmol/g tissue)	(%)	Group 3 (HCG, 10 days) (pmol/g tissue)	(%)	Group 4 (PMS, 20 days) (pmol/g tissue)	(%)	Group 5 (PMS, 10 days) (pmol/g tissue)
Polar zone (not identified)	5		28		48		42		29	
Testosterone	1	650	0.7	440	0.5	310	2	1100	1.5	930
5α-Androstanediol†	36	23,250	16	10,000	12	7500	6	3300	3	1900
Isoandrosterone‡	—		—		—		—		2	1200
Androstenedione (unconverted)	16		12		7		18		25	
5α-Androsterone‡	38	24,500	36	22,600	25	15,700	17	9200	24	14,850
5α-Androstanedione‡	2	1300	4	2500	2	1250	9	4900	9	5600
Less polar than And (not identified)	—		3		7		4		3	

* Percentage of conversion of $[^3H]$-androstenedione to the indicated metabolites.
† Percentage of conversion estimated on the basis of computations explained in paragraph "c" under "Computations".
‡ Percentage of conversion based on the radioactivity recovered after the first chromatography in Bush A system (paragraph "b" under "Computations").

Table 4. Metabolism of [^{3}H]-testosterone in incubates of testicular tissue

Metabolites	(%)*	Group 1 (control) (pmol/g tissue)	(%)	Group 2 (HCG, 20 days) (pmol/g tissue)	(%)	Group 3 (HCG, 10 days) (pmol/g tissue)	(%)	Group 4 (PMS, 20 days) (pmol/g tissue)	(%)	Group 5 (PMS, 10 days) (pmol/g tissue)
Polar zone (not identified)	8		33		25		36		27	
Testosterone (unconverted)	3		1.4		1.1		6		2	
5α-Androstanediol†	49	35,900	10	6700	16	11,300	6	4000	9	8000
Isoandrosterone‡	2	1500	—		—		—		9	8000
Androstenedione	3	2200	7	4900	4	2800	7	4650	36	32,150
5α-Androsterone‡	33	24,200	31	21,500	42	29,650	22	14,600	14	12,500
5α-Androstanedione‡	—		11	7600	4	2800	13	8600	2	1800
Less polar than And (not identified)	—		4		—		9		—	

* Percentage of conversion of [^{3}H]-testosterone to the indicated metabolites.

† Percentage of conversion estimated on the basis of computations explained in paragraph "c" under "Computations".

‡ Percentage of conversion based on the radioactivity recovered after the first chromatography in Bush A system (paragraph "b" under "Computations").

Tamaoki[16] and Noumura *et al.*[17], have demonstrated that the steroid biosynthetic pathways in immature rats testes are markedly different from those of mature animals. Steinberger and Ficher[6] have shown a characteristic biphasic pattern of conversion of progesterone to testosterone related to the stages of testicular differentiation. High conversion was observed in testes of newborn rats, it diminished progressively as the testes developed reaching the lowest point in testicular tissue from 20-day-old rats. As the conversion to testosterone decreased, the capacity to produce 5α-reduced androgens gradually increased. Ficher and Steinberger[7, 8] demonstrated the *in vitro* formation of 5α-reduced androgens in significant amounts by the 13th day, and speculated that the diminished capacity of the testes from 17 to 40-day-old rats to form testosterone (or the increased capacity to form 5α-reduced androgens) was related to changes in pituitary gonadotropin levels occurring during development.

Investigation of bioconversion of androstenedione and testosterone by testicular tissue from rats at three different ages (1, 20 and 90-day-old) revealed that tissue from newborn and adult rats (1 and 90-day-old) actively metabolized androstenedione, testosterone being produced as the main metabolite; testosterone, on the other hand, was poorly metabolized. Conversely, incubation of androstenedione or testosterone with testicular tissue from 20-day-old rats resulted in accumulation of large amounts of 5α- reduced androgens [9]. These results further suggested that testicular tissue from newborn and adult rats exhibits low 5α-reductase activity.

In studies reported here no evidence was obtained to support the hypothesis that an increase in gonadotropin levels occurring during early development is related to an increase in 5α-reductase activity. In the experiments utilizing [^{3}H]-progesterone or [^{3}H]-androstenedione as substrates, the gonadotropins failed to alter the low degree of accumulation of testosterone in incubates of testicular tissue from 20-day-old rats, despite the sharp increase in testicular weight and an increase in number and size of Leydig cells. The activity of 5α-reductase was not blocked but greatly reduced: a quantitative change in the formation of the various 5α-reduced androgens was observed in all three experiments (incubation of the testicular tissue with [^{3}H]-progesterone, [^{3}H]-androstenedione and [^{3}H]-testosterone). Formation of 5α-androstanediol and 5α-androsterone was diminished possibly because of inhibitory effects of HCG and PMS, not only upon 5α-reductase but also on 3α-hydroxysteroid dehydrogenase, enzyme that synergistically contributes, together with 5α-reductase, to the formation of 5α-reduced androgens. Another possible explanation for the decline in the formation of 5α-reduced androgens in the incubation with [^{3}H]-progesterone (Group 2 of rats) could constitute the presence of 20α-hydroxysteroid dehydrogenase, enzyme responsible for the formation of 20α-dihydroprogesterone. This enzyme directs the conversion of progesterone to 20α-dihydroprogesterone rather than to 17α-hydroxyprogesterone. Further studies have to be performed in order to thoroughly investigate the specific activity of those enzymes and to determine selectively which of them, and to what degree, are affected by the presence of exogenous gonadotropins.

Another interesting observation derived from the present studies is that both gonadotropins (HCG and PMS) exert different effects upon the enzymes involved in the testicular steroid biosynthetic pathways; this observation confirms early reports from studies with hypophysectomized rats [18]. These different effects, evidenced by the different rates of conversion to the various metabolites, could be due to the fact that HCG exerts an ICSH-like effect whereas PMS exerts a combined effect similar to that of ICSH and FSH together.

There were also significant quantitative differences in the conversion rates in relation to the animal's age at which the daily injections of gonadotropins were started. These differences may reflect continuing changing patterns in steroid metabolism observed in immature rat testes.

In summary, results from this study show that the metabolic pattern of testicular steroidogenesis observed during development remains qualitatively unchanged under the influence of exogenous gonadotropins, but that there are substantial quantitative modifications, especially in the formation of 5α-reduced androgens in the testes of 20-day-old rats.

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