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EFFECTS OF HYPERPROLACTINEMIA ON ACTIVITIES OF 17-HYDROXYLASE, 17β-OL-DEHYDROGENASE AND 5α-REDUCTASE IN NEONATALLY GRAFTED AND HOST TESTES IN MICE

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Summary—Male and female (WB × C57BL/6)F_1 hybrid mice were used. Two pituitaries from 60–80-day-old female mice were grafted under the capsule of the left kidney of 60–80-day-old male mice. One week after grafting, 2 testes from neonatal mice were grafted under the capsule of the right kidney of the grafted mice and 70–90-day-old intact male mice. The grafted and host testes, in groups of 10–26, were removed 15, 30, 40, 60 and 120 days after transplantation of the neonatal testes. Testicular homogenates were incubated with [^{3}H]progesterone or [^{14}C]4-androstene-3,17-dione, and enzyme activities per g tissue were estimated. Significantly elevated prolactin levels, slightly lower LH levels and normal testosterone levels were found in the mice with pituitary grafts, compared with those in the mice without pituitary grafts. Activities of 17-hydroxylase and 17β-ol-dehydrogenase increased clearly with age in the grafted testes in the mice without pituitary grafts, though the increases were inhibited significantly by the pituitary grafts. However, the pituitary grafts had no significant effect on activities of 17-hydroxylase and 17β-ol-dehydrogenase in the host testes under similar gonadotrophic stimulation. 5α-Reductase activities in the grafted and host testes were unaffected by the pituitary grafts. These results show that hyperprolactinemia may directly inhibit increases in activities of 17-hydroxylase and 17β-ol-dehydrogenase with testicular age in neonatally grafted testes in mice.

INTRODUCTION

Prolactin has been reported to potentiate the effect of LH on testis growth, spermatogenesis and steroidogenesis [1–3]. On the other hand, it has been shown that hyperprolactinemia in man is associated with hypogonadism, infertility and impotence [4, 5]. However, the mechanism by which hyperprolactinemia leads to hypogonadism is not well understood. In male rats, hyperprolactinemia induced by pituitary grafts or drugs led to decreased LH secretion [6–8], reduced [9] or normalized [6, 8, 10] serum testosterone levels and increased testicular LH receptors [8, 11]. Hyperprolactinemia in male rats also appeared to sensitize the hypothalamic–pituitary axis to the negative feed back effect of androgen [12].

Hyperprolactinemia has also been reported in woman to be associated with anovulation and hypofunction of the ovary. The suppressive effects by hyperprolactinemia are readily reversed by the lowering of prolactin levels by bromocriptine [4]. In rat ovaries, an excess of prolactin reduces the production of 4-androstene-3,17-dione in the interstitial and theca cells by inhibition of cholesterol side-chain cleavage [13], 17-hydroxylation and C_{17-21}-side-chain cleavage [14] and by stimulation of 5α-reductase activity [14]. Furthermore, prolactin inhibits the formation of estrogen from androgen by the granulosa cells [15–17]. The evidence of a direct inhibition by an excess of prolactin on ovarian cells suggests that anovulation and hypogonadism found in hyperprolactinemia may be induced via decreased follicular estrogen production. However, effects of hyperprolactinemia on testicular enzymes involved in testosterone production are not known in detail.

Recently we found that increases in specific activities of 17-hydroxylase, C_{17-21}-lyase and 17β-ol-dehydrogenase with testicular age in neonatally grafted testes in male, female and castrated mice were similar to those in normal mouse testes [18]. In the present study, we investigated the effect of hyperprolactinemia induced by pituitary grafts in adult male mice on the increases in these enzyme activities with age in neonatally grafted testes. The effect of hyperprolactinemia on the activities of these enzymes in host testes was examined, and the effect of hyperprolactinemia on testicular 5α-reductase activity and on serum LH and testosterone levels was also investigated.

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EXPERIMENTAL

Animals

Male and female (WB × C57BL/6)F_1 hybrid mice were raised in our laboratory from parental strains described previously [19]. Two pituitaries from 60–80-day-old female mice were grafted under the capsule of the left kidney of 60–80-day-old male mice. One week after grafting, 2 testes from mice on day 0 after birth were grafted under the capsule of the right kidney of the grafted mice and of 70–90-day-old intact male mice. The grafted testes and host testes, in groups of 10–26, were removed 15, 30, 40, 60 and 120 days after transplantation of the neonatal testes. Blood samples were obtained for the estimation of hormone levels.

Chemicals

(1,2,6,7-^{3}H]Progesterone (112 Ci/mmol) and [4-^{14}C]4-androstene-3,17-dione (52 mCi/mmol) obtained from New England Nuclear Corporation, Boston, U.S.A. were purified by paper chromatography using the hexane–formamide system [20] just before use. The purified radioactive substrates contained very small amounts ($\leq 0.1\%$) of contaminating radioactive steroids (see Tables 1 and 2). Non-radioactive steroids were obtained from Steraloids, Inc., U.S.A. and Ikapham, Israel. Other reagents were of analytical grade.

Incubation procedure

Testicular tissues were homogenized, and the homogenates (5–30 mg tissue) were incubated with purified [^{3}H]progesterone (2 nmol; 1 μCi/tube) or [^{14}C]4-androstene-3,17-dione (7.7 nmol; 0.4 μCi/tube) and NADPH in air at 34°C for 30 min in 0.8 ml incubation mixture, as previously described [18, 21, 22]. The incubation mixture consisted of 0.15 M potassium phosphate buffer (pH 7.4), 0.13 M sucrose, 0.03 M nicotinamide, 1 mM $MgCl_2$, 0.5 mM EDTA and 1.5 mM NADPH.

Estimation of activities of 17-hydroxylase, 17β-ol-dehydrogenase and 5α-reductase

Methods for the estimation of activities of 17-hydroxylase, 17β-ol-dehydrogenase and 5α-reductase and for the formation of C_{19}-steroids from progesterone were previously described [18, 23–25]. The 17-hydroxylase activity and the formation of C_{19}-steroids were expressed, respectively, as the sum of all 17-hydroxy-C_{21}-steroids plus C_{19}-steroids (3 steroids shown in Table 1) and the sum of all C_{19}-steroids formed from progesterone. (The estimation of 5α-products was omitted, since no significant amounts of 5α-C_{21}-17-OH- and 5α-C_{19}-steroids were formed from progesterone by grafted or host testes under the present incubation conditions.) The activities of 17β-ol-dehydrogenase

Table 1. Formation of ^{3}H-steroids from [^{3}H]progesterone by 5 mg of host and grafted testes of mice with or without pituitary grafts, expressed as percent of substrate

	Pituitary graft (−)		Pituitary graft (+)		
	Host testes	Grafted testes	Host testes	Grafted testes	Buffer
Progesterone (unchanged)	63.1	88.9	66.3	92.4	97.1
17-Hydroxy-4-pregnene-3,20-dione	17.8	2.7	9.1	0.3	0.0
4-Androstene-3,17-dione	3.2	2.6	1.8	0.6	0.1
Testosterone	9.5	1.1	12.7	0.3	<0.1

Homogenates of testes obtained 40 days after grafting were incubated with [^{3}H]progesterone (2 nmol; 1 μCi/tube) and NADPH at 34°C for 30 min in 0.8 ml incubation mixture. Values were obtained after recrystallization to constant specific activity. Treatments of animals are shown in Table 3. Mean values of two estimations under the same conditions are given, since the two estimations showed similar values.

Table 2. Formation of [^{14}C]steroids from [^{14}C]4-androstene-3,17-dione by 10 mg of host and grafted testes of mice with or without pituitary grafts, expressed as percent of substrate

	Pituitary graft (−)		Pituitary graft (+)		
	Host testes	Grafted testes	Host testes	Grafted testes	Buffer
4-Androstene-3,17-dione (unchanged)	61.2	89.3	60.5	96.2	99.4
Testosterone	32.7	8.0	36.9	2.2	0.0
5α-Androstane-3,17-dione	0.0	0.1	0.1	0.2	0.0
17β-Hydroxy-5α-androstan-3-one	0.0	<0.1	<0.1	<0.1	<0.1
Androsterone	<0.1	0.1	0.0	0.1	0.0
3β-Hydroxy-5α-androstan-17-one	0.1	0.3	0.1	0.2	0.0
5α-Androstane-3α,17β-diol	0.1	0.0	0.1	<0.1	0.0
5α-Androstane-3β,17β-diol	0.0	0.0	0.0	<0.1	0.0

Homogenates of testes obtained 40 days after grafting were incubated with [^{14}C]4-androstene-3,17-dione (7.7 nmol; 0.4 μCi/tube) and NADPH at 34°C for 30 min in 0.8 ml incubation mixture. Values were obtained after recrystallization to constant specific activity. Treatments of animals are shown in Table 3. Mean values of two estimations under the same conditions are given, since the two estimations showed similar values.

and 5α-reductase were expressed as the sum of all 17β-ol-steroids (4 steroids shown in Table 2) and the sum of all 5α-reduced steroids (6 steroids shown in Table 2) formed from 4-androstene-3,17-dione. These assays were quantitative when at least 50% of the substrate remained and the incubation time did not exceed 30 min. Under the assay conditions used, the rate of production of 17-OH-C_{21}-products plus C_{19}-products, C_{19}-products, 17β-ol, or 5α-products was proportional to the amount of tissue and incubation time. The enzyme activities were expressed as nmol of steroids formed per g tissue/h. Because the concentrations of substrate (2.0 or 7.7 nmol/5–30 mg tissue) were supraphysiological, the assumption was made that the endogenous levels of the steroid intermediates can be disregarded when estimating enzyme activity (for example, endogenous levels of progesterone and testosterone estimated by radioimmunoassay (RIA) in grafted and host testes were less than 0.003 nmol/10 mg tissue and less than 0.004 nmol/10 mg tissue, respectively).

Assays of prolactin, LH and testosterone

Mice were killed quickly by decapitation and blood was immediately collected in tubes. Serum prolactin levels were determined with a double antibody RIA using materials and protocols supplied by the National Institute of Arthritis, Metabolism, and Digestive Diseases Pituitary Hormone Distribution Program (Bethesda, MD) [26].

Since the mouse anterior pituitary extract shows a parallel inhibition curve to the rat standard LH in rat LH RIA system, mouse serum samples were assayed with the NIADDK rat LH RIA kit (NIADDK rat LH 1–5 for radioiodination, NIADDK anti-rat LH serum S-5, and NIADDK rat LH PR-1) according to the double antibody method of Monroe *et al.*[27] with minor modifications [28]. The assay results were expressed as ng equivalent of NIH-LH-S1. The average interassay coefficient of variation in the range of 0.5–8 ng S1/ml was 3.1% and interassay coefficient of variation was 11.5%.

Methods for extraction and RIA of serum testosterone were previously described [29]. The intra- and interassay coefficients of variation obtained from 10 assays were 8.0–8.8% and 10.0–11.2%, respectively.

RESULTS

Weights of testes and seminal vesicles and serum levels of prolactin, LH and testosterone

Mean weights of neonatally grafted testes, host tests and seminal vesicles of mice with or without pituitary grafts 15–120 days after transplantation of the neonatal testes are shown in Table 3. Weights of the seminal vesicles in the mice with pituitary grafts were significantly higher than those in the mice without pituitary grafts. Weights of host testes were generally slightly higher in the mice with pituitary grafts than in those without grafts.

Concentrations of serum prolactin, LH and testosterone were determined 40 and 120 days after transplantation of neonatal testes (Table 4). Pituitaries were grafted 1 week before the transplantation of

Table 3. Mean weights of both host testes, neonatally grafted testes and seminal vesicles of mice with or without pituitary grafts

	Without pituitary grafts			With pituitary grafts		
Days† after grafting	Host testes	Grafted testes	Seminal vesicles	Host testes	Grafted testes	Seminal vesicles
	Mean ± SD (mg); n = 5–8			Mean ± SD (mg); n = 6–13		
15	192 ± 21	11 ± 2	73 ± 10	252 ± 24**	18 ± 5**	106 ± 17**
30	202 ± 19	29 ± 6	78 ± 13	230 ± 30	28 ± 9	119 ± 8**
40	199 ± 25	26 ± 6	82 ± 9	240 ± 29*	33 ± 7	128 ± 6**
60	203 ± 18	34 ± 8	80 ± 12	231 ± 43	30 ± 8	128 ± 25**
120	209 ± 17	34 ± 7	77 ± 14	263 ± 19**	37 ± 17	138 ± 20**

Two pituitaries from 60–80-day-old female mice were grafted under the capsule of the left kidney of 60–80-day-old male mice. One week after grafting, 2 testes from mice at day 0 after birth were grafted under the capsule of the right kidney of the grafted mice and of 70–90-day-old intact male mice. The weights of both host testes, 2 grafted testes and the seminal vesicles were examined 15–120 days after transplantation of the neonatal testes. Blood samples were obtained for the estimation of hormone levels shown in Table 4.

Differences from non-grafted mice (P): * < 0.05, ** < 0.01 (t-test).

†Days after transplantation of neonatal testes.

Table 4. Mean serum levels of prolactin, LH and testosterone in mice with or without pituitary grafts

	Without pituitary grafts			With pituitary grafts		
Days† after grafting	prolactin (ng/ml)	LH (ng NIH-LH-S1/ml)	Testosterone (ng/ml)	Prolactin (ng/ml)	LH (ng NIH-LH-S1/ml)	Testosterone (ng/ml)
		Mean ± SD; n = 4			Mean ± SD; n = 4	
40	8.0 ± 2.6	0.74 ± 0.58	2.3 ± 0.9	16.8 ± 3.3**	0.62 ± 0.60	2.3 ± 1.8
120	6.3 ± 3.3	0.83 ± 0.31	2.2 ± 0.4	16.8 ± 4.9*	0.59 ± 0.18	3.0 ± 1.3

Treatments are shown in Table 3. Differences fron non-grafted mice (P): * < 0.05, ** < 0.01 (t-test).

†Days after transplantation of neonatal testes. The neonatal testes were grafted 1 week after the transplantation of pituitaries.

neonatal testes. Significantly elevated prolactin levels were found in the mice with pituitary grafts, compared with those in the non-grafted mice. There was no significant difference in the serum level of LH or testosterone between the mice with and without pituitary grafts.

Effects of pituitary grafts on activities of 17-hydroxylase, 17β-ol-dehydrogenase and 5α-reductase in noenatally grafted testes of different ages

Activity per g tissue of 17-hydroxylase in neonatally grafted testes in adult male mice without pituitary grafts increased with age of testes, and the most conspicuous increase was found from 15 to 30 days of age. The increase with age was markedly inhibited in mice with pituitary grafts. However, the 17-hydroxylase activity at 15 days of testicular age was significantly higher in the mice with pituitary grafts than in those without grafts, since 17-hydroxylase activity decreased from 15 to 30 days of age in mice with pituitary grafts (Fig. 1). Changes in the formation of C_{19}-steroids from progesterone with age in the neonatally grafted testes in the male mice with and without pituitary grafts followed a similar pattern to those in 17-hydroxylase activity (Fig. 2). Table 1 shows representative metabolic patterns of [^{3}H]progesterone used for these estimations.

Activity of 17β-ol-dehydrogenase in the neonatally grafted testes in mice without pituitary grafts also increased with age of testes, though the most conspicuous increase was found from 30 to 40 days of age. The increase with testicular age was again inhibited significantly but less evidently by pituitary grafts. 17β-ol-Dehydrogenase activity at 15 days of age was higher in the mice with pituitary grafts than in those without grafts, though the difference was not statistically significant (Fig. 3). In contrast with the increases in 17-hydroxylase and 17β-ol-dehydrogenase activities with age, 5α-reductase activity was highest at 30 days of age in the neonatally

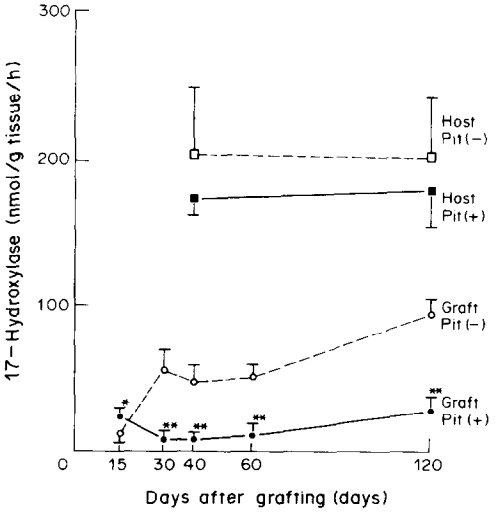

Fig. 1. Effect of hyperprolactinemia induced by pituitary grafts (Pit. +) on activities of 17-hydroxylase in host testes (Host) and in neonatally grafted testes (Graft) 15–120 days after grafting. Treatment of animals and incubation conditions are shown in Tables 3 and 1, respectively. Each point is the mean ± SD of 4 separate estimations. Differences from non-grafted mice (Pit. −) (P): * < 0.05, ** < 0.01 (t-test).

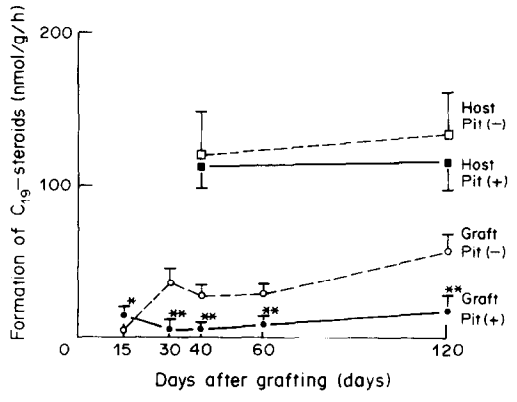

Fig. 2. Effect of hyperprolactinemia on the formation of C_{19}-steroids from progesterone in host testes (Host) and in neonatally grafted testes (Graft) 15–120 days after grafting. Treatment of animals and incubation conditions are shown in Tables 3 and 1, respectively. Each point is the mean ± SD of 4 separate estimations. Differences from non-grafted mice (Pit. −) (P): * < 0.05, ** < 0.01 (t-test).

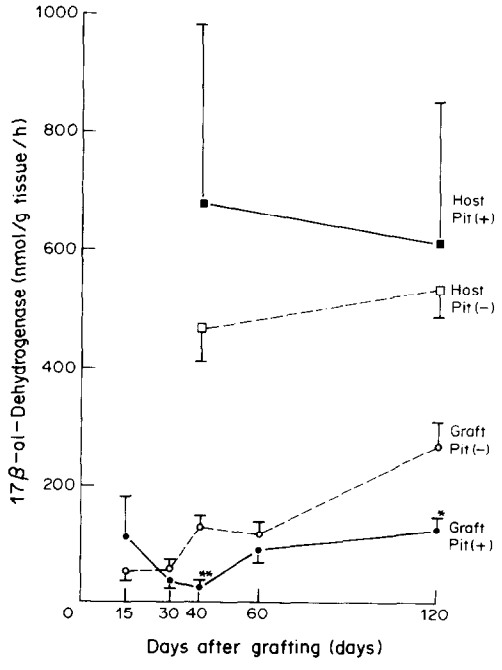

Fig. 3. Effect of hyperprolactinemia on activities of 17β-ol-dehydrogenase in host testes (Host) and in neonatally grafted testes (Graft) 15–120 days after grafting. Treatment of mice and incubation conditions are shown in Tables 3 and 2, respectively. Each point is the mean ± SD of 4 separate estimations. Differences from non-treated mice (Pit. −) (P): * < 0.05, ** < 0.01 (t-test).

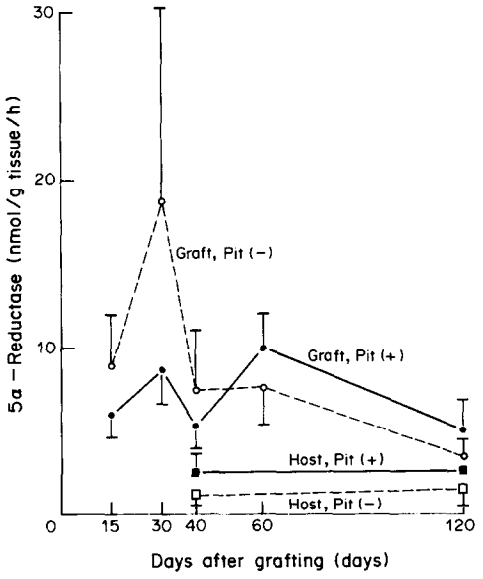

Fig. 4. Effect of hyperprolactinemia on activities of 5α-reductase in host testes (Host) and in neonatally grafted testes (Graft) 15–120 days after grafting. Treatment of mice and incubation conditions are shown in Tables 3 and 2, respectively. Each point is the mean ± SD of 4 separate estimations.

grafted testes, in mice without pituitary grafts and was not affected in mice with pituitary grafts (Fig. 4). Table 2 lists representative metabolic patterns of [^{14}C]4-androstene-3,17-dione used for the estimation of these enzyme activities. Testosterone was obtained as the major 17β-OH-product. 3β-Hydroxy-5α-androstan-17-one and 5α-androstane-3,17-dione were obtained as the major 5α-products.

Effects of pituitary grafts on activities of 17-hydroxylase, 17β-ol-dehydrogenase and 5α-reductase in host testes

Although hyperprolactinemia induced by pituitary grafts significantly inhibited the increases in activities of 17-hydroxylase and 17β-ol-dehydrogenase with age in the neonatally grafted testes, pituitary grafting had no significant effect on these enzyme activities in the host testes. The activities of these enzymes in the host testes were very high in both grafted and non-grafted mice (Figs 1–3). 5α-Reductase activities which remained very low in the host testes, were also unaffected by hyperprolactinemia (Fig. 4).

DISCUSSION

Our previous findings [18] in mice showed that activities of 17-hydroxylase and 17β-ol-dehydrogenase increase significantly with testicular age in normal testes and in neonatally grafted testes in males, females and castrated males, whereas 5α-reductase activity in normal and grafted testes reaches peak values at 30 days of testicular age. The present findings in the neonatally grafted testes in males confirm some of the previous results [18] and also indicate that the increases in activities of 17-hydroxylase and 17β-ol-dehydrogenase with age in the neonatally grafted testes were inhibited significantly by hyperprolactinemia induced by pituitary grafting, though the grafts had no significant effect on these enzyme activities in the host testes under similar gonadotrophic stimulation. These results suggest that hyperprolactinemia may directly inhibit the increase in these enzyme activities with age in neonatally grafted testes in mice. Although 17-hydroxylase and 17β-ol-dehydrogenase activities in the grafted testes were evidently lower in the mice with pituitary grafts than in those without grafts 30–120 days after grafting, the activities at 15 days after grafting were higher in the mice with pituitary grafts than in those without grafts (Figs 1–3). These findings seem to be explained by potentiating [1–3] and inhibiting [4, 5, 13–17] effects of prolactin on gonadotrophic stimulation.

It has been reported in male rats that hyperprolactinemia induced by pituitary grafts results in significant suppression of LH secretion [6–8]. Despite these reduced LH levels, plasma testosterone levels are normal [6, 8, 10]. These results are in agreement with the observation that prolactin enhances the sensitivity of the testis for LH [8, 11]. The present results on LH and testosterone levels in mice with or without pituitary grafts are not inconsistent with these previous findings [6–8, 10], because serum LH levels were slightly but not significantly lower in the mice with pituitary grafts than in those without grafts, though testosterone levels did not differ significantly. Weight of the seminal vesicles increased significantly under hyperprolactinemia (Table 3). The weight increase seems to be due to the direct effect of prolactin on the seminal vesicles, since serum testosterone concentrations remained almost unchanged under hyperprolactinemia. It has been reported that prolactin enhances the effect of androgens upon DNA synthesis and growth of the accessory sex organs such as the seminal vesicles [30, 31].

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