Effect of Hyperprolactinemia Induced by Prolactinoma (MtT/F84) on the Accessory Sexual Organs of Male Rat

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ABSTRACT

A new transplantable prolactinoma, designated MtT/F84 has been serially passaged in female F344 rats. Persistently high levels of serum prolactin could be achieved in male F344 rats by MtT/F84 inoculating under the skin.

This investigation deals with the effects of hyperprolactinemia upon the accessory sexual organs of male rats during puberty. The weights and the concentrations of dihydrotestosterone (DHT) in the dorsal prostate increased significantly in rats with moderate hyperprolactinemia (756 ± 179 ng/ml), but they in rats with marked hyperprolactinemia (3612 ± 1089 ng/ml) were similar to those of control rats. In contrast, serum testosterone levels (0.52 ± 0/17 ng/ml) in those of hyperprolactinemic rats were significantly decreased compared to that of controls (1.11 ± 0.13 ng/ml).

These results suggested that the growth-promoting effect of prolactin on the rat prostate mediated through the action of androgen varied according to the degree of hyperprolactinemia.

There have been demonstrated that hyperprolactinemia act synergistically with testosterone for the enhancement of the growth of rat prostate[6,8,16]. It is suggested that prolactin may influence testosterone metabolism, eg, by increasing the prostatic DHT concentration[12,15]. The lateral prostate is more sensitive by the effect of prolactin than the ventral or dorsal lobes[6,10,11,13,15]. This synergism occur mainly during sexual maturation and no such effects have been noted in adult rats[3,13,16]. In these studies, increase of plasma prolactin levels have been achieved by grafting the pituitaries under the renal capsule with several folds of control values. It is still unclear on the mechanism of the interaction between prolactin and testosterone at the time of high levels of prolactin. In the present study we have investigated the effects of hyperprolactinemia on the weights and DHT contents of the rat accessory sexual organs during puberty.

MATERIALS AND METHODS

Animals. Fifteen F344 male rats were obtained from Charles River Japan Co., Ltd., Kanagawa. At 5 weeks of age male rats (about 90%) were divided into control (group I) and 2 experimental groups (group II and III). They were maintained at 24 ± 2°C with 12hr light: 12 hr darkness, and chow and water were available ad libitum throughout the experiment.

A MtT/F84 growth in a female rat was grafted in 3 sites under the skin. Monodispersed tumor cells were inoculated 3.3 × 10⁵ cells/site in the group II and 9.8 × 10⁵ cells/site in the group III. At 3 weeks after transplantation,

"tumor take" was confirmed by palpating the grafted sites and a week later all rats were sacrificed under ether anesthesia. At autopsy, testis, seminal vesicle, ventral and dorsolateral prostate were quickly removed, weighed and frozen in liquid nitrogen. Blood samples were kept on ice and after centrifugation, sera were stored at $-20°C$ until assayed for testosterone and prolactin.

Transplantation of MtT/F84. Tumor tissue was minced in MEM and gently shaked for 2 hr (in 300 units collagenase/ml Worthington Biochemical Co., N.J. containing MEM solution) at $37°C$ in water bath. After washing out enzyme and debris with fresh MEM, tumor cell suspension was treated with 4 μg deoxyribonuclease I/ml solution (Sigma Chemical Co., Mo.). Monodispersed tumor cells were scored with trypan blue exclusion test. Appropriate numbers of viable tumor cell suspension in 0.03 ml were mixed with an equal amount of 50% brain homogenate, which was separately obtained from syngeneic rats and homogenized in MEM. A mixture of 0.06 ml tumor cell and brain homogenate was inoculated into 3 different sites (back of neck and both sides of lower lateral abdomen) at subcutaneous fat pads per rat.

RIA for serum prolactin and testosterone. Serum prolactin levels were measured using NIADDK rat prolactin kit. Six hundred μl of 0.1% BSA-PBS, 100 μl of anti-rPRL (anti-rPRL-S-9) diluted with 2% normal rabbit serum-PBS to 1 : 1000, 100 μl of ^{125}I-prolactin (New England Nuclear, Massachusetts, S.A. 20—50 μCi/μg) and 100 μl of reference standard of prolactin (NIADDK-rPRL-RP-3, 0.1—50 ng) or of serum sample suitably diluted with 0.1% BSA-PBS were incubated at room temperature for 20 hr, and then added 100 μl of sheep antirabbit IgG diluted to 1 : 20, and incubated at room temperature for 20 hr. The radioactivity of the supernatants was counted.

Serum testosterone levels were determined using Testosterone Direct RIA-kit (Commissariat A L'Energie Atomique, Italy) with 7.2% cross-reactivity to DHT. Since testosterone was not separated from DHT before assay, evaluated testosterone levels may contain small amounts of DHT.

Prostatic DHT content. It was measured using Testosterone/Dihydrotestosterone RIA kit

(Amersham, Buckinghamshire, England). Briefly, individual prostatic lobes were homogenized in 1 ml of 50 mM Tris buffer (pH 7.4). The ether extract was evaporated and suspended in distilled water. The oxidation step was carried out for assays of DHT levels. The solution was extracted again. The dried extracts were used for DHT radioimmunoassay.

STATISTICAL ANALYSIS

All numerical values were expressed as mean ± standard deviation (SD). The student's t-test was used to compare differences in values between the means of the three experimental groups.

RESULTS

Effects of prolactinoma on body weights and serum hormone levels (Table 1). At 4 weeks after tumor grafting, the tumor weights of MtT/F84 per 3 sites amounted to 2.66 ± 0.78 g in group II and to 9.28 ± 0.46 g in group III. Mean body weights were not significantly different among the three groups. The significant increase of serum prolactin levels were noted in group II (756 ± 179 ng/ml) and III (3612 ± 1090 ng/ml) compared to group I ($p < 0.05$). The serum testosterone levels were similar in group I (1.38 ± 0.14 ng/ml) and II (1.11 ± 0.13 ng/ml), but it significantly declined ($p < 0.01$) in group III (0.52 ± 0.17 ng/ml).

Effects of hyperprolactinemia on the genital organs (Table 2). No significant difference between the three groups were discernible in the testicular weights. The seminal vesicle weights were significantly increased ($p < 0.01$) in group II (372 ± 36 mg) and III (413 ± 74 mg) in comparison with group I (265 ± 35 mg). The weights of dorsolateral lobes in prostate were significantly increased ($p < 0.01$) in group II (235 ± 11 mg) in comparison with group I (190 ± 20 mg), whereas, the weights of ventral lobes were significantly increased ($p < 0.05$) in group II (215 ± 24 mg) in comparison with group I (178 ± 18 mg).

Effects of hyperprolactinemia on prostatic DHT contents (Table 3). DHT contents of ventral lobes were not significantly different among the three groups. DHT concentrations in dorsolateral lobes were significantly elevated ($p < 0.01$) in group II (7.12 ± 1.14 ng/g w.w.)

Table 1. Effects of prolactinoma (MtT/F84) grafts on body weights and serum hormones levels at 4 weeks after tumor grafting

Group	No. of rats	No. of tumor cells per site	No. of grafted sites	Tumor weights at 3 sites (g)	Body weight (g)	Serum hormones levels (ng/ml)	
						Testosterone	Prolactin
I	5	—	—	—	203 ± 12[a]	1.38 ± 0.14	45.4 ± 6.2[d]
II	5	3.3×10^5	3	2.66 ± 0.78	196 ± 20	1.11 ± 0.13[b]	756 ± 179[e]
III	5	9.8×10^5	3	9.28 ± 0.46	213 ± 6	0.52 ± 0.17[c]	3612 ± 1090

a : mean ± SD
b vs. c ; d vs. e : significantly different by $p < 0.01$

Table 2. Effects of hyperprolactinemia on genital organs at 4 weeks after tumor grafting

Group	Testis (g)	Seminal vesicle (mg)	Prostate (mg)	
			Ventral lobe	Dorsolateral lobe
I	2.28 ± 0.09[a]	265 ± 35[b]	178 ± 18[d]	190 ± 20[f]
II	2.43 ± 0.13	372 ± 36[c]	202 ± 26	235 ± 11[g]
III	2.34 ± 0.14	413 ± 74	215 ± 24[e]	171 ± 18

a : mean ± SD
b vs. c ; f vs. g : significantly different by $p < 0.01$
d vs. e : significantly different by $p < 0.05$

Table 3. Effects of hyperprolactinemia on DHT contents in ventral and dorsolateral lobes of prostate

Group	DHT concentration in prostate (ng/g w.w.)	
	Ventral love	Dorsolateral lobe
I	2.04 ± 0.53[a]	4.34 ± 0.52[b]
II	2.51 ± 0.46	7.12 ± 1.14[c]
III	2.47 ± 0.19	3.07 ± 1.01[d]

a : mean ± SD
b vs. c : significantly different by $p < 0.01$
b vs. d : significantly different by $p < 0.05$

over group I (4.34 ± 0.52 ng/g w.w.), but it was significantly decreased ($p < 0.05$) in group III (3.07 ± 1.01 ng/g w.w.) compared with in group I.

DISCUSSION

MtT/F84 is a tumorous growth of acidophils in the anterior pituitary. MtT/F84 grafted from a female donor to male rats was acknowledged of tumor take at 3 weeks after transplantation, and secreted large quantities of prolactin whereas no LH and FSH was produced[7,17]. Furthermore, increasing of serum prolactin levels were reflected with the number of tumor cells. In MtT-bearing male rats, the somatotropic effect was more prominent in contrast with that in females in which the mammotropic effects were conspicious[1,17]. In the present study,

however, MtT/F84 in male rats did not exhibit prominently somatotropic effect. These facts enabled us to study the influence of persistent hyperprolactinemia on male genital organs in male rats.

In the present study, serum testosterone levels decreased in markedly hyperprolactinemic rats, but did not change in moderately hyperprolactinemic rats. Prolactin by itself had no effect on testosterone production despite its ability on partial maintenance LH receptors[18]. Prolactin potentiated the effect of LH on both the synthesis and the release of testosterone by the testis[2,4,5]. It seemed that persistent excess of prolactin may down-regulate LH receptor and may inhibit testosterone synthesis in the testis during puberty.

After exposure of male rats to hyperprolactinemia, testicular growth was not observed as similar as of many investigations.

The present study showed that hyperprolactinemia stimulated the growth of seminal vesicles during the sexual maturation as other studies[9,14]. On the other hand, a growth difference in response to prolactin between ventral and dorsolateral lobe of prostate was depended upon the degree of serum prolactin levels. It is known that prolactin acts synergistically with testosterone to stimulate the growth of rat prostate[12,13]. This synergistic action is better demonstrated in the lateral lobe than the dor-

sal or ventral lobe of the prostate[3,6]. These effects of prolactin appeared to be dependent on the presence of DHT[8,15]. It is suggested that the elevated serum prolactin level is responsible for increasing the turn over of DHT in the lateral lobe, and that this event is related to the increased growth in that lobe exclusively[15]. Also the recent study does not show that hyperprolactinemia can facilitate DHT retention in the lateral prostate[11,15]. It is presented that DHT content in the lateral lobe was decreased by accelerated consumption of DHT[11], but the result in this study is presumed that DHT production is much faster than the turning over in the lateral lobe of moderately hyperprolactinemic rats during puberty.

Lee and Coert reported that hyperprolactinemia did not influence testosterone metabolism in the ventral lobe[3,11]. In the present study, the weight of ventral lobe increased in markedly hyperprolactinemic rats, though DHT content in this lobe was not changed. These evidences suggested that the effect of excessive prolactin on the growth of the ventral prostate is not mediated through testosterone action.

ACKNOWLEDGEMENTS

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