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Regulation of 3β-Hydroxysteroid Dehydrogenase Activity in Rat Testis under Hyperprolactinemia and Excessive 17β-Estradiol

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ABSTRACT

The effects of hyperprolactinemia on the activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) in rat testis were studied during excessive 17β-estradiol (E2) administration. Without E2 treatment, 3β-HSD activity did not change significantly in moderate hyperprolactinemic rats (756 ± 179 ng/ml). However, this enzyme activity was significantly decreased in marked hyperprolactinemic rats (3612 ± 1090 ng/ml) compared to that in control rats (45.4 ± 6.2 ng/ml). Under the excessive E2 administration, this enzyme activity was insignificantly decreased in slight hyperprolactinemic rats (218 ± 42 ng/ml), and inhibited in moderate hyperprolactinemic rats (566 ± 77 ng/ml) as refer to control level.

It may be concluded that inhibition of 3β-HSD activity in testis due to the excess administration of estrogen is promoted by the transplantation of MtT/F84 which produces prolactin at the limited level.

3β-hydroxysteroid dehydrogenase (3β-HSD) is one of the enzyme involved in the conversion of pregnenolone to androgens. In particular, 3β-HSD activity is necessary for the conversion of pregnenolone to progesterone. Prolactin appears to maintain the production of androgens in the rat synergistically with luteinizing hormone (LH)[4,7]. It is because prolactin has been shown to regulate the storage and metabolism of cholesterol in the testis[1]. Whereas, administration of prolactin alone to the LH deficient rats by hypophysectomy did not change the levels of 3β-HSD activity[3]. It is suggested that during sexual maturation the testicular biosynthesis of androgens may proceed with the help of age-dependent 3β-HSD[12]. It is well known that excess estrogen suppresses testicular androgen production. 17β-estradiol (E2) has an inhibitory effect on 3β-HSD plus isomerase activity[10,13]. It is still unclear that E2 inhibitory effect on testicular 3β-HSD activity is influenced by the alteration of the serum prolactin levels. In the present study we have measured the 3β-HSD in rat testis of during puberty after 1 month exposure to hyperprolactinemia and excessive E2.

MATERIALS AND METHODS

Animals. Twenty five F344 male rats were obtained from Charles River Japan Co., Ltd., Kanagawa. The rats aged 4 weeks were divided 5 experimental groups on arrival. The groups were itemized, i.e., group I was control rats, group II and IV were hyperprolactinemic rats, and group III and V were exposed to both

hyperprolactinemia and E2. The rats were housed 5 per cage, given chow and water ad libitum and maintained on a schedule of 12hr light and 12hr dark throughout the experiment.

Induction of hyperprolactinemia. Persistently high levels of circulating prolactin in male rats were achieved by grafting the transplantable prolactinoma designated MtT/F84. Transplantations of MtT/F84 from a female donor to male rats were performed according to the recent reports[6,8].

MtT/F84 was grafted in 3 sites under the skin of the rats aged 5 weeks. Monodispersed tumor cells were inoculated 3.3×10^5 cells/site in the group II and III, in addition 9.8×10^5 cells/site in the group IV and V. At the same time, the rats in the group III and V were received 2.5 mg of E2 pellet. At 3 weeks after transplantation, "tumor take" of MtT/F84 was evaluated by palpating the grafted sites and a week later all rats were sacrificed under ether anesthesia. At autopsy, the testes were quickly removed, weighed and frozen in liquid nitrogen until measurement of 3β-HSD. The sera were stored at −20C for hormone determination.

RIA for serum prolactin and testosterone. Serum prolactin levels were measured using NIADDK rat prolactin kit and testosterone levels were determined using Testosterone Direct RIA-kit (Commissariat A L'Energie Atomique, Italy) according to recent report[8].

Assay procedure for 3β-HSD[9]. Ten mg of testicular tissue was homogenated in 0.1 ml of 0.25 M sucrose with 20mM Tris (pH7.4). The homogenate was added into incubation medium which contained 10mM $NaHCO_3$, 135μM NAD and 0.154M KCl. The incubation was carried out at 37C for 30 min in addition to 2μCi of ^{3}H-pregnenolone (New England Nuclear, Massachusetts. S.A. 13.0 Ci/m mol). A 0.1 ml aliquot of the incubation mixture was removed and added to 1.0 mg of pregnenolone in 0.9 ml of absolute ethanol (carrier tube) and mixed. The radioactivity of 0.1 ml of aliquots taken from the carrier tube was counted. A volume of 0.9 ml of 1% digitonin in 50% ethanol was added to the remaining volume of the incubation mixture in the carrier tube, mixed thoroughly, and allowed to stand at room temperature for 1hr. The tubes were centrifuged and the radioactivities in 0.2 ml of the supernatants were counted.

Enzyme activity is derived as follows: A= radioactivity (dpm) / 0.1 ml aliquot without incubation. B= dpm / 0.2 ml following digitonin addition without incubation. C= dpm / 0.1 ml in the 30-min incubation sample. D= dpm / 0.2 ml from the 30-min incubation sample following digitonin addition. D − C (B/A)/C (1 − B/A) × nmoles pregnenolone added / wet weight of tissue (mg) = activity of 3β=HSD / nmoles / 30 min / mg w.w..

STATISTICAL ANALYSIS

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

RESULTS

Effects of hyperprolactinemia and E2 on testicular weights and serum hormone levels (Table 1). No significant difference among the rats without E2 pellet group I (2.28 ± 0.09 g), II (2.43 ± 0.13 g) and IV (2.34 ± 0.14 g) was discernible in the testicular weights. However it was markedly decreased in group III (0.36 ± 0.03 g) and V (0.63 ± 0.02 g) with E2 pellet. The significant increase of serum prolactin lev-

Table 1. Effects of hyperprolactinemia with or without E2 treatment on testicular weights and serum hormone levels

Group	No. of rats	Testicular weight(g)	Serum hormone levels (ng/ml)	
			Prolactin	Testosterone
I	5	2.28 ± 0.09[a]	45 ± 6[b]	1.38 ± 0.14
II	5	2.43 ± 0.13	756 ± 179	1.11 ± 0.13[d]
III	5	0.36 ± 0.03	218 ± 42[c]	0.12 ± 0.04
IV	5	2.34 ± 0.14	3612 ± 1090	0.52 ± 0.17[e]
V	5	0.63 ± 0.02	566 ± 77	0.29 ± 0.11

a: mean ± SD

b vs c; d vs e: significantly different by $p<0.01$

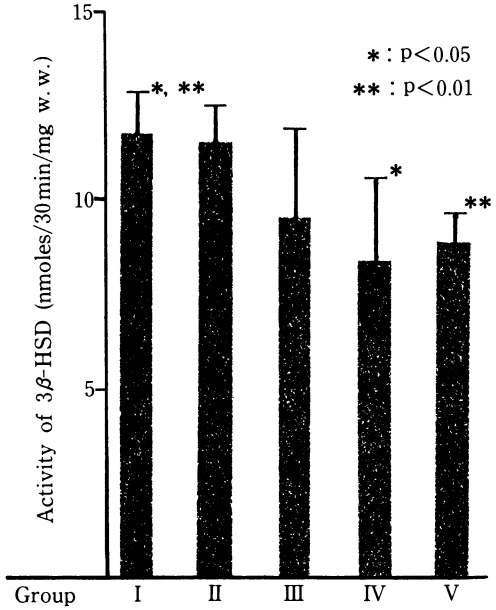

Fig. 1. 3β-hydroxysteroid dehydrogenase activity in testicular tissue

els was noted in the four groups grafted with MtT/F84 compared with 45.4 ± 6.2 ng/ml in group I ($p<0.01$), i.e., the levels amounted to 756 ± 179 ng/ml in group II, to 218 ± 42 ng/ml in group III, to 3612 ± 1090 ng/ml in group IV and to 566 ± 77 ng/ml in group V. 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 (0.12 ± 0.04 ng/ml), group IV (0.52 ± 0.17 ng/ml) and group V (0.29 ± 0.11 ng/ml).

Regulation of 3β-HSD activity by hyperprolactinemia and E2 in testicular tissue (Fig. 1). The activities of 3β-HSD in the testicular tissues were not significantly different among group I (11.67 ± 1.29 nmoles/30 min/mg w.w.), II (11.45 ± 0.91 nmoles/30 min/mg w.w.) and III (9.49 ± 2.54 nmoles/30 min/mg w.w.). It was seen that the activities of this enzyme in group IV (8.36 ± 2.24 nmoles/30 min/mg w.w.) and V (8.81 ± 0.83 nmoles/30 min/mg w.w.) significantly decreased in comparison with that in group I ($p<0.05$). Whereas, no significant difference between group IV and V were notes in this activity.

DISCUSSION

It was suggested that prolactin induced the increase of the testicular stores of esterified cholesterol, in activity of 3β- and 17β-hydroxysteroid dehydrogenase in testis, and in testicular binding LH[2]. In contrast, administration of prolactin to the hypophysectomized rats did not change the levels of 3β-HSD activity[3]. These results demonstrated the synergistic action of prolactin and LH on 3β-HSD activity[3,5]. In addition, increase of this enzyme activity was observed in immature rats[11].

By the way, it is well noted that E2 has an inhibitory effect on 3β-HSD and isomerase activities which are necessary for the conversion of androstenediol to T in the biosynthetic pathway leading to T from DHA[13]. Marked decrease of serum T levels were in the E2 treated rats (group III and V) in the present study were met our expectations. Moreover, 3β-HSD activities existed in the E2 treated rats. It is still unclear whether 3β- HSD activity in rats was influenced by the circulating prolactin levels during E2 treatment. In the present study, 3β -HSD activity did not change in moderate hyperprolactinemic rats (group II). However, it was significantly decreased in marked hyperprolactinemic rats (group IV) compared with in control rats. The result indicated that persistent excess of prolactin may inhibit 3β -HSD activity in the testis during puberty. Under the condition of excessive E2, 3β -HSD activity was insignificantly decreased in slight hyperprolactinemic rats (group III) compared to that of control rats. However, it was inhibited in moderate hyperprolactinemia in group V.

The results are summarized that hyperprolactinemia can not supress 3β -HSD activity in the circumstance in which the activity of this enzyme was declined by E2 treatment in the rat testis during puberty.

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