NOTE

Roles of Prepubertal Androgen, Estrogen or Androgen Plus Prolactin on Androgen-Induced Proliferative Response of Seminal Vesicles in Adult Mice

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Abstract

Male mice castrated on day 0 after birth were pretreated daily with testosterone propionate (TP, 4 μg/g body weight), 17β-estradiol (E2, 0.2 μg/g body weight) or vehicle for 21 days starting from day 20. In another experiment, male mice were castrated on day 25; two pituitaries from 60-day-old females were immediately grafted under the capsule of the left kidney in one group. The castrated mice with or without grafts were pretreated daily with TP (4 or 20 μg/g body weight) for 36 days starting from day 25, and the left kidney was removed on day 60. Daily TP injections (4 μg/g body weight) were started again at 30 days after the end of pretreatments to examine androgen-induced proliferation, and incorporation of 5-[125I]iodo-2'-deoxyuridine into the whole seminal vesicles was used as an index of proliferation. In the neonatally castrated mice, both TP and E2 pretreatments given during the prepubertal period significantly increased seminal vesicle weight even long after the end of the pretreatments. However, androgen-induced proliferative response found in the neonatally castrated adult mice (poor response; long duration with a low peak) was changed to that found in mice castrated at adulthood (good response; short duration with a high peak) by the TP pretreatment only but not at all by the E2 pretreatment. In the mice castrated on day 25, a pharmacological dose of TP or TP plus hyperprolactin could not enhance or change the adult castration type of androgen-induced proliferation induced by physiological prepubertal androgens, although both treatments significantly enhanced the prepubertal growth of the seminal vesicles.

Castration at adulthood causes rapid involution of the seminal vesicle, and the injection of androgen restores the weight and function of this organ (Allen, 1958; Morley et al., 1973; Okamoto et al., 1982a). The growth response of the seminal vesicle to androgen in mice at adulthood has been shown to be enhanced by exposure to androgen during the neonatal and pre-
pubertal periods (Bronson et al., 1972; Okamoto et al., 1982a). This early action of androgen on the development of the seminal vesicle is considered to be irreversible even after androgen removal, and has been referred to as neonatal and prepubertal “imprinting”. We recently found that testicular androgens secreted during the prepubertal period (days 20-40 after birth) but not during the neonatal period play an indispensable role in the production of so-called imprinted cells in the mouse seminal vesicle (Yamane et al., 1987).

The seminal vesicles of neonatally castrated adult mice are characterized by low weight before androgen treatment and long duration of androgen-induced proliferation with a low peak (neonatal castration type; poor response), compared to those of mice castrated at adulthood (adult castration type; good response). The poor response to androgen found in neonatally castrated adult mice is induced by a defect in the formation of so-called imprinted cells of the seminal vesicles due to the absence of prepubertal testicular androgens. In the neonatally castrated adult mice, daily injections of testosterone propionate (TP) or 17β-estradiol (E2) increased both the weight and DNA synthesis of the seminal vesicles and most of the cells, of which proliferation was induced by TP or E2, survived in the seminal vesicle, both in the presence and absence of TP or E2 (Yamane et al., 1986). Therefore, the defect in “imprinting” effects on androgen-induced proliferative response of mouse seminal vesicle cells due to the absence of prepubertal testicular androgens may be compensated at least in part by estrogens given during the prepubertal period.

On the one hand, it is well known that pharmacological doses of androgens induce much larger seminal vesicles than those induced by physiological testicular androgens and that hyperprolactinemia significantly enhances the androgen-induced increase in the weight and DNA content of the mouse seminal vesicles (Keenan et al., 1981). Therefore, “imprinting” effects of prepubertal physiological androgens on the mouse seminal vesicles may be enhanced by pharmacological doses of androgens or hyperprolactin given during the prepubertal period.

It is generally accepted that some androgen-responsive mouse seminal vesicle cells, that can survive even in the absence of androgens, are formed by neonatal and prepubertal testicular androgens. In the present study, we examined the effects of high, but not physiological, doses of androgen, estrogens or androgen plus high prolactin given during the prepubertal period on the formation of such seminal vesicle cells.

**Materials and Methods**

**Animals**

(C57BL/6×DBA) F₁ mice raised in our laboratory were used. Male mice castrated on day 0 after birth were pretreated daily with TP (4 μg/g body weight), E₂ (0.2 μg/g body weight) or vehicle during the prepubertal period (days 20-40); TP or E₂ was suspended in 0.05 ml vehicle (0.9% NaCl, 0.4% polysorbate 80, 0.5% carboxymethylcellulose, and 0.9% benzyl alcohol) and injected subcutaneously. In another experiment, male mice were castrated on day 25; two pituitaries from 60-day-old female mice were immediately grafted under the capsule of the left kidney in one group. The castrated mice with or without pituitary grafts were given daily injections of TP (4 or 20 μg/g body weight) for 36 days starting from day 25, and the left kidney with or without grafts was removed on day 60.

**Determination of 5-[¹²⁵I]iodo-2'-deoxuryridine ([¹²⁵I]IdUrd) retained in the seminal vesicles**

The castrated mice (on day 0 or 25) pretreated with TP, E₂, TP plus high prolactin or vehicle were given daily injections of TP (4 μg/g body weight) for 20 or 15 days starting 30 days after pretreatments to examine the androgen-induced
proliferative response. On various days after starting the TP injections, the incorporation of $^{125}\text{I}\text{dUrd}$ into the whole seminal vesicles was determined as an index of proliferation. Details of this method have been described (Okamoto et al., 1982b; Yamane et al., 1986; Yamane et al., 1987). Results are expressed as the mean percentage of the injected radioactivity retained in both lobes of the seminal vesicles.

**Histology**

Some seminal vesicles were fixed in 10% buffered formalin (pH 7.2) and embedded in paraffin. Cross sections (5 μm thick) from the middle portion of the seminal vesicle were stained with hematoxylin and eosin. The inside of the mouse seminal vesicle contained no branch on day 0 after birth. The formation of main branches by testicular androgens was found during the first 40 days; then second and third branches were formed. Numbers of main branches were compared by Student’s t-test.

**Results**

*Androgen-induced increases in $^{125}\text{I}\text{dUrd}$ uptake and weight of the seminal vesicles in neonatally castrated adult mice pretreated during the prepubertal period with TP or E₂*

Neonatally castrated mice were given daily TP, E₂ or vehicle for 21 days starting on day 20. The TP and E₂ pretreatments markedly and moderately increased seminal vesicle weight, respectively, both in the presence and absence of TP or E₂ in neonatally castrated mice (Table 1). These findings suggest that not only TP pretreatment but also E₂ pretreatment given during

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Weight (mg)*</th>
<th>No. of main branches*</th>
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</thead>
<tbody>
<tr>
<td>(Days 20-40)</td>
<td>Day 40</td>
<td>Day 70</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.6±0.1</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>TP (4 μg/g body weight/day)</td>
<td>36.4±0.4†</td>
<td>9.4±0.2†</td>
</tr>
<tr>
<td>E₂ (0.2 μg/g body weight/day)</td>
<td>9.6±0.2†</td>
<td>2.2±0.1†</td>
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</table>

Male mice castrated on day 0 were pretreated with TP or E₂ as shown in Materials and Methods and in Table 1.
† Values are the mean±SE for 6-7 mice.
† P<0.01, compared to the value for vehicle.

**Table 2. Weight of seminal vesicles in adult mice castrated on day 25 after birth and pretreated daily with TP, TP plus high prolactin or high TP.**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Weight+ (mg)</th>
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<tbody>
<tr>
<td>(Days 25-60)</td>
<td>Day 60</td>
</tr>
<tr>
<td>TP (4 μg/g body weight/day)</td>
<td>39.3±1.4</td>
</tr>
<tr>
<td>TP (4 μg/g body weight/day)+prolactin</td>
<td>56.2±2.1†</td>
</tr>
<tr>
<td>TP (20 μg/g body weight/day)</td>
<td>58.2±2.0†</td>
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</tbody>
</table>

Male mice castrated on day 25 were pretreated with TP, TP+high prolactin or high TP as shown in Materials and Methods and in Table 2.
† Values are the mean±SE for 6-9 mice.
† P<0.01, compared to the value for TP (4 μg/g body weight/day).
the prepubertal period may induce an "imprinting" effect on androgen-induced proliferation of the seminal vesicles. However, the poor response type (long duration with a low peak; neonatal castration type) of androgen-induced proliferation found in neonatally castrated adult mice was changed to the good response type (short duration with a high peak; adult castration type) only by the TP pretreatment but not at all by the E2 pretreatment (Fig. 1). The difference seems to be explained by

Fig. 1. Androgen-induced increases in \([^{125}\text{I}]\) IdUrd uptake and weight of seminal vesicles in neonatally castrated adult mice pretreated with TP or E2 during prepubertal period. Mice castrated on day 0 were pretreated daily with TP, E2 or vehicle for 21 days starting on day 20, as shown in Table 1. Daily injections of TP (4 µg/g body weight/day) for 20 days were started again on day 70 to examine the androgen-induced proliferative response. Each point represents the mean±SE for 6–7 mice in TP (○), E2 (□) or vehicle ( ●) pretreated group.

Fig. 2. Androgen-induced increases in \([^{125}\text{I}]\) IdUrd uptake and weight of seminal vesicles in adult mice castrated on day 25 after birth and pretreated daily with TP, TP plus high prolactin or high TP. Mice castrated on day 25 were pretreated daily with TP, TP plus high prolactin or high TP for 36 days starting on day 25, as shown in Table 2. Daily injections of TP (4 µg/g body weight/day) for 15 days were started again on day 90. Each point represents the mean±SE for 5–14 mice in TP (○), TP plus high prolactin ( ●) or high TP ( ●) pretreated group.
the difference in the morphology of TP and E₂ pretreated seminal vesicles. Although both pretreatments increased seminal vesicle weight, the main branches found inside the seminal vesicles were induced only by the TP pretreatment but not at all by E₂ pretreatment (Table 1).

**Effects of pretreatment with pharmacological dose of TP or physiological dose of TP plus high prolactin on androgen-induced proliferative response of the seminal vesicles**

The pretreatment with a pharmacological dose of TP or a physiological dose of TP plus high prolactin markedly stimulated the increase in seminal vesicle weight, which reached significantly higher values than those induced by physiological doses of TP (Table 2). However, the adult castration type of androgen-induced proliferative response found in mice pretreated with a physiological dose of TP was not enhanced or changed at all by these pretreatments (Fig. 2).

**Discussion**

The seminal vesicles of neonatally castrated mice show poor response to androgen due to the absence of prepubertal testicular androgens. In the neonatally castrated mice, pretreatments with androgen and estrogen during the prepubertal period induced different "imprinting" effects on the seminal vesicles. The androgen pretreatment resulted in increases in the weight and folds even in the absence of androgen, and the poor response to androgen (neonatal castration type) was changed to the good response type (adult castration type) by androgen pretreatment; these findings in the present study confirm previous observations (Yamane et al., 1987). The estrogen pretreatment during the prepubertal period resulted in an increase in the seminal vesicle weight even in the absence of androgen but did not induce folds, and the poor response to androgen was not changed at all by the estrogen pretreatment. Furthermore, the present findings demonstrate that the adult castration type of androgen-induced proliferative response of the mouse seminal vesicles, which was induced by prepubertal testicular androgen, cannot be enhanced or changed at all even by pharmacological doses of androgen or androgen plus hyperprolactin given during the prepubertal and pubertal periods.

**References**


to androgen and estrogen, assayed by incorporation of $[^{125}\text{I}]$iododeoxyuridine. *Endocrinology* 110, 1796–1803.
