NOTE

Deciduoma Formation in Rats Ovariectomized and Androgenized during Neonatal Life

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Synopsis

When rats ovariectomized at 3 days of age and given a single injection of 1.25 mg testosterone propionate on the next day became 60 days old, they were given 3 daily injections of 0.2 µg estradiol-17β followed by 7 daily combined injections of 2 mg progesterone and 0.2 µg estradiol. Incidence of deciduomata in reaction to uterine trauma applied on the 4th day of the progesterone-estradiol injections was almost as high as that in neonatally ovariectomized, non-androgenized rats, but the response was significantly smaller in size in androgenized rats than in non-androgenized animals. If females similarly operated on were given injections of 0.1 µg estradiol for 30 days prior to 7 daily injections of progesterone-estradiol, deciduoma formation in androgenized rats was markedly reduced in both incidence and size of the response. In non-androgenized group, deciduoma formation was not significantly affected by chronic administration of estradiol. Accordingly, it is likely that, although androgen injected during neonatal life is responsible for the reduction of uterine responsiveness in androgen-sterilized rats (Takewaki and Ohta, 1974), continued exposure of the uterus to estrogen may play a co-operative role in the event.

We have recently reported that incidence of deciduomata in response to uterine trauma is definitely reduced in neonatally androgenized, constant-estrous rats given combined injections of progesterone (P) and estradiol-17β (ED) after ovariectomy as compared with that in non-androgenized control animals similarly treated. Since the interruption of the constant estrus by ovariectomy for a period as long as two months in such rats prior to the start of the treatment with P plus ED could not restore the uterine responsiveness to normal, it was concluded that the lowered uterine sensitivity in the rats was largely ascribable to the effect of the single injection of 1.25 mg testosterone propionate (TP) given during neonatal life rather than to the influence of the continued exposure to ovarian estrogen (Takewaki and Ohta, 1974).

We have also pointed out that female rats given injections of estrone for the first few days after birth showed, when adult, a syndrome of sterility characterized by acyclicity and anovulation which was superficially indistinguishable from that in animals receiving TP during neonatal life, but the capacity to form deciduomata in response to uterine trauma was very different between the two groups of rats. These findings reinforce the conclusion that the steroid administered neonatally is primarily concerned in the altered uterine responsiveness in adulthood (Ohta and Takewaki, 1974).

In the present report, the conclusion previously arrived at is further substantiated and the possible contribution of continued estrogen secretion to the decrease in uterine
responsiveness in androgen-sterilized rats is suggested.

Materials and Methods

Female rats of the T strain used in these studies were raised in an artificially illuminated (14 hr of light, 10 hr of darkness), temperature-controlled room. All animals were allowed to take food and water ad libitum.

In the first study, 17 female rats ovariectomized at 3 days of age under cold anesthesia (Group I) were given a single subcutaneous injection of 1.25 mg TP in 0.05 ml sesame oil on the day following operation. When the animals reached 60 days of age, they were injected subcutaneously with 0.2 μg ED in 0.05 ml sesame oil for 3 consecutive days. Two days after the last ED injection, the rats were divided into 2 groups of 8 (Group Ia) and 9 (Group Ib) and given subcutaneous injections of 2 mg P plus 0.2 μg ED and 5 mg P plus 0.2 μg ED, respectively, for 7 consecutive days. In both subgroups, daily doses were dissolved in 0.15 ml sesame oil. On the 4th day of the injection period, the endometrium of the right uterine horn of each animal was traumatized by the method similar to that described in the previous paper (Takewaki and Ohta, 1974).

Eighteen other rats (Group II) were likewise ovariectomized at 3 days of age but given no TP on the next day. At 60 days of age, the animals were subdivided into 2 groups of 8 (Group IIa) and 10 (Group IIb) and given 3 daily injections of 0.2 μg ED followed by 7 daily injections of 2 mg P plus 0.2 μg ED and 5 mg P plus 0.2 μg ED, respectively, commencing 2 days after the last ED injection. Uterine traumatization was applied as in Group I animals.

In the second study, 19 rats were ovariectomized at 3 days of age. On the next day, 10 of them (Group III) were given a single subcutaneous injection of 1.25 mg TP in 0.05 ml sesame oil, while the remaining 9 received none at all (Group IV). When the rats became 30 days old, daily injection of 0.1 μg ED in 0.05 ml oil was commenced and continued for 30 consecutive days. All the rats were then injected subcutaneously with 2 mg P together with 0.2 μg ED in 0.15 ml oil for 7 consecutive days beginning 2 days after the last injection of ED. The right uterine horn of each rat was traumatized on the 4th day of the period of P-ED injections.

In animals having acquired the vaginal orifice before the start of the experiment, vaginal smears were taken daily for about 10 days prior to and during the experiment until the day before sacrifice. If the vagina was not open at the beginning, daily inspection for vaginal opening was carried out and smearing commenced immediately after the apertur appeared.

All the animals were sacrificed on the day following the last P-ED injection, i.e., 4 days after uterine traumatization. After uterus was checked for gross evidence of deciduomata, traumatized and intact horns of each animal were weighed separately and fixed in Bouin's solution. Doubtful traumatized horns were studied histologically for microscopical deciduomal nodules. The weight of the traumatized horn bearing deciduomata was taken as an estimate of the size of the response. The results were analyzed by the Student's t test.

Results

At the start of the priming with ED at 60 days of age, all of the 17 Group I rats receiving TP after ovariectomy had vaginal orifice, whereas only 2 of the 18 Group II rats given no TP after ovariectomy had the vagina open. Vaginal smears from all those having the vaginal aperture were invariably leucocytic. Three daily injections of 0.2 μg ED in the rats without vaginal aperture caused opening of the vaginæ on the day after the last injection with vaginal smears of the proestrous or estrous type. Those animals which had had the diestrous type vaginæ at the beginning of the ED administration also exhibited proestrous or estrous smears after 3 daily injections of ED.

When the injection of P plus ED was begun, 2 days after the last injection of ED, vaginal smears from all the rats were predominantly leucocytic and remained so during the 7-day period of injection, although a good many nucleated epithelial cells and a few cornified cells occasionally appeared in the smears. However, as reported previously (Takewaki and Ohta, 1974), the state of the vagina was not well correlated with the uterine reaction to traumatization. Copious mucus sometimes occurred in leucocytic vaginal smears toward the end of the injection period.

Comparison of the mean weights of the traumatized uterine horns between Group
Table 1. Deciduoma formation in rats ovariectomized and androgenized during neonatal life.

<table>
<thead>
<tr>
<th>Group</th>
<th>Final treatment daily dose</th>
<th>Positive response</th>
<th>Weight (M. ± S.E. mg) of traumatized horn</th>
<th>intact horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal ovariectomy, androgenization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia</td>
<td>2 mg P + 0.2 µg ED</td>
<td>7/8</td>
<td>181.1 ± 41.0 (114)*</td>
<td>72.8 ± 3.5</td>
</tr>
<tr>
<td>Ib</td>
<td>5 mg P + 0.2 µg ED</td>
<td>9/9</td>
<td>524.6 ± 68.0</td>
<td>95.7 ± 6.6</td>
</tr>
<tr>
<td>Neonatal ovariectomy, no androgenization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iia</td>
<td>2 mg P + 0.2 µg ED</td>
<td>8/8</td>
<td>366.4 ± 29.2</td>
<td>72.1 ± 3.8**</td>
</tr>
<tr>
<td>Iib</td>
<td>5 mg P + 0.2 µg ED</td>
<td>8/10</td>
<td>357.7 ± 39.7</td>
<td>76.3 ± 3.6</td>
</tr>
<tr>
<td>Neonatal ovariectomy, androgenization, chronic ED treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>2 mg P + 0.2 µg ED</td>
<td>2/10</td>
<td>142, 146</td>
<td>74.4 ± 3.8</td>
</tr>
<tr>
<td>IV</td>
<td>2 mg P + 0.2 µg ED</td>
<td>8/9</td>
<td>259.6 ± 57.3 (79)</td>
<td>66.0 ± 2.3</td>
</tr>
</tbody>
</table>

ED estradiol-17β, P progesterone.
* Weights or mean weight of traumatized uterine horns without deciduomata are given in parentheses.
** In one rat of this subgroup, deciduomata were found in the intact horn weighing 154 mg. This value was not included in calculating the mean.

Ia and Group IIa rats indicates that, although the incidence of deciduomata was high in both groups, the uterine response was significantly smaller in size in Group Ia rats which had been androgenized after ovariectomy than in Group IIa animals likewise ovariectomized but not androgenized (Table 1, p<0.01). If the daily dose of P was increased to 5 mg, the effect of TP injected during neonatal life upon the uterus was no longer evident (Groups Ib vs IIb).

In 6 of the 10 Group III rats ovariectomized and androgenized neonatally, the vaginae were canalized before 30 days of age when injection of 0.1 µg ED was commenced. Vaginal smears from these 6 rats were initially leucocytic but became cornified within 4 days after the start of the ED injections and remained predominantly so until the day after the 30th injection. The 9 rats given no TP after ovariectomy (Group IV) invariably lacked the vaginal orifice at 30 days of age. However, vaginal canalization occurred within 3 days after the start of ED administration followed by epithelial cornification on the next day in all the rats of both groups which had had the vagina closed at the beginning. Thenceforth, vaginal estrus persisted until the day after the last (30th) injection of ED, although a small number of leucocytes occasionally appeared in vaginal smears. Vaginal smears became leucocytic 2 days after withdrawal of ED and remained so during the 7-day period of P-ED administration. However, a good many nucleated epithelial cells sometimes made their appearance during this period.

The uterine response to trauma was markedly inhibited in Group III rats which had been androgenized after ovariectomy. By contrast, Group IV animals given no TP after ovariectomy exhibited much higher incidence (2/10 vs 8/9) and better development of deciduomata than did Group III rats (Table 1).

The size of the uterine response as estimated by the weight of the traumatized horns appeared to be smaller in Group IV receiving chronic administration of ED than in Group IIa not given the treatment. However, the difference is not statistically
Discussion

The 9 rats ovariectomized 3 days after birth invariably lacked the vaginal orifice at 30 days of age (Group IV) and 16 of the 18 rats similarly operated on still had the vagina closed when 60 days old (Group II). If a single injection of 1.25 mg TP was given on the day following ovariectomy carried out at 3 days of age, 6 of the 10 rats (Group III) formed the vaginal aperture by 30 days of age and all of 17 other rats (Group I) by 60 days. These findings are in good agreement with the report of Justo et al. (1970) that vaginal opening was delayed in rats ovariectomized neonatally and hastened by a single injection of TP after ovariectomy.

Comparison of the data from Group Ia and Group IIa demonstrates that the uterine response to trauma was much smaller in size in the neonatally ovariectomized, androgenized rats than in the neonatally ovariectomized, non-androgenized animals, under similar hormonal conditions supportive of the development of deciduomata.

The data from Group III animals show that continued exposure to estrogen caused a further reduction of the uterine capacity to differentiate into a deciduoma in rats ovariectomized and androgenized during neonatal life. By contrast, ovariectomized rats receiving no TP as neonates, continued administration of ED exerted no significant effects on both size and incidence of deciduomata following uterine traumatization (Compare Group IV and Group IIa).

Accordingly, as discussed previously (Takewaki and Ohta, 1974; Ohta and Takewaki, 1974), it seems highly probable that, in androgenized, persistent-estrous rats, the androgen administered during neonatal life is primarily responsible for the decrease in uterine reactivity to trauma. However, continued secretion of ovarian estrogen appears to co-operate with the androgen to evoke a further reduction of the uterine responsiveness.

Takewaki and Ohta (1974) reported that a group of androgen-sterilized adult rats given daily injections of 2 mg P plus 0.2 µg ED beginning 2 days after ovariectomy invariably failed to form deciduomata in response to uterine trauma, whereas in the groups ovariectomized 1 or 2 months prior to the start of similar P-ED administration, a few rats reacted positively to trauma. These findings may be accounted for by this hypothesis.

References