
Altered Sensitivity of Uterus to Progesterone-Estradiol in Rats Treated Neonatally with Androgen and Ovariectomized as Adults

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Synopsis

Female rats were made persistent-estrous and anovulatory by giving a single injection of 1.25 mg of testosterone propionate 4 days after birth. The rats were ovariectomized when adult and given 7 daily injections of progesterone plus estradiol in amounts sufficient to condition the uteri for formation of deciduomata in response to trauma. The administration of the hormones was commenced on the day following ovariectomy, or 32 or 62 postoperative days after priming with estradiol for 2 days. Incidence of deciduomata following uterine traumatization on the 4th day of the injection period was always much higher in non-androgenized controls than in androgen-sterilized rats. It seems likely that neonatal androgenization results in a permanent or at least a long-lasting reduction of uterine sensitivity to progesterone-estradiol. The longer the interval between ovariectomy and the beginning of progesterone-estradiol administration, the less marked was the mucification of the vaginal epithelium. There was no correlation between the degree of vaginal mucification and the development of deciduomata.

Treatment of female rats with androgen or estrogen during a critical neonatal period results in anovulatory, infertile animals exhibiting persistent vaginal cornification when they reach adulthood. It is now established that the acyclic male pattern of gonadotropin secretion results from permanent damage to hypothalamic centers normally responsive to steroid feedback (Takewaki, 1962; Jacobsohn, 1965; Dörner, 1972).

However, permanent disruption of normal function is also observed in organs other than the brain in steroid-sterilized rats. Anterior pituitary and uterine tissue are less responsive to estrogen (Hayashi, 1967; Wrenn et al., 1969; Lobl et al., 1974). Unusual reactivity of uterine mucosa is also shown by the metaplasia and hypertrophy of the epithelium following estrogen injections (Takewaki, 1968; Takewaki and Kawashima, 1967). Moreover, it has been reported that estrogen-binding sites are reduced in uterus, pituitary and hypothalamus of sterilized rats (Flerkó et al., 1969; Anderson and Greenwald, 1969). This may probably account for the decreased estrogen sensitivity in the organs.

Deciduoma formation in response to trauma in uterus acted upon by progesterone alone or in combination with a small amount of estrogen in rats treated with androgen neonatally has received less attention. Burin et al. (1963) reported that, in androgen-sterilized rats, deciduomata were elicited following uterine traumatization applied on the 5th day of 8 daily injections of 5 mg progesterone. By contrast, Zeilmaker (1964) could not induce deciduoma formation in a group of neonatally androgenized rats which had been ovariectomized after injections of HCG, by traumatizing the endometrium on the 4th day of an 8-day period of injections of 5 mg progesterone together with 0.5 μg estrone.

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The present paper deals with the experiments showing that the capacity of endometrium to differentiate into a deciduoma in response to trauma in the presence of an appropriate combination of estradiol and progesterone is markedly reduced in androgen-sterilized rats as compared with that in normal animals and does not restore to normal for at least 2 months after ovariectomy.

Materials and Methods

Female rats of the T strain used in this study were raised in a temperature and light (14 h light/day) controlled room.

Thirty-four rats were given a single subcutaneous injection of 1.25 mg testosterone propionate in 0.05 ml sesame oil 4 days after birth. When they were 60 days old, vaginal smears were taken for at least 10 days to ensure that persistent estrus was established. All the rats were ovariectomized after this period, at 70–80 days of age. The polyfollicular ovaries containing no corpora lutea from the 34 animals weighed 38.5 ± 1.43 mg.

The rats were randomly assigned to 4 groups. Three of the 4 groups were given subcutaneous injections of 2 mg progesterone plus 0.2 µg estradiol-17β in 0.15 ml sesame oil for 7 consecutive days, from the day following removal of the ovaries in Group I, and after postoperative periods of 32 and 62 days in Groups II and III, respectively. The animals of these 2 groups received subcutaneous injections of 0.2 µg estradiol for 2 days prior to the commencement of progesterone-estradiol administration. Group IV rats were injected with 5 mg progesterone plus 0.2 µg estradiol for a 7-day period from the day after ovariec-tomcy.

The control (non-androgenized) rats of comparable ages were ovariectomized during vaginal estrus after 3–5 estrous cycles determined by vaginal smear examination. The ovaries from 37 controls invariably contained follicles and corpora lutea, weighing 104.3 ± 2.80 mg. The rats were likewise divided into 4 groups and a series of 7 daily subcutaneous injections of 2 mg progesterone plus 0.2 µg estradiol was given in 3 groups (Groups V, VI and VII), commencing 1, 32 and 62 days after ovariectomy, respectively. The rats of Groups VI and VII were primed with injections of 0.2 µg estradiol for 2 days prior to the progesterone-estradiol administration. Group VIII animals received injections of 5 mg progesterone plus 0.2 µg estradiol for 7 days starting on the day after operation.

On the 4th day of the 7-day period of progesterone-estradiol injections, endometrium of the right uterine horn of each rat was scratched along the entire length by a needle with a bent point inserted into the uterine lumen via a small incision made near the cervical end of the horn (Takewaki, 1969). Daily vaginal smears were taken throughout the injection period.

On the day following the last injection, the animals were sacrificed. After uteri were checked for gross evidence of deciduomata, traumatized and intact horns were weighed separately and fixed in Bouin’s solution for histological study. Vaginae were also fixed in Bouin’s solution, sectioned transversely and stained with alcian blue or by the PAS method for demonstration of mucus.

Mean weights of uterine horns of different groups were compared by Student’s t test.

Results

Deciduoma Formation in Response to Uterine Trauma

Table 1 summarizing the results of the experiments shows that 9 control rats ovariectomized at estrus and given daily injections of 2 mg progesterone together with 0.2 µg estradiol starting on the day following the operation (Group V) invariably formed deciduomata in response to uterine trauma, while 10 androgen-sterilized rats receiving similar schedule of treatment always failed to respond to trauma (Group I), although the traumatized horns were usually heavier than the contralateral intact horns (p < 0.01). These results suggest that the uteri were at least less sensitive to the daily injections of progesterone-estradiol in androgen-sterilized rats than in the normal controls.

With these findings in mind, an investigation was initiated with a larger dose of progesterone. Eight androgen-sterilized rats were given injections of 5 mg progesterone plus 0.2 µg estradiol for 7 consecutive days beginning on the day after ovariectomy (Group IV). Uterine traumatization elicited deciduo-mata in 5 of 8 animals. Thus, the incidence of deciduomata was markedly elevated by increasing the daily dose of progesterone. Yet, in a control group of 10 non-androgenized rats given similar injections, all the animals re-
Table 1. Deciduoma formation in response to uterine trauma in androgen-sterilized and control rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Final body weight (g)</th>
<th>Positive response</th>
<th>Weight (M. ± S.E. mg) of traumatized horn</th>
<th>Weight (M. ± S.E. mg) of intact horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgenized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>224.5 ± 6.01</td>
<td>0/10</td>
<td>(132.5 ± 8.25)*</td>
<td>103.2 ± 4.31</td>
</tr>
<tr>
<td>II</td>
<td>270.0 ± 8.37</td>
<td>2/7</td>
<td>364, 207</td>
<td>82.7 ± 5.15</td>
</tr>
<tr>
<td>III</td>
<td>326.0 ± 13.61</td>
<td>1/9</td>
<td>184 (95.3 ± 9.64)</td>
<td>79.1 ± 5.65</td>
</tr>
<tr>
<td>IV</td>
<td>199.8 ± 8.02</td>
<td>5/8</td>
<td>224.8 ± 41.86 (142.0 ± 16.20)</td>
<td>118.0 ± 13.42</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>221.1 ± 7.16</td>
<td>9/9</td>
<td>490.0 ± 47.67</td>
<td>134.1 ± 7.85</td>
</tr>
<tr>
<td>VI</td>
<td>263.0 ± 3.19</td>
<td>7/8</td>
<td>307.9 ± 54.65 (105)</td>
<td>93.5 ± 2.65</td>
</tr>
<tr>
<td>VII</td>
<td>274.1 ± 4.28</td>
<td>5/10</td>
<td>131.0 ± 5.54 (117.6 ± 4.88)</td>
<td>86.9 ± 2.97</td>
</tr>
<tr>
<td>VIII</td>
<td>212.4 ± 2.88</td>
<td>10/10</td>
<td>735.8 ± 68.03</td>
<td>137.5 ± 9.09**</td>
</tr>
</tbody>
</table>

Groups I, II, III, V, VI and VII were given injections of 2 mg progesterone and 0.2 μg estradiol for 7 days, while Groups IV and VIII received injections of 5 mg progesterone and 0.2 μg estradiol for 7 days.

* Mean weights of traumatized uterine horns without deciduomata are given in parentheses.

** Two horns with deciduomata weighing 189 and 202 mg, respectively, are not included in this mean.

Responded to endometrial trauma by forming well developed deciduomata (Group VIII). The uterine horns bearing deciduomata were significantly larger in weight in the control rats than in the androgen-sterilized rats (Group IV vs Group VIII: p < 0.001). In 2 of the control rats, deciduomata were noted not only in the traumatized horns but also in the intact horns. The uteri of the control rats appeared to have been so strongly sensitized that weak mechanical stimuli due to manipulation in a horn evoked deciduoma formation in the opposite horn.

Effects of Time Interval between Ovariectomy and Commencement of Progesterone-Estradiol Injections on Uterine Reaction

In order to determine whether the uteri of neonatally androgenized rats recover the normal sensitivity to progesterone-estradiol combinations after blockade of persistent estrus by ovariectomy, 2 groups of androgen-sterilized rats were ovariectomized before being given the standard schedule of treatment.

Prior to starting injections of 2 mg progesterone and 0.2 μg estradiol at intervals of 32 and 62 days after surgery, respectively, all the rats were given subcutaneous injections of 0.2 μg estradiol for 2 days (Groups II and III). Two groups of non-androgenized rats similarly treated served as controls (Groups VI and VII).

During the 30- or 60-day postoperative interval, the genital tract underwent progressive atrophy in both the androgen-sterilized and control animals. This was reflected in the mean final weights of the intact uterine horns which were significantly smaller in these groups as compared with those of the animals given similar injections of progesterone-estradiol from the day after ovari-
A reduction of incidence of deciduomata following endometrial trauma was evident in the control rats. A few rats of the androgen-sterilized groups given progesterone-estradiol after prolonged postoperative intervals formed deciduomata in response to uterine traumatization. However, the incidence was always lower in the androgen-sterilized rats (2 of 7 and 1 of 9, Groups II and III) than in the controls (7 of 8 and 5 of 10, Groups VI and VII).

Vaginal Mucification

In both androgen-sterilized and normal control rats, marked mucification of the vaginal epithelium took place following injections of 2 mg progesterone together with 0.2 μg estradiol for 7 consecutive days commencing on the day after ovariectomy (Groups I and V). An increase in daily dose of progesterone to 5 mg did not result in any marked difference in degree of the vaginal reaction (Groups IV and VIII).

If the commencement of progesterone-estradiol administration was postponed until 32 days after surgery, vaginal mucification was definitely reduced at least in some animals. In one rat of Group II and 3 rats of Group VI, mucification was restricted to areas of the epithelium lining the bottom regions of folds of the vaginal mucosa, the remainder of the epithelium being stratified and squamous. Vaginal smears from such animals consisted mainly of nucleated epithelial cells and occasional cornified cells. In one animal of Group II and 2 rats of Group VI, the vaginal epithelium exhibited an overall squamatization, having no mucified areas.

Suppression of vaginal mucification was still more marked when the interval between ovariectomy and the commencement of hormone administration was lengthened to 62 days, strong and mild mucification taking place only in 3 out of 9 androgen-sterilized rats (Group III) and 4 out of 10 control animals (Group VII). In one sterilized rat, the vaginal epithelium lacked mucified areas.

There was no correlation between the degree of vaginal mucification and the development of deciduomata. Three rats showing no mucified areas in the vaginal epithelium had well developed deciduomata, while 4 animals with strongly mucified vaginae totally failed to respond to uterine trauma.

Discussion

The present data show that incidence of deciduomata in response to uterine trauma was markedly reduced in rats androgenized neonatally and ovariectomized as adults, even if they were given daily injections of progesterone and estradiol in quantities sufficient to condition the uteri for development of deciduomata in reaction to trauma. Two of the 7 androgen-sterilized rats given injections of 2 mg progesterone plus 0.2 μg estradiol daily after a 32-day postoperative period responded positively to uterine trauma. However, prolongation of the interval to 62 days yielded no better results. In view of these findings, the lowered uterine sensitivity in androgen-sterilized rats appears to have been brought about by the single neonatal injection of androgen rather than by the continued exposure to endogenous estrogen.

Under the conditions of the present experiments, mucification of the vaginal epithelium following injections of progesterone and estradiol was not perceptively different in degree between neonatally androgenized and control rats. In both kinds of rats, mucification was weaker when the hormone administration was begun 32 or 62 days after ovariectomy than when commenced on the day following the surgery. The vaginal epithelium was squamous with little or no sign of mucification in some rats receiving the injections after prolonged postoperative intervals. There was no correlation between the
degree of vaginal mucification and the development of deciduomata. Takewaki (1971) reported similar findings in a different kind of experiments. The uterus is capable of responding to progesterone-estradiol independently of the vagina.

Androgen-sterilized adult rats bearing ovaries induced to ovulate do not exhibit pseudopregnancy in response to procedures which are effective in eliciting luteal activity in normal female rats, such as a single injection of reserpine on the first day of diestrus (Kikuyama, 1963; Zeilmaker, 1964; Duluc and Mayer, 1967) and stimulation of the uterine cervix during estrus (Zeilmaker, 1964; Duluc and Mayer, 1967). Radioimmunoassays of blood prolactin concentrations appear to suggest that this failure of luteal activation is related to changes in the central mechanisms involved in the regulation of prolactin secretion in androgen-sterilized rats (Neill, 1972; Mallampati and Johnson, 1973a, b).

The present findings indicate that neonatal androgenization results in persistent changes in reactivity of the uterus to progesterone-estradiol in addition to permanent modifications of hypothalamic activities. Accordingly, deciduoma formation is not always reliable as a criterion for the luteal function in these animals.

References


