THE EFFECT OF UNILATERAL OVARIECTOMY IN THE NEONATAL MOUSE ON FOLLICULAR DEVELOPMENT

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SUMMARY

The remaining ovary of a 14-day-old mouse after unilateral ovariectomy (ULO) on day 2 showed the same number and stages of follicles as an ovary in an intact animal of the same age. No increase in the number of developing follicles nor development to more advanced stages occurred. Hemispaying in the neonatal period did not alter the number of follicles that started growth nor their speed of development in the subsequent 12 days. However, premature follicle fluid accumulation and a widening of the theca layer in early growing follicles were characteristically found in the remaining ovary. The failure of the remaining ovary to increase its complement of large follicles is probably due to the fact that those follicle stages (i.e. large follicles) which are able to react to stimulation by follicle-stimulating hormone in the prepubertal and adult ovary are as yet lacking in the early infant ovary. However, reaction to luteinizing hormone can already be expressed and is shown by premature follicle fluid accumulation and widening of the theca layer. The changes seen are interpreted as a reaction of the remaining ovary to a relative increase in an unchanged amount of available gonadotrophin rather than to a change in the pituitary–gonadal feedback mechanism.

INTRODUCTION

Unilateral ovariectomy (ULO) in adult mice and other species leads to compensatory hypertrophy of the remaining ovary (Arai, 1920; Greenwald, 1961; McLaren, 1963; Hermreck & Greenwald, 1964; Brinkley & Young, 1969). This is primarily due to increased gonadotrophin secretion (Grady & Greenwald, 1968; Benson, Sorrentino & Evans, 1969; Welschen, 1970). Whether removal of an ovary in a newborn animal alters the growth pattern of follicles in the remaining ovary has not yet been determined. It has recently been reported that ULO in the neonatal rat leads to an increase in weight in the remaining ovary within 10–15 days (Gerall & Dunlap, 1971). It was implied that a change in the pituitary–ovarian feedback mechanism was responsible for an ovarian hypertrophy.

The present investigations were undertaken in mice to determine what effect
hemispaying during the neonatal period has on follicle growth in the remaining ovary. The question to be answered was, whether after ULO on day 2 the number of growing follicles found in the remaining ovary on day 14 differed quantitatively or qualitatively from those found in intact animals of the same age.

MATERIAL AND METHODS

Twenty-three female Bagg mice (2-day-old) were used. They belonged to eight litters. In each litter some of the young were hemispayed, some remained intact as controls. The 15 experimental animals were anaesthetized by putting them for 4 min into a beaker containing ice cubes. Through a dorsal incision the right ovary was then removed. The wound was closed with two stitches. The young remained under a lamp for 2–3 h before they were returned to the mother mouse. Some of the eight control mice were anaesthetized and warmed in the same way before they were given back to the mother mouse.

All mice were killed at the age of 14 days by cervical dislocation. The ovaries were removed, fixed in Bouin solution, serially sectioned at 5 μm and stained with haematoxylin–eosin or Heidenhain’s azan. The ovaries were examined histologically and evaluated quantitatively and qualitatively. To determine the follicle number differentially, the follicles were divided into small and growing follicles (Peters & Levy, 1964). The growing follicles were further subdivided into different types according to the number of granulosa cells seen in the largest cross-section (Pedersen & Peters, 1968). It soon became evident that in the hemispayed animals follicle fluid formation often occurred prematurely, follicles without and with fluid were therefore scored separately (Fig. 1). A probability level of 0.01 was employed in Student’s t-test.

RESULTS

The number of follicles present in one ovary of 14-day-old control mice was determined and compared with the number of follicles in the remaining ovary after ULO on day 2 (Table 1). The number of small follicles was comparable in the two groups. Also the number of all types of growing follicles (Type 3b to 5) did not differ in the remaining ovary of hemispayed animals from that in one ovary of the control mice. However, there were significantly more (P < 0.01) early growing (Type 4) follicles with antrum formation and follicle fluid accumulated in the hemispayed than in the intact animals. Many of these follicles had theca layers that were broader and richer in cells than those in follicles of the same size in the controls. These follicles were healthy in that neither the oocytes nor granulosa cells showed signs of degeneration.

Except for the larger number of fluid-containing early follicles the ovaries in the two groups looked similar. There was no increase in follicle degeneration, the amount of stroma, and the development of the vascular system was not remarkably different.
Neonatal hemispaying and follicle development

Fig. 1. Classification of ovarian follicles in mice.
Table 1. Number of follicles in one ovary on day 14 in 8 control animals and 15 experimental mice after unilateral ovariectomy (ULO) on day 2 (means ± S.E.M.)

(See Fig. 1 for classification of ovarian follicles.)

<table>
<thead>
<tr>
<th>State of mice</th>
<th>Small follicles</th>
<th>Type 3b</th>
<th>Without fluid</th>
<th>With fluid</th>
<th>All</th>
<th>Type 3b–5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5510 ± 621</td>
<td>86 ± 5</td>
<td>95 ± 7</td>
<td>11 ± 3</td>
<td>106 ± 8</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>ULO</td>
<td>4957 ± 362</td>
<td>92 ± 12</td>
<td>77 ± 8</td>
<td>28 ± 4*</td>
<td>105 ± 11</td>
<td>8 ± 2</td>
</tr>
</tbody>
</table>

* P < 0.01 compared with control group.

DISCUSSION

Neither an increase in the number of growing follicles nor a faster development of follicles was noted 12 days after ULO. The number of small follicles, i.e. the pool of non-growing follicles, was the same in one ovary of experimental and control animals. This suggests that hemispaying on day 2 did not alter the start of follicle growth (number leaving the pool) nor the speed of growth for the next 2 weeks.

However, a change in the appearance of some of the growing follicles was characteristic in the remaining ovary 12 days after ULO. A considerable number of growing follicles (Type 4) showed an early accumulation of follicle fluid. The follicles were also characterized by a widened theca layer. Similar changes have been found to occur in ovaries of 14-day-old mice whose endogenous gonadotrophin had been blocked continuously from birth with anti-gonadotrophins but who had received simultaneously substitution therapy with follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Eshkol, Lunenfeld & Peters, 1970). Since such changes were not seen after substitution therapy with FSH alone, premature follicle fluid accumulation and early stimulation of theca was interpreted as a response to an overstimulation by LH in the presence of FSH. Price & Ortiz (1944) reported a widening of the theca layer in 10-day-old rats after injecting gonadotrophins for 6 days. Theca cells have been found to be a specific target for LH (Rajaniemi & Vanha-Perttula, 1972). Plasma FSH and LH levels are high in normal, immature female rats (Weisz & Ferin, 1970) with peaks reported for FSH on day 12 and for LH in 10-day-old animals (Ojeda & Ramirez, 1972).

The premature fluid formation in early follicle development and the widening of the theca layer seen in the present experiments suggests that the remaining ovary is affected by unilateral ovariectomy during the neonatal period. Ojeda & Ramirez (1972) found no increase in plasma gonadotrophins after ULO at 10 days of age until the rats reached 25 days. It is therefore likely that the effects observed in the present experiments arose from an enhanced reaction of the remaining ovary to an unchanged gonadotrophin level. This would suggest that in early infancy, in contrast to adulthood (Grady & Greenwald, 1968; Benson et al. 1969; Welschen, 1970) a ‘consumption mechanism’ (Zarrow, Sundaram & Stob, 1965; McLaren, 1966) is in operation in which the remaining ovary ‘reacts sensitively to an increased proportion of
Neonatal hemispaying and follicle development

an unchanged amount of gonadotrophin’ (Welschen, 1970). The question might be raised why a reaction only to LH becomes evident. This might be explained by the fact that those follicle stages which in the prepubertal and adult ovary are able to react to FSH (i.e. Type 5 and larger follicles) are as yet lacking in the ovaries during early infancy.

Our results thus indicate that the increase in ovarian weight after ULO at birth reported by Gerall & Dunlap (1971) is most likely due to early follicle fluid production and accumulation stimulated by the action of LH on the remaining ovary.

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REFERENCES


