Effects of Multiple LH Injections on the Secretion of Estrogens from Polycystic Ovaries of Androgen-Sterilized Rats

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Abstract

Differences in the secretion of estrogens by follicular polycystic ovaries of androgen-sterilized rats and by normal follicular ovaries of early proestrous rats were compared. Some rats were injected i.v. with LH 30 min before bleeding. This injection of LH did not influence the secretion of estrogens by normal ovaries, but greatly increased that by polycystic ovaries, suggesting that there was abnormal steroidogenesis in cystic ovaries. In the ovaries of such androgen-sterilized rats, two types of enlarged abnormal follicles were seen. One of these was truly cystic with few or no granulosa cells (1st type). The other had a hyperplastic and infolded layer of granulosa cells with a papillary appearance (2nd type).

Because it is known that the preovulatory LH surge is not found in androgen-sterilized rats, a classical approach was taken to circumvent the probable deficit in cyclic release of LH by giving an i.v. injection of LH every 4 days for 16 days, and ovarian venous blood was collected 4 days after the last injection. In consequence the 2nd type of abnormal follicle disappeared as did the abnormalities of estrogen production. These results suggest that the abnormalities of estrogen production by the polycystic ovaries of androgen-sterilized rats may be due to the 2nd type of abnormal follicle.

The high estrogen-producing ability of polycystic ovaries has been amply documented (Weisz and Lloyd, 1965; Sawada and Ichikawa, 1978), but the mechanism which induces abnormal estrogens has never been fully explained. It is well known that administration of androgen to neonatal female rats results in infertility characterized by follicular polycystic ovaries (Barraclough, 1961). It is thought that this syndrome may result from disorders of the hypothalamus and the subsequent change in the cyclic release of LH from the pituitary gland (Barraclough, 1966; Wagner et al., 1966). Since the structural changes in the ovaries of androgen-sterilized rats are not the result of intrinsic ovarian abnormalities and are reversible, it seemed worth while to examine these ovaries for the presence of a change in steroidogenesis. Here, I studied effects of multiple injections of LH with regard to secretion of estrogens from polycystic ovaries of androgen-sterilized rats. The acute effects of LH on secretion of estrogens in such rats were also studied. The acute steroidogenic effect of LH may vary ac-
Materials and Methods

Animals

Sprague-Dawley rats bred in this laboratory were used. They were kept at 24 ± 1°C under a 14-hr photoperiod (lights on from 05:00 to 19:00 hr), and maintained on water and commercial standard food pellets.

Polycystic ovaries were induced by an s.c. injection of 1.25 mg of testosterone propionate (Sigma Chemical Co., St. Louis, MO, U.S.A.) at 5 days of age. Uninjected littersmates served as controls. Daily vaginal smears were taken when the rats were 3 months old. Ovarian venous blood was collected from rats which showed a cornified vagina for at least 10 consecutive days and from control rats which showed three consecutive 4-day cycles. Some androgen treated rats which showed persistent estrus were given multiple i.v. injections of 25 µg of bovine LH (NIH-LH-B7) in 0.2 ml physiological saline. The multiple injections were given at 14:00 hr every 4 days for 16 days. Ovarian venous blood was collected 4 days after the last injection. Every 24 hours after the injection of LH, some rats were sacrificed and their oviducts were examined under a dissecting microscope for the presence of ova.

Collection of ovarian venous blood

Ovarian venous blood was collected between 10:00 and 12:00 hr by a method described previously (Ichikawa et al., 1970). Polyethylene tubing (0.58 mm inner diameter and 0.97 mm outer diameter; Intramedic PE 50; Clay-Adams, Division of Becton, Dickinson and Company, NJ, U.S.A.) was used for the cannulation of the ovarian vein. Heparinized arterial blood was infused into the femoral vein at a rate of 10.6 ml/hr in order to maintain the blood volume during collection. Blood used for the transfusion was obtained from the aorta of male rats. To stimulate the secretion of steroids, half of the rats had 2 µg of bovine LH injected into the external jugular vein 30 min before blood was collected. Ovarian blood was collected for 30 min and used for the assay of estrogens. Plasma was separated by centrifugation and stored at -20°C until use.

The ovaries of rats from which blood samples were obtained were removed immediately after blood collection and fixed in Bouin’s fluid. Fixed ovaries were serially sectioned (10 µm thick) and stained with hematoxylin and eosin. Sections of each ovary were examined under the microscope.

Steroid assay

Amounts of estrogens in 0.5 ml ovarian venous plasma were determined by the radioimmunoassay (RIA) technique described previously (Sawada and Ichikawa, 1978). This method permitted measurement of as little as 50 picogram (pg) per 0.5 ml of each estrogen with average recovery rates within the 80-118% range. The intra-assay coefficients of variation were 12%, while the interassay coefficients of variation were <16%.

Results

Effects of multiple LH injections on vaginal smear and ovulation in androgen-sterilized rats

A single injection of LH every 4 days induced irregular estrous cycle in most of the treated rats, whereas the non-treated group continually showed a persistent estrous smear. The effects of cyclic administration of LH on ovulation in such androgen-sterilized rats are shown in Table 1 (groups 1–4). The control group failed to

Table 1. Effect of a single and cyclic administration of LH on ovulation in androgen-sterilized rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (µg)</th>
<th>Total no. of doses administered</th>
<th>No. of rats ovulated (%)</th>
<th>No. of eggs recovered (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>1</td>
<td>0/5 (0)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>1</td>
<td>7/7 (100)</td>
<td>8 ± 1.2</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>1</td>
<td>6/6 (100)</td>
<td>9 ± 1.3</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>5</td>
<td>7/7 (100)</td>
<td>10 ± 1.5</td>
</tr>
</tbody>
</table>
Table 2. Concentrations of estrogens in ovarian venous plasma from normal proestrous rats, androgen-sterilized rats and androgen-sterilized rats given multiple i.v. injections of 25 μg LH at 14:00 hr every 4 days for 16 days. Values are means±SE.

<table>
<thead>
<tr>
<th>Group no. and treatment</th>
<th>No. of rats</th>
<th>Estrone (pg/ml)</th>
<th>Estradiol-17β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal proestrous rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. No acute stimulation</td>
<td>6</td>
<td>544±72#</td>
<td>2916±608#</td>
</tr>
<tr>
<td>6. Acute stimulation</td>
<td>7</td>
<td>514±62</td>
<td>2936±473</td>
</tr>
<tr>
<td>Androgen-sterilized rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. No acute stimulation</td>
<td>5</td>
<td>229±33</td>
<td>1161±234</td>
</tr>
<tr>
<td>8. Acute stimulation</td>
<td>6</td>
<td>578±127*</td>
<td>2593±431*</td>
</tr>
<tr>
<td>Androgen-sterilized rats given multiple i.v. injection of LH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. No acute stimulation</td>
<td>8</td>
<td>372±95</td>
<td>1634±442</td>
</tr>
<tr>
<td>10. Acute stimulation</td>
<td>8</td>
<td>483±89</td>
<td>1942±501</td>
</tr>
</tbody>
</table>

*P<0.05 compared with no acute steroid stimulation controls.
#P<0.05 compared with group 7.
For acute stimulation of steroid secretion, LH (2 μg) was injected i.v. 30 min before bleeding.

ovulate (group 1), but ovulation was observed within 24 hours after last injection of LH (groups 2–4). The mean numbers of eggs recovered from groups which received cyclic treatment were almost similar to those for the groups which received a single injection (groups 2 vs. 3 or 4).

Estrogens from polycystic ovaries of androgen-sterilized rats

Concentrations of estrogens in ovarian venous plasma of normal and androgen-sterilized rats are shown in Table 2 (groups 5–8). Amounts of estrogens from non-stimulated polycystic ovaries of androgen-sterilized rats were about a half those from non-stimulated normal ovaries of proestrous rats (group 5 vs. 7). An injection of LH markedly increased the secretion of estrogen by polycystic ovaries (group 7 vs. 8), whereas the response of estrogen secretion by normal ovaries showed no significant change (group 5 vs. 6).

Effects of multiple LH injections on the secretion of estrogens from polycystic ovaries of androgen-sterilized rats

Concentrations of estrogens in ovarian venous plasma from androgen-sterilized rats given multiple i.v. injections of LH are shown in Table 2 (groups 9 and 10). The multiple injections of LH in androgen-sterilized rats slightly increased the secretion of estrogens to more than those from ovaries of untreated androgen-sterilized rats (group 7 vs. 9). An injection of LH before sample collection in such androgen-sterilized rats had no significant effect on the secretion of estrogens (group 9 vs. 10).

Histology

Mature preovulatory follicles, such as are found in normal rats at proestrus, were absent from sections of ovaries from androgen-sterilized rats (Fig. 1A). Two types of enlarged abnormal follicles were seen. One of these was truly cystic with few or no granulosa cells. The other had a hyperplastic and infolded layer of granulosa cells with a papillary appearance. In the ovaries of rats following multiple LH treatments, however, these abnormal follicles disappeared and luteinization of cystic follicles and ovulatory corpora lutea were observed (Fig. 1B).

Discussion

In normal cyclic rats, generally, estrogen
reaches its peak level on the morning of proestrus (Naftolin et al., 1972; Butcher et al., 1974). The peripheral estrogen level in androgen-sterilized rats has been reported to be the same as (Cheng and Johnson, 1973/74) or less than (Naftolin et al., 1972) those seen on the morning of proestrus in the normal cycle. The concentrations of estrone and estradiol-17β from polycystic ovaries found here in androgen-sterilized rats were lower than values for normal rats at proestrus.

On the morning of proestrus, no significant change occurred in estrogen secretion during the first 3 hr after LH injection (Hori et al., 1969). In the present study, LH administered intravenously 30 min before sample collection did not influence secretion of estrogens by normal ovaries. However, this treatment greatly increased the secretion of estrogens by polycystic ovaries of androgen-sterilized rats, suggesting that there was abnormal steroidogenesis in cystic ovaries. The high estrogen-producing ability of polycystic ovaries of androgen-sterilized rats has been also found in in vitro experiments by Weisz and Lloyd (1965). In the ovaries of such androgen-sterilized rats, two types of enlarged abnormal follicles such as observed by Cortes et al. (1971) were seen. The first type is truly cystic with few or no granulosa cells and is not very active endocrinologically. The second type, however, has the appearance of an endocrinologically active structure and ovulates after treatment with LH. These facts led me to investigate the interrelationship between the abnormal secretion of estrogens and the presence of the second type of abnormal follicle in the polycystic ovaries of androgen-sterilized rats. Because it is known that the preovulatory LH surge is not found in androgen-sterilized rats (Barracough, 1966; Wagner et al., 1966), a classical approach was taken to circumvent the probable deficit in cyclic release of LH by giving an i.v. injection of LH every 4 days. In the present study the full effective doses of LH which would induce ovulation in androgen-sterilized rats (Cortes et al., 1971; Sawada & Kosaka, 1981) were used in multiple injections. In consequence in the ovaries of androgen-sterilized rats the second type of enlarged abnormal follicle disappeared and luteinization of cystic follicles and ovulatory corpora lutea were observed. The mean secretion of estrogens from the ovaries of such androgen-sterilized
rats was similar to that from normal ovaries, and the abnormal acute effect of LH on the secretion of estrogens disappeared. These results suggest that the abnormalities of estrogen production by the polycystic ovaries of androgen-sterilized rats may be due to the second type of abnormal follicle.

**Acknowledgements**

The author wishes to thank the Endocrinology Study Section, NIADDK, Bethesda, MD, U.S.A. for their gift of LH. Excellent technical assistance was provided by Dr. K. Toda. The author is grateful to Dr. S. Ichikawa for his help with part of these experiments. This study was supported by Research Grant No. 466154 from the Ministry of Education, Science and Culture, Japan.

**References**


