ACTION OF TESTOSTERONE PROPIONATE ON THE GONADOTROPHIC FUNCTION OF THE PITUITARY GLAND IN THE CYCLIC FEMALE RAT

By
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ABSTRACT

Four-day cyclic female rats were injected with 5 mg testosterone propionate (TP) at dioestrus II at 10.00. A blockade of ovulation was observed on the morning of oestrus in most of these animals. No LH surge occurred in the afternoon of pro-oestrus. By contrast the size of follicles exceeding 400 μm in diameter did not differ in the afternoon of pro-oestrus in TP-treated and control females. Moreover both the number of follicles and the blood FSH concentration appeared to be higher at 14.00 on pro-oestrus in TP-treated than in control females. The effects of TP in vivo are thus in agreement with the observations which showed that TP caused a blockade of LH release and the maintenance of FSH release in vitro.

Few experiments have been devoted to the action of testosterone propionate (TP) on the gonadotrophic function of the pituitary gland during the oestrous cycle in the rat. TP was shown to block ovulation in 4-day cyclic rats when injected either in the afternoon of dioestrus I or on the morning of dioestrus II (Aron & Asch 1963; Aron et al. 1967). Follicular growth appeared to be inhibited following TP injection on dioestrus I but remained unchanged after TP administration at dioestrus II (Aron & Asch 1963; Aron et al. 1967). Later work (Achille et al. 1971) showed that TP was even capable of accelerating follicular growth when injected at dioestrus II. Such a result is in keeping with the observations of Kerdelhie et al. (1977) which revealed that TP caused FSH
surge in castrated female rats. Labrie et al. (1977) also indicated that testosterone increased FSH release from incubated gonadotrophic pituitary cells when stimulated by LH-RH. Inversely LH release appeared to be inhibited under these experimental conditions. It thus seemed of interest to determine whether changes in LH and FSH blood concentration in the afternoon of pro-oestrus would correspond to the modifications of ovarian function as those observed following TP treatment at dioestrus II in the rat. The aim of this work was to investigate this question.

**MATERIAL AND METHODS**

**Animals**

Fifty female Wistar rats of the WI strain from our colony, weighing 180–200 g, were used. Vaginal smears were taken on each morning 6 days a week for the control of oestrous rhythm. Only those females which exhibited 2 or 3 successive 4-day cycles were used. They were kept on the normal rhythm of natural lighting and fed with a commercial laboratory food and water *ad libitum*. They were divided into two groups. A first group comprised 40 animals. One half of the females served as control. The other half of the group was injected with testosterone propionate (TP) on dioestrus II at 10.00–11.00. Half of the controls and of the injected females were sacrificed by decapitation at 14.00 and the remaining half at 17.00 at pro-oestrus. The blood was collected for the radioimmunoassay of FSH and LH. The ovaries were removed for histological study.

A second group of 10 animals was used as a control for the blockade of ovulation in TP injected females. TP was injected at dioestrus II at 10.00–11.00 as above and the ovaries were extirpated on the morning of oestrus.

**Method for the study of ovulation and of follicular growth**

The ovaries were stained with haematoxyline-eosine and examined either on the morning of oestrus for signs of ovulation or in the afternoon of pro-oestrus for the determination of the state of follicular development. Only follicles without picnotic granulosa cells the diameters of which exceeded 400 μm were counted and measured using an ocular micrometer. Previous work has shown that follicles able to ovulate were recruited from a pool of follicles exceeding 400 μm the number of which appeared to be constant from the morning of dioestrus II to the afternoon of pro-oestrus (Buffler 1973; Buffler & Roser 1974). The section showing the largest dimension of each follicle was selected for measuring its diameter in two directions at right angles to each other. The mean of the two measurements was calculated and the mean diameter of the follicles in each animal was estimated. From these data the mean diameter of the follicles was calculated for each group of animals.

**LH and FSH radioimmunoassays**

Blood was allowed to clot overnight at 4°C. All blood samples were centrifuged at 4°C and sera were stored at -25°C until assayed for LH and FSH using previously described radioimmunoassay and labelling procedure (Kerdelhue et al. 1969, 1972, 1973). FSH assay was performed using NIAMDD anti-rat-FSH-S6. The NIAMDD rat FSH-RP-1 (2.1 × NIH-FSH-S6) was used as standard and a purified laboratory rat FSH preparation was used as tracer.
LH assay was performed using an antiserum against ovine LH-β and a laboratory rat LH preparation (1.2 × NIH-LH-S1) as standard and tracer. The sensitivity of LH and FSH was as follows: LH = 0.024 ng in terms of NIH-LH-S1; FSH = 4.2 ng in terms of NIH-FSH-S1. The variability within the assays was 8 and 9% for LH and FSH, respectively. The determinations were made in triplicate and the results averaged.

Statistical procedures
Two-way analysis of variance was performed for each criterion, that is follicular size, number of follicles, and, after logarithmic transformation, LH or FSH blood concentrations. The significance of the effects of TP and of the time of autopsy, on the one hand, and the significance of their interaction, on the other hand, was established. When analysis of the factorial plan showed a significant interaction, Newman-Keuls' method was used for the comparison of the data.

RESULTS
Action of TP on ovulation
Blockade of ovulation was observed in 8 out of the 10 animals injected with 5 mg TP on dioestrus II of 4-day cycles.

Action of TP on follicular growth
As shown in Table 1 follicular size did not differ in controls and TP-treated females (F139 = 0.00). Neither was a difference observed between 14.00 and 17.00 in controls as well as in TP-injected females (F139 = 0.00). No interaction

Table 1.
State of development of ovarian follicles exceeding 400 μm in 4-day cyclic female rats treated with testosterone propionate (TP) on the morning of dioestrus II.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage of sacrifice on pro-oestrus</th>
<th>Mean diameter of follicles</th>
<th>Mean number of follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>14.00</td>
<td>508.79 ± 13.94*</td>
<td>12.40 ± 1.09</td>
</tr>
<tr>
<td></td>
<td>17.00</td>
<td>509.89 ± 12.36</td>
<td>12.70 ± 0.53</td>
</tr>
<tr>
<td>TP</td>
<td>14.00</td>
<td>508.72 ± 6.33</td>
<td>14.60 ± 0.68</td>
</tr>
<tr>
<td></td>
<td>17.00</td>
<td>511.03 ± 6.55</td>
<td>14.30 ± 0.59</td>
</tr>
</tbody>
</table>

* Ten animals per group.
* * Standard error.
Table 2.
Changes in peripheral blood FSH and LH levels in 4-day cyclic female rats injected with 5 mg testosterone propionate (TP) on the morning of dioestrus II.

<table>
<thead>
<tr>
<th>Treatment1)</th>
<th>Controls</th>
<th></th>
<th>TP-treated rats</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSH</td>
<td>LH</td>
<td>FSH</td>
<td>LH</td>
</tr>
<tr>
<td>Blood withdrawal on pro-oestrus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 14.00</td>
<td>190.8 ± 13.62)</td>
<td>0.102 ± 0.0023)</td>
<td>267.9 ± 22.9</td>
<td>0.100 ± 0.000</td>
</tr>
<tr>
<td>at 17.00</td>
<td>360.3 ± 42.1</td>
<td>17.2 ± 3.9</td>
<td>302.5 ± 35.0</td>
<td>0.557 ± 0.2094)</td>
</tr>
</tbody>
</table>

1) Ten animals per group.
2) Mean in ng/ml ± sem (2.1 x NIH.FSH-S1).
3) Mean in ng/ml ± sem (1.2 x NIH.LH-S1).
4) One animal partially escaped the blocking effect of TP.

was noted (F136 = 0.00). A significant increase in the number of follicles exceeding 400 µm was observed in TP-injected females (F136 = 6.22; P < 0.025). No time effects were observed in both groups of animals (F136 = 0.00; interaction: F136 = 0.00).

Action of TP on blood FSH and LH concentrations at pro-oestrus

For both hormones, two-way analysis of variance showed significant interaction (FSH = F136 = 18.37; P < 0.001; LH: F136 = 6.78; P < 0.025). Analysis was then performed by the Newman-Keuls' method.

The results shown in Table 2 indicate that the LH level sharply increased from 14.00 to 17.00 in control females (P < 0.001). The blood LH level also appeared to be significantly higher at 17.00 in controls than in TP-injected females (P < 0.001). A very slight increase in blood LH was observed in TP-injected females between 14.00 and 17.00 (P < 0.05).

It also appears that blood FSH level significantly increased in the controls from 14.00 to 17.00 (P < 0.05). An increase in FSH level was also observed at 14.00 in TP-treated females as compared to control animals (P < 0.05).

Discussion

The present results are in keeping with previous observations which showed that TP was able to suppress ovulation when administered either at dioestrus I or at dioestrus II in 4-day cyclic female rats (Aron & Asch 1963; Aron et al. 1967). They also confirm that the state of follicular development as based on
follicular diameter was not modified in the afternoon of pro-oestrus following TP treatment on the morning of dioestrus II (Aron et al. 1967). Indeed Achille et al. (1971) observed a speeding up in follicular growth in the above mentioned experimental conditions. Our observations did not rule out the possibility that FSH might have influenced the growth of the ovarian follicles. A greater number of follicles exceeding 400 µm was observed at 14.00 and 17.00 on pro-oestrus in TP-treated that in control females. Moreover FSH release was higher at 14.00 at pro-oestrus in the former that in latter. Given that the number of follicles appeared to be increased at that time it would be of interest to determine whether FSH levels were also high before the afternoon of pro-oestrus in TP treated females. However, we are in agreement with Kerdelhue et al. (1977) who described an enhanced FSH release in ovariec
tomized female rats under the influence of testosterone. Inversely the LH surge in the afternoon of pro-oestrus was prevented by the injection of TP. This may account for the suppression of ovulatory phenomena in most of the TP treated females. The very slight increase which occurred from 14.00 to 17.00 in the TP-injected females may be explained by the fact that LH release was not completely blocked at 17.00 in one of the TP-injected females. The fact that the size attained by follicles exceeding 400 µm in the afternoon of pro-oestrus was similar in controls and TP-injected females rendered improbable the hypothesis that the anti-ovulatory effect of TP may be due to follicular refractoriness to LH. Moreover there is no conclusive evidence to our knowledge of a direct effect of androgens on the ovary in experimental animals. Experiments performed in vitro (Labrie et al. 1977) demonstrated that testosterone exerted a direct action on the pituitary gonadotrophic cells by preventing LH release caused by LH-RH. By contrast LH-RH-induced FSH release was not impaired by testosterone. An enhanced FSH secretion was even observed under these circumstances (Labrie et al. 1977). The effects of TP in vivo are in agreement with these observations by showing a blockade of LH release and the maintenance of FSH release by TP. However, the question arises as to whether the pituitary constitutes in vivo a target for the inhibitory effects of TP as it does similar to that in vitro. We have to take into account the possibility that the hypothalamus is involved. Further experiments are needed to clarify this situation.

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REFERENCES


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