MODE OF ACTION OF TESTOSTERONE PROPIONATE ON THE SECRETION AND RELEASE OF LUTEINIZING HORMONE (LH) IN THE CASTRATED RAM

By

Jean Pelletier

ABSTRACT

The mode of action of a single injection of testosterone propionate on LH secretion and release has been studied in the castrated ram. In the first experiment, six castrated rams were injected intramuscularly with 400 mg of testosterone propionate, and the plasma LH levels were assayed by radioimmunoassay: on the first day after treatment, the plasma LH levels decreased significantly ($P < 0.001$), then remained at a very low level for 5–7 days; initial LH levels were restored by day 9. Testosterone propionate thus blocks the discharge of LH.

A second experiment was then performed. Castrated rams in groups of 3 were killed either without treatment (control group D0), or 2, 7 or 9 days after the injection of testosterone propionate (groups D2, D7 and D9). In addition to the plasma LH, hypophyseal LH and hypothalamus LRF (LH releasing factor) were measured: the LRF activity was assessed by the quantity of LH released in vitro from the hypophysis of castrated rats blocked with testosterone propionate. The LH released into the incubation medium and the hypophyseal LH of the ram were measured by the ovarian ascorbic acid method and by radioimmunology.

The results of the plasma LH assays were comparable to those in the first experiment: plasma LH was significantly decreased in group D2, increased in group D7 and was restored in group D9. Hypophyseal levels, on the other hand, increased in groups D2 and D7, but were comparable to the controls in group D9, indicating that the injection of testosterone propionate had little or no effect on LH secretion.

Finally, the LRF activity was considerably reduced in group D2 ($P < 0.001$) as compared with the controls (D0), but similar to the control values in group D9. Although secretion appeared normal, the decreased plasma LH
levels in group D₃ indicated that LRF had not been released; consequently the decrease in LRF observed can be interpreted as being due to an inhibition of its synthesis under the influence of testosterone propionate.

The inhibitory role of testosterone on hypophyseal LH activity has been widely demonstrated: chronic injections of this androgen considerably lower the hypophyseal and plasma LH levels in the castrated rat (Greep & Chester Jones 1950; Paesi et al. 1958; Gans 1959; Ramirez & McCann 1965); under these conditions LH synthesis is reduced. However, Boyd & Johnson (1968) have shown that castrated rats repeatedly injected with testosterone propionate (TP) for one week only, show a concomitant increase in hypophyseal LH and a decrease in plasma LH, indicating that the discharge of LH is blocked earlier than its synthesis.

It thus seems that short-term treatment primarily affects the discharge of LH, while a longer treatment also inhibits its synthesis. The mechanism of this dual action with time, already proposed for oestradiol by Ramirez & McCann (1963) is not at present understood. We decided to examine the question again, studying first the mode of action of a single injection of the androgen. At various times after the administration of TP, we therefore measured hypophyseal and plasma LH in rams that had been castrated, in order to exclude any testicular steroids. We also measured hypothalamic LRF, since it is known that steroid implants into the region of the median eminence or the hypothalamic arcuate nucleus interfere with hypophyseal function (Lisk 1960; Davidson & Sawyer 1961; Chowers & McCann 1963).

In a preliminary experiment, the action of TP on plasma LH was studied in order to select the time intervals after which the animals were to be killed following the steroid injection.

MATERIAL AND METHODS

Experiment I

Six adult rams (Ile-de-France breed), castrated one week previously, were injected im with 400 mg of TP in solution (Steraloids inc.). Blood samples were collected over heparin before the injection and then every day for 9 days; the plasma samples obtained after centrifugation were kept frozen until assayed by the radioimmunological technique described previously (Pelletier et al. 1968). This technique, which has a sensitivity of 0.5–2 ng/ml in plasma, is suitable for quantitative estimations in the ram (Pelletier 1968). The specificity of this method with regard to TSH has been confirmed (Freychet et al., in press). The results are expressed as ng of LH-M₁, a purified ovine hormone with an activity of 1.8 times the standard NIH-LH-S₁ (Jutisz & Courte 1968).

Experiment II

12 adult rams of the same breed comparable in all respects with the previous animals were divided into four groups of 3: D₀, D₂, D₇, and D₉.
The animals in group D₀ were killed without treatment. Groups D₂, D₇ and D₉ were killed 2, 7 and 9 days respectively after the injection of 400 mg of TP. Blood samples were taken from all animals, and the hypophysis and hypothalamus were removed. Measurements of plasma LH were made under the same conditions as in Experiment I.

**Assay of hypophyseal LH**

The hypophyses were crushed in distilled water, then freeze-dried. At the time of assay, the powders were extracted for 3 h in physiological saline. After centrifugation LH was estimated in the supernatant by biological and radioimmunological methods.

- **Biological method.** We used the ovarian ascorbic method (Parlow 1958) adapted to Wistar laboratory rats (Pelletier 1963). Each of the hypophyseal extracts was tested at two dose levels against the hormone NIH-LH-S₈ provided by the American National Institute of Health. The statistical calculations were carried out according to Bliss (1952) and Guillemin & Sakiz (1963).

- **Radioimmunological method.** The technique was the same as that used for plasma, each hypophyseal extract being tested at two dose levels.

**Measurement of hypothalamic LRF activity**

Immediately after killing, the hypothalamic region surrounding the median eminence (size of fragments about 3 × 3 × 2 mm) and the hypophysal stalk of each animal were crushed in 0.1 N HCl (2 ml HCl/hypothalamus). These extracts were kept for 20 min in a boiling water bath, then centrifuged and frozen. The LRF activity was assessed from the quantity of LH released by rat hypophyses in vitro, in the presence of hypothalamic extracts; the extracts were neutralized with NaOH. Control hypophyses were incubated with neutralized 0.1 N HCl only. The doses of hypothalamic extract tested were equivalent to 0.1 and 0.5 of a hypothalamus. The conditions of incubation in Krebs-Ringer solution plus bicarbonate were identical to those described by Jutisz et al. (1966); we have previously shown the validity of this technique for measuring ram LRF (Pelletier & Ortavant 1968). In the present experiment, the hypophyses came from male rats castrated 3 months previously; the rats were injected sc with 15 mg of TP in oil solution 2 days before the incubation.

The LH released into the incubation medium was assayed in one of two ways; in the first the ovarian ascorbic acid technique was used in which the hypophyses were incubated with 0.5 of a hypothalamus and each of the incubation media was tested at two dose levels, using NIH-LH-S₈ hormone as a standard. For the radioimmunological assay, the cross-reaction between rat LH and antiserum against ovine LH was used. The various incubation media were tested at successive dilutions, in order to establish the doses at which the curve for rat LH – anti-ovine LH was identical to the standard curve for sheep LH – anti-ovine LH. All the incubation media were also tested at two dose levels.

All the results were expressed as LH-M₁; those obtained by comparison with the standard NIH-LH-S₈ were divided by the factor 2.25 which expresses the ratio of activities LH-M₁/NIH-LH-S₈.

**RESULTS**

**Experiment I**

Fig. 1 shows that the plasma LH decreases sharply \((P < 0.001)\) on the first
Effect of injection of 400 mg of testosterone propionate on plasma LH level in the castrated ram (mean ± standard error).

day following TP injection. The level remains low for 5 to 7 days, then rises rapidly: on day 8, in particular, the rise in blood LH varies in different animals (Table 1): it occurs between the days 4–8, when a very marked rebound effect is found in some cases, while others show levels still lower than in group D₀. This is reflected by the amplitude of the standard error of the mean.

The periods of time between TP injections and killing of groups D₂, D₇ and D₀ were selected on the basis of these results. Two days after steroid ad-

Table 1.
Individual variations of plasma LH following testosterone propionate injection in rams.

<table>
<thead>
<tr>
<th>Ram number</th>
<th>Days after TP ng LH/ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>134</td>
<td>14.0</td>
</tr>
<tr>
<td>135</td>
<td>4.8</td>
</tr>
<tr>
<td>139</td>
<td>9.4</td>
</tr>
<tr>
<td>156</td>
<td>7.6</td>
</tr>
<tr>
<td>159</td>
<td>11.0</td>
</tr>
<tr>
<td>165</td>
<td>10.2</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>± 1.0</td>
</tr>
</tbody>
</table>

n. d.: not detectable.
ministration the concentration of plasma LH was reduced in all animals, while after 7 days it differed according to the animals. On day 9, the experimental animals were again comparable to controls.

**Experiment II**

Table 2 shows that plasma LH levels were comparable to those in experiment I: 2 days after the injection, the plasma LH was significantly diminished ($P < 0.01$). On day 7, there were differences between the animals: the circulating LH was very low in ram 148, almost normal in ram 156, and normal in ram 159.

**Assay of hypophyseal LH.** It is immediately apparent from Table 3 that the injection of TP leads to a very marked increase in hypophyseal LH levels. The level is already high in group D2, and is maximal in group D7. Nine days after the injection of the androgen, the level has become comparable to that in the hypophyses of the control group D0.

**Influence of testosterone propionate on LRF activity of the hypothalamus.** All the hypothalamic extracts caused a discharge of LH into the incubation medium, that differed significantly from that liberated by the control hypophysis (Table 4). However, the quantity of LH liberated in the presence of hypothalamic extracts from group D2 was significantly less ($P < 0.001$) than that liberated under the influence of the hypothalamic extracts of group D0. This activity was slightly higher for D7 and tended to return to the initial level in the D9 group.

**Study of animals in group D7.** Table 2 shows that the plasma LH levels in group D7 animals were very different: the low level in ram 148 was close to...
Table 3.
Effect of testosterone propionate on hypophyseal LH concentrations in rams 2, 7, 9 days after injection (groups D₂, D₇, D₉).

<table>
<thead>
<tr>
<th>Group</th>
<th>LH concentrations x</th>
<th>Biological assay λ = 0.255</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Radioimmuno-assay</td>
<td></td>
</tr>
<tr>
<td>D₀ (Control)</td>
<td>4.0 ± 0.2</td>
<td>7.75</td>
</tr>
<tr>
<td>D₂</td>
<td>7.2 ± 0.7***</td>
<td>12.4</td>
</tr>
<tr>
<td>D₇</td>
<td>9.3 ± 0.1***</td>
<td>14.7*</td>
</tr>
<tr>
<td>D₉</td>
<td>4.25 ± 0.3</td>
<td>8.8</td>
</tr>
</tbody>
</table>

x μg equivalents of LH-M₁/mg dry weight of anterior hypophyseal tissue. Group hypophyseal weights were identical.

λ = Index of precision.

* P < 0.05 when compared with the control group values.

*** P < 0.001.

Table 4.
Effect of testosterone propionate on LRF hypothalamic activity in rams 2, 7, 9 days after injections (groups D₂, D₇, D₉).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of hypothalamic fraction x</th>
<th>LH released in the incubation medium expressed in terms of the LH standard</th>
<th>Biological assay λ = 0.367</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Radioimmunoassay ng LH/mg hyp./2 h</td>
<td>Biological assay λ = 0.367 ng LH/mg hyp./2 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D₀ (Control)</td>
<td></td>
<td>31 ± 1</td>
<td>79.5</td>
</tr>
<tr>
<td>D₂</td>
<td>0.1</td>
<td>245 ± 7</td>
<td>463</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>339 ± 9</td>
<td>318</td>
</tr>
<tr>
<td>D₇</td>
<td>0.1</td>
<td>136 ± 6***</td>
<td>169 ± 10***</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>228 ± 6***</td>
<td>180 ± 7***</td>
</tr>
<tr>
<td>D₉</td>
<td>0.1</td>
<td>233 ± 5</td>
<td>228 ± 6***</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>251 ± 9***</td>
<td>233 ± 5</td>
</tr>
</tbody>
</table>

x Extract equivalent to 0.1 or 0.5 hypothalamic fragment added into the incubation medium.

*** P < 0.001 when compared with the control group values.

λ = Index of precision.
the levels found in group D₂, while the high level in ram 1959 was more like the value found in group D₉, and ram 156 was intermediate between the two. It was therefore of interest to compare hypothalamic LRF activity and the hypophyseal LH levels in these three animals.

Table 5 shows the results of assays carried out individually by radioimmunoassay; like the animals of group D₂, ram 148 had a high level of hypophyseal LH, but the lowest LRF activity. Conversely, ram 159 had a low level of hypophyseal LH but a high LRF activity; lastly, the hypophyseal LH and hypothalamic LRF levels in ram 156 were intermediate between those of animals 148 and 159.

**DISCUSSION**

The results obtained by radioimmunoassay and bioassay are parallel. However, the absolute values expressed in terms of the standard are always lower in the radioimmunoassay. A possible decrease in activity of the standard NIH-LH-S₈ obtained 5 years ago may account for an over-estimation in the results of bioassay, the figures being calculated from the original theoretical value of the standard.

From the sum of assays, it is possible to consider two aspects of hypothalmo-hypophyseal function – i.e. those of secretion and excretion. While measurement of a hormonal level represents the equilibrium between synthesis and hormonal release, plasma LH levels can be considered as reflecting the rate of excretion of this hormone.

The measurements of plasma LH in Experiment I clearly show that the injection of 400 mg of TP blocks the discharge of LH into the bloodstream for 5–7 days. This blockade, which is evident on the first day, appears quite

<table>
<thead>
<tr>
<th>Group D₇</th>
<th>Plasma LH*</th>
<th>Hypophyseal LH*</th>
<th>Hypothalamic LH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ram Number</td>
<td>ng/ml plasma</td>
<td>µg/mg hyp.</td>
<td>/µg LH released/mg hyp./2 h</td>
</tr>
<tr>
<td>148</td>
<td>3.6 ± 0.4</td>
<td>13.8 ± 0.4</td>
<td>119 ± 3</td>
</tr>
<tr>
<td>156</td>
<td>10.4 ± 0.6</td>
<td>9.8 ± 0.4</td>
<td>211 ± 4</td>
</tr>
<tr>
<td>159</td>
<td>18.0 ± 0.1</td>
<td>7.1 ± 0.4</td>
<td>243 ± 11</td>
</tr>
</tbody>
</table>

* Mean ± SEM.
suddenly; similar results have also been obtained in man by an injection of testosterone propionate (Franchimont 1968).

Plasma LH levels are of the same order in Experiment II, but in this case the determinations of hypophyseal LH clearly indicate that secretion continues, despite the inhibition of excretion. When the excretion of LH is no longer blocked, the level of plasma LH rises and the hypophyseal level becomes comparable to that of controls: this return to the initial level must be related to the increased liberation of LH found on day 8 of the preceding experiment.

Since the demonstration of Moszkowska (1959) and McCann et al. (1960) it has been accepted that the discharge of LH is under the control of hypothalamic LRF; in particular Ramirez & Sawyer (1965) and Chowers & McCann (1965) have shown that when plasma LH increases in the blood of female rats at prooestrus, a diminution of hypothalamic LRF occurs, interpreted as a liberation of this factor.

In our experiments, the concomitant diminution of hypothalamic LRF and plasma LH does not justify the same conclusion. The possibility that TP inhibits the action of LRF on the hypophysis in unacceptable, as it is most unlikely that testosterone would stimulate the discharge of LRF, while at the same time blocking its action at the target organ level.

Similarly, because of the diminution of plasma LH found in group D2, it is not possible to explain the decrease in hypothalamic LRF activity by a negative short feed-back system between LH and LRF, such as proposed in the rat by David et al. (1966) and Corbin & Cohen (1966).

The most likely explanation is therefore that TP inhibits LRF synthesis.

Piacsek & Meites (1966) have also shown that in castrated rats, TP diminishes hypothalamic LRF activity; from our results – particularly the assays of plasma LH – we have been able to elucidate the mode of action of testosterone.

As observed by Meites et al. (1966), these results must be attributed to the action of injected testosterone on the hypothalamus, and not to that of testosterone being fixed by the hypothalamus, and acting on the hypophyses in vitro; under our experimental conditions, the addition of 1 mg of testosterone propionate to the incubation medium did not modify either the spontaneous discharge of LH by the hypophyses in vitro, or the action of a hypothalamic extract on these hypophyses.

To sum up, our experimental results clearly demonstrate that testosterone acts on LH release by inhibiting the synthesis of LRF; they do not, however, explain the way in which testosterone injected over long periods affects LH synthesis, but constitute an essential step in this investigation.

REFERENCES


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