TEMPORAL ASPECTS OF OESTROGEN
STIMULATED INCREASES IN PLASMA AND PITUITARY
FOLLICLE STIMULATING HORMONE

By
Donald C. Johnson

ABSTRACT

Plasma and pituitary follicle stimulating hormone (FSH) were studied in immature female rats treated with oestradiol benzoate (OB), testosterone propionate (TP) or a combination. Plasma FSH changes were evaluated by use of an endogenous augmented ovarian weight assay.

A single dose of 1 µg OB caused an increase in plasma and pituitary FSH within 24 h. Glandular level then fell, as plasma level rose to a peak at 60 h. After 60 h the amount in plasma decreased to a low point at 84 hours and remained unchanged until 156 h. A second dose of OB at 60 h produced an increase in pituitary content, without lowering the plasma level, within 12 h. After this rise, pituitary content decreased rapidly to a low level, but plasma FSH continued to rise to another peak within 48 h. After this peak, plasma content fell to that of controls while pituitary content remained very low. TP (100 µg) in a single dose did not cause any profound changes in pituitary content. When given with 1 µg OB the glandular content remained at control level for 96 h, while plasma content rose a moderate amount. The amount stored decreased at 120 h in animals receiving the double treatment. TP stimulated FSH synthesis in OB treated animals even when given 60 h later, but it had no effect upon plasma FSH in these animals. The results demonstrate that oestrogen has a distinct positive feedback effect upon pituitary FSH and stimulates both synthesis and release of this hormone.

Both oestradiol (Corbin & Daniels 1969) and testosterone (Zarrow et al. 1969) can advance puberty in the immature female rat. Furthermore, a single in-
jection of either steroid can increase the output of follicle stimulating hormone (FSH) from the pituitary (Naqvi & Johnson 1969). Testosterone propionate, but not oestradiol benzoate, appeared to stimulate a cyclic release pattern in FSH, as measured by augmented ovarian weight (Johnson & Naqvi 1969). Pituitary FSH level was not altered by the androgen treatment, but it was greatly reduced three days after a single injection of oestradiol benzoate (Naqvi & Johnson 1969). The question arose: Does oestradiol stimulate FSH synthesis and release, or is the short term increase in output seen in immature females simply the result of an induction of a puberal change in storage level? While oestrogen has often been associated with a positive feedback effect on pituitary luteinizing hormone (reviewed by Mess & Martini 1968) it has not been associated with a positive feedback effect upon FSH. The results of the present experiments clearly demonstrate that oestradiol can increase FSH synthesis and release, regardless of the storage level of hormone in the pituitary.

**MATERIAL AND METHODS**

Female rats of the Holtzman strain were used. They were housed in air-conditioned, and light controlled (lights on from 6 a.m. to 8 p.m. CST) quarters with free access to laboratory chow and tap water. Animals were received in the laboratory when 21 days old. On the following day, large groups (about 100 animals) were injected subcutaneously with 1 µg of oestradiol benzoate, or 100 µg of testosterone propionate, or both hormones. The steroids were dissolved in 0.1 ml of sesame oil. With double treatment, the injection sites were separate.

**FSH Assay**

For evaluating changes in circulating FSH the augmented ovarian weight method was used in an endogenous assay. This has been described in detail previously (Johnson & Naqvi 1969). It involves the injection of 25 IU of human chorionic gonadotrophin (HCG) at various times after the first steroid injection, followed exactly 12 h later by a second injection of 25 IU of HCG, and autopsy, with determination of ovarian weight, exactly 24 h after the second injection of HCG. Since previous work has been shown that more than 24 h is necessary for an ovarian weight response to HCG + FSH, regardless of the dose of FSH, we assume the FSH in the blood at, or near, the time of the first HCG injection accounts for most of the ovarian weight obtained. Therefore ovarian weights were plotted against the time from the steroid injection (time 0) when the first HCG injection was given. For example, the ovarian weight registered at 60 h in Fig. 1 was obtained by injecting animals with HCG 60 h after giving oestrogen, with a second injection of HCG at 72 h and autopsy at 96 h. Controls for this assay consisted of animals treated with oil and given the augmenting doses of HCG at various times. Control ovarian weights over the entire experimental period have been obtained in several previous experiments and only representative times were chosen for checking in the present study. The average ovarian weight for 168 controls (0–144 h) was 32.5 ± 0.09 mg, with no indication of any differences resulting from injections at various times of the day. There is of course a tendency for ovaries to increase in size as the animals get older and thus
augmented ovarian weight at 22 days was found to be 28.1 ± 1.6 mg while at 28 days it was 35.2 ± 2.2 mg.

Pituitary FSH was evaluated using a modified Steelman-Pohley assay (Steelman & Pohley 1953; Johnson & Naqui 1970). Groups of 6 or 7 animals were killed with chloroform at various times after receiving steroid (they did not receive the augmentation doses of HCG). Their pituitaries were homogenized in saline and stored frozen until assayed. At the time of assay they were thawed, and brought to a concentration of 1 pituitary per ml of saline. To this solution was added 50 IU of HCG. Immature (24 day) females were injected with 0.8 ml of this solution at 9 a.m. and with 0.7 ml about 7 h later. Fifty-five hours after the first injection the animals were killed with chloroform and their ovaries weighed on a torsion balance. In order to test the response of the animals in each assay, standard FSH (NIH-FSH-S7, generously supplied by the Endocrinology Study section of the National Institutes of Health) was used to construct a log dose-response curve. The responses to the standards remained remarkably constant in the three assays and gave the following ovarian weights: 0 = 41.7 ± 2.0 mg; 25 µg FSH = 55.3 ± 5 mg; 50 µg FSH = 90.4 ± 4.5 mg; 100 µg FSH = 124.3 ± 4.0 mg.

The ovarian weights obtained with the equivalent of one pituitary were not converted to FSH values because we were interested only in changes in FSH, not absolute amounts. For this reason, all of the groups of a particular series were assayed simultaneously so that the resulting ovarian weights could be compared. The pituitaries were assayed in three consecutive assays, and since the responses for the standards did not differ from assay to assay, the ovarian weights obtained in the various series can be compared with each other.

**RESULTS**

The effect of 1 µg of oestradiol benzoate (OB) on plasma FSH, as measured by ovarian weight changes following augmentation, are shown in Fig. 1. After a small initial increase at 24 h, and a fall at 36 h, there was a steady increase until 60 h. A decline was noted at 72 h with a drastic drop by 84 h; the ovaries were no larger than in controls at 84 h. No significant changes in ovarian weight were recorded between 84 and 156 h after the oestrogen. If a second 1 µg dose of OB was given at the peak of the response (60 h) ovarian weight was not changed 12 h later (72 h, Fig. 1) but by 24 h it was significantly elevated (84 h, Fig. 1) in comparison with animals receiving only a single injection. Ovarian weights increased at 96 h and reached a peak at 108 h (i.e. 48 h after the second injection). After 108 h the weights declined again and reached control level by 144 h (84 h after the second injection). In another series, not shown in Fig. 1, the second injection was delayed until 72 h after the first, and the pattern of response was very similar to that obtained with the second injection at 60 h. The weights were: 84 h = 41.3 ± 3.4; 96 h = 50.5 ± 3.9; 108 h = 83.5 ± 2.1; 120 h = 48.4 ± 3.5; 132 h = 44.6 ± 1.3 mg.

Testosterone propionate (TP) (100 µg) injected 60 h after oestrogen did not
Changes in plasma FSH, as reflected by augmented ovarian weight in immature female rats. All animals were given 1 µg oestradiol benzoate (EB) at 0 time. In some groups another 1 µg was injected at 60 h (EB × EB) and in a third series animals received 100 µg testosterone propionate (TP) at 60 h (EB × TP). Each point is the average of at least 6 animals and the standard error is indicated by the vertical lines. Control ovarian weight averages 32.5 ± 0.09 mg with a range from 28.1 at time 0 to 35.2 at 144 h.

induce any drastic changes in plasma FSH (Fig. 1). The ovarian weights continued to decline 12 and 24 h after the androgen just as they had with the oestrogen alone. There was a tendency for an increase in ovarian weight after 84 h, but this was significantly different from oestrogen alone only at 108 h.

Administration of androgen and oestrogen simultaneously produced a different response than when either hormone was given separately. In the upper part of Fig. 2 the patterns obtained with a single dose of either oestrogen or androgen alone are shown. The data for the latter was taken from previous experiments (Johnson & Naqvi 1969) and is included only to illustrate the pattern obtained with androgen. When the steroids were combined, the large
Plasma FSH changes in animals treated with 1 µg oestradiol benzoate (EB) and 100 µg testosterone propionate (TP). The pattern for each steroid singly is shown in the upper figures, using a different ovarian weight scale and a different time scale. Each point is the average of at least 6 animals with standard errors indicated by vertical lines.

Fig. 2.

 output of FSH found with oestrogen alone was not obtained; maximal ovarian weight was about 50 mg rather than about 70 mg. After an initial increase at 24 h the level of FSH appeared to remain constant until 72 h and then fell at 84 h (Fig. 2). Following this drop there were two distinct peaks at 96 and 120 h with nadirs at 108 and 132 h. A small insignificant rise at 144 h was followed by a considerable drop to control level at 156 h.

Changes in pituitary FSH potency are shown in Fig. 3. Only two values for controls are indicated; at time 0 and 156 h. However, several determinations have been made at intermediate times but they do not show any changes from these values; 128.5 ± 2.9 mg ovaries for 6 groups of 6 animals each. This would correspond closely to the value of 124 µg of FSH/pituitary reported by Kragt & Ganong (1968). In animals given a single dose of oestrogen there was
Pituitary FSH potency expressed as ovarian weight in recipient females injected with the equivalent of one gland. Donors received 1 µg oestradiol benzoate (EB) at time 0, or 1 µg at time 0 and another 1 µg at 60 h (EB × EB 0–60), or 1 µg EB at time 0 and 100 µg testosterone propionate at 60 h (EB × TP 0–60), or 1 µg EB at 0 time as well as 100 µg TP at 0 time (EB × TP 0–0). Control values at the start and end of the experimental period are indicated by squares. Each point is the average of at least 6 animals and standard errors are indicated by vertical lines.

no change in pituitary FSH 12 h later, but by 24 h the amount of stored hormone had increased significantly. This part of the curve was repeated for the double injection series, and the rise at 24 h was again found. Following this initial rise there was a steady fall with a low point reached at 84 h. There was a slight rise at 96 h but by 144 h the level was the same as at 84 h. At 156 h and 168 h pituitary FSH was elevated slightly but still far below control level. If a second injection of OB was given at 60 h there was a distinct significant rise in pituitary FSH 12 h later, but the level quickly fell to a low point at 96 h, remained steady through 108 and 120 h and declined slightly (to lower limit of the assay) at 132 h. There was a rise at 144 h, but the amount of stored hormone was then only about the same as in animals treated with only a single dose of oestrogen.

Pituitary FSH was also altered within 12 h in oestrogen treated animals that received a dose of TP at 60 h (Fig. 3). The pituitary content was in fact the same as in animals receiving a second dose of OB at 60 h. However, glandular
content did not fall as much in androgen treated animals: At 108 h there was a great deal more FSH in the pituitaries of animals that had received androgen, compared with those that had either a single, or a double, dose of oestrogen. However, at 132 and 156 h the stored FSH was about the same as in animals that had not received the androgen, and it was far below control level.

When both oestrogen and androgen were given simultaneously at time zero, pituitary FSH remained at control level until at least 96 h (Fig. 3). There was a distinct drop at 120 h but changes after this time were not studied.

In previous studies we have not been able to demonstrate any changes in pituitary FSH potency despite cyclic changes in plasma FSH following a single injection of testosterone propionate (Naqvi & Johnson 1969). In the present experiment we used 13 groups of 7 animals each, killed between 24 and 168 h of the androgen injection. We chose to study one day in greater detail in order to possibly pick up changes which might occur about the time of a nadir (60 h) or a peak (72 h) in plasma FSH. The results are shown in Table 1.

### Table 1.

Pituitary FSH potency in animals treated with 100 µg of Testosterone Propionate.

<table>
<thead>
<tr>
<th>Time (h) after TP</th>
<th>Ovary wt. (mg) in recipient rat</th>
<th>Time after TP</th>
<th>Ovary wt. of recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>141.3 ± 8.3</td>
<td>78</td>
<td>138.8 ± 18.6</td>
</tr>
<tr>
<td>36</td>
<td>139.1 ± 7.3</td>
<td>84</td>
<td>130.1 ± 10.2</td>
</tr>
<tr>
<td>58</td>
<td>162.3 ±14.8</td>
<td>96</td>
<td>139.2 ± 12.5</td>
</tr>
<tr>
<td>60</td>
<td>151.4 ± 7.8</td>
<td>120</td>
<td>139.7 ± 10.5</td>
</tr>
<tr>
<td>62</td>
<td>172.0 ±13.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>152.3 ± 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>136.4 ± 6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>144.5 ± 9.9</td>
<td>168</td>
<td>147.6 ± 14.3</td>
</tr>
</tbody>
</table>

± standard error; 6 animals per group.

The ovary weights were generally about 140 mg. There was a tendency for the glands removed at a time of low plasma FSH (i.e. 60 h) to have more FSH than those obtained near a peak in plasma FSH (i.e. 72 h). If the values obtained with 58, 60 and 62 h animals were combined and compared with those of 70, 72 and 74 combined, there was a statistically significant difference ($P < .05$). However, there certainly was no profound changes in pituitary FSH concomitant with plasma changes.
DISCUSSION

The rationale for using augmented ovarian weight changes as an indicator for plasma FSH has been extensively discussed before (Johnson & Naqvi 1969; Naqvi & Johnson 1969) and will not be taken up again here. The method obviously does not quantitate plasma FSH in absolute terms, nor can it indicate exactly how long a certain level of hormone has been maintained. However, we are interested in changes in FSH output, and ovarian weight increases of as much as 200% are certainly the consequence of significant increases in FSH. From our experience with the test system, using closely spaced injections, we believe that the time of the output is accurate to within 4–6 h of the time shown in the figures.

The large changes in FSH obtained with a single injection of oestrogen were similar to those reported previously (Johnson & Naqvi 1969) but in the present study the effect was followed for a longer period of time. No significant changes occurred after 84 h. Could the big outpouring, which reached its peak at 6 h, be the result of a massive discharge coincident with a puberal change in storage level? Bradbury (1947) was the first to demonstrate that a single injection of oestradiol benzoate could reduce pituitary gonadotrophin potency within 72 h. He also showed that the ovaries enlarged at this time, and therefore the gonadotrophin appeared to pass into the blood. Using a slightly different design than that presently used, and sampling only every 24 h, we (Naqvi & Johnson 1969) found that the amount of pituitary FSH declined between 48 h and 72 h after a single injection of OB. The present study demonstrated that the early effect of oestrogen was to cause a rise in pituitary FSH, with a concomitant rise in plasma FSH (Figs. 1 and 3, 24 h). Both plasma and pituitary FSH began to fall between 24 and 36 h but the pituitary level continued to decline until 84 h, while the amount in the plasma continued to rise from 36 to at least 60 h, and then fell quickly to a low point at 84 h. This suggests that oestrogen stimulated synthesis and release of FSH, at least initially, but the apparent large outpouring of hormone might be accounted for on the basis of a puberal adjustment in storage level hastened by the oestrogen. To test this further we followed the effects of a second injection of oestrogen. After this injection there was an immediate pituitary response which resulted in a significant increase in the amount of stored hormone 12 h later (72 h, Fig. 3): A duplicate of the effect seen after the first injection except there was no 12 h lag period. Plasma FSH did not fall at this time, the ovarian weight being elevated to the same extent as it was in the single dose animals (Fig. 1), which suggests that release of FSH was not inhibited. Within another 12 h pituitary FSH fell (84 h, Fig. 3) but plasma FSH was higher than in animals treated with only a single dose of oestrogen. The amount of hormone synthesized between 60 and 72 h, and released about 24 h
later may have been responsible for the increased plasma level at 84 h. The amount of hormone in the pituitary decreased a little further over the next 12 h, while the amount in the plasma increased. The large amount of FSH in the circulation at 108 h could not be accounted for by any changes in pituitary content. Therefore we concluded that a good deal of FSH was being synthesized and released from a pituitary which had a very low storage level of hormone.

The time between the first increase in plasma FSH, in the single dose animals (24 h) and the peak level was 48 h and the time between the second injection and the second peak was also 48 h. Moreover, the time between the peaks in pituitary FSH and the peaks in plasma FSH was 36 h. Does this timing have any significance? We attempted to answer this by giving the second injection of oestrogen at 72 h rather than at 60 h. We expected the peak in plasma FSH to shift by 12 h; i.e. to occur at 120 rather than at 108 h. However, this did not happen, and the peak still came at 108 h. Apparently the mechanism responsible for synthesis and release was primed by the first injection and it was able to respond again in 48 h. The second injection somehow triggered the mechanism. This has considerable fundamental importance if oestrogen induces an FSH cyclic mechanism with a period of 48 hours.

A single injection of 100 μg TP causes cyclic increases in plasma FSH (Johnson & Naqvi 1969). The period of the cycles is about 24 h, and the last distinct peak in FSH output occurs at 96 h (see Fig. 3). If a second injection of androgen is given at 48 h the cyclic pattern is continued for another three periods (Johnson & Naqvi, unpublished data). Pituitary FSH does not fall following androgen treatment but is maintained at, or above, control level (Table 1).

When 100 μg TP was injected at 60 h, into animals pre-treated with oestrogen at time zero, plasma FSH was not altered to any extent (Fig. 1). This suggests that the mechanism by which oestrogen stimulates release of FSH differs from the mechanism by which androgen does so: The second injection of oestrogen could trigger the release of a second outpouring of FSH. However, a stimulatory effect of the androgen on pituitary FSH was evident, but certainly the effect was blunted (Fig. 3). Within 12 h of the androgen injection, pituitary FSH was elevated (same level as with a second injection of oestrogen) but even the control level could not be maintained, and by 60 h after the injection (132 h, Fig. 3) pituitary FSH was very low.

When the oestrogen and androgen were given simultaneously, at time zero, the pattern of FSH output did not follow that of either oestrogen or androgen. There was a gradual increase in FSH during the first 24 h and then it remained only moderately elevated until 72 h and then it fell at 84 h (Fig. 2). Oestrogen alone would have produced ovaries weighing about 70 mg by 60 h, but with the double treatment they did not get much above 46 mg. With either oestrogen or androgen alone the plasma responses were over by about 84 h;
the last peak with androgen at 96 h is small. However, the double treatment resulted in two distinct peaks after 84 h; one at 96 and one at 120 h, plus a smaller peak at 144 h. This complex pattern is difficult to interpret, but could mean that neither steroid could dominate during the first three days, and that some sort of average output resulted; oestrogen caused increased output at 60 h, but in androgen treated animals this is a time of a nadir. A peak output in androgen treated animals occurred at 72 h, when the level is falling in the oestrogen treated animals. At 84 h the cycles of both types of animals are in phase at a nadir and this is also seen in the double treatment series. Essentially the full androgenic effect was found after 84 h. The meaning of this is unclear, but this delayed effect was also found in animals treated with progesterone plus testosterone at time zero (Naqvi & Johnson 1970).

Even though there were profound changes in plasma FSH during the first 96 h in the animals receiving both oestrogen and androgen, pituitary FSH did not change. However, it did fall at 120 h, the time of the last peak in plasma FSH activity.

In conclusion the present study has placed added emphasis on the view that plasma changes in gonadotrophin cannot be accurately indicated by the study of pituitary changes. Quite obviously, profound alterations in plasma level occur without any change in the amount of hormone stored. Furthermore, production and release of FSH does not appear to be related to the amount of hormone present in the pituitary.

REFERENCES


Received on April 21st, 1970.