PITUITARY FOLLICLE-STIMULATING HORMONE IN IMMATURE GUINEA-PIGS AND HAMSTERS AND IN FEMALE RATS AFTER NEONATAL TREATMENT WITH TESTOSTERONE

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(Received 13th November 1970)

Summary. The pituitary content of FSH was equivalent to approximately 50 μg NIH-FSH s1/gland in female hamsters aged 26 days and fell to 12 μg/gland by 40 days. Male hamsters had similar levels at 26 days but the hormone content rose to 105 μg/gland by 40 days. This contrast between males and females is similar to that found in rats during sexual maturation. In guinea-pigs, pituitary FSH fell in both males and females from means of 45 and 40 μg/gland at 18 days to 15 and 12 μg/gland at 88 days. Testosterone treatment of female rats on the 5th day of life reduced pituitary FSH to 65% of normal at 28 days of age but caused no significant changes at 22, 25, 34 or 40 days.

INTRODUCTION

Changes in the pituitary content of FSH in laboratory rats during sexual maturation follow a different pattern in males and females. In our colony, the FSH content in females is high at the age of 22 days but falls to about one tenth of this level by 40 days (Fawke & Brown, 1970). The content in males, on the other hand, rises as sexual maturation approaches (Pearce & Brown, 1970). These changes result in a strikingly higher pituitary FSH content in mature males than in mature females. These differences, and possibly the patterns of change that lead to them, may result from the difference in the steroids controlling FSH secretion, oestrogen but not androgen being potent in suppressing synthesis of the hormone. Before accepting such a hypothesis, it seemed advisable to examine other rodent species to see if their pituitary FSH content changes in the same way as in laboratory rats, and hence to see whether the changes found in the rat represent a general pattern. The guinea-pig, Cavia porcellus, and the golden hamster, Mesocricetus auratus, were chosen. Female rats treated with testosterone shortly after birth were also studied to see if this type of masculinization altered the levels of pituitary FSH.

MATERIALS AND METHODS

Porton rats were derived from our own colony and managed as described...
previously (Fawke & Brown, 1970). Hamsters were obtained from M. Buckley (Hadfields-via-Hyde). They were either sent by the dealer when 24 days old for use at the age of 26 days and older, or were bred in this Department using stock from the same source, weaned at 21 days and used when 22 or 26 days old. They were housed and managed in the same way as the rats. Albino guinea-pigs were obtained from A. Tuck (Rayleigh) and sent by the dealer when 16 days old. They were maintained on diet SG1 with additional fresh hay and ascorbic acid.

Animals were killed with chloroform at the ages shown in the tables and their anterior pituitary tissue was homogenized in 0.9 % NaCl solution and frozen at −20 °C until assay. Male genital organs were weighed after fixation in formol-saline. Pituitary homogenates were assayed for fSH by a modification of the method of Steelman & Pohley (1953), based on the findings of Parlow & Reichert (1963). Details of the method of storage and assay have been described previously (Fawke & Brown, 1970). Ovine NIH.fSH s6 and s8 were used as standards but results are expressed in terms of NIH.fSH s1. All assays were of at least (2+2) design unless otherwise indicated in the tables and, in all cases, parallelism was entirely satisfactory. Pituitary glands of males and females of the same age, or of androgen-treated and control rats of the same age, were assayed in the same multiple assays.

Female rats selected for androgen treatment were injected subcutaneously on the 5th day of life with 1.25 mg testosterone propionate (TP) in arachis oil containing 10 % benzyl alcohol. In the first experiment, all the females in alternate litters were injected with TP, the remaining litters serving as untreated controls. In a second experiment, the female rats of each litter were split into two groups, one treated with TP and one with arachis oil containing 10 % benzyl alcohol. The rats were then grouped separately according to treatment and returned to foster mothers in all-female groups of ten.

RESULTS

Hamsters

The results are summarized in Tables 1 and 2. At 26 days of age, the body weight and pituitary fSH content of females obtained directly from the dealer were similar to those of females bred in the Department, but the ovaries and uteri of the former were significantly heavier. In males aged 26 days, the body weight and pituitary fSH level were similar in animals from the two sources, though here again gonadal weight was significantly greater in the dealer’s animals. However, in view of the similarity of fSH levels, it seems reasonable to consider results from the two sets of animals as comparable for the present purpose.

Considered in this way, the results show a rise in pituitary fSH content in the males as sexual maturation is approached and a fall in content in the females over the same period.

Guinea-pigs

The results are summarized in Table 3. In both males and females, there was an overall fall in pituitary fSH content as sexual maturity approached.
Androgen-treated female rats

In the first experiment (Table 4), androgen treatment produced the expected reduction in ovarian weight but the only statistically significant change in pituitary FSH content was at 28 days of age. This change was a reduction to 66% of the control level but because there was no consistent reduction at other ages, it was thought that this might be a chance finding. A second experiment was performed in which glands from androgen-treated rats were assayed against those of litter-mates treated with the vehicle. At 25 days of age, the glands of treated rats were 84% (95% fiducial limits: 54 to 133%) as potent as those of controls in the assay of FSH. At 28 days of age, the glands of androgen-treated rats were 64% (45 to 92%) as potent as controls. This confirms the result of the first experiment.

**Table 1**

MEAN BODY AND ORGAN WEIGHTS AND PITUITARY FSH (NIH.FSH 51) CONTENT IN FEMALE HAMSTERS

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Hamsters bred in Department</th>
<th>Hamsters obtained from dealer</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>26</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Body weight (g)*</td>
<td>±6.22 ± 7.66</td>
<td>±5.10 ± 5.04</td>
</tr>
<tr>
<td>Combined ovarian weight (mg)*</td>
<td>6.98 11.4</td>
<td>15.5 20.7</td>
</tr>
<tr>
<td>Uterine weight (mg)*</td>
<td>±1.31 ± 3.02</td>
<td>±3.22 ± 4.25</td>
</tr>
<tr>
<td>FSH/gland (µg) (Fiducial limits, P = 0.95)</td>
<td>38 (27 to 54)</td>
<td>53 (38 to 74)</td>
</tr>
</tbody>
</table>

* Mean and standard deviation.
† Result of a (1+2) assay: all other assays of at least (2+2) design.

**Table 2**

MEAN BODY AND ORGAN WEIGHTS AND PITUITARY FSH (NIH.FSH 51) CONTENT IN MALE HAMSTERS

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Hamsters bred in Department</th>
<th>Hamsters obtained from dealer</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>16</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Body weight (g)*</td>
<td>±6.48 ± 6.60</td>
<td>±3.90 ± 5.63</td>
</tr>
<tr>
<td>Right testis (mg)*</td>
<td>85.4 144</td>
<td>184 356</td>
</tr>
<tr>
<td>Seminal vesicles† (mg)*</td>
<td>9.63 25.1</td>
<td>29.9 67.8</td>
</tr>
<tr>
<td>FSH/gland (µg) (Fiducial limits, P = 0.95)</td>
<td>17 (12 to 25)</td>
<td>54 (38 to 75)</td>
</tr>
</tbody>
</table>

* Mean and standard deviation.
† With coagulating glands.
DISCUSSION

In female rats between the ages of about 20 and 40 days, there is a striking decline in pituitary FSH content at a time when ovarian weight is rising and sexual maturation occurs (Kragt & Ganong, 1968a; Fawke & Brown, 1970). In both the hamster and the guinea-pig, there was a slight rise in pituitary content after weaning but this was followed by a substantial decline in FSH content while ovarian growth was occurring. The females of both these species, therefore, appear to show a similar pattern to that in the rat, though there are differences both in the absolute levels of FSH and in their rates of decline. In all three of these rodent species, the secretion of FSH which contributes to sexual maturation of the female seems to occur at a rate which exhausts the stores and outrips the synthetic activity of the gland. The pituitary FSH levels found in the oldest group of hamsters are a little lower than those found by Keever & Greenwald (1967) in mature hamsters; the levels found in the oldest guinea-pigs are slightly lower than those found by d'Angelo (1966) in adult females.

### Table 3

Mean Body and Organ Weights and Pituitary FSH (NIH.FSH SI) Content in Guinea-Pigs of Various Ages

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>18</td>
<td>34</td>
<td>50</td>
<td>66</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)*</td>
<td>182</td>
<td>±16-3</td>
<td>261</td>
<td>±17-9</td>
<td>328</td>
<td>±14-8</td>
</tr>
<tr>
<td>Anterior pituitary (mg)*</td>
<td>4-82</td>
<td>±0-52</td>
<td>5-89</td>
<td>±1-01</td>
<td>6-50</td>
<td>±38-0</td>
</tr>
<tr>
<td>Combined ovaries (mg)*</td>
<td>27-4</td>
<td>±10-3</td>
<td>60-8</td>
<td>±14-4</td>
<td>62-9</td>
<td>±0-98</td>
</tr>
<tr>
<td>Uterus (mg)*</td>
<td>102</td>
<td>±18-0</td>
<td>138</td>
<td>±38-7</td>
<td>480</td>
<td>±40-0</td>
</tr>
<tr>
<td>Pituitary FSH (µg)</td>
<td>46</td>
<td>±4-3</td>
<td>53</td>
<td>±9-80</td>
<td>25</td>
<td>±16-3</td>
</tr>
<tr>
<td>(Fiducial limits, P = 0-95)</td>
<td>(32 to 67)</td>
<td>(32 to 89)</td>
<td>(15 to 40)</td>
<td>(15 to 35)</td>
<td>(8-4 to 22)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>18</td>
<td>34</td>
<td>50</td>
<td>66</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)*</td>
<td>186</td>
<td>±9-80</td>
<td>267</td>
<td>±21-2</td>
<td>362</td>
<td>±30-2</td>
</tr>
<tr>
<td>Anterior pituitary (mg)*</td>
<td>4-85</td>
<td>±0-72</td>
<td>6-73</td>
<td>±0-98</td>
<td>7-23</td>
<td>±1-58</td>
</tr>
<tr>
<td>Right testis (mg)*</td>
<td>77-9</td>
<td>±17-0</td>
<td>194</td>
<td>±69-5</td>
<td>430</td>
<td>±95-2</td>
</tr>
<tr>
<td>Seminal vesicles (mg)*</td>
<td>55-5</td>
<td>±13-0</td>
<td>130</td>
<td>±92-0</td>
<td>508</td>
<td>±305</td>
</tr>
<tr>
<td>Pituitary FSH (µg)</td>
<td>52</td>
<td>±9-50</td>
<td>33</td>
<td>±11-5</td>
<td>25</td>
<td>±11-5</td>
</tr>
<tr>
<td>(Fiducial limits, P = 0-95)</td>
<td>(36 to 76)</td>
<td>(21 to 53)</td>
<td>(16 to 40)</td>
<td>(6-3 to 17)</td>
<td>(11 to 29)</td>
<td></td>
</tr>
</tbody>
</table>

* Mean and standard deviation.
† Result of a (1+2) assay; all other assays of at least (2+2) design.
The pituitary FSH content of male rats in our colony rises continuously until at least 70 days of age (Pearce & Brown, 1970) and during this time, steady growth of the testis occurs. The FSH levels found by Kragt & Ganong (1968b) and Labhsetwar (1970) differ from our values in some respects, such as the timing of the changes and the absolute levels, but agree to the extent that major increases in pituitary FSH content occur during rapid testicular growth. In the male hamster, the general pattern of a rising FSH content was found to occur during maturation.

### Table 4

**Mean body and organ weights and pituitary FSH (NIH.FSH s1) content in female rats treated with testosterone compared with those of untreated rats**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Body wt (g)*</th>
<th>Anterior pituitary (mg)*</th>
<th>Paired ovaries (mg)*</th>
<th>Uterus (mg)*</th>
<th>Pituitary FSH content (µg/gland)</th>
<th>Pituitary FSH content of TP-treated rats as % of control (Fiducial limits, P = 0.95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>TP</td>
<td>16</td>
<td>48.4 ± 1.55</td>
<td>1.88 ± 0.11</td>
<td>23.5 ± 0.57</td>
<td>23.5 ± 0.57</td>
<td>227 ± 1.61</td>
<td>(161 to 325)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>46.5 ± 2.39</td>
<td>1.42 ± 0.15</td>
<td>13.1 ± 1.84</td>
<td>30.6 ± 1.84</td>
<td>276 ± 1.95</td>
<td>(195 to 390)</td>
</tr>
<tr>
<td>28</td>
<td>TP</td>
<td>18</td>
<td>56.0 ± 3.15</td>
<td>1.75 ± 0.16</td>
<td>9.41 ± 0.71</td>
<td>29.5 ± 5.36</td>
<td>124 ± 1.16</td>
<td>(91 to 168)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>57.1 ± 2.77</td>
<td>2.29 ± 0.12</td>
<td>17.1 ± 1.16</td>
<td>34.6 ± 2.93</td>
<td>188 ± 1.23</td>
<td>(132 to 265)</td>
</tr>
<tr>
<td>34</td>
<td>TP</td>
<td>20</td>
<td>72.8 ± 3.84</td>
<td>2.56 ± 0.21</td>
<td>17.0 ± 1.20</td>
<td>70.7 ± 12.0</td>
<td>116 ± 1.12</td>
<td>(74 to 180)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>16</td>
<td>75.6 ± 4.05</td>
<td>2.43 ± 0.14</td>
<td>19.3 ± 1.26</td>
<td>57.6 ± 9.80</td>
<td>124 ± 1.12</td>
<td>(80 to 192)</td>
</tr>
<tr>
<td>40</td>
<td>TP</td>
<td>20</td>
<td>110 ± 3.15 3</td>
<td>4.66 ± 0.28</td>
<td>26.8 ± 2.11</td>
<td>197 ± 10.5</td>
<td>53 ± 1.03</td>
<td>(33 to 87)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>16</td>
<td>111 ± 3.40 3</td>
<td>5.11 ± 0.21</td>
<td>36.2 ± 2.80</td>
<td>166 ± 10.6</td>
<td>43 ± 1.03</td>
<td>(26 to 69)</td>
</tr>
</tbody>
</table>

The results of a further experiment of similar nature are reported in the text.

* Mean ± standard error.

as in the rat, though again the absolute levels and the extent of the increase differ in the two species. Here it seems that the increase in FSH secretion contributing to sexual maturation in the male is exceeded by the synthetic activity of the gland. In the guinea-pig, however, pituitary FSH was found to decline during gonadal growth in much the same way as it did in the female of that species. The limited observations recorded here prevent generalization about the balance of FSH synthesis and secretion during sexual maturation in male rodents.

Treatment of female rats with TP on the 5th day of life did not cause a gross alteration in the pituitary FSH content. There was, however, a repeatable and
statistically significant reduction in content at the age of 28 days. The physiological significance of this reduction is not clear and at other ages studied, the levels did not differ markedly from the normal. There was nothing to suggest that the levels in the masculinized females tended towards the male pattern.

Pituitary homogenates from rats, hamsters and guinea-pigs all gave valid assays against ovine FSH: results from two homogenates were used at only one dose-level because the material was of low potency. In general, these results confirm the finding of Parlow & Reichert (1963) and Reichert (1967) that preparations of rFSH from various species give log dose-response lines of similar slope in the assay of Steelman & Pohley (1953) as long as the test material is injected at least twice daily. It should be noted, however, that routine assays of the type used in the present investigation do not constitute a very rigorous test of the identity of dose-response lines. Greenwald, Keever & Grady (1967) also report valid (2 + 2) assays of hamster pituitaries in terms of ovine FSH in this assay and the results of d’Angelo (1966) imply parallel log dose-response lines for homogenates of guinea-pig pituitaries and ovine FSH.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Population Council and the Smith Kline and French Foundation. I am grateful to the National Institutes of Health for supplying FSH standards and to Mr A. C. Wyatt, Mr L. G. Cowley and Mr M. Pearce for breeding and managing the animals.

REFERENCES


