Effects of in-vivo administration of GnRH on the release of gonadotrophins in the female rabbit

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Summary. Developing female rabbits were studied weekly from Day 22 of life to Day 100. At all ages GnRH (1.5 μg/kg) induced a large increase in LH release 15 min later. By contrast, FSH was significantly increased only on Days 22, 29 and 72 and no significant increase was detected up to 2 h after GnRH administration at other ages. Functional corpora lutea were absent at the start of all treatments as indicated by circulating concentrations of progesterone <2 ng/ml. It is concluded that the immature rabbit pituitary is functionally capable of responding to GnRH with an increase in LH secretion, whereas the control of FSH secretion may be regulated by other factors.

Keywords: LH; FSH; GnRH; age-dependent changes

Introduction

As part of our continuing studies to elucidate the mechanisms by which female rabbits become sexually mature, we have observed that there is a large increase in circulating gonadotrophin concentrations between Days 30 and 50 of life (YoungLai, 1986), which is several weeks before the rabbit becomes sexually receptive (Hulot et al., 1982; DeTurckheim et al., 1983; Kamwanja & Hauser, 1983). A similar elevation in gonadotrophin concentrations is seen in the prepubertal female rat before the onset of puberty (Ramaley, 1979). This increase in rabbit gonadotrophins was associated with maximum opiate binding in the hypothalamus (Wilkinson & YoungLai, 1986).

In spontaneously ovulating animals such as the rat (Ojeda & Urbanski, 1988), monkey and human (Plant, 1988) a rise in LH pulse frequency is associated with puberty and this seems to be related to a self-priming effect of previous GnRH stimulation. The distribution of GnRH neurones in the rabbit hypothalamus is very diffuse with 5 major fields of activity compared to the rat in which only a dual GnRH pulse generating and surge-generating system seems to exist (Ramirez & Beyer, 1988). It is therefore possible that differences in pituitary control mechanisms occur between spontaneously ovulating species and the rabbit, a reflex ovulator. The objective of this study was to determine the in-vivo response of the maturing female rabbit pituitary to maximum stimulation with GnRH.

Materials and Methods

New Zealand White rabbits were obtained from local breeders and kept in individual cages on a 12-h light:12-h dark schedule (lights on 07:30 h E.S.T.) with food and water available ad libitum.

Female rabbits, 21 days of age, were purchased. On Day 22, 6 rabbits (body weight 350 ± 15 g) were bled via the middle ear artery (1.5 ml) and injected s.c. with GnRH (Factrel: Ayerst Laboratories, Montreal, Canada), 1.5 μg/kg, and 15 min later a 1.5-ml blood sample was taken. For this age group only 2 blood samples were taken to avoid the stress of multiple sampling. On Day 29, the other 6 rabbits (body weight 519 ± 30 g) were treated in an identical manner but two additional blood samples were taken at 30 and 45 min. On all subsequent days blood samples were taken at 0, 15, 30, 45, 60 and 120 min. Plasma was stored at −15°C until assayed. At least 2 weeks elapsed before each
group of rabbits was treated again. This method of blood sampling allowed a weekly analysis to be performed. This study was completed during the months of March, April and May.

Radioimmunoassays. LH and FSH were measured by radioimmunoassays using established techniques (Moor & YoungLai, 1975; Armstrong et al., 1978). The LH standard used was WP 360A (Dr A. F. Parlow), 1 ng of which was equivalent to 30 pg pure rabbit pituitary LH (EX 130 GB, Dr L. E. Reichert) and guinea-pig anti-rabbit LH 7F GPaLH (Dr R. Scaramuzzi). The antigen used for iodination was LER-1056-C2 (Dr L. E. Reichert). All LH results are expressed in terms of the pure pituitary LH standard. The sensitivity of the assay was 42 pg with intra- and inter-assay coefficients of variation of 4-8% and 18% respectively.

Reagents for the assay of FSH were provided by Dr A. F. Parlow. The antigen AFP-9688-C was used for iodination and standards. The antibody used, AFP-4-7-21-76, was prepared in guinea-pigs. The sensitivity of the assay was 80 pg with intra- and inter-assay coefficients of variation of 13.2% and 21% respectively.

To ensure that the GnRH administered to rabbits younger than 72 days did not induce ovulation, samples of plasma were analysed for progesterone by radioimmunoassay (YoungLai, 1985). Concentrations of <2 ng/ml were taken as presumptive evidence for the absence of functional corpora lutea.

Statistics. Results were analysed by Student's paired t test to detect changes 15 min after GnRH administration. Values obtained after 15 min were not used since they had already started declining by 30 min. Analysis of variance and Duncan's multiple range test were also used. A P value of 0.05 was considered significant.

Results

As shown in Table 1, the profile of LH concentrations at zero time indicates that there was a significant increase in basal LH concentrations on Days 43, 50 and 58 while basal FSH values on Days 22–72 were significantly higher than those on Day 100. The administration of GnRH led to a prompt and significant increase in LH concentrations at all ages studied. While this increase was most dramatic at 15 min, by 30 min levels had already started to decline in rabbits aged 29–72 days (Table 1). At 86 and 100 days, there was a more sustained increase in LH concentrations for 2 h. By contrast, analysis of variance for FSH values indicated that only on Days 22, 29 and 72 were there significant interactions among the mean values before and after GnRH administration.

Table 2 shows that there was a normal weight gain as the animals matured. Analysis of variance indicated that all weight increases were significantly different from each other except between 86 and 100 days. The net changes in LH 15 min after GnRH administration were also significantly different by analysis of variance. Duncan's multiple range test indicated that the net LH change induced by GnRH at 100 days was significantly greater than those at 86, 43, 50, 22 and 29 days of age. On the other hand, the greatest net changes in FSH induced by GnRH were on Days 22, 29 and 72. Progesterone values in all animals before GnRH injection were <2 ng/ml.

Discussion

The results of the present investigation indicate that the rabbit pituitary is capable of responding to GnRH with an increase in LH secretion at all ages studied from Day 22 to Day 100. This suggests that the hypothalamo–pituitary axis is already functional at an early age. The net change in the LH response to GnRH was not significantly different among the various age groups except on Day 100 when the high and sustained LH response would be expected to cause ovulation. Since these animals were not examined later, this could not be confirmed. In previous studies (Armstrong et al., 1978) it was found that an LH value of >20 ng/ml invariably was associated with an ovulatory response. By contrast, a single injection of GnRH to female rabbits at 12 weeks of age failed to induce ovulations although ovulatory increases in LH, i.e. concentrations >20 ng/ml were observed in all animals (YoungLai, 1985). It is therefore unlikely that GnRH injections 2 weeks apart would have induced ovulations in any of the rabbits younger than 86 days old. Moreover, mature antral follicles are not present in these younger rabbits (Erickson et al., 1974). Further evidence for this conclusion is seen in the progesterone data (Table 2) for which all values were <2 ng/ml, indicating that GnRH had not induced ovulation in these rabbits. These results also
Table 1. Changes in peripheral gonadotrophin concentrations in female rabbits after a single s.c. injection of GnRH

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Time (min)</th>
<th>LH*</th>
<th>FSH*</th>
<th>LH*</th>
<th>FSH</th>
<th>LH*</th>
<th>FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>0-60 ± 0.01</td>
<td>8.20 ± 1.17</td>
<td>3.42 ± 0.30</td>
<td>7.67 ± 0.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>0-60 ± 0.01</td>
<td>6.72 ± 0.80</td>
<td>4.03 ± 0.41</td>
<td>2.42 ± 0.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>0-60 ± 0.01</td>
<td>9.13 ± 0.98</td>
<td>6.73 ± 0.73</td>
<td>4.92 ± 0.51</td>
<td>3.08 ± 0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>1.25 ± 0.23</td>
<td>10.10 ± 1.52</td>
<td>6.92 ± 0.75</td>
<td>5.45 ± 0.30</td>
<td>3.32 ± 0.51</td>
<td>2.00 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>1.62 ± 0.29</td>
<td>8.16 ± 1.82</td>
<td>4.96 ± 0.99</td>
<td>4.32 ± 0.72</td>
<td>3.24 ± 0.34</td>
<td>2.80 ± 0.67</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>1.74 ± 0.80</td>
<td>18.88 ± 6.62</td>
<td>15.05 ± 3.29</td>
<td>9.98 ± 2.23</td>
<td>6.92 ± 1.14</td>
<td>2.37 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>0.75 ± 0.15</td>
<td>17.82 ± 3.87</td>
<td>14.78 ± 1.35</td>
<td>11.83 ± 1.59</td>
<td>9.53 ± 0.76</td>
<td>3.47 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>0.60 ± 0.01</td>
<td>10.90 ± 2.73</td>
<td>12.50 ± 2.65</td>
<td>10.82 ± 1.99</td>
<td>13.40 ± 2.24</td>
<td>10.78 ± 3.12</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.68 ± 0.08</td>
<td>25.70 ± 6.67</td>
<td>30.10 ± 4.70</td>
<td>23.30 ± 3.87</td>
<td>24.48 ± 4.46</td>
<td>21.08 ± 6.38</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for 6 rabbits.
*The means of these values for different times were significant (P < 0.05) by one-way analysis of variance.

Table 2. Changes in body weight and mean differences in gonadotrophin concentrations before and 15 min after GnRH administration and progesterone concentrations before GnRH administration

<table>
<thead>
<tr>
<th>Days of age</th>
<th>Weight (g)</th>
<th>ΔLH (ng/ml)</th>
<th>ΔFSH (ng/ml)</th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>350 ± 15</td>
<td>8.17 ± 1.17</td>
<td>4.33 ± 0.75</td>
<td>1.03 ± 0.15</td>
</tr>
<tr>
<td>29</td>
<td>519 ± 30</td>
<td>6.25 ± 0.78</td>
<td>4.08 ± 1.22</td>
<td>0.51 ± 0.11</td>
</tr>
<tr>
<td>36</td>
<td>815 ± 37</td>
<td>9.58 ± 1.00</td>
<td>0.92 ± 0.44</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>43</td>
<td>1189 ± 49</td>
<td>9.00 ± 1.53</td>
<td>2.08 ± 1.67</td>
<td>0.90 ± 0.13</td>
</tr>
<tr>
<td>50</td>
<td>1536 ± 42</td>
<td>7.75 ± 1.57</td>
<td>-1.30 ± 1.45</td>
<td>1.07 ± 0.17</td>
</tr>
<tr>
<td>58</td>
<td>1866 ± 40</td>
<td>17.08 ± 5.64</td>
<td>2.83 ± 1.39</td>
<td>1.02 ± 0.13</td>
</tr>
<tr>
<td>72</td>
<td>2250 ± 127</td>
<td>17.42 ± 3.79</td>
<td>2.92 ± 0.40</td>
<td>0.95 ± 0.21</td>
</tr>
<tr>
<td>86</td>
<td>2770 ± 73</td>
<td>9.17 ± 2.72</td>
<td>0.75 ± 0.17</td>
<td>1.21 ± 0.13</td>
</tr>
<tr>
<td>100</td>
<td>3065 ± 132</td>
<td>21.25 ± 6.70</td>
<td>0.83 ± 0.38</td>
<td>1.48 ± 0.14</td>
</tr>
</tbody>
</table>

The net changes in LH values induced by GnRH were significantly different from 0 values by Student’s paired t test. For the corresponding FSH changes, significant differences were only found on Days 22, 29 and 72.

suggest that the 2-week interval between GnRH injections did not have any self-priming effect on advancing follicular maturation.

It was surprising that an increase in sensitivity to GnRH was not found as the animals matured. It is possible that the interval of testing was too long to detect such a change. However, the sustained elevations in LH for 2 h in rabbits at 86 and 100 days of age suggest that, at these ages,
the pituitary responds differently to GnRH than it does at earlier ages. With an in-vitro system, isolated rat pituitaries were shown to be more sensitive to GnRH between 20 and 30 days of age with puberty being about Day 35 (Wilkinson & Moger, 1981). The LH response of isolated and superfused pituitaries to GnRH was also greatest on Days 15 and 20 (Naish et al., 1986). The decline in sensitivity of the pituitary to GnRH was interpreted as the first step in the initiation of sexual maturation. Although we observed a decrease in the net change in LH following GnRH administration in vivo on Day 86 (Table 1), this was not statistically significantly different from Days 58 and 72 (Duncans’ multiple range test).

The dose of 1.5 μg GnRH/kg per rabbit used in our in-vivo studies is comparable to the 1.7 μg/kg dose used by Caillol et al. (1986) to show a seasonal variation in the LH response to GnRH in the brown hare but much lower than the 0.5 μg/100 g dose used by Debeljuk et al. (1972) to show a response in the rat. Kanematsu et al. (1974) have previously demonstrated that 250 ng/kg was the minimal ovulating dose of GnRH required to induce an LH peak in rabbits. In the mature domestic rabbit there is a definite seasonal variation in the activity of the GnRH pulse generator (Ramirez et al., 1986) and progesterone can influence its activity if given in a pulsatile manner (Lin & Ramirez, 1988). Although significant differences in endogenous progesterone concentrations were found among the various age groups, these could not be correlated with variations in LH response to GnRH.

A more surprising finding was the age-related differential FSH response to GnRH (Table 1). At all age groups except Days 22, 29 and 72, GnRH failed to induce a 15-min increase in FSH secretion. The differences in FSH response to GnRH cannot be explained by changes in route of GnRH administration since all animals received a single bolus injection. Although the parallel increase in basal values of LH and FSH suggest co-ordinate regulation, the larger increase in circulating FSH concentrations between Days 36 and 58 with an LH:FSH ratio of approximately 0.1 compared to a ratio of > 5 in coitus- (YoungLai & Armstrong, 1981) or GnRH- (YoungLai, 1985) induced LH surges suggest that these high levels are probably not GnRH-mediated unless the high values are due to infrequent, randomly occurring synchronization of low-frequency GnRH pulses, as postulated by Ojeda & Urbanski (1988) for the rat. Support for the non-involvement of GnRH is seen in the study of Mills et al. (1983) in which the post-ovulatory peak in FSH secretion after coitus in the rabbit could not be prevented with a GnRH antagonist. It remains to be determined whether these increased FSH concentrations are functional.

Another possibility to account for the selective increase in FSH is maturation of the pituitary–gonadal axis with the secretion of an FSH-releasing factor from the ovary. Evidence for the presence of such a factor has been obtained (Ling et al., 1986; Vale et al., 1986). As the ovary produces more mature follicles they could produce inhibin as well as 3α-hydroxy pregn-4-en-20-one to inhibit FSH secretion selectively at maturity (Wiebe & Wood, 1987). An ovarian peptide called ‘maturin’ and associated with sexual maturity may also be involved in controlling ovarian function (Washenik & Dunbar, 1988).

It is also possible that the developing female rabbit pituitary undergoes changes in cell composition such that monohormonal cells producing FSH dominate during the period from Day 35 to 60 of life. Evidence for the existence of such cells, as well as monohormonal cells producing LH and multihormonal cells producing both LH and FSH, has been provided for rats (Lloyd & Childs, 1988) and could be tested in rabbits.

In summary, our results suggest that there is no decrease in pituitary sensitivity to GnRH before sexual maturation and that FSH and LH are differentially regulated in the immature female rabbit.

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References


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