Pulsatile GnRH treatment of the ovariectomized rat and release of LH and FSH

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Abstract. The effect of pulsatile GnRH administration on the levels of LH and FSH was investigated in rats that had been ovariectomized 2 weeks earlier. Also the asynchronous occurrence of endogenous and GnRH-induced LH and FSH pulses was analysed. A small pulse dose of GnRH (1.25 ng/100 g) was given iv at a frequency of once every 60 min or once every 120 min during 24 h. A larger dose of 5 ng/100 g was given once every 60 or 120 min during either 24 h or 96 h. Blood was sampled arterially every 5 min around the two first and last GnRH injections and LH and FSH were measured. Only the treatment with the larger GnRH pulse dose resulted in a change of LH and FSH plasma levels. LH levels declined under all circumstances, whereas FSH was found to be increased temporarily after 24 h of treatment. The pituitary LH response to pulses of GnRH (5 ng/100 g) decreased irrespective of the frequency or duration with which GnRH was administered. There was a marked asynchronicity between LH and FSH pulses and almost every injection of GnRH (5 ng/100 g) resulted in clear LH pulses but not in FSH pulses.

GnRH injection was given within 15 min after a preceding endogenous LH pulse.

Since the response to GnRH was reduced on the last day of the experiment, the effect of a lower dose (1.5 ng/100 g body weight) has now been investigated, too. Moreover, effects of both doses of GnRH on FSH were included. It was also studied to what extent endogenous and GnRH-induced pulses of LH and FSH were synchronous.

Materials and Methods

Experimental animals and cannulation
OVX Wistar rats (14 days after ovariectomy) weighing approximately 200 g and derived from the inbred laboratory colony were used. They were kept at controlled lighting (14 h light and 10 h darkness) and temperature (22°C). At the beginning of each experiment the animals were anesthetized with ether and supplied with two cannulas: one was inserted into the left carotid artery for taking blood samples and the other was placed into the right external jugular vein for administration of GnRH. The two cannulas were led outside the cage in such a way that both administration of GnRH and sampling of blood could take place without disturbing the animals.

GnRH
GnRH (Beckman, lot No. B10321) was dissolved in saline containing 3 kIU of heparin/l. A pulse was given...
by iv injecting 0.1 ml of the solution during a period of 2 min. An electronically timed Braun-Melsungen pump (Tübingen, West-Germany) was used for this purpose.

**Treatment groups**

The experiment included six groups of animals which received pulsatile GnRH treatment varying in pulse dose, and/or pulse frequency and/or duration of treatment.

A small pulse dose of GnRH, 1.25 ng/100 g, was given at a frequency of once every 60 min (N = 5) or once every 120 min (N = 5) during 24 h. When this pulse dose is given to phenobarbital-blocked OVX rats, LH pulses are induced which are of similar magnitude as those spontaneously occurring in untreated castrated rats (van Dieten & van Rees 1983; Lambalk et al. 1987a).

A larger GnRH pulse dose of 5 ng/100 g was given once every 60 min (N = 11) or once every 120 min (N = 10) during 24 h, or once every 60 min (N = 6) or 120 min (N = 6) during 96 h.

**Blood sampling**

Blood samples (0.4 ml) were taken from the arterial cannula. Every blood sample was replaced by 0.4 ml of a suspension of red blood cells in saline. Subsequently the cannula was filled with saline containing 250 kIU/l of heparin. Plasma was collected and frozen until assayed.

On day 1 of each experiment blood samples were taken at 5-min intervals, starting 15 min before administering the first pulse of GnRH. In case of a 60-min pulse interval, sampling was continued until 15 min after the second pulse. When the interval was 120 min, no samples were taken from 50 min after the first GnRH pulse until 15 min before the second. Exactly 24 or 96 h later the blood sampling procedure was repeated with respect to the last two GnRH pulses.

**Radioimmunoassay**

LH and FSH contents of the plasma samples were estimated in duplicate by radioimmunoassay (Welschen et al. 1975). For this purpose, specific anti-ovine LH and anti-ovine FSH from rabbits were generously donated by Dr J. Dullaart and Dr J. Th. J. Uilenbroek (Erasmus University, Rotterdam). Rat-LH-I-5, rat FSH-I-3, LH-RP-1 and FSH-RP-1, kindly provided by NIADDK, were used for iodination and as standard, respectively. The sensitivity of both assays, defined as the amount of standard required to suppress binding of iodinated hormone to 85% of the amount occurring in the absence of unlabelled hormone, was estimated as 2.3 ng/tube for LH and 5.8 ng/tube for FSH. Each tube

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of</th>
<th>Treatment</th>
<th>LH</th>
<th>FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH pulse</td>
<td>animals</td>
<td>day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ng/100 g per interval (min)</td>
<td>(N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.25/ 60 (24 h)</td>
<td>5</td>
<td>1</td>
<td>437 ± 39</td>
<td>1576 ± 80</td>
</tr>
<tr>
<td>2</td>
<td>418 ± 25</td>
<td>1561 ± 80</td>
<td></td>
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</tr>
<tr>
<td>1.25/120 (24 h)</td>
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<td>1</td>
<td>475 ± 47</td>
<td>1732 ± 59</td>
</tr>
<tr>
<td>2</td>
<td>408 ± 68</td>
<td>1717 ± 66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/ 60 (24 h)</td>
<td>11</td>
<td>1</td>
<td>545 ± 45</td>
<td>1593 ± 90</td>
</tr>
<tr>
<td>2</td>
<td>404 ± 22*</td>
<td>1676 ± 81*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/120 (24 h)</td>
<td>10</td>
<td>1</td>
<td>453 ± 26</td>
<td>1548 ± 73</td>
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<tr>
<td>2</td>
<td>391 ± 19*</td>
<td>1622 ± 67*</td>
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</tr>
<tr>
<td>5/ 60 (96 h)</td>
<td>6</td>
<td>1</td>
<td>721 ± 100</td>
<td>1634 ± 151</td>
</tr>
<tr>
<td>5</td>
<td>541 ± 53*</td>
<td>1611 ± 125</td>
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<tr>
<td>5/120 (96 h)</td>
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<td>1</td>
<td>606 ± 85</td>
<td>1481 ± 84</td>
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<tr>
<td>5</td>
<td>501 ± 77*</td>
<td>1411 ± 116</td>
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</table>

* Significantly different compared with value of day 1 (paired t-test P < 0.05).
contained 50 µl of plasma. The intra-assay coefficient of variation for LH and FSH, 8.1% and 5.2%, respectively, were both calculated from 200 OVX rat serum pool replicates. The inter-assay coefficient of variation was 11.7% for LH and 16.0% for FSH.

Pulse detection method
Hormone pulses were detected by a method described elsewhere (Lambalk et al. 1985), by which the intra-assay variation (SD) is calculated from duplicate samples in each separate assay. This variation is different at different hormone levels. Therefore SD was computed over different ranges of the assay, which were chosen in such a way that SD was calculated from 30 or more data.

The intra-assay coefficients of variation of LH calculated from SD ranged from 6.8% to 9.7% for concentrations between 2.3 and 15.0 ng/tube and from 3.0% to 6.7% for concentrations between 15.1 and 30.0 ng/tube. The intra-assay coefficients of variation of FSH ranged from 4.4% to 10.1% for concentrations between 5.8 and 15.0 ng/tube and from 3.5% to 6.1% for concentrations between 15.1 and 30.0 ng/tube.

A hormone pulse was indicated when the difference between the peak value and the value of the preceding nadir (i.e. the lowest value from the point where the ascending limb of the hormone pulse originates without altering the sign of the slope) exceeded a certain threshold. The nadir was used as a marker point of time. The threshold was set at $2 \times sd_{diff}$. The $sd_{diff}$ was calculated from SD and represented the standard deviation from the differences between means.

Statistical analysis
For each rat the means of all LH and FSH estimations were calculated on both the first and the last day of the experiment. The differences between the means were used for statistical analysis (paired t-test) in each treatment group.

In each rat the mean LH and FSH response (increment) to the exogenously administered GnRH pulses was measured on the first experimental day and compared with the mean response to the corresponding GnRH injection on the last day. The significances of the differences between these mean responses were analysed with the paired t-test. If means of unpaired observations had to be compared, analysis of variance followed by Duncan's New Multiple Range test was carried out, or the unpaired t-test in case of comparison of only two means.

For all statistics $P < 0.05$ was considered to indicate significance.

Table 2.
Mean increments (µg LH(RP-1)/l and µg FSH(RP-1)/l) ± SEM of LH and FSH in OVX rats to GnRH pulses during the first and last day of pulsatile GnRH treatment. The duration of the treatments is given in parentheses in the first column.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals (N)</th>
<th>Treatment day</th>
<th>LH</th>
<th>FSH</th>
</tr>
</thead>
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<tr>
<td>GnRH pulse</td>
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<td>ng/100 g per interval (min)</td>
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<td></td>
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<td>1.25/60 (24 h)</td>
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<td>1</td>
<td>98±32</td>
<td>88±23</td>
</tr>
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<td></td>
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<td>2</td>
<td>85±38</td>
<td>77±36</td>
</tr>
<tr>
<td>1.25/120 (24 h)</td>
<td>5</td>
<td>1</td>
<td>42±19</td>
<td>157±46</td>
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<td></td>
<td></td>
<td>2</td>
<td>59±26</td>
<td>78±49</td>
</tr>
<tr>
<td>5/60 (24 h)</td>
<td>11</td>
<td>1</td>
<td>367±54</td>
<td>149±67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>232±31*</td>
<td>81±23</td>
</tr>
<tr>
<td>5/120 (24 h)</td>
<td>10</td>
<td>1</td>
<td>286±31</td>
<td>106±28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>129±27*</td>
<td>64±16</td>
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<td>5/60 (96 h)</td>
<td>6</td>
<td>1</td>
<td>405±85</td>
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<td></td>
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<td>5</td>
<td>213±92*</td>
<td>154±69</td>
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<td>5/120 (96 h)</td>
<td>6</td>
<td>1</td>
<td>499±83</td>
<td>160±43</td>
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<td></td>
<td></td>
<td>5</td>
<td>170±56*</td>
<td>177±71</td>
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</table>

* Significantly different from value of day 1 (paired t-test $P < 0.05$).
Results

Pulsatile GnRH treatment and the levels of LH and FSH

Table 1 shows the mean LH and FSH levels at the beginning and the end in the animals of the different treatment groups. No changes in LH and FSH levels were found when GnRH was given at a dose of 1.25 ng/100 g either every 60 or 120 min during 24 h.

If 5 ng of GnRH/100 g was injected every 60 or 120 min during a period of either 24 h or 96 h, a significant decline in LH levels was observed. FSH levels were increased slightly but significantly after 24 h of treatment, but not after 96 h.

LH and FSH responsiveness to GnRH injections

Table 2 shows the mean LH and FSH increments to GnRH injections on the first and the last day of the experiment in each group. When GnRH was injected at a dose of 5 ng/100 g, the LH response after 24 h or 96 h was diminished, regardless of the frequency used. Although the increment of FSH also seemed to have decreased after 24 h treatment with 5 ng/100 g of GnRH, the difference was not significant. Moreover, as opposed to
LH, no decrease was seen at all after 96 h of treatment.

No change in LH and FSH responsiveness could be measured during treatment with GnRH pulses of 1.25 ng/100 g. No differences in LH and FSH response were observed between groups that received different frequencies of GnRH treatment.

Not all injections of GnRH were followed by a pulse of LH or FSH: with regard to LH, only after 35% of the injections of 1.25 ng of GnRH and after 85% of the injections of 5 ng of GnRH. Significant FSH pulses occurred after only 18% of the injections of both the lower and the higher GnRH pulse dose.

**Synchronicity between LH and FSH pulses**

Fig. 1 shows the LH and FSH data of one representative rat from each group treated with GnRH pulses of 5 ng/100 g. It can be seen that endogenous pulses of both LH and FSH occurred and that LH and FSH pulses were not synchronous. We counted all endogenous LH and FSH pulses in all rats of all experiments irrespective of the GnRH treatment given, which yielded a total of 247 and 186 spontaneous LH and FSH pulses, respectively. Only 66 occurred synchronously, i.e. that the nadirs occurred at the same time. This amounted to 35% of the FSH pulses and 27% of the LH pulses.

**Discussion**

The results show that pulsatile administration of 1.25 ng/100 g of GnRH had no effect on mean LH and FSH levels within 24 h. This dose also only infrequently induced LH and FSH pulses. However, it has been shown that when this amount was given to phenobarbital-blocked OVX rats, the LH pulses generated were of similar magnitude as those endogenously occurring in OVX rats (van Dieten & van Rees 1983). Phenobarbital pretreatment of OVX rats has been found to result in an increased response to GnRH (Lambalk et al. 1987a) which is possibly due to recovery of pituitary sensitivity to GnRH through removal of endogenous GnRH.

In the present experiment, administration of 5 ng/100 g regularly induced LH pulses. However, during the treatment both mean LH levels and LH pulse amplitudes decreased, indicating that the pituitary had become desensitized to some extent.

Mean FSH levels increased slightly but significantly after GnRH pulses of 5 ng/100 g during 24 h, both when injected every 60 and every 120 min. This increase was not found after 96 h. Thus, we could not observe the 'paradoxical' effect of a low frequency of GnRH administration increasing the plasma levels of FSH as described by Wildt et al. (1981) and Clarke et al. (1984). It should, however, be mentioned that those experiments were done with OVX monkeys and OVX ewes, respectively, with hypothalamic lesions. It might be that the present increase resulted from accumulation of FSH owing to its relatively long plasma half-life, and a further development of desensitization could be responsible for the disappearance of this effect at 96 h.

Spontaneous LH and FSH pulses remained present during the entire experiment. It has been shown that GnRH pulses induce an acute, short-lasting refractoriness of the pituitary gland (Lambalk et al. 1986) and that endogenous GnRH is less effective in inducing an LH pulse if the injection is given shortly (i.e. within 15 min) after a spontaneous LH pulse (Lambalk et al. 1988). However, in the present experiments the interval between GnRH injections was such that endogenous LH pulses were not fully suppressed.

In contrast to LH, not only the low but also the high dose of GnRH frequently failed to induce FSH pulses. Endogenous FSH pulses remained present throughout the experiment. They did not occur synchronously with the LH pulses, which is in agreement with previous findings (Lumpkin et al. 1984; DePaolo 1985; Condon et al. 1986; de Greef et al. 1987; Culler & Negro-Vilar 1987; Lambalk et al. 1987b). FSH pulses have also been shown to remain present under a variety of conditions in which endogenous GnRH activity was suppressed by administering an antiserum against GnRH or a GnRH antagonist or even extreme desensitization by continuous infusion of a high concentration of GnRH (DePaolo 1985; Culler & Negro-Vilar 1986, 1987; Lambalk et al. 1987b). It seems therefore that the pulsatile nature of plasma FSH in the OVX rat is largely independent of pulsatile GnRH.

It can be concluded that prolonged treatment of the OVX rat with pulsatile GnRH (5 ng but not 1.25 ng/100 g) at large intervals relative to the
endogenous GnRH pulse interval, lead to a decreased level of plasma LH and only a temporary small increase of plasma FSH levels. Under such circumstances, the pituitary LH responsiveness to exogenous GnRH is decreased. Furthermore, in accordance with the literature, in the OVX rat there was a marked asynchronicity between LH and FSH pulses, and the injection of small doses of GnRH resulted in clear LH pulses but only infrequently in FSH pulses.

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