Minor interference of low-dose pulses of GnRH with the positive effect of oestradiol on the pituitary gland


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Abstract. In the ovariectomized rat we investigated the effects of prolonged treatment with oestradiol and 'physiological' pulses of exogenous GnRH on the GnRH-responsiveness of the pituitary gland. Rats were for 20 h pre-treated with either GnRH (3-ng pulses of 3 min duration every 20 min) or saline and for 19 h with either oestradiolbenzoate (OB; 3 μg/sc injection) or oil. Then LH and FSH responses were evoked by continuous test infusions of GnRH at the rates of either 60, 100, 150 or 1000 ng/h, lasting 10 h. It appeared that, irrespective of pre-treatment with either GnRH or saline, OB caused an increase of the maximal LH and FSH responses (that is the responses to the maximal stimulus of 1000 ng GnRH/h), and an increase of the GnRH-sensitivity of the pituitary gland, as far as the FSH secretion is concerned. GnRH pulses caused, irrespective of pre-treatment with OB or oil, a decrease of the GnRH-sensitivity of the pituitary gland, as well as a decrease of the maximal FSH response and of the pituitary FSH content. It was concluded that low-dose pulses of exogenous GnRH desensitize the pituitary gland and deplete the pituitary FSH stores, but do not change the positive effect of oestradiol on the maximal gonadotropin response.

Prolonged exposure to oestradiol increases the LH response to GnRH (‘positive’ effect of oestradiol; Libertun et al. 1974; Vilchez-Martinez et al. 1974; Schuiling & Gnodde 1976b). A positive effect on the LH response is also observed, when, for a prolonged period of time, the influence of endogenous GnRH on the pituitary gland is diminished, e.g. by antibodies against GnRH or an antagonist of GnRH (van Rees et al. 1983; van Rees & van Dieten 1985). As oestradiol also suppresses the secretion of GnRH by the hypothalamus (Sarkar & Fink 1980), it is possible that the positive effect of oestradiol is simply due to the sensitizing effect of the diminished exposure of the pituitary gland to GnRH. However, it has also been observed that combined treatment with oestradiol and 'physiological' pulses of GnRH still increased the LH-response, albeit that relatively high doses of GnRH had to be used to show this effect (van Dieten & van Rees 1983). Yet, this study did not make clear whether under ‘normal’ conditions (i.e. during oestradiol treatment without exogenous GnRH pulses), sensitization by diminished exposure of the pituitary gland to GnRH also contributes to the positive effect of oestradiol. In the present study the putative role of the suppression of the secretion of GnRH by the hypothalamus in the genesis of the positive effect of oestradiol is further investigated.

Materials and Methods

Wistar rats were ovariectomized at the age of 3 months and used for experiments 2 weeks later, when they weighed 200–220 g. The rats were ovariectomized to exclude possible interference of the ovarian hormone production with the phenomena under investigation.
GnRH (a gift of Hoechst Holland) or saline was infused via an intrajugular cannula and 300-μl blood samples were taken from an intracarotid cannula. Cannulation was performed under light ether anaesthesia 2–3 h before the start of the experiment. Oestradiolbenzoate (OB), 3 μg in 0.2 ml of arachis oil, or arachis oil only was injected sc. The LH and FSH concentrations of the plasma samples and the LH and FSH contents of the pituitary extracts were measured by double antibody radioimmunoassay (Koiter et al. 1983; Welschen et al. 1975). LH-RP-1 and FSH-RP-1 standards as well as LH-1-5 and FSH-1-3 for iodination were donated by the NIADDK. The antibodies against LH and FSH were gifts of Drs Dullaart and Uilenbroek of Erasmus University, Rotterdam.

**Experimental design**

Rats were intermittently infused with GnRH for 20 h: pulses of 3 ng of GnRH, each lasting 3 min, were given at 20-min intervals (cf. van Dielen & van Rees 1983; Schuiling & Gnodde 1976a). This pattern of GnRH infusion might be called 'physiological' as these authors observed that exogenous GnRH pulses induce pulses of LH in the plasma of phenobarbitone-anaesthetized ovariectomized rats which are comparable both in magnitude and frequency with the LH pulses in untreated ovariectomized rats. Control rats received saline pulses. After 1 h of pulsatile infusion OB or oil was sc injected; after 20 h the pulsatile infusion of GnRH was stopped and gonadotropin responses were evoked by test infusions of GnRH. For this purpose GnRH was infused for 10 h at the constant rate of either 60, 100, 150 or 1000 ng/h (1000 ng GnRH/h represents a maximal stimulus for the secretion of LH and FSH). Experiments were carried out with 12 animals at a time, equally divided over the 4 treatment groups (GnRH or saline pulses; OB or oil). The 4 groups were tested with the same test infusion of GnRH. The experiment was repeated once for each of the 4 doses of GnRH.

To study the responsiveness of the pituitary gland, continuous infusion of GnRH may be a less 'physiological' method than (multiple) injections of GnRH (Schuiling & Gnodde 1976a; Gallo 1981; Knobil 1980). Yet, infusion has the advantage that it generates a simple signal (a constant GnRH concentration throughout the experiment; Koiter et al. 1982), which can readily be related to simultaneous release of LH and FSH. We consider this of particular importance in the present situation, in which responses to different doses of GnRH are compared. Thus, when GnRH is injected at a high dose, this will result not only in a higher initial concentration of GnRH in the plasma, but also in a longer duration of the resulting GnRH-stimulus than when GnRH is injected at a low dose. Infusion eliminates this aspect of variability in time from the test.

In a separate experiment, 4 groups of 4 rats were treated as described above, with the only difference that these rats were sacrificed after 20 h of pulsatile infusion of GnRH; the anterior pituitary lobes of the pituitary glands were removed, extracted in saline and stored frozen until assay.

**Parameters and statistics**

LH responses were judged according to the mean maximal plasma concentrations, which are reached after 2 h of constant rate infusion of GnRH. FSH responses were judged according to the mean maximal increments (that is the maximal plasma concentrations after 2–3 h of infusion, from which the pre-infusion plasma concentrations are subtracted). This procedure was used, because in the ovariectomized rat the non-stimulated (or autonomous) secretion of FSH is relatively high compared with the GnRH-stimulated secretion and may therefore interfere with a correct evaluation of the response to GnRH (Koiter et al. 1983). Next to the absolute responses, we also analysed the relative or percentage responses (i.e. the responses expressed as percentage of the maximal (= 100%) response, this maximal response being the response to the infusion at the maximally stimulating rate of 1000 ng/h). When for two or four groups the relative responses to the infusion at the lowest rate of 60 ng GnRH/h were different, and the ascending parts of the relative dose-response curve (interval 60–150 ng GnRH/h) were parallel, it was concluded that the groups had different dose-response curves.

Differences between 2 means were tested by the two-tailed Student's *t*-test and between 4 means by two-way analysis of variance. Tests for parallelism were performed according to the method described in Sokal & Rohlf (1969). Differences were considered significant when *P* was smaller than 0.05.

**Results**

OB was ineffective in rats which had comcomitantly been pre-treated for 20 h with GnRH pulses and were subsequently tested with GnRH at the rate of 60 ng/h (Fig. 1). However, in all other groups of rats, OB increased the release of LH and FSH during the initial 4–6 h of the test infusions.

Analysis of the dose-response curves of the GnRH infusion rates and the maximal plasma concentrations of LH (Fig. 2, left panel) or the maximal increments of the plasma FSH concentrations (Fig. 2, right panel), showed that, independent of GnRH or saline pre-treatment, OB increased the maximal response (*P* < 0.01 for both LH and FSH). On the other hand, independent of OB pre-treatment, the GnRH pre-
Fig. 1. Plasma LH (left panels) and FSH (right panels) during continuous infusion with GnRH (60 ng/h: upper panels; 1000 ng/h: lower panels) in ovariectomized rats pre-treated with oil (open symbols) or with OB (closed symbols) and with pulses of GnRH (––, ○–○) or with saline pulses (▼ –▼, ▲ –▲). N = 5 or 6. Means ± sem.

FSH response (P < 0.05). This reduction was associated with a decrease of the pituitary FSH content before the start of the GnRH test infusions (Table 1).

The 'percentage' gonadotropin responses to the test infusions at the rate of 60 ng GnRH/h were lower for the GnRH-pre-treated groups than for the saline-treated groups (P < 0.01 for both LH and FSH). As, furthermore, the slopes of the ascending parts of the 'percentage' LH and FSH curves (interval 60–150 ng GnRH/h) were not different, it was inferred that the pre-treatment with GnRH pulses induced a significant shift to the right of the dose-response curves. According to a similar analysis, OB pre-treatment induced a shift to the left of the 'percentage' dose-response curves of FSH, but not of LH.

Discussion

The present results show that the positive effect of oestradiol is largely due to an increase in the maximal LH and FSH responses to GnRH. As this effect of oestradiol was also observed in rats in which the secretion of GnRH, which had supposedly been suppressed by oestradiol (Sarkar & Fink 1980), was substituted with exogenous GnRH, it is suggested that the effect is exerted directly on the pituitary gland and that it is not the result of the suppression of the secretion of GnRH by the hypothalamus. The suggestion of such a direct effect of oestradiol on the pituitary gland is supported by observations of other investigators, using either a complete in vitro design with hemi-pituitary glands (de Koning et al. 1976)
Dose-response relationships between (the logarithms of) the GnRH-infusion rates and the maximal LH plasma concentrations (left panel) or the maximal increments of the FSH plasma concentrations (right panel) during continuous test infusions of GnRH in ovariectomized rats pre-treated with oil (open symbols) or OB (closed symbols) and with pulses of GnRH (●—●, ○—○) or with saline pulses (▼—▼, ▼—▼). N = 5 or 6 for each point. Means ± SEM.

Fig. 2.

Table 1.

LH and FSH pituitary contents (µg LH-RP-1 or µg FSH-RP-1) of ovariectomized rats treated with a pulsatile infusion of GnRH (3-ng pulses, each lasting 3 min, every 20 min) or saline (3-min pulses; every 20 min) for 20 h and with OB (3 µg sec) or oil 19 h previously. N = 4 for all groups. Values are means ± SEM.

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>LH</th>
<th>FSH</th>
<th>LH</th>
<th>FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB</td>
<td>482 ± 76</td>
<td>102 ± 15</td>
<td>533 ± 62</td>
<td>117 ± 11</td>
</tr>
<tr>
<td>Oil</td>
<td>484 ± 48</td>
<td>84 ± 5</td>
<td>582 ± 60</td>
<td>128 ± 12</td>
</tr>
</tbody>
</table>

* The pituitary FSH content of the groups of GnRH-treated rats is significantly lower than that of the saline-treated rats (P < 0.05; two-way analysis of variance).
or pituitary cells (Liu & Jackson 1984) or an in vivo design with rats in which the neural connections between the preoptic area and the medical basal hypothalamus had been cut (Higuchi & Kawakami 1982).

Next to oestradiol, GnRH can also modulate the maximal gonadotropin response: 'physiological' pulses of GnRH reduced the maximal FSH response. This reduction was associated with a decrease of the pituitary FSH content. These observations confirm and extend previous observations that continuous treatment with high doses of GnRH or an GnRH 'superagonist' decreases the maximal LH response and depletes the pituitary LH content (Koiter et al. 1983; Schuling et al. 1984). Furthermore, from this observation it can be inferred that the suppression of the hypothalamic GnRH secretion by oestradiol may facilitate the positive effect of oestradiol on the maximal FSH response.

Further analysis of the gonadotropin responses revealed that the pre-treatment with GnRH pulses also induced a shift to the right of the dose-response curves of both LH and FSH, indicating a decrease of the pituitary sensitivity to GnRH. This apparent desensitizing effect of low-dose pulses of GnRH might suggest that these pulses, although they maintain a more or less physiological pattern of release of LH (van Dieten & van Rees 1983; Schuling & Gnodde 1976a), are of a non-physiological nature. In this context it may be noted that the hormone is infused into the systemic circulation. This procedure necessarily has consequences for the pharmacokinetics of the peptide; under physiological circumstances, hypothalamic GnRH, having passed the pituitary gland, is very much diluted in the systemic circulation (Koch et al. 1973).

In the saline-treated rats, oestradiol induced a shift to the left of the dose-response curve of FSH, but not of LH. The absence of an effect on the LH system is probably due to the present experimental design, as in similar studies, in which oestradiol was administered in vivo and the pituitary responsiveness to GnRH was tested in vitro, a left shift of the dose-response curve of LH was found (Moes et al. 1984). Apparently, oestradiol increases the sensitivity of the pituitary gland to GnRH in the ovariectomized rat.

A change in sensitivity has been suggested to be due to a change of the number of receptors or of their affinity for the ligand (Kahn 1980). Indeed it has been demonstrated that GnRH can affect the number of its own receptors; high levels of GnRH ultimately lead to down-regulation (Clayton 1982; Loumaye & Catt 1982). Also in the present study the changes of the sensitivity may be explained by changes in the numbers of receptors: the GnRH pulses may decrease the number of GnRH-receptors, whereas oestradiol (via the suppression of the endogenous GnRH secretion) may induce an increase of that number. However, the changes in the $ED_{50}$ are not very large (increases and decreases of only about 30–40%) and may reflect even smaller fluctuations in numbers of receptors. This may explain the failure of other investigators to find any effect of oestradiol and/or GnRH pulses on the number (or the affinity) of the GnRH receptors (Marchetti et al. 1982; Duncan et al. 1986).

In summary, the present results show that in vivo the increase of the gonadotropin response to GnRH is mainly due to an 'own' direct effect of oestradiol. However, the withdrawal of the pituitary gland from the influence of GnRH, as caused by oestradiol, also plays a role and may (in particular in the case of FSH) by of importance under physiological conditions.

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References


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