Prolonged combined in vivo pre-treatment with luteinizing hormone-releasing hormone (LRH) and oestradiol benzoate causes long-lasting suppression of the autonomous and the LRH-stimulated secretion of luteinizing hormone and follicle stimulating hormone.

An in vitro study

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Abstract. The effect of a combined in vivo pre-treatment with luteinizing hormone-releasing hormone (LRH) and oestradiol benzoate (EB) on the autonomous and the 'supra-maximally' LRH-stimulated in vitro release of LH and FSH by pituitary glands of 2 weeks ovarietomized (OVX) rats was studied using a perfusion system. The concentration of LRH in the perfusion medium was 1 μg/ml.

Pre-treatment with LRH during 6 days was effected by means of sc implanted Alzet® osmotic minipumps (MP). Control rats received a piece of silastic with the dimensions of a minipump ('sham-pump'; Sh-P). EB, 3 μg/injection or solvent (arachis oil) was sc injected on days −3 and −1 (day of perfusion: day 0).

Of the pituitary glands of EB-injected, Sh-P-implanted rats both the autonomous and the LRH-stimulated secretion of LH and the LRH-stimulated secretion of FSH were significantly higher than those of the oil-injected, Sh-P-implanted rats without EB administration. Pre-treatment with LRH for 6 days had a suppressing effect on the autonomous and the LRH-induced depletion of the pituitary LH and FSH stores. In combination with EB, the suppressing effect of LRH pre-treatment on the LRH-stimulated secretion of LH and FSH was still greater: the pituitary gland appeared to be fixed in a relatively unresponsive state with very low autonomous LH and FSH secretion.

It is discussed that increase of pituitary LRH-responsive-ness due to EB demands withdrawal of the pituitary gland from the influence of LRH, an effect which is in vivo achieved by the negative feedback of oestrogen on the hypothalamus.

In the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) an autonomous (i.e. non-stimulated; cf. Pasteels et al. 1977; Sheridan et al. 1979; Jenner et al. 1983) and an LRH-stimulated component (see for reviews: Schally 1978; Guillemin 1978) can be distinguished; the autonomous secretion of FSH is in contrast to that of LH, relatively high in proportion to the maximal LRH-stimulated secretion (Moes et al. 1983).

The secretion of LH and FSH can be modified by gonadal factors, notably oestrogens, which, in vivo, exert a biphasic effect on the pituitary gonadotrophs: during the first 9 h after injection of oestradiol benzoate (EB) the LRH-responsiveness of these cells is depressed (Schuiling & Gnodde 1977) but thereafter it increases (Schuiling & Gnodde 1976).

In the present study we investigated whether this phenomenon of increased responsiveness is due to an oestrogen-induced, time-dependent sequence of changes in the pituitary gland only, or whether
oestrogen-induced changes (suppression) in the hypothalamic LRH output (Bogdanove 1964) also play a role. For this purpose we investigated in vitro (perifusion) the autonomous and the LRH-stimulated secretion of LH and FSH after a 6 day in vivo LRH exposure of moderate intensity (250 ng/h; Moes et al. 1983) as well as after either a combined pre-treatment with LRH and EB or after a pre-treatment with EB alone.

Materials and Methods

Wistar rats were ovariectomized at the age of 3 months and used for experiments 2 weeks later. Ovariectomy was performed to eliminate the influence of ovarian hormones. In part of the animals infusion of LRH, 250 ng/h during 6 days was effected by means of sc implanted Alzet® osmotic minipumps (model 2001) which pump at the rate of 0.1 µ/h. Control rats received a silastic 'sham-pump', i.e. a silastic implant of similar dimensions.

Fig. 1.
LRH-stimulated release of LH (A) and FSH (B) and autonomous release of LH (C) and FSH (D) from perifused anterior pituitary tissue derived from 2 weeks ovariectomized rats implanted with either an Alzet® osmotic minipump (MP) for an infusion with LRH (250 ng/h during the 6 days preceding perifusion) or with a silastic 'sham-pump' (Sh-P), and injected either with oestradiol benzoate (3 µg/injection), 72 and 27 h before perifusion, or with oil. Group a: Sh-P; oil (in A and B: n = 7; in C and D: n = 4). Group b: Sh-P; EB (in A and B: n = 6; in C and D: n = 6). Group c: MP; oil (in A and B: n = 8; in C and D: n = 6). Group d: MP; EB (in A and B: n = 5; in C and D: n = 4).
According to the protocol apparent from Results, part of the rats were injected sc with either oestradiol benzoate (EB), 3 µg, in a volume of 0.2 ml of arachis oil, or only with oil.

Pituitary glands were perifused as described in Moees et al. (1983). In short: after removal of the glands they were cut in half and for 15 min washed in a 37°C 95% O\textsubscript{2}/5% CO\textsubscript{2} gassed perfusion medium (Krebs-Ringer-bicarbonate-glucose-1% BSA, KRBG; Edwardson & Gilbert 1976). Thereafter they were transferred to the perfusion chambers with 2 hemi-pituitaries per chamber. Through the chambers (volume about 300 µl) (gassed) medium (37%) was pumped at the rate of 134 ± 1.4 µl/min using an Ismatec peristaltic pump. After a 90 min pre-perfusion with medium only, medium containing LRH at the 'supra-maximally' stimulating concentration of 1 µg/ml (de Koning et al. 1976) was pumped through the chambers. Samples were automatically taken at 5 min intervals.

LH and FSH were measured in the media and, after perfusion, in the pituitary glands by double antibody radioimmunoassay with NIADDK-rat LH-RP-1 and FSH-RP-1 as reference preparations. From an additional number of rats implanted with either a minipump or a sham-pump and injected with either EB or oil, the pituitary LH and FSH contents were measured at a time corresponding to that of starting perfusion in the other experiments.

Differences between means were analysed by the unpaired, two-tailed Student’s t-test.

**Results**

**Effects of LRH- and EB-pre-treatment on the autonomous and the LRH-stimulated secretion of LH and FSH**

Fig. 1 shows the courses of the LRH-stimulated (A and B) and the autonomous (C and D) secretion of LH and FSH. After pre-treatment with 2 EB injections over 72 h the LRH-stimulated secretion of both hormones by pituitary glands of Sh-P-implanted 2 weeks OVX rats (groups b) was significantly higher than after pre-treatment with oil (groups a). EB increased also the autonomous secretion of LH (but not of FSH). Furthermore, like the LRH-stimulated LH- and FSH secretion, the autonomous secretion showed in both series a tendency to decrease with increasing perfusion time.

In the LRH- (minipump) implanted rats another pattern of LRH-stimulated LH- and FSH secretion was seen: after pre-treatment with oil only (groups c) the secretion of LH and FSH was depressed, an effect corresponding with the (partial) depletion of the gonadotrophin stores (see in Table 1: pituitary content at the beginning of perfusion). After a combined LRH- and EB-pre-treat-

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**Table 1.**

The autonomous (A) and 1 µg LRH-stimulated (St) LH and FSH secretion, the total quantity of hormone released during perfusion (data correspond with Fig. 1) and the pituitary LH and FSH content before perfusion.

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Pituitary content before perfusion (µg/pituitary) (n)</th>
<th>Autonomous secretion (A)</th>
<th>1 µg LRH-stimulated secretion (St)</th>
<th>Total quantity released (µg/pituitary)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LH</td>
<td>FSH</td>
<td>LH</td>
<td>FSH</td>
</tr>
<tr>
<td>Sh-P; oil</td>
<td>752 ± 10a</td>
<td>96 ± 4a</td>
<td>729 ± 110a</td>
<td>345 ± 45</td>
</tr>
<tr>
<td>Sh-P; EB</td>
<td>640 ± 22c</td>
<td>85 ± 5a</td>
<td>2144 ± 295b</td>
<td>434 ± 31a</td>
</tr>
<tr>
<td>MP; oil</td>
<td>255 ± 30b</td>
<td>29 ± 10e</td>
<td>523 ± 64</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>MP; EB</td>
<td>267 ± 88b</td>
<td>25 ± 10e</td>
<td>382 ± 58d</td>
<td>111 ± 18b</td>
</tr>
</tbody>
</table>

A and St: maximal LH or FSH secretion/pituitary gland (ng/5 min).

Statistical comparisons in each column: P (a vs b); (b vs d); (e vs d) < 0.001; P (a vs e); (a vs c); (c vs d) < 0.01.

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ment (groups d) the autonomous and the LRH-stimulated secretion of LH and FSH was not enhanced (cf. groups b) but generally still further depressed.

Discussion

After a 6 day in vivo pre-treatment with LRH, analysis of the LH and FSH secretion in a pituitary perfusion system showed that for both LH and FSH both the pituitary content and the autonomous and the maximally LRH-stimulated secretion rates were lowered significantly. Consequently, also the total quantities of LH and FSH released during perfusion were smaller after LRH pre-treatment, which might well be due to the LRH-induced depletion of the pituitary LH/FSH stores.

These observations confirm that though constant exposure of the pituitary gonadotrophs to LRH may cause ‘desensitization’ or ‘down-regulation’ of the processes underlying LH and FSH release (Schuiling & Gnodde 1976; Catt & Dufau 1977) a pituitary gland, desensitized by a given (non-maximal) LRH concentration, is still able to respond to a higher LRH concentration: ‘LRH-induced desensitization of the gonadotrophs is not ‘absolute’, but relative and pituitary LH-responses to staircase stimulation patterns are ‘additive’ (Koiter et al. 1981a). Similar observations were done by Badger et al. (1983) during their in vitro studies on the pattern of LH release induced by exposure to LRH of dispersed pituitary cells.

Koiter et al. (1981a) also demonstrated that during prolonged LRH stimulation the LH secretion rate and the (decreased) pituitary LH content stabilize after 2 days at levels which are then maintained for at least another 6 days, suggesting the development of an equilibrium between LH release and LH synthesis. The present results suggest the development of a similar situation for FSH. The existence of such an equilibrium suggests that next to the release of LH and FSH, LRH controls also the synthesis of two gonadotrophins and that a pituitary gland, once depleted by LRH, will not be repleted until cessation of LRH stimulation.

Whilst in non-LRH-pre-treated rats EB caused a marked increase of the pituitary in vitro LRH-stimulated secretion of LH and FSH as well as of the autonomous secretion of LH, it did not do so in rats implanted with LRH releasing minipumps. Of these latter rats lower secretion rates might be expected because of depletion of LH and FSH due to the LRH-pre-treatment (see above), but if in these minipump-implanted animals the LRH-responsiveness of the pituitary gland would be affected by EB the way it was affected in the sham-pump-implanted rats, the responses would have been considerably higher and larger, and in any case higher and larger than in the oil-treated, minipump-implanted animals. Instead of this effect the LRH-responsiveness of the pituitary gland was still further depressed by EB.

Schuiling & Gnodde (1977) suggested that for an oestrogen-induced increase of the pituitary LRH-responsiveness to develop, the gland should, at least to a large extent, be withdrawn from the influence of LRH. In vivo, EB-induced reduction of LRH exposure is achieved by depression of the hypothalamic LRH secretion by the so-called indirect negative feedback of oestrogen (Bogdanove 1964; Sarkar & Fink 1980), a feedback effect which, probably, has also been exerted in the present EB-injected rats but which in the minipump-implanted animals would have been completely ‘overruled’ by the present pharmacological treatment with exogenous LRH.

These observations support the suggestion that lowering of LRH is a prerequisite for increase of the pituitary LRH-responsiveness after administration of oestrogen and suggest that this increase is not a phenomenon which appears always when a certain period of time has elapsed after administration of the steroid, but only when certain conditions are fulfilled. Of these conditions lowering of LRH is one.

Yet, from the reported experiments it can not be inferred whether lowering of LRH is not only a necessary, but also a sufficient condition for the LRH-responsiveness to increase. After all, increase of LRH-responsiveness has been observed by Koiter et al. (1981b) in non-oestrogen-primed O VX rats after cessation of continuous stimulation for 3, 12, 24 or 72 h with exogenous LRH, and the process may therefore simply reflect adaptation of the LH and FSH secretory mechanisms to low LRH concentrations.

The complex nature of the processes which lead to oestrogen-induced changes in the pituitary LRH-responsiveness is also apparent from recent work of van Dieten & van Rees (1983), who demonstrated that maintenance of pulsatile LH secretion in phenobarbitone-blocked O VX rats by pulsatile
infusion of LRH (LRH-pulses: 1.25 ng every 20 min) did not completely abolish the development of a ‘positive’ effect of oestrogen on the pituitary LRH-responsiveness, although such a positive effect was only observed when after a 24 h period of 1.25 ng LRH-pulses, the pituitary gland was stimulated at a higher intensity. From these results the authors concluded that with an administration of LRH which can maintain plasma LH levels at a height and pattern similar to that in otherwise untreated OVX rats EB can induce no LH surges. Contrary to the these results, Fraser & McNeilly (1982) observed in the ewe the neutralization of LRH with LRH-antibodies prevented the induction of LH and FSH surges by oestrogen.

The difference between the results of van Dieten & van Rees (1983) and ours may be caused by the well-known fact that in contrast to continuous exposure to LRH, pulsatile exposure to LRH does not cause desensitization of the pituitary gland (Belchetz et al. 1978): apparently, when the pituitary gland is exposed to a more or less physiological pattern of (exogenous) LRH stimulation, it does not loose its capacity to increase its LRH-responsiveness after administration of EB, whilst after continuous exposure to LRH this capacity gets lost and the gland becomes desensitized. Therefore, cells being desensitized or not may be of crucial significance for the development of the positive oestrogen effect: in a desensitized pituitary gland oestrogen can not induce the sequence of events which leads to increase of the LRH-responsiveness, and the view of Badger et al. (1983) who claim that ‘the major difference between cells desensitized with LHRH and non-desensitized cells is that desensitized cells require a larger dose of LHRH to elicit a given L.H response’ may only be true when cells are not exposed to oestrogens (and possibly to other gonadal hormones).

In any case, the combined treatment with LRH and oestrogen appears to be very effective in suppressing both the autonomous and the LRH-stimulated secretion of LH and FSH, and it can be concluded that the pituitary gland, when exposed to elevated levels of LRH and oestrogen, is ‘fixed’ in the relatively unresponsive state which resembles that following prolonged treatment with potent agonistic analogues of LRH, and which is characterized by low autonomous secretion of LH and FSH so that induction of even a moderate increase of the LH/FSH secretion rate demands a high level of LRH-stimulation (Moes et al. 1983).

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References


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