Effect of pretreatment of long-term ovariectomized (OVX) rats with a competitive LRH antagonist on FSH release in vitro

J. A. M. J. van Dieten and G. P. van Rees

Department of Pharmacology of the University of Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands

Abstract. The effect of a single sc injection of an LRH antagonist ((Ac-D-p-Cl-Phe^{1,2},D-Trp^{3},D-Phe^{6},D-Ala^{10})-LRH, Org 30093) into OVX rats on FSH release 24 h later was studied. Plasma FSH was decreased but pituitary FSH content was not changed. Incubation of the pituitary glands during 4 h resulted in a decreased basal release. FSH release induced by a low concentration of LRH (1 ng/ml) was depressed but that of a high concentration (10 000 ng/ml) was augmented in comparison to FSH release induced in control glands. However, pretreatment with the antagonist had no specific effect on FSH release in vitro induced by high K+ or high K+ plus mbcAMP and theophylline, indicating that the changes of pituitary responsiveness to LRH are not caused by those parts of the secretory mechanism which are stimulated by these secretagogues. Moreover, it is concluded that the changes of pituitary LH release induced by administration of an LRH antagonist also concern FSH.

In a previous study (Van Rees & Van Dieten 1985) we described the effect of a purely competitive antagonist of LRH (Org 30093) on LH release in vitro. It was found that a single sc injection of this compound into OVX rats resulted in lowered plasma LH levels 24 h later, although pituitary LH content was unchanged. When the pituitary glands of such animals were incubated with increasing concentrations of LRH, LH release incubated by low concentrations of LRH was depressed, but that caused by high concentrations of LRH was augmented. LH release in vitro induced by other secretagogues (mbcAMP and theophylline, or a high K+ concentration of the incubation medium) was not appreciably affected.

It was concluded that the inhibited LH release observed during incubation with low concentrations of LRH was the result of the continued presence of the antagonist in the pituitary glands but that the augmented response caused by high concentrations of LRH was the result of a mechanism similar to that induced by treating OVX rats with an antiserum to LRH (Van Rees et al. 1983). Since the increased pituitary responsiveness caused by an antiserum against LRH not only concerned LH but also FSH, we also investigated the effect of an injection of Org 30093 into OVX rats on pituitary FSH release in vitro 24 h later.

Materials and Methods

Wistar-derived rats were ovariectomized and used 14 days later. The animals received a single sc injection of Org 30093 (100 µg/100 g body weight) or an equal volume (0.2 ml) of solvent and were decapitated 24 h later. In experiment 1 trunk blood was collected and the pituitary glands were removed and weighed. After clotting the blood sera were collected and frozen for FSH assay. The pituitary glands were extracted in saline and the extracts were also frozen until assay. In experiment 2 the pituitary glands were incubated for 4 h in the presence of the following concentrations of LRH: 0, 1, 10, 100, 1000 or 10 000 ng/ml. In experiment 3 the glands
were incubated for 4 h in the presence of mbcAMP (1 mM) plus theophylline (10 mM), or elevated K+ (50 mM) or mbcAMP plus theophylline and elevated K+ in the same concentrations.

Chemicals used: the LRH antagonist Org 30093 was suspended in a solution of gelatin A (0.5%)/mannitol (5%) in water, according to the directions given by Organon International, to a final concentration of 1 mg/ml. LRH (Beckman), mbcAMP and theophylline were dissolved in medium TC 199. The elevated K+ concentration of the medium was reached by adding KCl to the medium. No changes were made with regard to the osmolarity of the medium since previous experiments had shown that they were not necessary (De Koning et al. 1978).

Incubations were carried out by adding two pituitary halves to 1 ml of ice-cold medium TC 199 and incubating them under continuous shaking at 37°C under 95% O2 plus 5% CO2. After 30 min the media were exchanged for fresh medium in which the various secretagogues had been dissolved and the incubation was continued under the same conditions for 4 h.

Estimation of FSH was made by a radioimmunoassay developed by Drs. J. Th. J. Uilenbroek and J. Dullaart and described by Welschen et al. (1975). Specific rabbit anti-ovine FSH was a gift from Drs. Uilenbroek and Dullaart. Rat FSH-I-4 was used for iodination and FSH-RP-1 as a standard preparation. These preparations were kindly provided by NIAMDD (Bethesda, MD, USA) and Dr. A. F. Parlow.

Statistical evaluation: significance of differences was calculated by Student's t-test when appropriate. In other cases analysis of variance was applied, if necessary followed by Duncan's multiple comparison test.

Results

Experiment 1 (see Table 1)

Twenty-four h after a single sc injection of Org 30093 serum FSH had decreased considerably. Pituitary FSH content, however, was not changed.

Experiment 2 (see Fig. 1)

The injection of Org 30093 caused a decrease of basal release of FSH in vitro which was of a similar magnitude as the decrease of serum FSH which was observed in experiment 1. Exposure to increasing concentrations of LRH caused a dose-related FSH release both in the experimental and in the control group. Although in both cases the slopes of the response curves significantly differed from

---

**Table 1.**

Effect of a single injection of Org 30093 (100 µg/100 g, sc) or solvent on pituitary and plasma FSH 24 h later (mean ± SEM).

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Pituitary weight (mg)</th>
<th>Pituitary FSH (µg FSH-RP-1/mg)</th>
<th>Serum FSH (ng FSH-RP-1/ml)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Org 30093</td>
<td>10.8 ± 0.3</td>
<td>10.348 ± 0.560</td>
<td>963 ± 59</td>
<td>6</td>
</tr>
<tr>
<td>Solvent</td>
<td>10.7 ± 0.2</td>
<td>11.313 ± 1.010</td>
<td>2282 ± 227</td>
<td>6</td>
</tr>
</tbody>
</table>

\[ t = 0.277 \quad \text{and} \quad t = 0.836 \quad \text{and} \quad t = 5.624 \]
**Fig. 2.** FSH release during 4 h incubation (mean ± SEM) by control glands (A, A') or in the presence of mbcAMP + theophylline (B, B'), high K⁺ (C, C') or high K⁺ and mbcAMP + theophylline (D, D'). (n = 4). The rats had been pretreated 24 h earlier with a sc injection of Org 30093 (hatched bars) or solvent (open bars). • Indicates a significant difference between C and D vs A or between C' and D' vs A'. ○ Indicates a significant difference between A vs A', B vs B', C vs C' and D vs D'.

zero (controls: F₁,₂₄ = 13.98; experimental group = F₁,₂₄ = 205.62), the dose-response relationship of LRH and FSH release was markedly altered by pretreatment with the antagonist. As opposed to controls, 1 ng/ml of LRH did not cause a significant FSH release, but the slope of the dose-response curve induced by higher concentrations of FSH was significantly steeper. This resulted in about an equal release of FSH in both groups induced by 100 ng/ml of LRH and a significantly larger release of FSH by 10 000 ng/ml after pretreatment with the antagonist.

**Experiment 3 (see Fig. 2)**

The effects of Org 30093 as observed on pituitary response to LRH were not seen when other secretagogues were used. In both groups cAMP plus theophylline had no effect, but high K⁺ had and the effect of high K⁺ was further increased when high K⁺ was combined with mbcAMP plus theophylline. As in the previous experiment, basal release of FSH was lower when the animals had been pretreated with the antagonist. This difference remained when the glands were exposed to the secretagogues. However, the absolute increases of FSH release induced remained of the same order of magnitude in both groups.

**Discussion**

As stated in the Introduction, the experiments were carried out to investigate whether the effects of pretreatment with a competitive antagonist in vivo on pituitary FSH release in vitro induced by LRH and mbcAMP plus theophylline and/or high K⁺ were comparable to those on LH release as reported before (Van Rees & Van Dieten 1985). In that publication it was also shown that the antagonist used (Org 30093) has the properties of a pure competitive antagonist to LRH, as it had no effect on the LH release by supramaximally active concentrations of LRH and only shifted the dose-response curve to the right without affecting its slope. In vitro the antagonist had similar effects on LRH-induced FSH release (data not shown).

In interpreting the data it should be taken into account that FSH release in vitro by pituitary glands of OVX rats differs from LH release in that basal release, i.e., release in the absence of any secretagogue, closely follows FSH secretion in vivo at the time of decapitation, whereas basal LH release is not or hardly affected. This difference was found in earlier experiments in which pituitary LH and FSH release in vitro was studied after pretreatment with an antiserum to LRH 24 or 48 h earlier (Van Rees et al. 1983). The cause of this difference is not known.

Injection of the antagonist into OVX rats had similar effects on FSH in vivo as on LH (Van Rees & Van Dieten 1985), since plasma FSH was decreased considerably but pituitary FSH content was not altered significantly, indicating that suppression of the action of endogenous LRH results in a decrease of the synthesis of FSH.

The effects of pretreatment of the animals with Org 30093 on pituitary FSH release in response to
LRH were also comparable with those on LH release if the effect of the pretreatment on basal release of FSH is taken into account. Thus, as was the case with LH, FSH release in response to low concentrations of LRH was depressed but that in response to a high concentration of LRH was augmented. The depressed response to low concentrations may be ascribed to the continuing presence of the antagonist due to its binding to LRH receptors in the gonadotrophic cells, whereas the augmented response could be due to the withdrawal of the action of endogenous LRH, similar to that seen when endogenous LRH is suppressed by injection of an antiserum against LRH (Van Rees et al. 1983). In the present case, this latter effect can only be demonstrated in the presence of high concentrations of LRH because of the competitive nature of the interaction between LRH and the antagonist.

The response to secretagogues such as high K+ alone or combined with mbcAMP plus theophylline did not show such a specific change. In both groups, mbcAMP plus theophylline was an insufficient stimulus to induce FSH release, but it potentiated the response to high K+ as has been found for LH release (Van Rees & Van Dieten 1985). However, if the lower basal release of FSH after pretreatment with the antagonist is taken into account, no effect of the pretreatment on FSH release induced by these agents was found. This indicates that the increased responsiveness of the pituitary gland to LRH as induced by pretreatment with the antagonist is not due to changes of that part of the release mechanism which is stimulated by high K+ or cAMP.

It has been demonstrated (Jenner et al. 1983) that the mechanism of action of LRH in inducing FSH release is similar to that by which release of LH is induced. The present results further extend the similarity between these two mechanisms, without denying that other parts of FSH release can be quite different from those of LH release.

Acknowledgment

We thank Organon International for providing us with a generous supply of the LRH antagonist, Org 30093.

References


Received on February 27th, 1985.