DOSE-RESPONSE STUDY WITH A NEW LH-RH ANALOGUE, D-Ser (TBU)\(^6\) LH-RH 1-9 (EA)\(^{10}\) DURING THE FOLLICULAR PHASE OF THE MENSTRUAL CYCLE

By

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ABSTRACT

D-Ser (TBU)\(^6\) LH-RH 1-9 (EA)\(^{10}\) (HOE 766) a highly active LH-RH analogue, was studied with regard to its effects on the release of follicle stimulating hormone (FSH), luteinizing hormone (LH) and oestradiol-17\(\beta\) (O\(\varepsilon\)\(\varphi\)) during the follicular phase of the menstrual cycle. Forty-two regularly menstruating women were allowed to five different treatment groups with different doses (1.25 \(\mu\)g; 2.5 \(\mu\)g; 5.0 \(\mu\)g; 10.0 \(\mu\)g; 20.0 \(\mu\)g) of HOE 766 given as intravenous bolus injections and the plasma concentrations of FSH, LH and O\(\varepsilon\)\(\varphi\) were measured up to 24 h after injection using specific radioimmunoassays. In the majority of cases, peak values of both FSH and LH occurred 4 h after injection being significantly different from pre-injection levels \((P < 0.02\) in the 1.25 \(\mu\)g treatment group, \(P < 0.005\) for the other treatment groups). Statistical analysis of maximum values as well as the absolute and relative increase in the different treatment groups revealed a dose-dependent effect of HOE 766. Maximum values of O\(\varepsilon\)\(\varphi\) occurred 8 h after injection and were found to be significantly different from pre-injection levels \((P < 0.005)\). However,
no dose dependent effect was observed. It was concluded that HOE 766 is a potent and long-acting stimulator of FSH, LH and Oe2 release in women. The effect of HOE 766 is dose dependent for FSH and LH but not for Oe2.

Since synthetic luteinizing hormone-releasing hormone (LH-RH) became available, attempts have been made to utilize this decapeptide for the induction of ovulation in the human. From the balance of evidence it appears, that ovulation induction by LH-RH is still an unsolved problem (Kastin et al. 1971; Schneider et al. 1972; Zarate et al. 1974; Keller 1973; Nillius et al. 1975). Elevation of circulating levels of FSH and LH after a single injection of LH-RH is of such short duration that administration of 500 μg LH-RH at 6 h intervals has been suggested for therapeutic purposes (Nillius et al. 1975). The pregnancy rate that was achieved by means of a variety of LH-RH treatment regimes, however, has been disappointing. It appears therefore that the efficacy of LH-RH as a fertility promoting agent in women is rather limited.

Recently, several long-acting and more potent analogues of LH-RH have been studied (Arimura et al. 1974; Kuhl et al. 1976; Sandow et al., in press; Wiegelmann et al. 1976) and it is the purpose of this report to present results of an inter-individual dose-response study performed with D-Ser(TBU)6 LH-RH 1-9 (EA)¹⁰ (HOE 766), a nonapeptide synthesized by Hoechst AG (König et al. 1975). The effects of different doses of HOE 766 on plasma concentrations of FSH, LH and oestadiol-17β (Oe2) were measured on days 5–7 of the follicular phase of the menstrual cycle in order to obtain “normal values” of the time course of the effects of this nonapeptide and to select a proper regime for its therapeutic administration.

**Material and Methods**

Forty-two regularly menstruating women in their reproductive age and without hormonal therapy for at least 3 months were allocated at random to five groups of treatment. Age, height and body weight of these volunteers as well as the doses of HOE 766 that were administered in each treatment group are listed in Table 1. HOE 766 was given as a single bolus injection intravenously on day 5, 6 or 7 of the menstrual cycle. Venous blood was obtained at 15 min, and 0 min before injection as well as 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h after injection. The plasma was stored at -20°C before being assayed.

Plasma LH and FSH were measured by specific radioimmunoassays as described by Joel et al. (1974). LER 960 (HLH) and LER 1366 (HFSH) were used for iodination, LER 907 as standard reference preparation and anti-HLH (batch 1) as well as anti-HFSH (batch 4) as antisera. Specific radioimmunoassay for Oe2 was developed in our laboratory.

Pre-injection levels were compared with the values reached at 4 h for LH and FSH
Table 1.
Clinical data regarding the case material.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of HOE 766</th>
<th>No. of volunteers</th>
<th>Age in years mean ± sd</th>
<th>Height in cm mean ± sd</th>
<th>Body weight in kg mean ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.25</td>
<td>6</td>
<td>22.67 ± 2.88</td>
<td>170.17 ± 7.63</td>
<td>59.67 ± 6.92</td>
</tr>
<tr>
<td>2</td>
<td>2.50</td>
<td>8</td>
<td>28.50 ± 6.05</td>
<td>168.00 ± 6.28</td>
<td>52.75 ± 6.30</td>
</tr>
<tr>
<td>3</td>
<td>5.00</td>
<td>10</td>
<td>24.00 ± 4.64</td>
<td>172.10 ± 5.76</td>
<td>60.00 ± 4.05</td>
</tr>
<tr>
<td>4</td>
<td>10.00</td>
<td>9</td>
<td>25.44 ± 5.77</td>
<td>168.50 ± 6.54</td>
<td>58.56 ± 5.32</td>
</tr>
<tr>
<td>5</td>
<td>20.00</td>
<td>9</td>
<td>26.89 ± 8.77</td>
<td>165.88 ± 5.03</td>
<td>67.31 ± 10.77</td>
</tr>
</tbody>
</table>

and those reached at 8 h for Oe₂ using the Wilcoxon test (Wilcoxon 1945). The dose-response relationship was evaluated using the non-parametric analysis of variance of Kruskal & Wallis (1952). The Nemeniy test was used to detect differences between each pair of treatment groups. The absolute increase was defined as maximum minus pre-injection values and the relative increase was defined as the ratio of maximum response value/pre-injection level. Statistical evaluation of the time distribution of maximum values of all parameters was performed according to Stucky & Vollmar (1975).

Fig. 1.
Time course of the release of LH after injection (Rx) of different dosages of D-Ser (TBU)⁶ LH-RH 1–9 (EA)¹⁰ as mean ± sem,
RESULTS

1) LH

The plasma concentrations of LH calculated as means ± SEM for each treatment group and at each time point are shown in Fig. 1. The single bolus injection of HOE 766 was followed by a biphasic pattern of LH release: Plasma levels of LH were found to increase until 20 min after injection, followed by a plateau between 20 and 60 min, which was again followed by a rapid increase to peak values which were observed at 4 h after injection in 26 cases and at 2 h in the remaining 16 cases. Maximum values were reached earlier (2 h) with the lower doses, namely 1.25 µg and 2.5 µg of HOE 766, than with the higher doses. The difference in time distribution was statistically significant between the groups (P < 0.028).

Plasma concentrations of LH at 4 h were significantly different from pre-injection levels at P < 0.02 in the 1.25 µg group and at P < 0.005 for the other treatment groups. The LH response increased with the augmentation of the dose up to 10 µg of HOE 766 and ceased off when 20 µg was given. With regard to a dose-dependent effect of the LH response significant differences (P < 0.05) between the groups were revealed by the Kruskal-Wallis-test (Kruskal & Wallis 1952). The 10 µg and 20 µg group were found to be significantly different from the 1.25 µg group with regard to maximum values, absolute

Fig. 2.
Ordinate: relative increase (maximum value/pre-injection value) of LH after injection of HOE 766; abscissa: time of maximum increase of LH-concentrations (t_max).
and relative increase in LH (Nemeniy-test). The relative increase of LH concentrations, expressed as the ratio of maximum levels/pre-injection levels, is shown in Fig. 2. At 8 h LH concentrations were still elevated in the 10 μg and 20 μg group and returned to pre-injection levels in all the treatment groups after 24 h.

2) FSH

The time course of FSH (mean ± SEM) after injection of HOE 766 for each group is shown in Fig. 3. There was a similar biphasic pattern of release as with LH and again the majority of peak values (81%) was observed 4 h after injection of HOE 766 with the 4 h values being significantly different from the basal levels (P < 0.02 for the 1.25 μg group, P < 0.005 for all other groups). Again there was an increase of the stimulating effect with the higher doses of HOE 766. Elevation of FSH levels appeared to be even more prolonged than that of LH. With regard to a dose-response relationship significant differences among the treatment groups were observed for the relative and absolute increases in the FSH concentrations (P < 0.05). The absolute and relative increase in the 10 μg and 20 μg group was significantly different from the 1.25 μg group and the relative increase in the 2.5 μg group was different from the relative increase in the 10 μg group (Nemeniy-test). The relative increases in each group are illustrated in Fig. 4.

![Image](image_url)
Stimulating effect of HOE 766 on FSH

Fig. 4.
Ordinate: relative increase of FSH after injection of different doses of HOE 766; abscissa: time of maximum increase of FSH concentrations ($t_{\text{max}}$).

Fig. 5.
Time course of the release of oestradiol (Oe$_2$) after injection (Rx) of different doses of D-Ser (TBU)$_6$ LH-RH 1–9 (EA)$_{10}$ as mean $\pm$ SEM.
Stimulating effect of HOE 766 on E2

Fig. 6.
Ordinate: relative increase of oestradiol (Oe2) after injection of HOE 766; abscissa: time of maximum increase of Oe2 concentrations (tmax).

3) Oestradiol

The effect of HOE 766 on plasma concentrations of Oe2 is shown in Fig. 5. Eighty-one per cent of maximum values were observed after 8 h with the 8 h values significantly different from pre-injection levels. Elevation of circulating concentrations of Oe2 was still present at 24 h after injection. However, no dose-response relationship was observed. The relative increase of Oe2 is illustrated in Fig. 6.

DISCUSSION

The present study provides evidence for a dose-dependent LH and FSH release after injection of different doses of the nonapeptide HOE 766 during the follicular phase of the menstrual cycle. Peak values of FSH and LH, which in the majority of cases occurred 4 h after injection, were significantly different from pre-injection levels. The highest response of both FSH and LH was seen in the 10 µg treatment group. Statistical analysis of the dose-response relationship, however, revealed that the absolute and relative responses of the 10 µg and 20 µg group were significantly different only from the 1.25 µg group for LH and from the 2.5 and 1.25 µg group for FSH, respectively. The time course of FSH and LH release as well as the maximum plasma concentrations of both hormones, induced by a single bolus injection of HOE 766 differ markedly from results obtained with comparable doses of the decapeptide LH-RH. It is noteworthy that HOE 766 is a potent stimulator of both
FSH and LH release whereas the decapeptide LH-RH predominantly releases LH.

The biphasic pattern of LH response, however, is strikingly similar to the effect of constant infusions of LH-RH during the early follicular phase of the menstrual cycle (Yen et al. 1976). In contrast to our findings in the female, HOE 766 did not cause a biphasic pattern of LH release when administered to normal men (Kuhl et al. 1976; Wiegelmann et al. 1976).

From experiments in the rat (Sandow et al., in press) it appears that the plasma elimination rate of HOE 766 is similar to that of LH-RH, but the time course of organ distribution shows much higher tissue/plasma ratios in the anterior pituitary, liver and kidney for HOE 766 than for LH-RH, thus indicating a slower breakdown of the nonapeptide. It is tempting to speculate that by prolonged binding to pituitary receptor sites HOE 766 not only causes the secretion of the “immediately releasable pool” (Yen et al. 1976) of LH but also stimulates de novo synthesis of pituitary LH. The latter would account for a second increment of LH as observed in our study.

From the significant rise in Oe2 levels it is obvious that ovarian stimulation up to 24 h can be achieved by a single injection of low doses of the nonapeptide. The ovarian response does not seem to be dose-dependent within the range of 1.25 μg to 20 μg.

It is concluded that HOE 766, being a potent and long-acting stimulator of FSH, LH and Oe2 release in women, should offer major advantages over LH-RH as a fertility promoting agent. Clinical trials are presently being carried out to evaluate the ability of HOE 766 to induce ovulation in amenorrhoea.

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REFERENCES


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