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Acute Effects of a New Stimulatory Luteinizing Hormone-releasing Hormone Analogue D-Ser(TBU)^{6}-EA^{10}-LRH on the Gonadotrophin and Gonadal Steroid Secretion in Women with Amenorrhoea

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ABSTRACT

A new stimulatory luteinizing hormone-releasing hormone (LRH) analogue D-Ser(TBU)^{6}-EA^{10}-LRH was administered subcutaneously in a dose of 10 μg into 10 women with amenorrhoea. The injection resulted in a 20-fold increase of the LH level in blood and a 5-fold increase of the FSH level with maxima at 4 and 6 h after the administration, respectively. There was an evident biphasic pattern of LH release similar to that described after extended pituitary stimulation by constant infusions of LRH with early and late peaks of LH combined with a gradual, progressive FSH release. The duration of the effect on LH and FSH was at least 12 and 24 h, respectively. All the women responded with evident oestradiol increases in blood during the last 18 h of the 24 h study period. A comparison between the effects of a single subcutaneous injection of 10 μg of the LRH analogue and 500 μg of LRH showed that the initial FSH and LH release was similar. However, the FSH and LH release during the remainder of the study period was significantly greater after administration of the analogue and so was the oestradiol increase. Thus, this study in women confirms previous studies in men that D-Ser(TBU)^{6}-EA^{10}-LRH is a potent stimulatory LRH analogue with prolonged biological activity.

INTRODUCTION

Luteinizing hormone-releasing hormone (LRH) was isolated from porcine hypothalami, structurally determined and synthesized by Schally and coworkers in 1971 (14). During recent years numerous analogues of LRH have been synthesized in attempts to (*a*) develop inhibitors of LRH useful for birth control and (*b*) to find superactive stimulatory analogues with prolonged biological activity (15, review). Such stimulatory analogues should be more useful for therapeutic use than LRH itself, which has effects on the gonadotrophin secretion of short duration. The plasma clearance of exogenous LRH is very rapid with initial half-life of 4 min (2, 10). Present therapeutic regimens for treatment of hypogonadism in men and women therefore require 8-hourly injections of large doses of LRH over prolonged periods of time to be effective (6, 8, 9). There is need for a superactive LRH analogue with prolonged activity for simplifying treatment with LRH.

In the present study, acute effects of a new stimulatory LRH analogue D-Ser(TBU)^{6}-EA^{10}-LRH (Hoechst 766) on the gonadotrophin and gonadal steroid secretion were investigated in ten women with amenorrhoea. In five of these women the effects of the LRH analogue were compared with those obtained after a large dose of synthetic LRH.

PATIENTS AND METHODS

Patients. Ten 20–29-year-old women with amenorrhoea volunteered for this study. Nine of them had secondary amenorrhoea of at least 2 years' duration, while one woman had primary amenorrhoea due to Kallman's syndrome (hypogonadotrophic hypogonadism with anosmia). In seven of the women the amenorrhoea had occurred in a setting of self-imposed weight loss. Four of them had regained weight while three women were still underweight at the time of study. All the women had normal X-rays of the skull and pituitary fossa and their thyroid, adrenal, renal and hepatic function was normal. The patient with Kallman's syndrome was found to have a moderate hyperprolactinaemia (26 μg per l) while all the other women had normal prolactin levels in serum. The gonadotrophin levels in serum were low or normal and the endogenous oestrogen production was low.

LRH treatment. The LRH analogue D-Ser(TBU)^{6}-EA^{10}-LRH (Hoechst 766) was administered subcutaneously in a dose of 10 μg to all the women. Venous blood samples were obtained before and during the first 24 h after

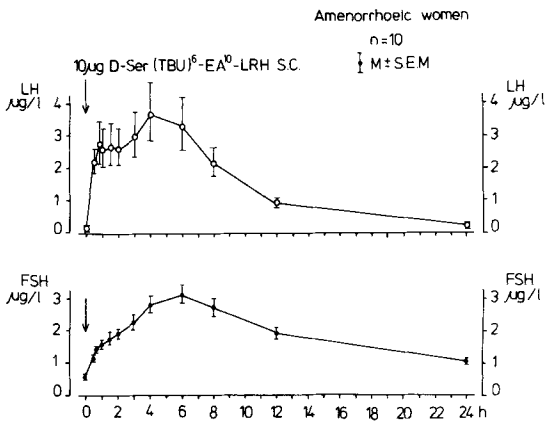

Fig. 1. Mean serum levels of LH and FSH before and after subcutaneous administration of 10 µg of D-Ser(TBU)⁶-EA¹⁰-LRH into 10 women with amenorrhoea.

the administration and assayed for FSH, LH, oestradiol and progesterone.

Five of the women were also studied by hormone assays before and after subcutaneous administration of 500 µg of LRH (Hoechst) which was given either one month before or after the administration of the analogue. Twenty-four hours before the administration of LRH or the analogue, the pituitary reserve capacity for gonadotrophin secretion was tested with 100 µg of LRH (7).

Hormone assay methods. Immunoreactive FSH and LH in serum were assayed by a radioimmunosorbent technique with indirectly coupled antibodies (17). LH in serum was measured by utilizing human pituitary LH (12) labelled with ^{125}I and rabbit anti-human pituitary LH. The LH preparation had a biological activity of 9400 IU (2nd IRP-HMG) per mg. FSH in serum was measured by utilizing human pituitary FSH (11) labelled with ^{125}I and guinea pig anti-human pituitary FSH. The FSH preparation had a biological activity of 12000 IU (2nd IRP-HMG) per mg. The results are expressed in µg per l.

Immunoreactive oestradiol in serum was measured by a radioimmunological technique using an antiserum to an oestradiol-6-oxime-BSA conjugate (5). Progesterone was assayed by a similar radioimmunological technique.

Statistical methods. The hormone values were transformed into logarithms in the statistical calculations. The mean values given in the text are geometric means. For calculation of differences between mean values, formulas based on the Student's *t*-distribution were used.

RESULTS

Mean LH and FSH levels in serum before and after subcutaneous administration of 10 µg of D-Ser(TBU)⁶-EA¹⁰-LRH intor the ten women with amenorrhoea are shown in Fig. 1. The LH and FSH results from the preceding intravenous LRH test are shown in Fig. 2.

The mean pretreatment LH level (0.18 µg per l) was below the normal range for women of fertile age. All the women responded to the LRH analogue with a rapid increase of the LH level in blood with a first peak (2.7 µg per l) at 45–60 min after the administration. The LH level remained unchanged for the next hour and then increased again to a second peak with the highest mean value (3.7 µg per l) at 4 h after the administration. After that the LH level slowly declined. The mean LH level was still significantly elevated over the pretreatment level at 12 ($p<0.001$) but not at 24 h ($p>0.05$) after the administration of the analogue.

The mean pretreatment FSH level (0.60 µg per l) was within the lower normal range for women of fertile age. FSH progressively increased after the injection of the LRH analogue and reached a peak (3.1 µg per l) at 6 h after the administration. Then the FSH level slowly declined. The mean FSH values at 12 (1.9 µg per l) and 24 (1.1 µg per l) were significantly higher ($p<0.001$) than the mean pretreatment value.

The mean oestradiol level in serum before and after administration of the LRH analogue is shown in Fig. 3. The mean pretreatment level (162 pmol per l) was similar to that found in the early follicular phase of the menstrual cycle. All the women responded with evident oestradiol increases in blood after 6 h with maximum values of between 230 and 1930 pmol per l (mean 610) at 12 h after the administration of the analogue. The mean oestradiol level at 24 h after the administration (405 pmol per l) was 2.5 times higher than the pretreatment level. The mean progesterone levels before and at 12 and 24 h after the administration of the analogue were low (1.3, 1.4 and 1.2 nmol

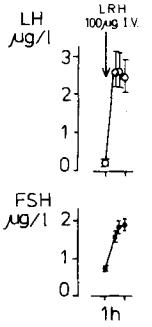

Fig. 2. LH and FSH results from intravenous LRH tests in the 10 amenorrhoeic women before the administration of the LRH analogue.

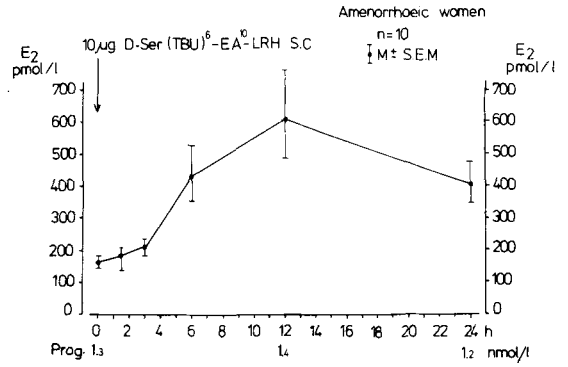

Fig. 3. Mean oestradiol (E_2) levels in blood before and after administration of 10 µg of D-Ser(TBU)^6-EA^{10}-LRH into 10 women with amenorrhoea.

per l, respectively) and did not differ significantly ($p>0.05$).

A comparison between effects on the gonadotrophin secretion of subcutaneous administration of 10 µg of D-Ser(TBU)^6-EA^{10}-LRH and 500 µg of LRH is illustrated in Fig. 4, which shows mean LH and FSH results from the five women who were given both LRH and the analogue on separate occasions. It can be seen from the figure that the results from the preceeding intravenous LRH tests did not differ on the two occasions.

After administration of 500 µg of LRH the LH level rapidly increased to a peak 45 to 60 min later. The LH level remained essentially unchanged for about four hours and then slowly declined. LH was still significantly higher than the pretreatment level at 8 h ($p<0.02$) but not at 12 h after the administration ($p>0.05$). The injection of the analogue resulted in a similar initial LH increase in blood, later followed by a second increase to a peak at 4 h after the administration. The LH level then slowly declined. Increased LH levels were observed at 12 ($p<0.001$) but not at 24 h after the administration of the analogue ($p>0.05$).

The FSH level progressively increased in similar fashion after injection of LRH and the analogue. Peak levels were reached after 4 and 6 h, respectively. The FSH levels at 6, 8, 12 and 24 h after the administration of the analogue were significantly higher ($p<0.001$) than those obtained after 500 µg of LRH. There were no significant differences ($p>0.05$) between the mean FSH levels during the first 4 h after the administration.

The mean oestradiol level in blood was signifi-

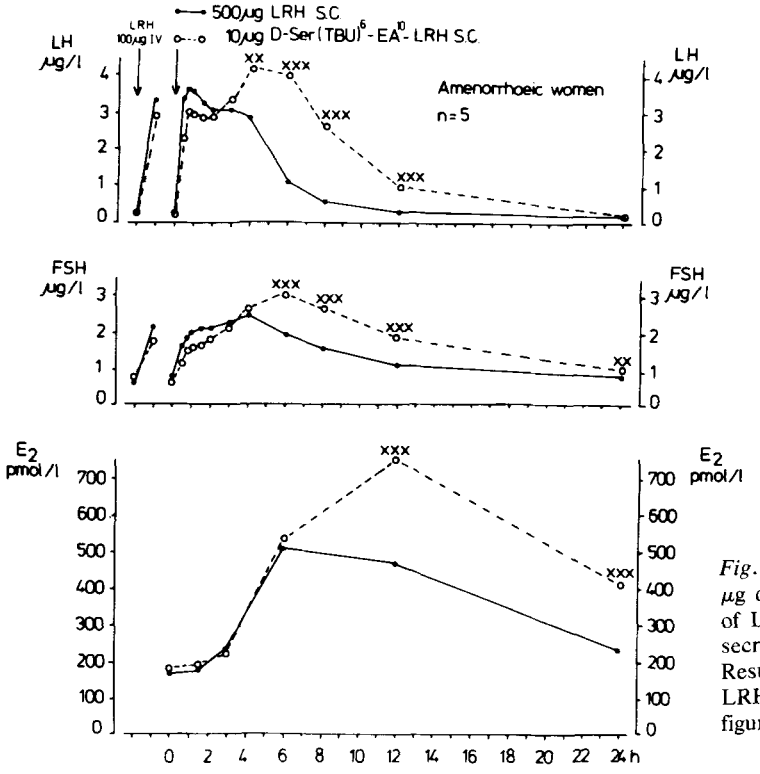

Fig. 4. A comparison between effects of 10 µg of D-Ser(TBU)^6-EA^{10}-LRH and 500 µg of LRH on LH, FSH and oestradiol (E_2) secretion in 5 women with amenorrhoea. Results from the preceding intravenous LRH tests are shown to the left on the figure. $\times\times p<0.01$, $\times\times\times p<0.001$.

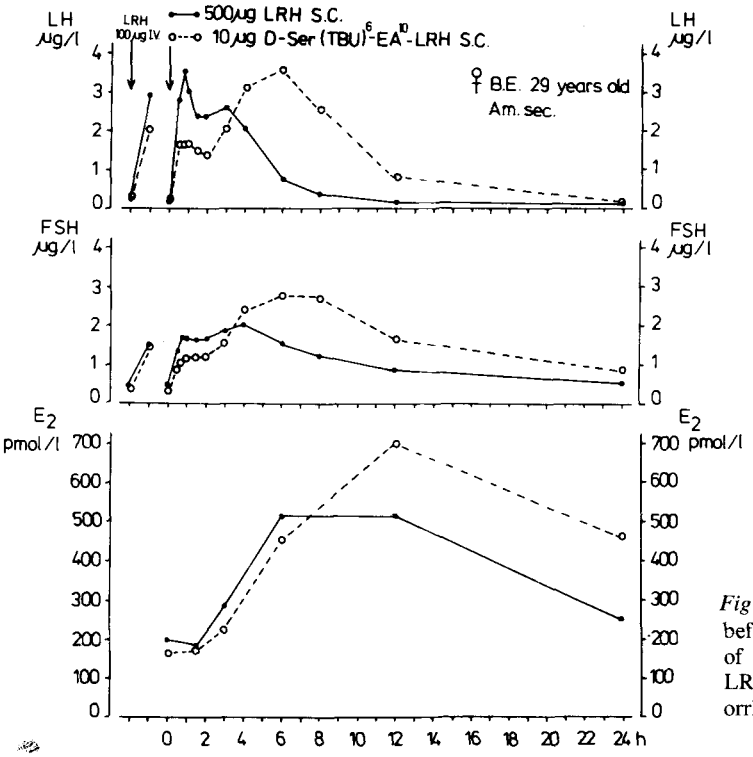

Fig. 5. LH, FSH and oestradiol (E_2) levels before and after administration of 10 µg of D-Ser(TBU)⁶-EA¹⁰-LRH and 500 µg of LRH in a 28-year-old woman with amenorrhoea.

cantly increased ($p<0.001$) over the pretreatment level at 6 h after the administration of both LRH and the analogue. Peak levels were reached after 6 and 12 h respectively. The oestradiol levels at 12 and 24 h after administration of the analogue were significantly higher ($p<0.001$) than those obtained after 500 µg of LRH. The mean oestradiol level at 24 h after administration of 500 µg of LRH was not significantly different ($p>0.05$) from the pretreatment level. There was no increase of the low pretreatment progesterone level at 12 and 24 h after the administration of either the analogue or LRH.

Blood levels of LH, FSH and oestradiol after administration of the analogue and LRH to an individual patient with amenorrhoea are shown in Fig. 5. The gonadotrophin responses to intravenous LRH were similar on the two occasions. The subcutaneous injection of 500 µg of LRH resulted in a rapid LH increase to a peak at 45 min after administration followed by a plateau-like level for 2–3 hours and then a decline down to the pretreatment level which was reached at 12 h after the administration. The initial LH peak reached at 30–60 min after subcutaneous injection of the analogue was lower than obtained after 500 µg of LRH. However, the LH level after the analogue injection increased further after 2 h and reached a second peak at 4–6 h after the administration followed by a slower fall down to basal levels.

The FSH increase after subcutaneous administration of 10 µg of the analogue was more pronounced than after 500 µg of LRH with a broad peak at 6–8 h and higher blood levels throughout the rest of the 24-hour-period. The oestradiol increase observed after injection of the analogue was also greater than that obtained after LRH (Fig. 5).

No side-effects were observed after the injection of LRH or the analogue. One of the women fainted in connection with venipuncture 3 h after administration of the analogue.

DISCUSSION

This study shows that the new LRH analogue D-Ser(TBU)⁶-EA¹⁰-LRH was capable of increasing the gonadotrophin secretion in amenorrhoeic women who had very low basal LH and low normal FSH levels. A single subcutaneous injection of 10 µg of the analogue evoked a 20-fold increase of the LH level in blood and a 5-fold increase of the FSH

level with maxima at 4 and 6 h after the administration respectively. This analogue has previously proved to be high effective in raising gonadotrophin secretion in experimental animals and normal male volunteers (3, 13, 18). Wiegelmann et al. (18) found significant LH increases after injection of 1 and 2.5 μg and dose-dependent LH increases after 5 and 10 μg of the analogue with peaks at 30 min after intravenous administration into normal men. FSH increases were only observed after the 5 and 10 μg doses with peaks after 120 min (18). Kuhl et al. (3) found that a subcutaneous injection of 5 μg of the analogue was as effective as 100 μg of LRH. However, the analogue had a depot effect and the subcutaneous dose of 5 μg was effective for 6–8 h in normal men (3). In the amenorrhoeic subjects of the present study the duration of the effect on FSH was at least 24 h and the LH level remained elevated over the basal level for at least 12 h after a single subcutaneous injection of 10 μg of the analogue.

There was an evident biphasic pattern of LH release after the single injection of the LRH analogue with early and late peaks of LH combined with a gradual, progressive FSH release. This pattern of gonadotrophin release is similar to that described by Bremner & Paulsen (1) after continous intravenous infusions of LRH into normal men. The results from their study suggested the existence of two functional pools of LH in the human pituitary, one that is acutely releasable and another that requires longer stimulation to be released (1). In women, this biphasic pattern of LH response to extended stimulation by constant infusion of LRH is most evident during the early follicular phase of the cycle (16).

The amenorrhoeic subjects had oestrogen levels similar to those found in the early follicular phase of the cycle. When they were given a single injection of the large dose of 500 μg of LRH, there was a pronounced first initial peak of acutely releaseable LH followed by continued LH release for the next 3–4 hours but no evident second peak of LH release was observed in contrast to what was found after administration of the analogue. A single injection of 10 μg of the LRH analogue resulted in a comparable initial LH peak but then it also evoked a great release of LH from the second pool. This pool may be regarded as a pituitary reserve with a component of yet unmeasurable amounts of newly synthesized gonadotrophins (4). The great activation of the second pool by the single dose of the LRH analogue shows that it exerts a more extended pituitary stimulation than a single large dose of LRH. This prolonged action of the LRH analogue may be due to a better binding to the pituitary receptors, a slower inactivation or a combination of both factors (15).

The gonadotrophins released into the blood by the LRH analogue stimulated the ovaries to an increased steroid secretion and all the women had evident oestradiol elevations in blood during the last 18 hours of the 24 h study period with maximum at 12 h after the administration. The gonadal response was more pronounced after administration of the analogue than after the large dose of LRH, another illustration of the more prolonged and potent action of the LRH analogue.

Thus, the present study in women confirms previous studies in men and shows that D-Ser(TBU)^{6}-EA^{10}-LRH is a potent stimulatory LRH analogue with prolonged biological activity. The marked acute effects on the gonadotrophin and gonadal steroid secretion in amenorrhoeic women suggest that this LRH analogue will be useful therapeutically. It might be possible to simplify the present therapeutic regimens by using this potent and long-acting LRH analogue for chronic LRH treatment.

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