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

Göran Skarin, Sven Johan Nillius and Leif Wide

Departments of Obstetrics and Gynaecology and Clinical Chemistry, University Hospital, Uppsala, Sweden

ABSTRACT

The agonistic analogue of luteinizing hormone-releasing hormone (LRH) D-Ser-(TBU)^{6}-EA^{10}-LRH was given subcutaneously or intranasally to 21 women with longstanding secondary amenorrhoea in an attempt to induce follicular maturation and ovulation. Seven women were given daily subcutaneous injections of 10 µg of the LRH analogue for 21 days. The pituitary and ovarian responses were monitored by follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol (E_2) and progesterone (P) in serum. A significant increase of the E_2 levels in blood was seen during the first three days of treatment but the E_2 elevations were not sustained. No signs of follicular maturation were detected during the treatment. Intranasal treatment with the LRH agonist was instituted in 14 women. Repeated daily low intranasal spray doses (1 - 10 µg) were given. The treatment was continued for 2 - 4 weeks. Intranasal doses of 5 µg and below were ineffective in increasing gonadotrophin and gonadal steroid secretion. An initial gonadotrophin release was observed after intranasal doses of 10 µg and there was a slight increase of the E_2 concentrations in serum during the first 2 - 3 days. The pituitary responsiveness to the agonist then decreased markedly during the treatment. None of the 14 women showed signs of follicular maturation during or after the treatment. The pituitary desensitization caused by this potent agonistic LRH analogue seems to limit its therapeutic use for treatment of anovulatory infertility.

INTRODUCTION

Stimulatory analogues of the gonadotrophin-releasing hormone LRH have been developed during recent years to simplify LRH treatment of anovulatory infertility (13). One of the most potent stimulatory analogues of LRH is D-Ser(TBU)^{6}-EA^{10}-LRH (11). We have previously shown that acute administration of this long-acting LRH agonist has a marked stimulatory effect on the gonadotrophin secretion in women with amenorrhoea (7). Here we report on chronic effects of this potent stimulatory LRH analogue on the gonadotrophin and

gonadal steroid secretion in amenorrhoeic women with low or absent endogenous oestrogen production.

PATIENTS AND METHODS

Twenty-one women, aged 17 - 32 years (mean 26 years), with long-standing amenorrhoea (more than 12 months) volunteered for the study. In 18 women, the amenorrhoea developed in connection with self-imposed weight-loss. Ten of them had regained weight while 8 were still underweight at the time of the study. They all had normal thyroid, adrenal and hepatic function. The prolactin levels in serum were normal. The serum gonadotrophin levels were low or normal. The endogenous oestrogen production was low, as judged by the lack of response with withdrawal bleeding after intramuscular progesterone injection and the serum levels of oestradiol.

LRH agonist treatment

The stimulatory LRH analogue D-Ser(TBU)^{6}-EA^{10}-LRH (Hoe 766, Hoechst AG, Frankfurt/Main, FRG) was administered either subcutaneously or intranasally.

Subcutaneous treatment.

Seven women were given daily subcutaneous injections of 10 µg of the LRH agonist for 21 days. Repeated venous blood samples were taken before and during the first 24 hours of treatment and then once daily, immediately before the LRH agonist injection.

Intranasal treatment.

Fourteen women were given repeated intranasal spray doses of the LRH agonist for 16 - 30 days. Four groups of three women were given intranasal doses of 1, 2, 5 and 10 µg, respectively, every fourth hour five times daily for 16 days. Frequent venous blood samples were taken before and during the first 24 hours of treatment. This was repeated after 14 days of treatment. Blood samples were also obtained every second or every fourth day before the first daily spray administration. Two women were given intranasal doses of 2 µg of the LRH agonist every second hour nine times daily for 15 days followed by 4 µg every second hour nine times daily for another 15 days. Venous blood samples were obtained as described above.

Hormone assay methods

Immunoreactive FSH and LH in serum were assayed by a radioimmunosorbent technique with indirectly coupled antibodies (14). The results were expressed in µg per l using highly purified FSH and LH preparations as reference standards (9,10). Immunoreactive oestradiol in serum was measured by a radio-

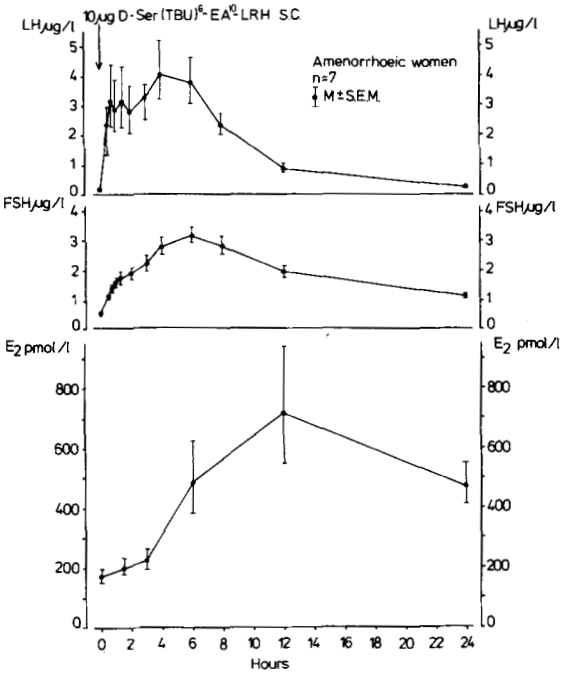

Fig. 1. Mean serum levels of LH and FSH before and after subcutaneous administration of 10 µg of D-Ser(TBU)^{6}-EA^{10}-LRH in seven women with amenorrhoea.

immunological technique using an antiserum to an oestradiol-6-oxime-BSA conjugate (4). Progesterone was assayed by a similar radioimmunological technique. The reference ranges for these hormone analyses at the time of the study were as follows: FSH (women of fertile age): 0.5 - 3.0 µg/l; LH (follicular and luteal phase): 0.4 - 3.0 µg/l, (midcycle peak): 5 - 20 µg/l; oestradiol (early follicular and late luteal phase): 60 - 200 pmol/l, (midcycle peak): 500 - 1300 pmol/l; progesterone: (follicular phase): 3 nmol/l, (at ovulation): 3 - 6 nmol/l, (luteal phase): 6 - 60 nmol/l.

Statistical methods.

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

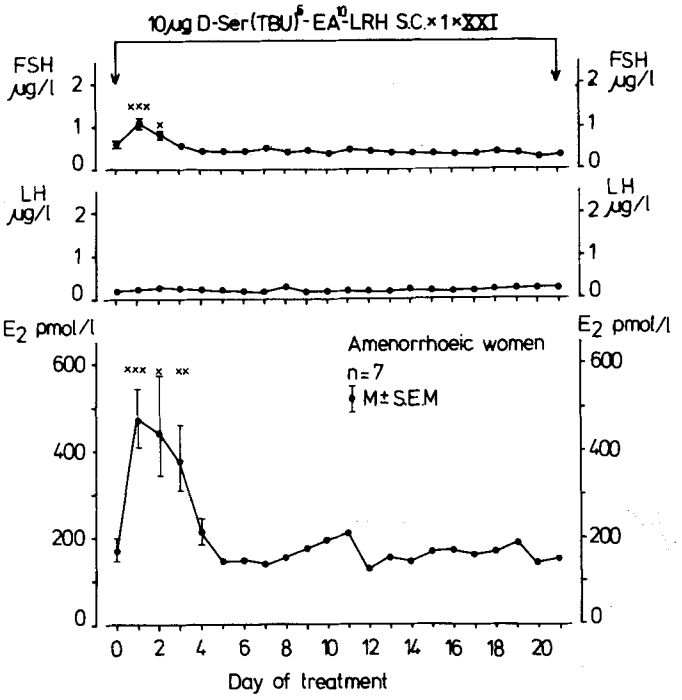

Fig. 2. Mean serum levels of FSH, LH and E_2 before and during daily administration of 10 µg of D-Ser(TBU)^6-EA^{10}-LRH for 21 days in seven women with amenorrhoea.

RESULTS

Subcutaneous agonist treatment.

Mean LH and FSH levels in serum before and during the first day of treatment with 10 µg of the LRH agonist given subcutaneously are shown in Fig. 1. There was a marked acute increase of both LH and FSH secretion. The stimulatory effects on the gonadotrophin and gonadal steroid secretion were not sustained (Fig. 2). Follicular maturation and ovulation did not occur in any of the women.

Intranasal agonist treatment.

The gonadotrophin responses during the first 24 hours after repeated administration of 1, 2 and 5 µg of the LRH agonist are shown in Fig. 3. There were no changes in either the FSH or LH levels in serum during the first 24 hours of the LRH agonist treatment. Nor were the gonadotrophin or E_2 levels affected during daily treatment with the same regimens for 16 days. This is illustrated by Fig. 4, which shows the results of daily treatment with repeated 5 µg doses of the LRH agonist.

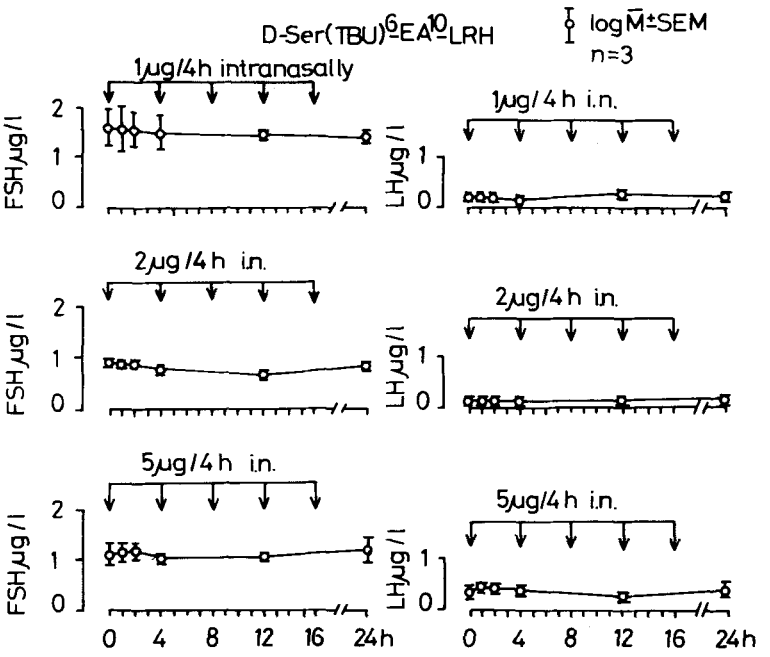

Fig. 3. Mean serum levels of FSH and LH before, during and after repeated intranasal administration of 1, 2 and 5 µg of D-Ser(TBU)^{6}-EA^{10}-LRH every 4 hours in women with amenorrhoea.

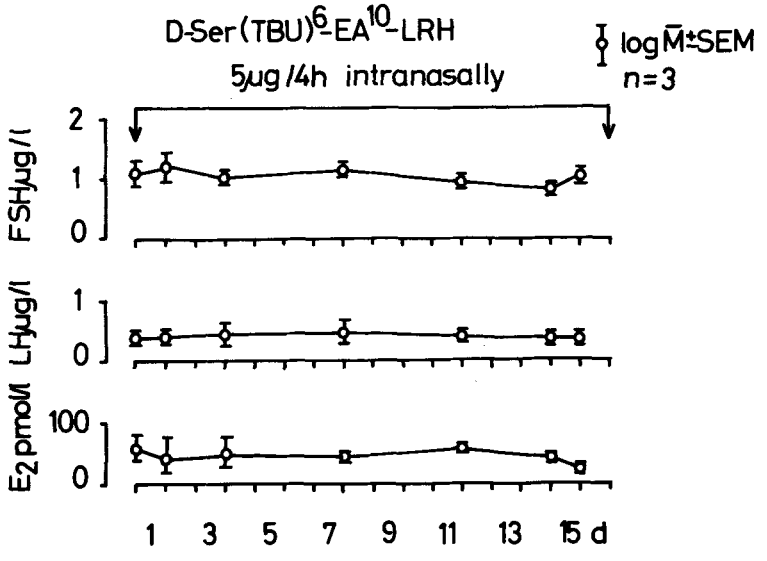

Fig. 4. Mean serum levels of FSH, LH and E_2 before and during daily intranasal administration of repeated 5 µg doses of D-Ser(TBU)^{6}-EA^{10}-LRH in three women with amenorrhoea.

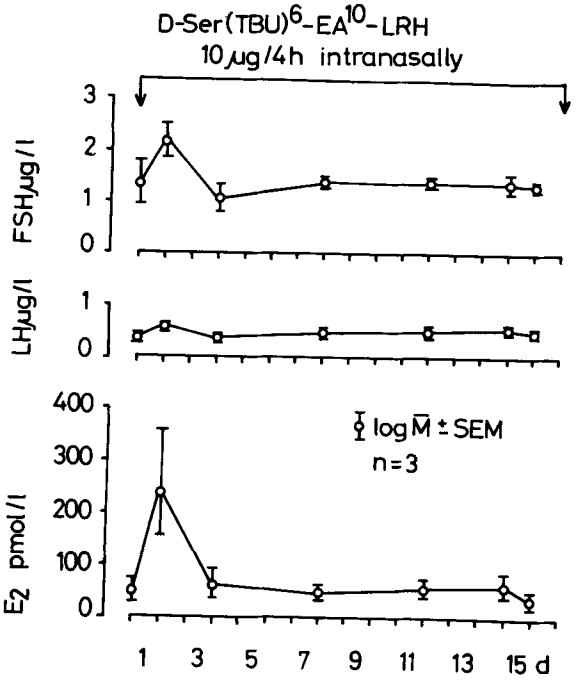

Fig. 5. Mean serum levels of FSH, LH and E_2 before and during daily intranasal administration of repeated 10 µg doses of D-Ser(TBU)^6-EA^{10}-LRH for 16 days in three women with amenorrhoea.

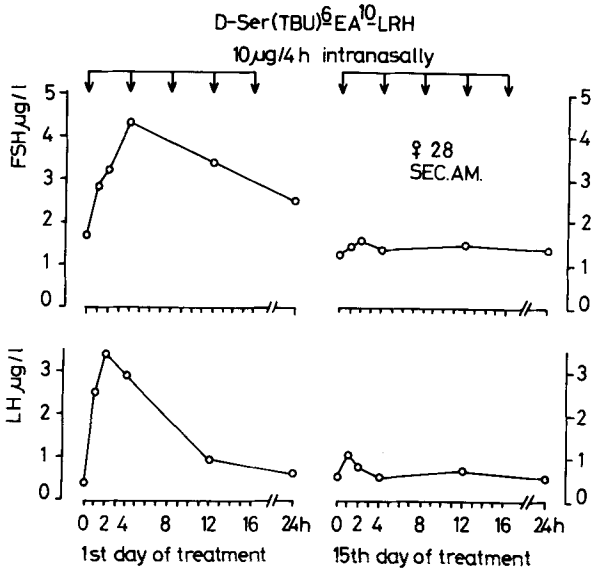

Fig. 6. FSH and LH levels in serum before and after repeated intranasal administration of 10 µg of D-Ser(TBU)^6-EA^{10}-LRH during the first and 15th day of treatment in a 28-year-old woman with amenorrhoea.

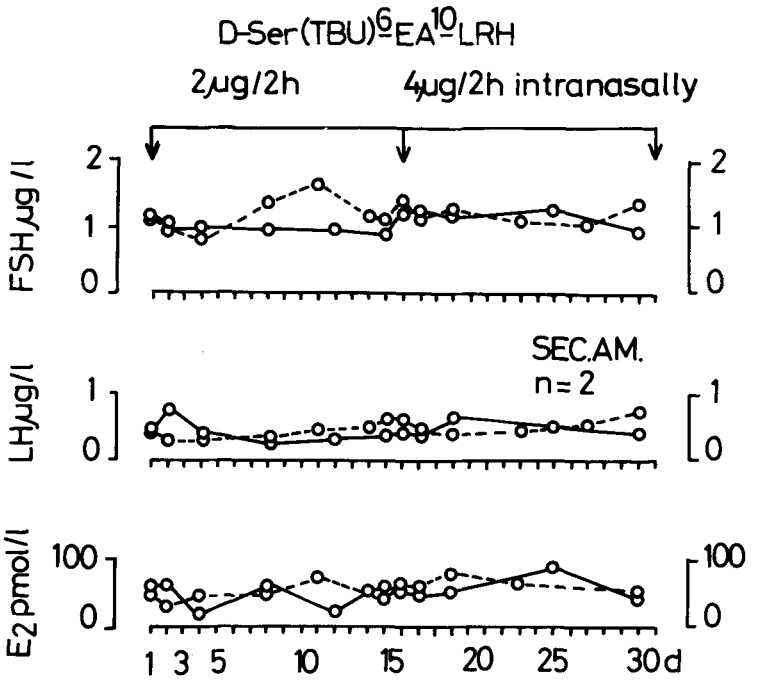

Fig. 7. FSH, LH and E_2 levels in serum before and during daily treatment with repeated intranasal doses of 2 µg of D-Ser(TBU)^6-EA^{10}-LRH for 15 days followed by repeated intranasal doses of 4 µg for another 15 days in two women with amenorrhoea.

Daily treatment with repeated intranasal doses of 10 µg of the LRH agonist resulted in an increase of the gonadotrophin and oestrogen levels in serum during the first 24 hours (Fig. 5). The stimulatory effects on the gonadotrophin and oestrogen secretion were not sustained during chronic treatment (Fig. 5). The gonadotrophin response to repeated 10 µg doses in one of the women during the first and 15th treatment day are shown in Fig. 6. There was a marked FSH and LH increase during the first day of treatment. After 14 days of treatment there was no evident effect on the gonadotrophin levels in serum by administration of the agonist (Fig. 6).

The gonadotrophin and E_2 levels in serum of the two women treated with 2 and 4 µg of the LRH agonist every second hour during daytime for 30 days are shown in Fig. 7. There were no changes in the hormone levels during the treatment.

None of the 14 amenorrhoeic women treated with repeated intranasal administration of low doses of the LRH agonist showed any signs of follicular maturation and ovulation during or after the treatment period.

DISCUSSION

The hypothalamic gonadotrophin-releasing hormone LRH was isolated by Schally and co-workers in 1971 (12). The hormone was expected to be useful for stimulation of fertility in hypogonadal men and women. Clinical trials were performed over several years before an effective therapeutic regimen was established. Chronic treatment with 8-hourly subcutaneous injections of 500 µg of LRH proved to be effective in inducing potency and spermatogenesis in hypogonadal men (5) and follicular maturation and ovulation in women with amenorrhoea (7). Potent and long-acting stimulatory analogues of LRH have been developed during recent years to facilitate the LRH treatment (13). In the present study one of these synthetic agonistic analogues of LRH was used in an attempt to induce follicular maturation and ovulation in women with secondary amenorrhoea. The stimulatory LRH analogue had previously proved to be very active when given acutely to such women (7). This was confirmed in the present study. However, the stimulatory effect on the gonadotrophin secretion was not sustained during chronic treatment and it was not possible to induce follicular maturation and ovulation in the amenorrhoeic women.

The LRH agonist was found to exert a prolonged effect on the pituitary gonadotrophin secretion similar to that seen during constant infusion of LRH. Normally, LRH seems to be secreted episodically from the hypothalamus (3). In rhesus monkeys with hypothalamic lesions, Belchetz and associates found that constant infusion of LRH failed to restore sustained gonadotrophin secretion but intermittent administration of LRH established normal pituitary gonadotrophin secretion (1). The authors suggested that the constant stimulation of the pituitary gonadotrophs by LRH caused a desensitization of the processes responsible for gonadotrophin release. It is possible that the long-acting stimulatory LRH analogue has a similar inhibitory effect on the pituitary gonadotrophin secretion when given daily in a large dose.

In an attempt to mimic the physiological pulsatile pattern of LRH secretion, low doses of the agonist were given repeatedly during daytime to women with amenorrhoea. This regimen of intermittent LRH administration was facilitated by the fact that the LRH agonist is active when given intranasally (11). However, this intermittent intranasal treatment also proved to be ineffective in inducing follicular growth and maturation. The intranasal dose which was effective in stimulating acute gonadotrophin release proved to cause pituitary desensitization during chronic treatment.

Thus, long-term treatment with the potent stimulatory LRH analogue D-Ser(TBU)^{6}-EA^{10}-LRH has so far only resulted in desensitization of the processes responsible for gonadotrophin release. This effect can be utilized for inhibition of ovulation in normally menstruating women, a new approach to contraception (8,2). An effective therapeutic regimen for LRH agonists

in amenorrhoea has not yet been established. Further studies are needed to define the proper place of stimulatory LRH analogues in the treatment of anovulatory infertility.

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Address for reprints:

Göran Skarin
Department of Obstetrics and Gynaecology
University Hospital
S-750 14 UPPSALA 14
SWEDEN