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Effects of Prolonged Luteinizing Hormone-releasing Hormone Therapy on Follicular Maturation, Ovulation and Corpus Luteum Function in Amenorrhoeic Women with Anorexia Nervosa

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ABSTRACT

Nine amenorrhoeic women with anorexia nervosa (AN) were given long-term treatment with 500 μg of synthetic luteinizing hormone-releasing hormone (LRH) every 8 h. All the women had impaired luteinizing hormone (LH) secretion and no evidence of endogenous oestrogen production. Three of them also had deficient follicle-stimulating hormone (FSH) secretion. The pituitary reserve capacity for gonadotrophin secretion was normal but the response pattern to LRH was similar to that described in prepubertal girls. The constant administration of LRH normalized basal LH and FSH secretion and induced a cyclical gonadotrophin secretory pattern with differential changes of the LH and FSH responses to LRH during the treatment. LRH-induced gonadotrophin secretion produced follicular growth and maturation in all the women. Presumptive ovulation also occurred during the 8 treatment courses in which only LRH was administered. However, inadequate luteal phases were observed during 6 of these 8 cycles. Combined therapy with LRH and human chorionic gonadotrophin (HCG) during 5 treatment courses resulted in normal ovulatory cycles with adequate corpus luteum function.

INTRODUCTION

Anorexia nervosa is characterized endocrinologically by an impairment of the gonadotrophin secretion from the anterior pituitary. The impairment is more marked for LH than for FSH secretion. The pituitary responsiveness to LRH is reduced in acute stages of AN and restored to normal after clinical improvement with regain of body weight (20, 28, 32, 3, 18). Repeated stimulation with LRH can also restore the pituitary reserve capacity for gonadotrophin secretion to normal (16, 19, 36). By long-term therapy with LRH it is possible to induce normal ovulatory menstrual cycles in amenorrhoeic women with AN (16, 19). However, cycles with luteal phase defects often occur during prolonged treatments with only LRH (16, 19).

Here we analyze 13 menstrual cycles induced by LRH in 9 amenorrhoeic women with AN in an attempt to disclose the mechanism(s) behind the luteal phase defects.

MATERIAL and METHODS

Patients

Nine women, aged 20-33 (mean 25.4) years, with symptoms of AN volunteered for the study. They all had secondary amenorrhoea of 8 months' to 13 years' (median 20 months) duration. Six women were judged by an experienced psychiatrist to fulfill the diagnostic criteria of true AN (4) while the other three were labelled as anorectic behaviour, considered as a mitigated form of AN (7). Their mean body weight 42.8 kg (range 36-47) which corresponds to 72% (range 63-88) of mean ideal body weight (8). No organic cause of the amenorrhoea and weight loss was found at the clinical investigation. Buccal smear chromatin complement showed a normal female pattern. X-ray examinations of the skull and pituitary fossa were normal. All the patients were euthyroid, as judged by clinical examination and thyroid function tests. The 24-hour urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids was normal in all except one patient, who had low basal levels which increased normally after metyrapone. Testosterone and prolactin levels in blood were within the normal ranges.

All the patients had low or absent endogenous oestrogen production, as judged both by indirect clinical methods for estimating oestrogenic activity on target organs (no withdrawal bleeding after intramuscular progesterone etc.) and direct measurements of oestrogen levels in blood.

LRH treatment

Intravenous LRH test (100 µg, Hoechst) were performed before, during and after the treatments. The FSH and LH response to LRH was defined as the difference between the mean of the two values at 30 and 45 minutes after the LRH injection and the mean of the two control values.

Five hundred µg of LRH (Hoechst) were administered subcutaneously or intramuscularly every 8 h during the treatment. Seven women were treated with only LRH during 8 treatment cycles. During treatment frequent determinations of FSH, LH, oestradiol (E_2) and progesterone in blood were made. Oestrogen monitoring was performed by estimation of the total urinary oestrogen (TE) excretion in all but two of the women. The treatment with LRH alone was continued until menstruation occurred.

Four women were treated with LRH in combination with HCG during 5 treatment cycles monitored by daily determinations of E_2 in blood or TE in urine. When oestrogens levels consistent with follicular maturation were reached, the LRH injections were interrupted and a single intramuscular injection of 6-9000 IU of HCG (Pregnyl^R, Organon) was administered. After that, 1-4 injections of 1500-6000 IU of HCG were given at intervals of 3-7 days.

Hormone assay methods

Immunoreactive FSH and LH in serum were assayed by the radioimmunosorbent technique with indirectly coupled antibodies (33). LH in serum was measured by utilizing human pituitary LH (22) labelled with ^{125}I and rabbit antihuman 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 (21) 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 were expressed in ng of the purified LH and FSH preparations per ml of serum. Comparisons between FSH and LH levels were made in relation to the geometric mean FSH/LH ratio (approximately = 1) of the normal menstrual cycle.

Immunoreactive E_2 was measured by a radioimmunological technique using an antiserum to oestradiol-6-oxime (11). Progesterone was assayed by a similar method (31). One pg of E_2/ml = 3.67 pmol/l and 1ng of progesterone/ml = 3.12 nmol/l.

RESULTS

Pretreatment gonadotrophin levels in serum before and after intravenous LRH are shown in Fig. 1. The basal LH levels were below the normal range in all the patients while only three of the patients had abnormally low basal FSH levels. All but one of the AN patients responded to LRH with evident release of LH and FSH. The mean LH response was similar to that of the control group of women in the early follicular phase of the menstrual cycle while the FSH response was 4 times larger (Fig. 1). The pretreatment FSH/LH ratio of the gonadotrophin responses to LRH was 1.04 in comparison with 0.22 for the control subjects.

Treatment with LRH alone

Five hundred µg of LRH was administered parenterally every 8 hours to 7 women until menstruation occurred after 24-36 (mean 29.5) days of treatment. Follicular growth and maturation, as judged by increased oestrogen levels in urine or blood, were induced during all the 8 treatment cycles. Ovulation, as judged by progesterone values of more than 3 ng/ml in single blood samples taken 3-10 days before menstruation (9), was also induced in all the cycles. Basal body temperatures were recorded during 5 treatment cycles and all the curves were biphasic. The maximum progesterone concentrations observed were less than 10 ng/ml in all but 2 cycles (26.4 and 13.8 ng/ml). In the four cycles were blood samples were obtained at least every 4th day, the maximum progesterone value was 7.7 ng/ml, suggesting insufficient corpus luteum function. The length of the luteal phase was 12-16 days.

Hormone levels during a presumptively ovulatory menstrual cycle induced by LRH alone are shown in Fig. 2. During the first three days of treatment the FSH responses were larger than the LH responses. Then the FSH responses

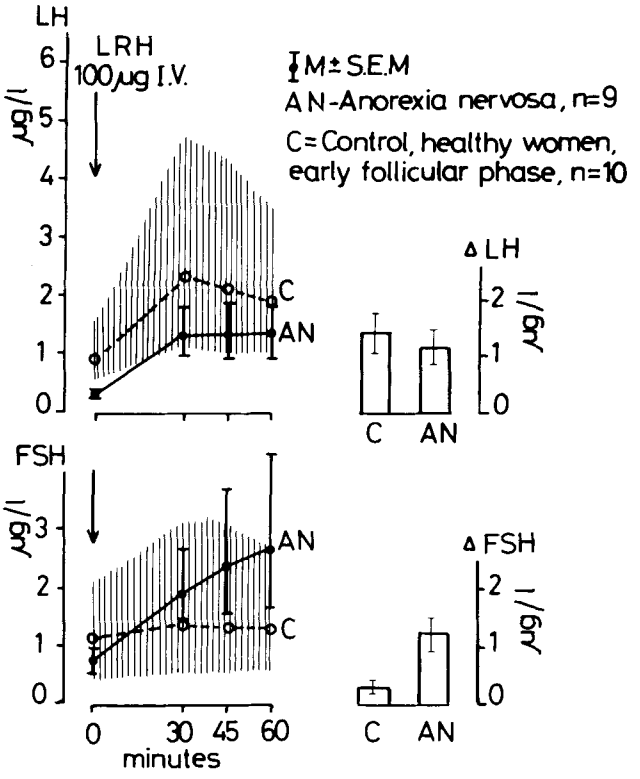

Fig. 1. Mean LH and FSH levels before and after intravenous LRH (left) and mean LH and FSH responses to LRH (right) before LRH treatment of 9 women AN.

decreased while the LH responses progressively increased. There was slow increase of E_2 in serum during the first week of treatment followed by a more rapid increase to a peak level (350 pg/ml) consistent with follicular maturation on treatment day 13. At that time LH in serum reached a maximum with a level similar to that seen during the midcycle peak in the normal menstrual cycle. The LH peak was not accompanied by an evident FSH peak. After the LH peak the E_2 level decreased somewhat and at the same time increased progesterone levels were observed, indicating that ovulation presumably occurred. Progesterone in blood slowly increased to reach a plateau of 1-14 ng/ml one week later. Increased progesterone levels in blood were seen for 12-14 days before menstruation.

One week later, a new treatment course was initiated (Fig. 3). The pretreatment LRH test resulted in small gonadotrophin increases from low basal values with a greater response of LH than of FSH. The FSH response increased slightly during the first days of treatment but the FSH levels after LRH

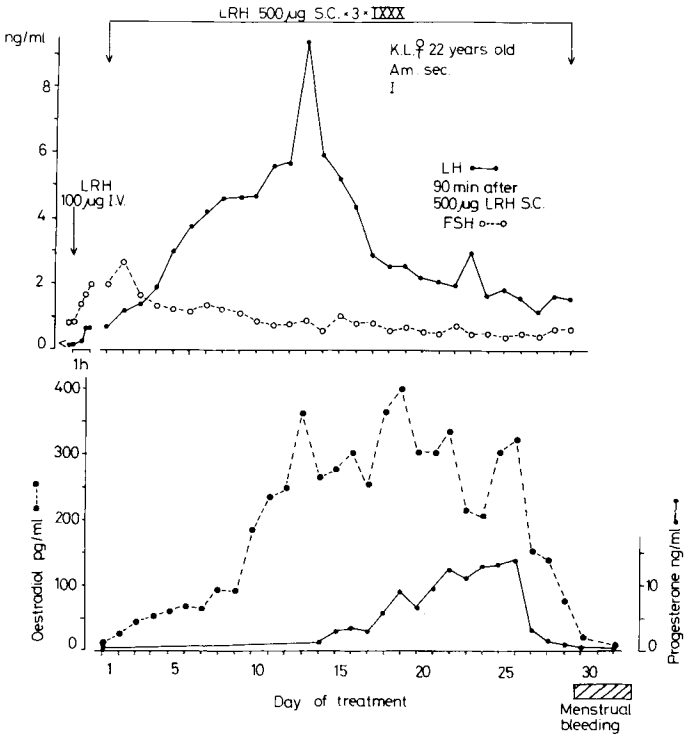

Fig. 2. Basal LH and FSH levels in serum as well as FSH and LH responses to LRH and serum levels of E_2 and progesterone before, during and after the first LRH treatment of a 22-year-old patient with AN.

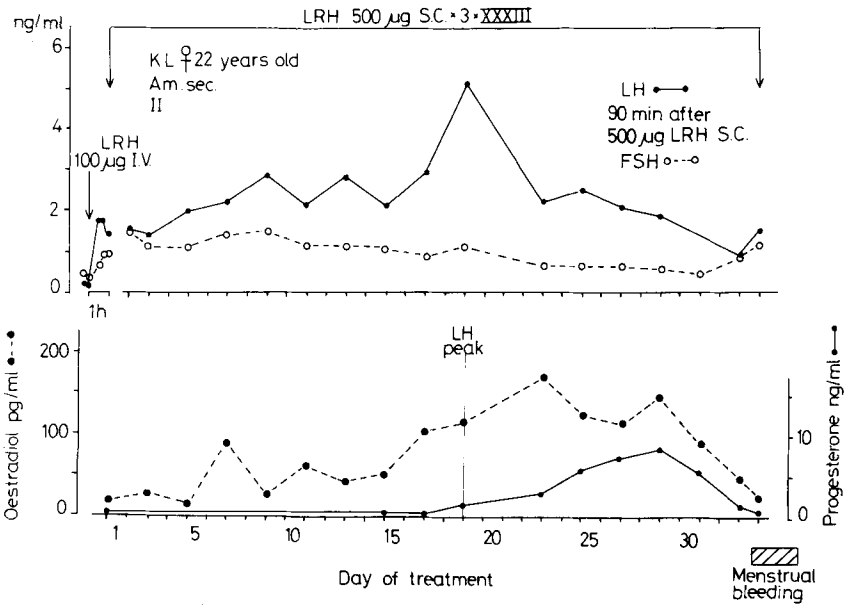

Fig. 3. LH, FSH, E_2 and progesterone levels in serum before, during and after the second LRH treatment of the AN patient KL from fig. 2.

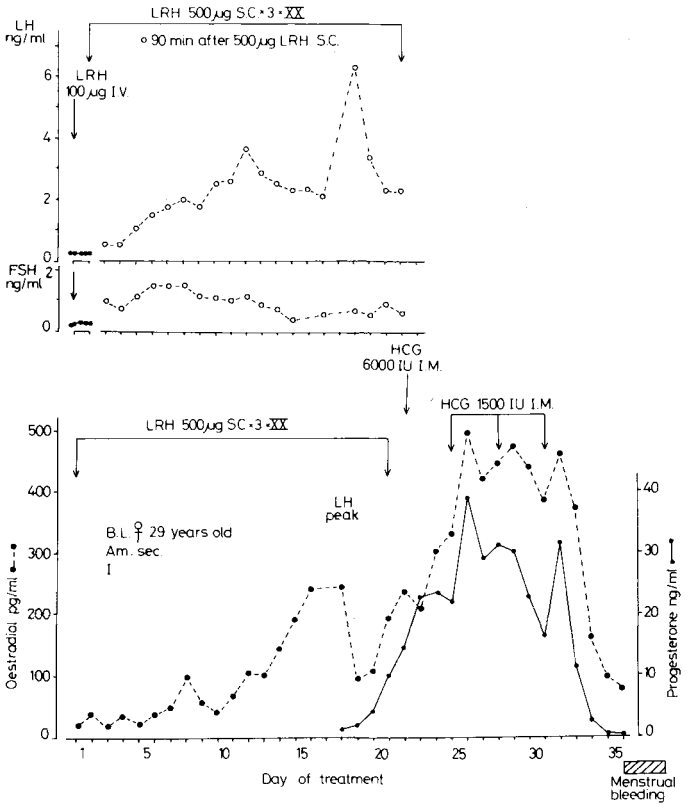

Fig. 4. Basal LH and FSH levels in serum as well as LH and FSH responses to LRH and serum levels of E_2 and progesterone before and during the first treatment with LRH in combination with HCG of a 29-year-old woman with AN.

remained below the LH levels throughout the treatment. There was a small and very slow E_2 increase and there was no evident midcycle E_2 peak. On treatment day 19, when the maximum LH response to LRH was observed, the E_2 levels was only 120 pg/ml. After the LH peak, there was a slow increase of progesterone with a maximum of 8.2 ng/ml on day 29.

Treatment with LRH in combination with HCG.

Follicular maturation and ovulation were induced by combined therapy with LRH and HCG in 5 treatment cycles. The corpus luteum function was adequate, as judged by high normal progesterone levels in blood. Results from a combined LRH-HCG treatment of an infertile amenorrhoeic patient with a complete lack of pituitary responsiveness to LRH before the treatment, are shown in Fig. 4. During the prolonged LRH treatment both LH and FSH responses to LRH appeared. The FSH response was more marked than the LH response during the first 3 days and a prepubertal-like FSH/LH ratio of the responses were seen during the first week of treatment. Then the LH response progressively increased while the FSH

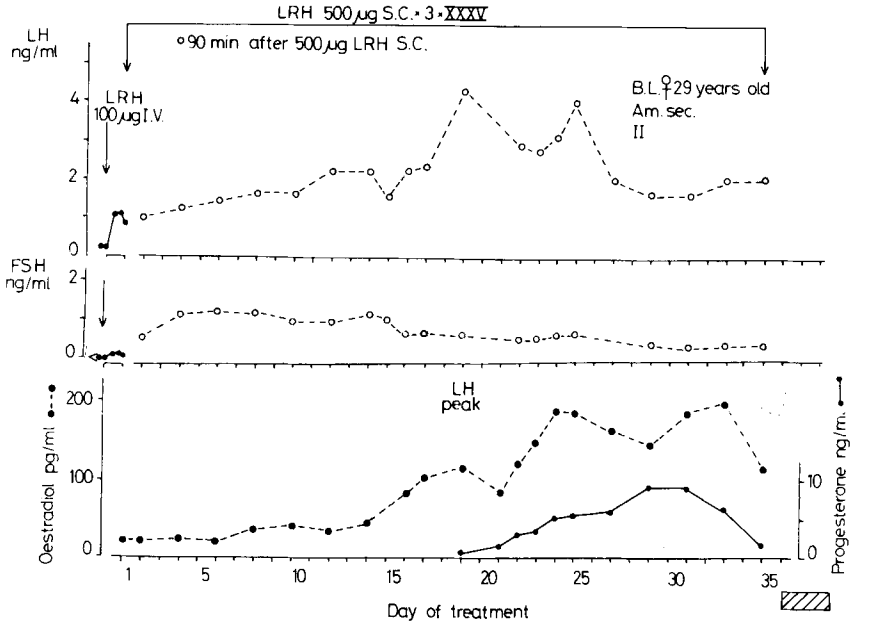

Fig. 5. LH, FSH, E_2 and progesterone levels in serum as well as LH and FSH responses to LRH before and during the second LRH treatment of the AN patient BL from fig. 4.

response decreased. At the same time, the E_2 secretion started to rise. On treatment day 18, the LH level reached a midcycle-like peak, which was followed by increased progesterone levels in blood. The characteristic fall of the E_2 level concomitant with the increase of the progesterone level, found at ovulation in the normal menstrual cycle, was seen during the LRH induced cycle of this patient. The progesterone concentration was 10.7 ng/ml when HCG was administered, indicating that ovulation had already occurred at that time. Repeated injections of HCG were then given during the luteal phase, which was slightly prolonged with high E_2 and high-normal progesterone levels.

On menstrual day 5 a new treatment with only LRH was instituted (Fig. 5). Before this second LRH treatment, there was only a small LH response but no evident FSH response to intravenous LRH. During the first day of treatment, the FSH response reappeared but the FSH levels after LRH never became higher than the LH levels. After about 10 days of treatment there was a very small and slow increase of E_2 in blood. When the maximal LH response to LRH was observed on treatment day 19, the E_2 level was only 119 pg/ml. Progesterone started to increase slowly and there was a further E_2 increase to a plateau with levels between 150 and 200 pg/ml. Increased progesterone levels in blood were observed for 13 days before menstruation with maximum of 8.5 ng/ml on treatment days 29 and 31.

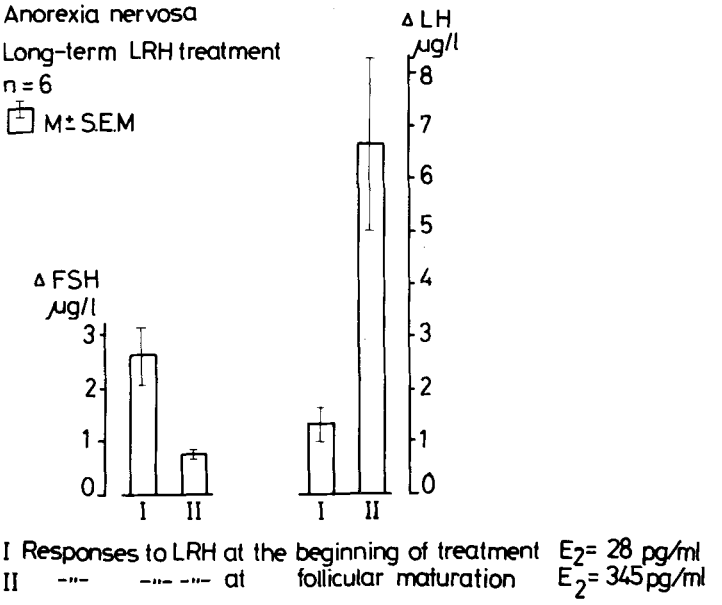

Fig. 6. Mean FSH and LH responses to LRH in 6 women with AN at the beginning of the prolonged LRH treatment and at follicular maturation during the treatment.

Changes in the pituitary responsiveness to LRH during prolonged LRH treatment.

The alterations in the pituitary gonadotrophin responses to LRH during chronic administration of LRH are summarized in Fig. 6, which is a composite illustration of FSH and LH levels after LRH administration at the beginning of and after 10-19 days of treatment with 500 µg of LRH every 8 hours in 6 of the women with AN. During the first days of treatment, at a mean serum E_2 level of 28 pg/ml, the FSH level after LRH was twice as high as the LH level, an inverted prepubertal-like response pattern. After 14 days of treatment, on average, at a mean E_2 level (345 pg/ml) consistent with follicular maturation, the situation had changed dramatically. The mean LH concentration in blood after LRH had risen to a level similar to that observed during the midcycle peak in the normal menstrual cycle. The LH level after LRH was 9 times higher than the FSH level, which had decreased considerably at that time.

The FSH and LH responses to LRH before, during and after two consecutive LRH-HCG treatments of one of the AN patients (Fig. 7) illustrate the changes in the pituitary responsiveness to LRH which take place during prolonged treatments with LRH. Before the first treatment course, the basal LH levels were very low but there was a normal response to LRH. The basal FSH level was normal and the FSH response to LRH was 10 times greater than the average FSH response in healthy women in the early follicular phase of the menstrual cycle. During the therapy with 500 µg of LRH every 8 hours, there was a progressive decrease of the great FSH response. At follicular maturation on treatment day 10, there was a slightly increased LH response but no longer any FSH response to LRH. HCG was

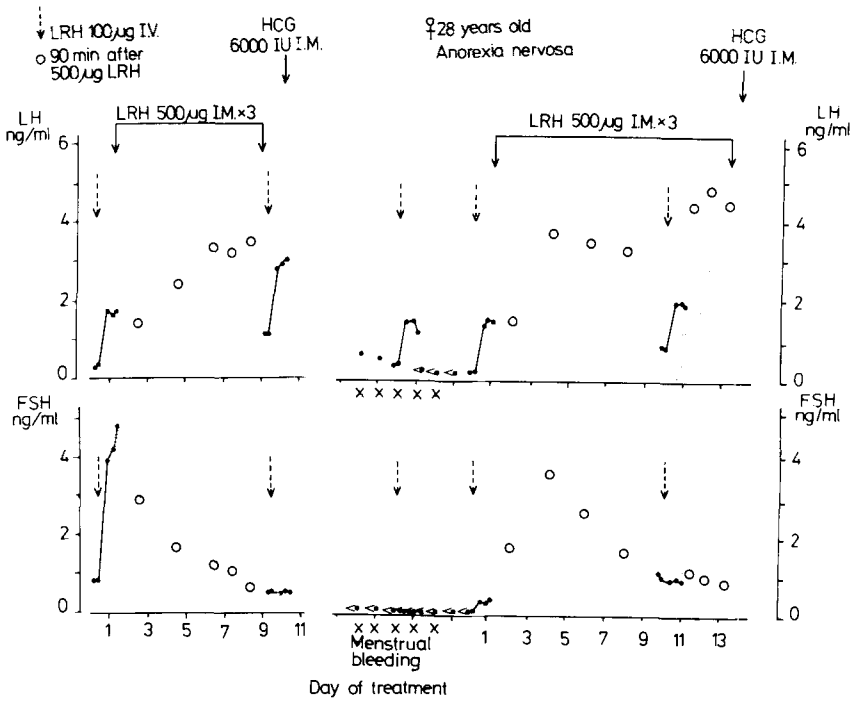

Fig. 7. Basal LH and FSH levels in serum as well as LH and FSH responses to LRH before, during and after two consecutive LRH treatments of a 28-year-old woman with AN.

then given to induce ovulation. A second HCG injection was given one week later to support corpus luteum function. Menstruation occurred 15 days after the first HCG injection.

After the first treatment, on menstrual day 3, the basal FSH levels were below the detection limit of the assay and there was no FSH response to LRH. Four days later, there was a very small but significant FSH release after LRH from unmeasurable basal levels. During the first days of LRH therapy, the FSH response to LRH increased markedly and reached a high maximum on treatment day 4, when the E_2 level was still low. The FSH response then progressively decreased. On treatment day 10 there was no longer any FSH response to LRH. The LH levels 90 min after the 500 µg dose of LRH increased during the treatment and were maximal after 12-14 days. However, these LH levels were apparently not high enough to induce ovulation as no progesterone increase was observed at that time. HCG was therefore given to induce ovulation and pregnancy occurred in this infertile patient with 13 years' of amenorrhoea. One healthy child was delivered at term.

Only two (patient KL and BL) of the other 8 women were involuntarily sterile. They were treated by LRH alone or in combination with HCG four times (Figs. 2-5) but did not conceive during the treatments.

DISCUSSION

This study shows that the impaired gonadotrophin secretion in women with AN can be restored to normal by long-term treatment with LRH. Constant administration of the single gonadotrophin-releasing hormone not only normalized basal FSH and LH secretion but also induced a cyclical gonadotrophin secretory pattern with differential changes of the LH and FSH responses to LRH during the treatment. LRH-induced gonadotrophin secretion initiated follicular growth and a normal ovarian cycle with follicular maturation and ovulation could be produced in amenorrhoeic women who were devoid of ovarian activity before the treatment. The results suggest that the impaired gonadotrophin secretion in amenorrhoeic women with AN is due to a supra-pituitary disturbance with deficient secretion of endogenous gonadotrophin-releasing hormone from the hypothalamus.

The LH secretion was reduced in all the 9 patients with AN but the pituitary reserve capacity for LH secretion was normal in all but one woman. The pituitary FSH secretion was unimpaired in most patients and the pituitary capacity to release FSH in response to LRH was, on average, four times greater than in healthy women in the early follicular phase of the menstrual cycle. The pretreatment FSH/LH ratio, both in the basal state and after stimulation with LRH, was therefore much greater than in healthy women of fertile age and similar to that described in prepubertal children (10, 23, 5). One of the AN patients had neither any LH nor FSH response to LRH before the treatment but her pituitary responsiveness was restored to normal after repeated stimulation with LRH. Thus, unresponsiveness in a diagnostic LRH test does not necessarily indicate primary pituitary failure but may be due to dysfunction at the hypothalamic level with insufficient hypothalamic stimulation of the pituitary gonadotrophs by endogenous gonadotrophin-releasing hormone. Nor does a lack of gonadotrophin response to acute LRH stimulation preclude successful results of long-term LRH therapy as shown by the induction of both follicular maturation and ovulation by LRH in the patient with no pretreatment gonadotrophin responses to LRH (Fig. 4).

Striking changes in the pituitary responsiveness to LRH were seen during the LRH treatments. The maximal FSH responses were obtained during the first days of treatment and at that time FSH levels which were greater than or equal to the LH levels after LRH were observed in most patients. However, the FSH responses rapidly decreased during the treatment and this decrease became more marked when the oestrogen secretion from the ovaries started to rise. The LH responses to LRH, on the other hand, progressivley increased during the treatment and, in most patients, reached maximal midcycle-peak levels at high oestrogen levels consistent with follicular maturation. After that increased progesterone levels began to appear in the blood, suggesting that ovulation occurred. During the luteal phase of the cycle, the LH responses decreased somewhat but

remained much greater than the FSH responses throughout the remainder of the cycle. Thus, the inverted pretreatment gonadotrophin responses to LRH changed during the prolonged LRH treatment and became similar to those seen in healthy adult women. The changes in the pituitary responsiveness to LRH may be due to the fact that LRH stimulates synthesis and release of predominantly LH and that modulatory feedback effects of the ovarian hormones, particularly E_2, then act at the pituitary level together with self-priming effects of LRH to cause a further marked increase of the LH responsiveness to LRH (25, 1, 35). These alterations are very similar to those seen in the normal female passing from the prepubertal to the postpubertal stage (10, 5). Similar endocrine and physical changes of puberty have been seen during prolonged LRH therapy in hypogonadotropic men (15). It could be considered to be analogous to taking these patients through puberty by the high dose LRH treatment.

Follicular growth and maturation were induced during the LRH treatment and presumptive evidence of ovulation, i.e. increased progesterone secretion, was also obtained. However, the progesterone values during the premenstrual period were rather low in 6 of the 8 cycles where only LRH was administered, suggesting insufficient corpus luteum function. The luteal phases were of normal length and the luteal phase defects were therefore more similar to that described as the inadequate luteal phase by Sherman & Korenman (27) than to the short luteal phase defect described by Strott et al. (29). Subnormal preovulatory FSH levels have been found during menstrual cycles with luteal phase defects (29, 26, 27). Strott and co-workers postulated that a relative FSH deficiency during the follicular phase results in abnormal follicular development and subsequent inadequate corpus luteum formation or function (29). Patients of the present study, who had inverted pretreatment response patterns with great FSH releases after LRH, responded promptly to the LRH treatment with marked E_2 increases consistent with full follicular maturation and they had normal corpus luteum function, as judged by the progesterone concentration in blood (e.g. patient KL, treatment I, Fig. 2). Patients with low FSH responses in combination with higher LH responses before the treatment responded slowly with much lower preovulatory oestrogen increases followed by inadequate luteal phases (e.g. patient BL, treatment II, Fig. 5). The results suggest that a possible explanation for the luteal phase defects might be an absolute or relative FSH deficiency which leads to defective follicular maturation and subsequent insufficient corpus luteum function.

An alternative explanation for abnormal follicular development might be the relatively high LH levels induced by LRH during the follicular phase of the treatment cycles. The LH/FSH ratio was similar to that described in women with the polycystic ovary syndrome (14, 34) where follicular maturation is imparied. The raised LH levels may stimulate the ovaries to an increased androgen secretion.

which inhibits follicular development. Ross and co-workers reported that small doses of HCG or LH to oestrogen-treated hypophysectomized immature female rats resulted in decreased granulosa cell proliferation and increased follicular atresia and showed that this inhibitory effect was mediated by local intra-ovarian effects of androgens, secreted by the ovary in response to HCG and LH (12, 13). In regularly menstruating women, elevation of LH activity in blood by HCG administration during the early follicular phase has been shown to cause luteinization of the theca interna with degeneration of tertiary follicles and delay or suppression of ovulation (30, 6). It can not be excluded that the LRH-induced LH elevations during LRH stimulation of follicular growth and maturation may have had deleterious effects on the follicular development and subsequent corpus luteum function.

Thirdly, it might be that during treatment with only LRH the LH peak levels at follicular maturation were not high enough for sufficiently long periods for normal ovulation and corpus luteum formation to occur. To secure an adequate preovulatory LH surge, HCG was therefore administered during five additional treatment cycles after induction of follicular maturation by LRH. All these LRH-HCG treatment cycles were ovulatory with normal luteal phases, as judged by the progesterone values. In two of the cycles (e.g. Fig. 4), the E_2 and progesterone patterns in blood suggested that ovulation had already occurred when HCG was given. During the postovulatory phase additional HCG injections were given to support corpus luteum function. In the cycles where only LRH was given, the luteal phase LH levels after LRH were presumably high enough for further support of the corpus luteum. However, it seems necessary to continue the LRH treatment throughout the luteal phase as we observed short luteal phases during treatment cycles where the LRH injections were interrupted during the postovulatory phase of the cycle (17).

One may question whether ovulation really occurred during the treatment cycles with signs of luteal phase insufficiency. The increased progesterone levels in blood are only indirect indices of ovulation and may be caused by luteinization of granulosa cells of the follicles without ovulation. In summing up results of treatment with LRH, Schally and co-workers concluded that although ovulation can be induced with LRH in sterile women the percentage of ovulations and pregnancies is relatively low (24). This might possibly be explained by a high percentage of cycles with luteal phase insufficiency. Only three of the nine women in the present study were involuntarily sterile. One of them became pregnant during her second LRH-HCG treatment (Fig. 7) and by that she proved that normal follicular maturation can be induced by treatment with LRH alone in women with impaired gonadotrophin secretion and absent pretreatment ovarian activity. For treatment of anovulatory infertility, it may be necessary to combine LRH with HCG or LH to secure normal ovulation and adequate corpus luteum function.

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REFERENCES

1. Aiyer, M.S., Chiappa, S.A. & Fink, G.: A priming effect of luteinizing hormone releasing factor on the anterior pituitary gland in the female rat. J Endocrinol 62:573-588, 1974.
2. Aono, T., Kinugasa, T., Yamamoto, T., Miyake, A. & Kurachi, K.: Assessment of gonadotrophin secretion in women with anorexia nervosa. Acta Endocrinol 80:630-641, 1975.
3. Beumont, P.J.V., George, G.C.W., Pimstone, B.L. & Vinik, A.I.: Body weight and the pituitary response to hypothalamic releasing hormones in patients with anorexia nervosa. J Clin Endocrinol Metab 43:487-496, 1976.
4. Bruch, H.: Changing approaches to anorexia nervosa. In: Anorexia and obesity. International psychiatry clinics, vol 7. (ed. C. Rowland), pp. 3-9. Little Brown & Co, Boston, 1970.
5. Franchimont, P., Becker, H., Ernould, Ch., Thys, Ch., Demoulin, A., Bourguignon, J.P., Legros, J.J. & Valcke, J.C.: The effects of hypothalamic luteinizing hormone releasing hormone (LH-RH) on plasma gonadotrophin levels in normal subjects. Clin Endocrinol 3:27-39, 1974.
6. Friedrich, F., Kemeter, P., Salzer, H. & Breinenecker, G.: Ovulation inhibition with human chorionic gonadotrophin. Acta Endocrinol 78:332-342, 1975.
7. Fries, H.: Secondary amenorrhoea, self-induced weight reduction and anorexia nervosa. Acta Psychiatr Scand (Suppl) 248:1-70, 1974.
8. Geigy Scientific Tables (1970): Average weights of adults (from insured person in the United States, Society of Actuaries. Build and Blood Pressure Study, 1:16, 1959, Ciba-Geigy, Basel.
9. Israel, R., Mishell Jr., D.R., Stone, S.C., Thorneycroft, I.H. & Moyer, D.L.: Single luteal phase serum progesterone assay as an indicator of ovulation. Am J Obstet Gynecol 112:1043-1046, 1972.
10. Job, J.C., Garnier, P.E., Chaussain, J.L. & Milhaud, G.: Elevation of serum gonadotropins (LH and FSH) after releasing hormone(LH-RH) injection in normal children and in patients with disorders of puberty. J Clin Endocrinol Metab 35:473-476, 1972.
11. Lindberg, B.S., Lindberg, P., Martinsson, K. & Johansson, E.D.B.: Radioimmunological methods for the estimation of oestrone, oestradiol-17β and oestriol in pregnancy plasma. Acta Obstet Gynecol Scand (Suppl) 32:5-19, 1974.
12. Louvet, J-P., Harman, M.S. & Ross, G.T.: Effects of human chorionic gonadotropin, human interstitial cell stimulating hormone and human follicle-stimulating hormone on ovarian weights in estrogen-primed hypophysectomized immature female rats. Endocrinology 96:1179-1186, 1975.
13. Louvet, J-P., Harman, M.S., Schreiber, J.R. & Ross, G.T.: Evidence for a role of androgens in follicular maturation. Endocrinology 97:366-372, 1975.
14. McArthur, J.W., Ingersoll, F.M. & Worcester, J.: The urinary excretion of interstitial-cell and follicle-stimulating hormone activity by women with diseases of the reproductive system. J Clin Endocrinol Metab 18:1202-1215, 1958.
15. Mortimer, C.H., McNeilly, A.S., Fisher, R.A., Murray, M.A.F. & Besser, G.M.: Gonadotrophin-releasing hormone therapy in hypogonadal males with hypothalamic or pituitary dysfunction. Br Med J 4:617-621, 1974.

16. Nillius, S.J. & Wide, L.: Gonadotrophin-releasing hormone treatment for induction of follicular maturation and ovulation in amenorrhoeic women with anorexia nervosa. Br Med J 3:405-408, 1975.
17. Nillius, S.J. & Wide, L.: Luteal function during menstrual cycles induced by gonadotrophin-releasing hormone in women with amenorrhoea. V International Congress of Endocrinology, Hamburg, July 18-24, 1976, Abstract 784, p. 323.
18. Nillius, S.J. & Wide, L.: The pituitary responsiveness to acute and chronic administration of gonadotropin-releasing hormone in acute and recovery stages of anorexia nervosa. In: Anorexia nervosa (ed. R.A. Vigersky), pp. 225-241. Raven Press, New York, 1977.
19. Nillius, S.J., Fries, H. & Wide, L.: Successful induction of follicular maturation and ovulation by prolonged treatment with LH-releasing hormone in women with anorexia nervosa. Am J Obstet Gynecol 122:921-928, 1975.
20. Palmer, R.L., Crisp, A.H., Mackinnon, P.C.B., Franklin, M., Bonnar,J. & Wheeler, M.: Pituitary sensitivity to 50 µg LH/FSH-RH in subjects with anorexia nervosa in acute and recovery stages. Br Med J I:179-182, 1975.
21. Roos, P.: Human follicle-stimulating hormone. Acta Endocrinol (Suppl) (Kbh) 131, 1-93, 1968.
22. Roos, P., Nyberg, L., Wide, L. & Gemzell, C.: Human pituitary luteinizing hormone. Isolation and characterization of four glycoproteins with luteinizing activity. Biochim Biophys Acta 405:363-379, 1975.
23. Roth, J.C., Kelch, R.P., Kaplan, S.L. & Grumbach, M.M.: FSH and LH response to luteinizing hormone-releasing factor in prepubertal and pubertal children, adult males and patients with hypogonadotropic and hypergonadotropic hypogonadism. J Clin Endocrinol Metab 35:926-930, 1972.
24. Schally, A.V., Kastin, A.J. & Arimura, A.: The hypothalamus and reproduction. Am J Obstet Gynecol 122:857-862, 1975.
25. Schally, A.V., Kastin, A.J. & Coy, D.H.: LH-releasing hormone and its analogues: Recent basic and clinical investigations. Int J Fertil 21:1-30, 1976.
26. Sherman, B.M. & Korenman, S.G.: Measurement of plasma LH, FSH, estradiol and progesterone in disorders of the human menstrual cycle: The short luteal phase. J Clin Endocrinol Metab 38:89-93, 1974.
27. Sherman, B.M. & Korenman, S.G.: Measurement of serum LH, FSH, estradiol and progesterone in disorders of the human menstrual cycle: The inadequate luteal phase. J Clin Endocrinol Metab 39:145-149, 1974.
28. Sherman, B.M., Halmi, K.A. & Zamudio, R.: LH and FSH response to gonadotropin-releasing hormone in anorexia nervosa: Effect of nutritional rehabilitation. J Clin Endocrinol Metab 41:135-142, 1975.
29. Strott, C.A., Cargille, C.M., Ross, G.T. & Lipsett, M.B.: The short luteal phase. J Clin Endocrinol 30:246-251, 1970.
30. Tamada, T. & Matsumoto, S.: Suppression of ovulation with human chorionic gonadotropin. Fertil Steril 20:840-848, 1969.
31. Thorneycroft, I.H. & Stone, S.C.: Radioimmunoassay of serum progesterone in women receiving oral contraceptive steroids. Contraception 5:129-146, 1972.
32. Warren, M.P., Jewelewicz, R., Dyrenfurth, I., Ans, R., Khalaf, S. & Vande Wiele, R.L.: The significance of weight loss in the evaluation of pituitary response to LH-RH in women with secondary amenorrhea. J Clin Endocrinol Metab 40:601-611, 1975.
33. Wide, L., Nillius, S.J., Gemzell, C. & Ross, P.: Radioimmunosorbent assay of follicle-stimulating hormone and luteinizing hormone in serum and urine from men and women. Acta Endocrinol (Suppl) 174, 1-58, 1973
34. Yen, S.S.C., Vela, P. & Rankin, J.: Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. J Clin Endocrinol 30:435-442, 1970.
35. Yen, S.S.C., Lasley, B.L., Wang, C.F., Leblanc, H. & Siler, T.M.: The operating characteristics of the hypothalamic-pituitary system during the menstrual cycle and observations of biological action of somatostatin. Recent Prog Horm Res 31:321-363, 1975.

36. Yoshimoto, Y., Moridera, K. Imura, H.: Restoration of normal pituitary gonadotropin reserve by administration of luteinizing-hormone-releasing hormone in patients with hypogonadotropic hypogonadism. New Engl J Med 292: 242-245, 1975.

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