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Effect of Synthetic Luteinizing Hormone Releasing Hormone (LH/FSH-RH) in Women with Menstrual Disorders

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Summary

Synthetic luteinizing hormone/follicle stimulating hormone-releasing hormone (LH/FSH-RH) (50 µg) was given intravenously to six women with oligomenorrhoea and to four women with secondary amenorrhoea. Peripheral venous blood was withdrawn at regular intervals over a 24-hour period. The concentrations of LH, FSH, and oestradiol-17β were determined by radioimmunoassay. In all subjects there was a variable rise in LH (3-16 times the mean basal level); in six a small rise in FSH (two to three times the mean basal level) and in seven a twofold to threefold rise in oestradiol three to eight hours after the rise in gonadotrophins.

Introduction

There is good evidence to believe that the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) is under a neuroendocrine control emanating from the hypothalamus (Guillemin and Schally, 1963). Evidence for a specific LH-releasing hormone (LH-RH) in rat hypothalamic extracts was first presented by McCann *et al.* (1960) and in sheep by Courier *et al.* (1961).

Recently Schally *et al.* (1971) showed that a purified extract of pig hypothalamus (LH-RH) has both LH and FSH releasing properties in rats. Human subjects given this purified porcine LH-RH also release LH and FSH (Kastin *et al.*, 1971), as do monkeys, sheep, and rabbits (Reeves *et al.*, 1970; Schally *et al.*, 1970). Kastin *et al.* (1970) also isolated two fractions from human hypothalamus with LH-RH and FSH-RH activity, and when given separately both produced a significant increase in FSH and LH after administration to volunteers.

Schally *et al.* (1971) proposed a provisional structure for porcine LH-RH, which was revised and found to be a decapeptide (Matsuo *et al.*, 1971). A product with this amino-acid sequence has now been manufactured by Hoechst Pharmaceuticals and was used in the present study.

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Patients and Methods

Patients selected for this study were all volunteers. They were admitted to hospital on the day before the test and were kept in bed for the duration of the study but were not fasting. A No. 16-gauge angiocath was inserted into an antecubital fossa vein and connected to an extension tube and three-way tap. The tube and angiocath were flushed with heparinized sterile distilled water between each sample. Blood was withdrawn to determine basal hormone levels at 8, 8.30, and 8.50 a.m. A 50-µg dose of synthetic LH-RH (HOE 471) was dissolved in 0.9% sterile saline solution and diluted to 10 ml in the same material. This was injected via the intravenous line at 9 a.m. Blood samples were then withdrawn at the following times after the injection: 5, 10, 15, 20, and 30 minutes; and 1, 1½, 2, 3, 6, 9, and 24 hours. The blood samples were centrifuged immediately, the plasma being separated and deep frozen at -25°C until assayed. FSH and LH were determined by radioimmunoassay using a pituitary standard LER 907 (Wide and Porath, 1966) and oestradiol-17β by radioimmunoassay using an anti-serum to oestradiol-6-carboxymethyl oxime-bovine serum albumin (Emment *et al.*, 1972).

The first six patients had oligomenorrhoea with cycles of six to eight weeks. These patients do not have the premenstrual rise in LH and FSH (Newton, 1972c; Ross *et al.*, 1970) which occurs in the normal menstrual cycle (Newton *et al.*, 1971a); the injection of LH-RH was given on the first day of bleeding. The last four patients had secondary amenorrhoea for not less than six months and had previously been investigated to assess their basal endocrine state (Newton 1972a, 1972b).

Results

The data in Tables I and II show the first significant rise of LH and FSH after the injection of synthetic LH-RH, the time at which the peak level was reached, the duration of raised gonadotrophin levels, and the factorial increase of LH and FSH over the mean basal sample (mean of three). As can be seen from Table I there was a rise in LH in all 10 patients. Three showed the first significant rise 5 minutes after the injection, five after 10 minutes, and two after 15 minutes. The peak value for LH was reached after 30 minutes in four patients, 45 in two patients, and 90 minutes in two patients. The duration of response varied from 45 minutes to 8 hours 50 minutes, with five patients between 2 hours 50 minutes and 2 hours 55 minutes. The

TABLE I—Change in Plasma LH after LH-RH

Case No.	Time of First Significant Rise in LH (Min)	Time of Peak Value (Min)	Duration of Raised Levels (Hr min)	Factorial Increase
1	10	30	8 50	× 7.6
2	10	90	2 50	× 8.9
3	5	60	2 55	× 7.3
4	15	30	0 45	× 3.5
5	15	45	1 45	× 16.3
6	5	60	2 55	× 6.0
7	10	90	2 50	× 8.3
8	10	30	1 20	× 7.2
9	10	45	1 50	× 9.4
10	5	30	2 55	× 14.8

TABLE II—Change in Plasma FSH after LH-RH

Case No.	Time of First Significant Rise (Min)	Time of Peak Value (Min)	Duration of Raised Levels (Hr Min)	Factorial Increase
1	10	180	2 50	× 2.9
2	15	15	—	× 2.2
3	15	30	2 45	× 2.3
4	No change	—	—	—
5	No change	—	—	—
6	No change	—	—	—
7	No change	—	—	—
8	15	30	—	× 2.0
9	10	60	1 20	× 2.0
10	10	60	2 50	× 2.4

factorial increase over the basal level ranged from 3.5 to 16.3, with a mean value of 8.9.

In Table II it may be seen that the FSH results are more variable; only six of the 10 patients showed an increase. In three of these the first rise was detectable 10 minutes after the injection and in the remaining three after 15 minutes. The peak value was seen at 15 minutes in one patient, 30 minutes in two patients, and 3 hours in one patient. In two patients there was no sustained rise of FSH, but in four the duration of response varied from 1 hour 20 minutes to a maximum of 2 hours 50 minutes. The factorial increase in FSH was lower than with LH, varying from 2.0 to 2.9, with a mean of 2.3. The basal and peak values for LH, FSH, and oestradiol are given in Table III.

TABLE III—LH, FSH, and Oestradiol Levels

Case No.	LH (mIU/ml)		FSH (mIU/ml)		Oestradiol (pg/ml)	
	Basal Value	Peak Value	Basal Value	Peak Value	Basal Value	Peak Value
1	7.27	55	5.2	15	32	98
2	8.99	80	14.8	32.5	41	78
3	22	160	10.7	24.5	38	108
4	22.86	80	12.8	No change	97	291
5	6.75	110	19.8	No change	55	79
6	6.25	37.5	8.6	No change	45	99
7	7.4	61.2	9.8	No change	—	—
8	6.0	43.2	11.3	22.5	—	—
9	19.15	183	9.8	20.5	—	—
10	7.2	106	11.5	27.6	50	95

The values for LH, FSH, and oestradiol obtained from a patient given 50 µg LH-RH are charted in Fig. 1. There was a rapid initial rise in LH which reached a plateau after 30 minutes and then gradually fell from a level of 55 mIU/ml to near basal levels nine hours later. Next morning there was a rise in LH, and 8 days and 24 days later the values were similar to those seen before the test. The FSH increase was much smaller; a plateau was reached after 15 minutes and showed very little change over the next nine hours. The results 8 days and 24 days later were also very similar to the pretreatment values. The level of plasma oestradiol tended to fall for the first 90 minutes, then increased, reaching a maximum at 6 hours. The concentration then fell over the next 18 hours to preinjection values. The level on day 8 was similar. On day 24 there was a rise to 99 pg/ml, which is within the normal luteal phase range. This patient showed a basal temperature shift 14 days after the injection of LH-RH but there was no conclusive proof of ovulation and she did not menstruate 28 days after the injection.

Fig. 2 shows the same results for LH and FSH plotted on a logarithmic scale. The release of LH was linear for the first 15 minutes after injection, the slope then changed, and for the next 6 hours the release was more gradual, but the levels did not return to pretreatment values. FSH showed a more variable response,

FIG. 1—LH-RH, Case 1. Hormone changes after 50 µg LH-RH.

FIG. 2—LH-RH, Case 1. Log plot of LH and FSH against time.

the levels fluctuating throughout the period of test but remaining above the preinjection level of 6 mIU/ml. This type of response, shown in Figs. 1 and 2, was seen in most cases; however, in two patients a biphasic release was observed. One of these is illustrated in Fig. 3. Initially there was a rapid rise in LH, reaching a peak after 10 minutes. Levels then fell, and a second peak occurred 1½ hours after the injection; the levels again fell over the next 5½ hours. The FSH rose, reaching a peak after 15 minutes, then decreased over the next 60 minutes and remained fairly constant for the rest of the test. The concentration of oestradiol started to increase 2 hours after the injection, reached a maximum value after 3 hours, and remained elevated for a period of 6 hours.

FIG. 3—LH-RH, Case 2. Hormone changes after 50 µg LH-RH showing biphasic pattern.

Discussion

The presence of LH-RH in the hypothalami of animals such as rats and pigs (Schally *et al.*, 1968) and of man (Schally *et al.*, 1967) has been described. Attempts have been made to purify this substance, which in pigs was found to have the following sequence of amino-acids (pyro): Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂ (Matsuo *et al.*, 1971). This decapeptide has been synthesized (Arimura *et al.*, 1972) and its biological activity found to be similar to natural LH-RH.

In this preliminary series of patients all showed an increase in LH after injection of 50 µg LH/FSH-RH, but only six showed an increase in FSH and only four showed a sustained increase in FSH. In all these patients the FSH increase was lower (mean 2.3) than for LH (mean 8.9). The radioimmunoassays for LH and FSH are specific, and this therefore seems to indicate that the main action of LH/FSH-RH in these patients is to release LH, and in some a smaller rise in FSH can also be expected. Further work in progress will help to elucidate whether LH/FSH-RH releases both FSH and LH or whether LH-RH and FSH-RH are in fact two different compounds. Oligomenorrhoeic patients were chosen for this initial study to avoid the natural increase in endogenous LH and FSH seen premenstrually and for the first few days of the menstrual cycle in normal patients with regular menstrual cycles.

There is a need for a pituitary function test that measures directly the release of LH and FSH. Previous work with clomiphene (Newton and Dixon, 1971) has indicated that it acts on the

hypothalamic-pituitary axis and that it is useful as a test of pituitary function (Newton *et al.*, 1971b). Hopefully, LH/FSH-RH will be capable of development as a test of pituitary function in patients suspected of having a disorder of the pituitary, and when used in combination with clomiphene will enable disorders of the hypothalamic-pituitary axis to be better understood. In addition ovulation induction, which has been carried out in golden hamsters with LH/FSH-RH (Arimura *et al.*, 1972), may be possible in patients and provide a useful form of treatment.

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