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LH–FSH/RH: an equally potent stimulus for the release of HLH and HFSH

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Blood was sampled continuously both before and after a single i.v. injection of 50 μg synthetic LH–FSH/RH in 5 normal women during the luteal phase of the cycle and in 5 women with hypoestrogenic secondary amenorrhea.

LH, but not FSH concentrations were significantly higher at the beginning than at the end of the control sampling period. This phenomenon can be ascribed to the stress occasioned by initiating the blood sampling procedure.

LH levels were lower, and FSH levels were higher in the group of women with amenorrhea than in the control group. These differences, however, were significant only after stimulation with LH–FSH/RH.

Production rates were calculated using a single compartment model. In both groups studied maximal production rates occurred after 10 min for LH and after 20 min for FSH. Although the LH concentration increased relatively much more than the FSH concentration, the production rates of both hormones increased in the same proportion. This seems to indicate that LH–FSH/RH is an equally potent stimulus for the release of both gonadotropins.

LH–FSH/RH; LH; FSH; gonadotropin production rates

Introduction

Recent studies (Schally, Nair, Redding and Arimura, 1971b; Schally, Arimura, Baba, Nair, Matsuo, Redding, Debeljuk and White, 1971a; Matsuo, Arimura, Nair and Schally, 1971) indicate that, in humans, synthetic LH–FSH/RH stimulates the release of both LH and FSH. The pituitary responsiveness is dose dependent (Rebar, Yen, Vandenberg, Naftolin, Ehara, Engblom, Ryan, Rivier, Amoss and Guillemin, 1973b; Thomas, Donnez and Ferin, 1972), modulated by sex steroid environment (Nillius and Wide, 1972a; Thomas, Cardon, Donnez and Ferin, 1973; Yen, Vandenberg, Rebar and Ehara, 1972b) and within certain limits proportional to the basal hormone concentrations (Nillius and Wide, 1972b).

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The rise in plasma concentration after rapid intravenous injection of LH–FSH/RH is much greater for LH than for FSH. The biologic activity of gonadotropins, however, is not only dependent on their concentrations, but also to a large extent on their half-lives (Parlow, 1972). Therefore we compared for both LH and FSH the basal production rate with the increase in production after LH–FSH/RH administration in normal and pathological conditions.

Materials and methods

In a preliminary experiment all women were studied under basal conditions. For the actual experiment two groups, five patients with secondary amenorrhea and five normal young women in the mid-luteal phase of the cycle, were selected. In the former

TABLE I Subjects: age, estrogen excretion, and basal plasma LH and FSH concentrations

Age		Duration of amenorrhea (mth)	Plasma gonadotropin concentration (mIU/ml)		Estrogen excretion (μg/24 h)
			LH	FSH	
Secondary amenorrhea					
B.V.	24	36	1.7	3.7	5.3
C.V.	28	48	1.3	5.4	4.2
D.M.	24	72	3.9	2.6	8.4
G.M.	33	60	1.5	3.6	6.2
V.O.M.	34	96	2.3	6.0	11.1
Mean±SEM	28.6±2.1	62.4±10.3	2.1±0.5	4.3±0.6	7.0±1.2
range	24–34	36–96	1.3–3.9	2.6–6.0	4.2–11.1
Luteal phase					
Mean±SEM	32.8±4.1	–	2.7±0.6	2.5±0.4	32.4
range	24–48		1.1–4.1	1.3–3.6	15–87*

* Brown et al., 1968.

group other endocrinopathies were excluded; the ovaries were normal or hypoplastic at laparoscopy and estrogen excretion and plasma gonadotropins were both below or at the lower limit of the normal range (Table I). In the latter group the luteal phase was determined by basal body temperature charts. The luteal phase of the cycle was selected for the control group, since this period is the most constant as far as sex steroid environment (Abraham, Odell, Swerdloff and Hopper, 1972) and gonadotropin response to LH–FSH/RH (Nillius and Wide, 1972a; Thomas et al., 1973; Yen et al., 1972b) are concerned. Blood was drawn continuously over a one-hour period, both before the rapid intravenous injection of 50μg synthetic LH–FSH/RH (Hoechst OP 25) and over a four-hour period following this injection by a modified technique of Alford (Alford, Baker, Culross and Chamley, 1972) with a constant blood-heparin ratio of 10 to 1 (Koninckx, De Moor and Brosens, 1974). A fraction collector divided the aspirated blood into 10- or 20-minute sampling periods. Plasma was separated and frozen until assay. All samples were assayed in duplicate for LH and FSH.

The assay method for LH and FSH was essentially that described by Midgley (1966, 1967). Total incubation time was 7 days at 0 °C, with tracer addition after two days and addition of the second antibody after six days. For the determination of LH a rabbit anti-HCG serum (AS/350 prepared by Organon) was used and for the FSH determinations the NIH provided a rabbit anti-HFSH serum. The HLH and HFSH tracers (^{125}I) were purchased form I.R.E. (Fleurus, Belgium) and repurified every fortnight on a Sephadex G-100 (60 x 1 cm) column. The bound and free hormone fractions were separated by a DASP (Double Antibody Solid Phase) system (Den Hollander and Schuurs, 1971) for reasons of convenience, absence of prozoning, and absence of non-specific serum effects (Koninckx, Bouillon and De Moor, 1975). Our DASP system was prepared as follows: goats were immunized with 5 mg rabbit gammaglobulin in complete Freund's adjuvant according to the method of Vaitukaitis (Vaitukaitis, Robbins, Nieschlag and Ross, 1971). The gammaglobulin fraction of serum was extracted and coupled to cyanogenbromide-activated microcrystalline cellulose (Merck) (Wide, 1969). The detection limit (Ekins, Newman and O'Riordan, 1968) varied from 0.3 to 0.6 mIU/ml for LH and from 0.16 to 0.55 mIU/ml for FSH. At the fifty-percent binding level, the within and between assay variance was respectively 2.3 and 8.1 percent for LH and 2.2 and 8.5 percent for FSH (Fig. 1). The results have been expressed in mIU/ml and the standards used were the MRC 68/40 and 68/39 for LH and FSH, respectively, and the NIH LER 907. The latter standard gave results which were in very good agreement with those obtained with the MRC standards.

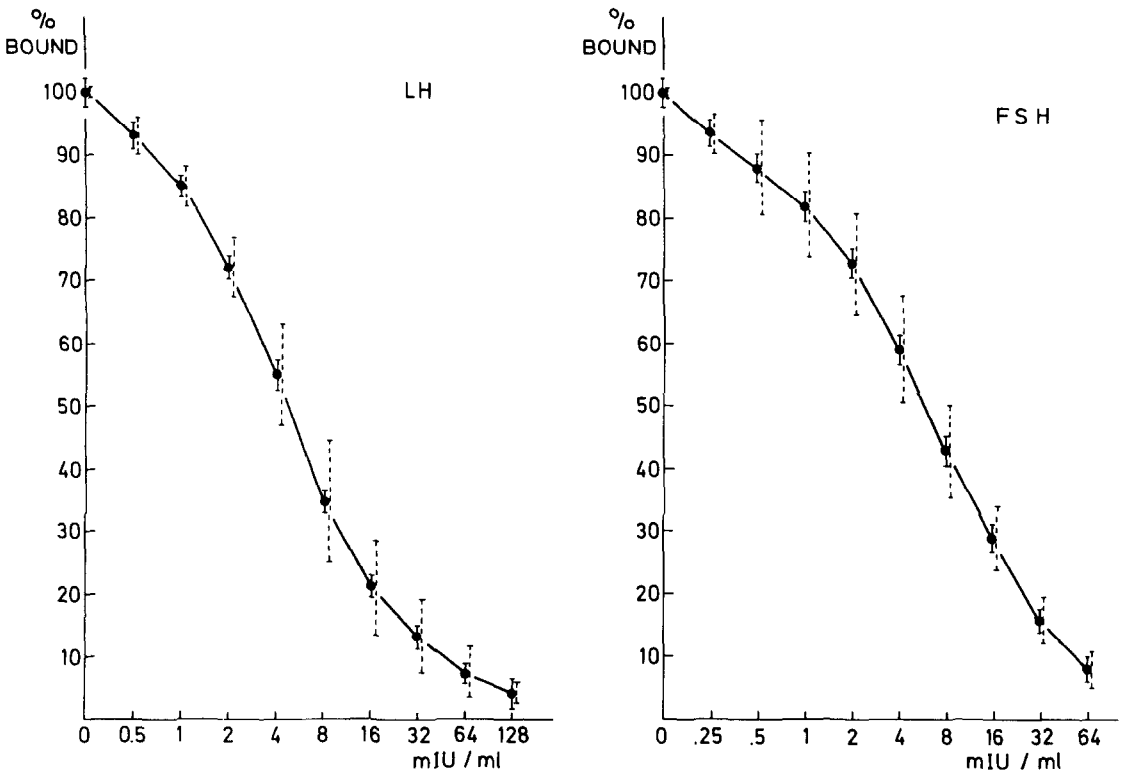

Fig. 1 Normalized standard curves of LH and FSH. Solid lines represent the within, and dotted lines the between assay variance.

Estrogen excretion was assayed by the method of Brown (Brown, McLeod, MacNaughtan, Smith and Smyth, 1968) on 24-hour urine specimens.

Production rates of LH and FSH were computed from successive plasma hormone concentrations assuming constant production and removal rates, and a single compartment model. Concentration changes would thus be expressed by the following differential equation (De Moor, De Backer, Hendrickx, Hinnekens and De Bock, 1960):

$$\frac{dC}{dt} = P - r \cdot C$$

where C = concentration in mIU/ml; P = production rate in mIU/ml/min; r = removal constant.

After transformation this became:

$$P = r \frac{C_{(t)} - C_{(o)} e^{-rt}}{1 - e^{-rt}}$$

For LH all experiments were calculated with an assumed half-life of 80 minutes and for FSH with an assumed half-life of 220 minutes. Distribution time was neglected and distribution volume assumed to be constant at 5000 ml (Rebar, Perlman, Naftolin and Yen, 1973a). The total amount released by LH–FSH/RH was found by multiplying production rate with time for the hour following the injection; the basal production was not subtracted.

Student's paired and unpaired t-tests were used for calculation of statistical significance.

Results

During the control period LH levels, but not FSH levels, showed a gradual decline both in the group of normal women and in the women with amenorrhea. Only the differences between the first (−60 to −40 minutes) and the third collection period (−20 to 0 minutes) were statistically significant (Table II).

TABLE II Basal LH and FSH levels during the control period in 5 women in the luteal phase of the cycle and 5 women with secondary amenorrhea; each experiment was done twice

	SAMPLE I −60 to −40 minutes	SAMPLE II −40 to −20 minutes	SAMPLE III −20 to 0 minutes	Significance of differences between samples I and III
LH (mIU/ml)				
Nl. luteal phase mean±SEM	2.9±0.5	2.6±0.4	2.3±0.3	$p < 0.05$
Sec. amenorrhea mean±SEM	2.6±0.5	2.4±0.6	2.0±0.4	$p < 0.025$
FSH (mIU/ml)				
Nl. luteal phase mean±SEM	2.5±0.3	2.3±0.2	2.2±0.2	NS
Sec. amenorrhea mean+SEM	2.7±0.4	2.8±0.4	2.5±0.3	NS

As shown in Table III the mean basal concentrations of LH and FSH were not significantly different between the groups. After rapid intravenous injection of 50 μg LH–FSH/RH, however, the maximum plasma concentrations of LH were significally lower ($P < 0.01$) whereas the maximum plasma concentrations of FSH were significantly higher ($P < 0.025$) in the women with amenorrhea in comparison with the normal women. The relative increases were not statistically different between both groups of women but more pronounced for LH than for FSH ($P < 0.001$). Moreover in the two groups of patients taken together, the correlation between basal concentration and maximum concentration obtained was highly significant for FSH ($P < 0.001$) but not significant for LH (Fig. 2). Qualitatively the response pattern to LH–FSH/RH was the same in both groups with maximum concentrations of LH and FSH obtained after 30–40 minutes. The FSH concentration diminished slowly thereafter whereas the LH concentration fell more rapidly. In the normal group we noted in four out of five cases a second increase in LH concentration about three hours after the LH–FSH/RH administration. This was not

TABLE III LH and FSH stimulation by rapid intravenous injection of 50 μg LH–FSH/RH in the luteal phase of the cycle and in secondary amenorrhea

	Basal plasma concentrations (mIU/ml)	Maximum plasma concentrations (mIU/ml)	Relative increases	Total amounts (IU) released by LH–FSH/RH
LH				
Nl. luteal phase mean±SEM	2.7±0.6	20.0±1.9	9.3±0.3	118.1±15.0
Sec. amenorrhea mean±SEM	2.1±0.5	11.6±1.9	6.1±1.5	70.3±10.8
	NS	$P < 0.01$	NS	$P < 0.025$
FSH				
Nl. luteal phase mean±SEM	2.5±0.4	4.7±0.7	1.9±0.1	15.7± 1.8
Sec. amenorrhea mean±SEM	4.3±0.6	9.4±1.6	2.3±0.3	32.6± 8.4
	NS	$P < 0.025$	NS	$P < 0.05$

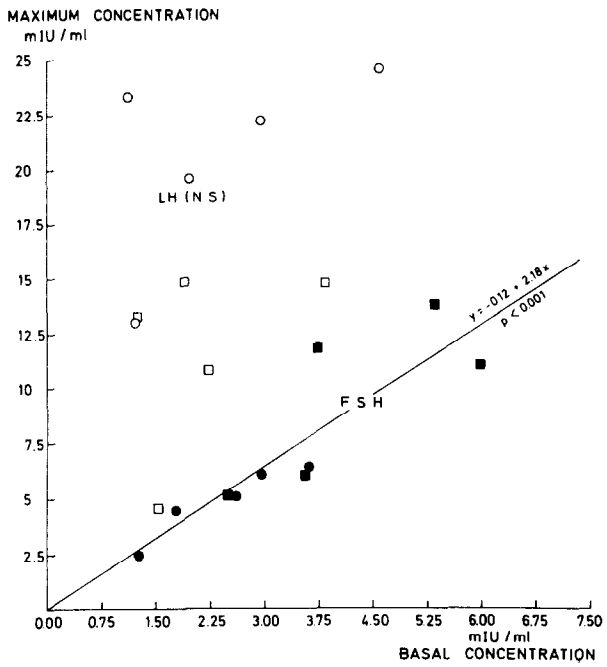

Fig. 2 Correlation between basal plasma concentration and maximum concentration after 50 μg LH–FSH/RH in 5 women in the luteal phase of the cycle (LH = 0, FSH = ●) and in 5 women with secondary hypoestrogenic amenorrhea (LH = □, FSH = ■).

seen in the group with secondary amenorrhea (Figs. 3 and 4). The total amount of LH released by LH–FSH/RH was lower ($P < 0.025$) whereas the total amount of FSH released by LH–FSH/RH was higher ($P < 0.05$) in the women with amenorrhea in comparison with the findings in the control group (Table II). Taking the two groups together the correlation between basal production rate, in mIU/min, and total amount released by LH–FSH/RH, in IU, was significant for FSH ($P < 0.01$) but not for LH (Fig. 5).

In contrast to the relative increase in concentration, the relative increase in production, measured by the ratio of total amount released over the basal production rate, of LH and FSH after 50 μg LH–FSH/RH was only slightly different for LH and FSH in both groups of women (Fig. 5).

Discussion

Continuous blood sampling was used to eliminate the interference of the pulsatile release of gonadotropins (Yen, Tsai, Naftolin and Ajabor, 1972a). Serial sampling would have been of little help in calculating production rates since the extreme hormone concentrations cannot be smoothed out mathematically (Rebar et al., 1973) in a meaningful way.

Production rates calculated from peripheral plasma concentrations are totally dependent upon the assumed removal rate and distribution volume. The latter varies between subjects from one to two times the plasma volume (Scrivastava, 1972). The fact that the apparent distribution volume exceeds the plasma volume can be interpreted as diffusion in the extravascular compartment or as an accumulation of hormone into certain organs (e.g. the ovaries). Distribution volume was assumed to be constant (5000 ml) in our calculations since differences between amenorrheic women and women in the luteal phase have not been reported. The half-lives of gonadotropins have been shown to be proportional to their sialic acid content (Yang and Papkoff, 1973) for which both hormones are microheterogeneous (Saxena, Rathnam and Römmler, 1973). By radioimmunoassay (RIA) disappearance of endogenous hormone is biexponential (Rebar et al., 1973a; Yen, Llerena, Little and Pearson, 1968), while disappearance of exogenous LH is monoexponential (Marshall, Anderson, Fraser and Harsoulis, 1973). However, nonnative hormone, with different biological characteristics but also measured by RIA, could be released during hypophysectomy, while exogenous hormone is necessarily purified. Therefore we have chosen a single compartment model and have used arbitrarily the longest half-life which did not give 'negative' production rates in our calculations. Our assumed half-lives of 80 minutes for LH and 220 minutes for FSH agree with reported values (Yen et al., 1968; Parlow, 1965).

The production rates of LH and FSH increased in the same proportion after intravenous injection of 50 μg of LH–FSH/RH; however, the concentration of LH increased much more than the concentration of FSH. The latter difference between production rate and concentration is explained by the fact that FSH has a much longer half-life than LH. Therefore we consider LH–FSH/RH an equally potent stimulus for the release of LH and FSH. No argument can thus be derived from LH–FSH/RH experiments to postulate the existence of two distinct gonadotropin-releasing hormones.

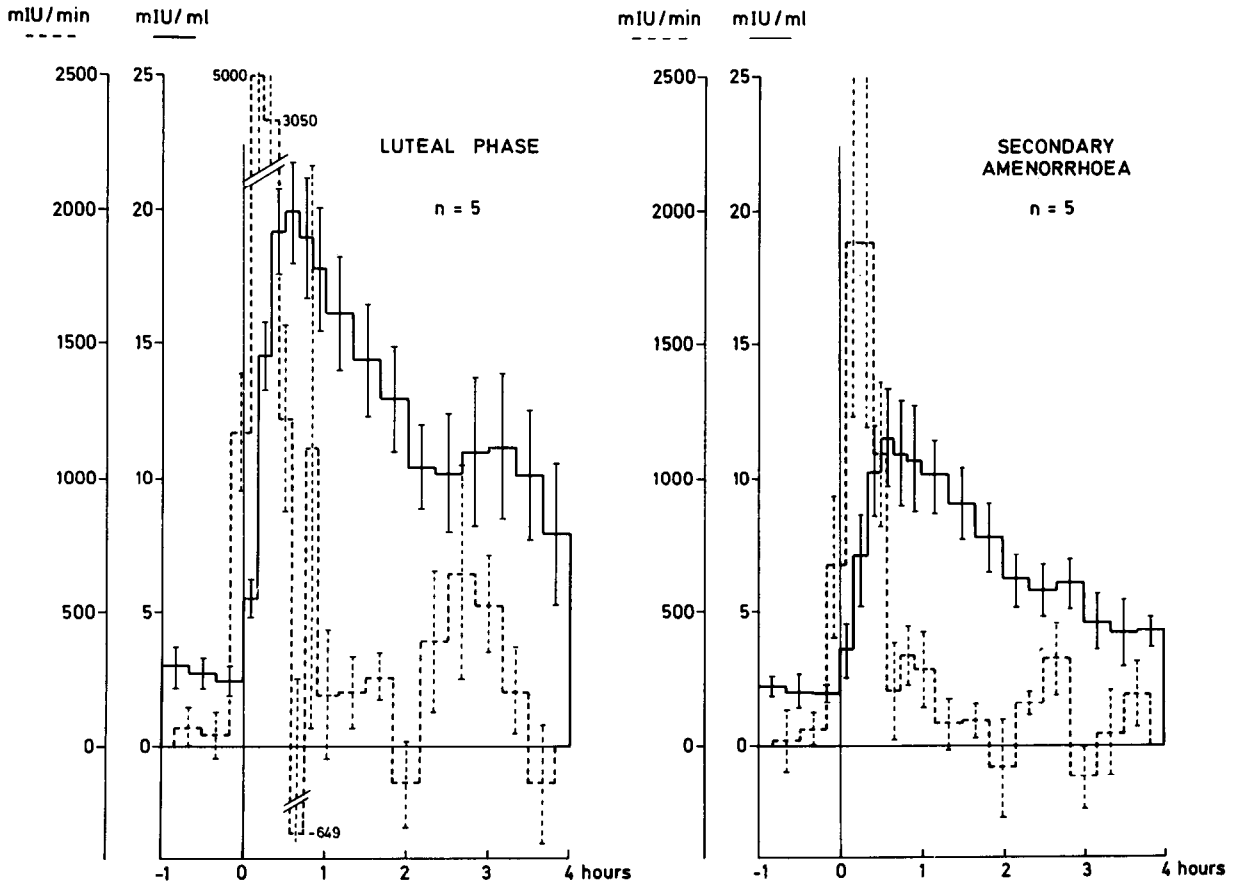

Fig. 3 LH plasma concentration (—), and production rate (---) in 5 women in the luteal phase of the cycle and in 5 women with hypoestrogenic secondary amenorrhea after i.v. injection of 50 μg synthetic LH–FSH/RH.

LH, but not FSH, levels decline significantly during the control period in both groups studied. This could be interpreted as an artefact due to the pulsatile nature of their release since the descending limb of a peak is much longer than the ascending, especially during the luteal phase (Yen et al., 1972). However, this pulsatile release is known to be absent in hypogonadotropic amenorrhea (Yen, Rebar, Vandenberg and Judd, 1973). Therefore we postulate that the stress of initiating the blood sampling procedure stimulates the secretion of LH and FSH, as described for other pituitary hormones (Noel, Suh, Stone and Frantz, 1972). The longer half-life of FSH makes this decline less apparent for the latter hormone.

This study confirms that patients with hypoestrogenic secondary amenorrhea are relatively hyperreactive to LH–FSH/RH in comparison with women in the early follicular phase of the cycle (Yen et al., 1973). Indeed we found a similar increase in patients with secondary amenorrhea as in the luteal phase which is known to be more reactive than the follicular phase (Nillius and Wide, 1972a; Thomas et al., 1973; Yen et al., 1972b).

The clinical usefulness, however, of gonadotropin assays and of LH–FSH/RH as a diagnostic tool is limited in patients with secondary amenorrhea. Basal concentrations overlap between the latter and normal conditions, whereas stimulation tests by rapid intravenous injection of LH–FSH/RH, although offering the advantage of an enlarged picture of the basal concentration, remain difficult to interpret (Aono, Minagawa, Kinusaga, Miyake and Kurachi, 1974) since the release of gonatropins is modulated by sex steroid environment.

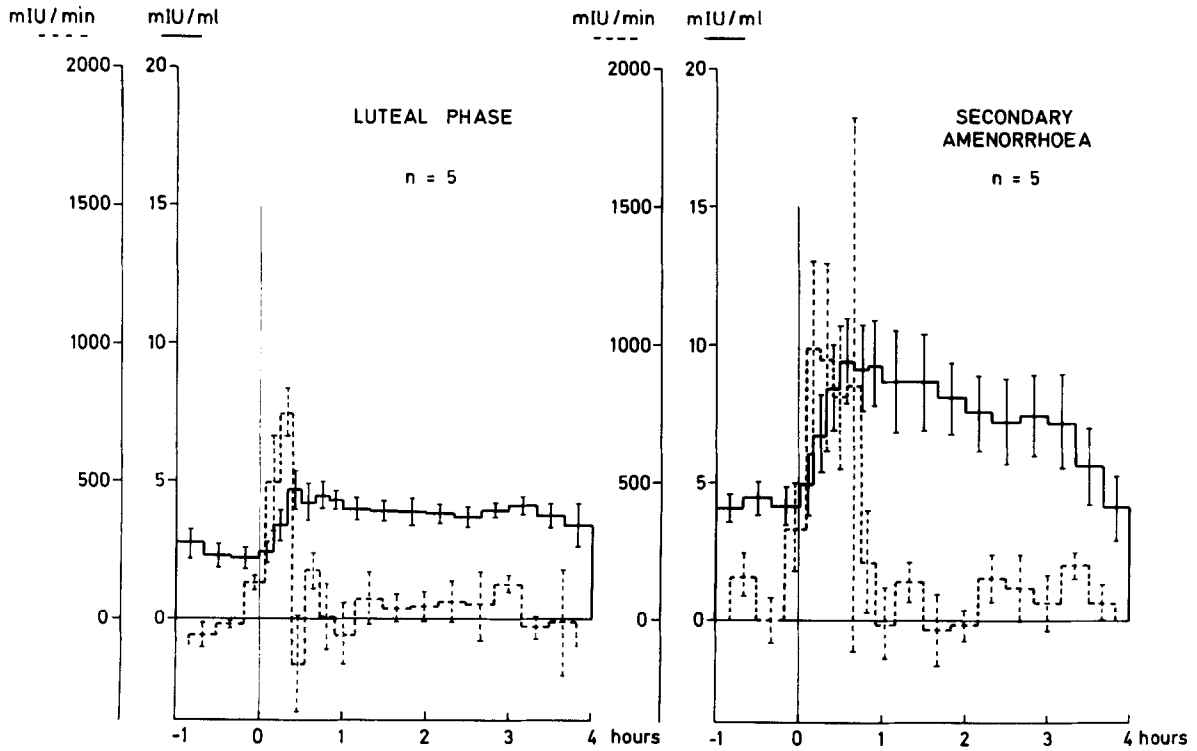

Fig. 4 FSH plasma concentration (—) and production rate (---) in 5 women in the luteal phase of the cycle and in 5 patients with hypoestrogenic secondary amenorrhea after i.v. injection of 50 μg synthetic LH–FSH/RH.

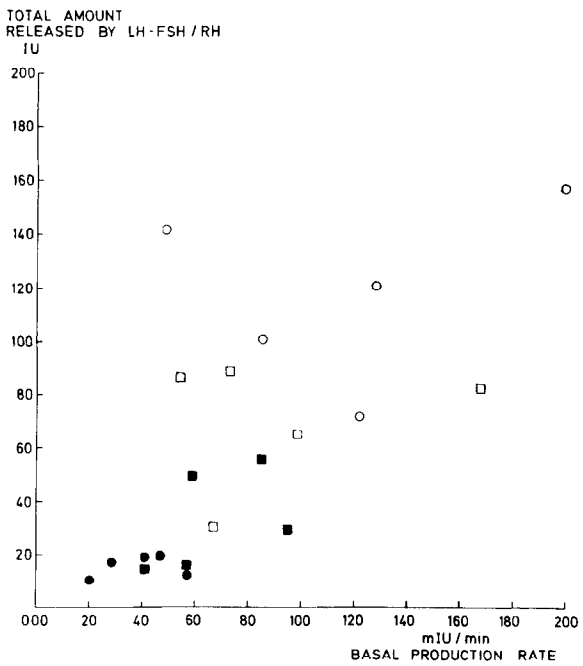

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Fig. 5 Basal production rate and total amount of LH and FSH released by 50 μg of synthetic LH–FSH/RH in 5 women in the luteal phase of the cycle (LH = ○, FSH = ●) and in 5 women with secondary hypoestrogenic amenorrhea (LH = □, FSH = ■).

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