The response to luteinizing hormone-releasing hormone in normal men

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Summary
Synthetic luteinizing hormone-releasing hormone (LH–RH) was given in doses of 11–100 μg to normal men. Doses of 50 and 100 μg caused a two- to ten-fold rise in serum LH in all subjects, but in only 40% was the serum follicle-stimulating hormone (FSH) elevated. A significantly higher LH response to LH–RH was seen in subjects in whom a rise in serum FSH could be detected. Although there was a wide variation in the serum gonadotrophic hormone response, the test was reproducible in individual subjects. A significant rise of serum LH was seen following the injection of 1 μg LH–RH and a linear log-dose response occurred over the range 0.25 to 4 μg. There was no rise in serum FSH with the lower doses of LH–RH. Two injections of LH–RH 90 min apart produced comparable rises of serum LH and FSH. The rise of serum LH following a second injection of LH–RH was blunted when the two injections were given 30 or 45 min apart.

Introduction
Luteinizing hormone releasing hormone (LH–RH) was first isolated by Schally and his colleagues (Schally et al., 1971a). It has subsequently been synthesized and has been shown to be active in man. A rise of serum LH regularly occurs following the injection of doses of 50–100 μg (Schally et al., 1971b; Besser et al., 1972; Yen et al., 1972; Nillius and Wide, 1972) and in a proportion of subjects follicle-stimulating hormone (FSH) is also released. The hormone has been evaluated in hypogonadal patients but, in the doses used, does not seem to be able to distinguish pituitary from hypothalamic disease (Mortimer et al., 1973).

In the present study, we have investigated the reproducibility of the changes in serum gonadotrophin following the administration of LH–RH in the same subjects, and determined the lowest dose to produce a response. We have also given consecutive injections of the hormone in the hope that the relative changes in serum LH and FSH might thereby be altered (Schally, Arimura and Kastin, 1973).

Subjects and methods
The subjects were normal male volunteers aged 19–42 whose consent was given after a full explanation of the nature of the project. LH–RH was synthesized by Dr Derek Schafer of Reckitt and Coleman (Schafer and Black, 1973). The tests were all begun between 9 a.m. and 10 a.m. An indwelling catheter was inserted into the forearm vein and two or three basal blood samples were withdrawn over the next 15–30 min. The LH–RH was then injected rapidly through the catheter and further blood samples withdrawn at frequent intervals.

Nine men were given doses of 50 and 100 μg at least 1 week apart. In a second experiment, five men received three injections of the same dose of hormone at weekly intervals, two of 50 μg and three of 100 μg. A third experiment consisted of the administration of two successive injections of 100 μg LH–RH, 90 min apart, to six men, three of whom were later re-tested with doses 45 min apart and another two with doses 30 min apart. Finally, five men received doses of 0.25, 1 and 4 μg at hourly intervals.

Measurements of LH and FSH were undertaken in all serum samples. Serum LH was measured with a double-antibody radioimmunoassay. Purified human pituitary (Fraction IRC 2, kindly donated by Dr A. Stockell Hartree, Cambridge) was used for labelling with 181I. Anti-human pituitary LH (70/229) was supplied by the Medical Research Council. Results were expressed in terms of a human pituitary LH standard (MRC 68/40) ascribed an arbitrary value of 20 u/ampoule. The smallest
amount of LH which could be measured was approximately 0·2 mu/ml serum.

Serum FSH was assayed by a double-antibody radioimmunoassay as described previously (Groom et al., 1971). The second International Reference Standard Human Menopausal Gonadotrophin (2nd IRP-HMG) (potency 40 iu/ampoule) was used as a standard. In the assay, the MRC-FSH standard 69/104 has a potency of 16·8 iu FSH/ampoule in terms of the 2nd IRP–HMG. The lower limit of sensitivity of the assay was 1 mu/ml serum and intra-assay variation was 10%.

Results

Serum LH

There was a two- to ten-fold rise of serum LH following doses of 50 and 100 μg of LH–RH (Fig. 1). The response to the two doses was similar and the maximum rise in serum LH in individuals showed no significant difference using the paired t test (P > 0·5). The response in individuals tested on three occasions proved to be reproducible (Fig. 2). The maximum rise of serum LH after 100 μg LH–RH showed a variance ten times higher between subjects than within the same subject (P < 0·01). The mean rise of serum LH following a second injection of LH–RH, 90 min after the initial injection was 2·15 mu/ml (9 ± 0·34 s.e.m.) and was similar to the rise of 2·49 mu/ml (±0·45) seen after the first injection (Fig. 3). When two doses of LH–RH were given 45 min apart, the maximum rise in serum LH after the two injections was 2·95 mu/ml (±0·62) and 0·28 mu/ml (±0·13). Injections of the hormone at 0 and 30 min produced rises in serum LH of 1·3 mu/ml (±0·45) and 0·12 mu/ml (±0·03) respectively. Only two of the five subjects given 0·25 μg LH–RH showed more than a doubling of their basal LH levels though a small response was seen in the other three (Fig. 4). Following 1 and 4 μg LH–RH, the serum LH was significantly elevated above the levels seen immediately before each injection (P < 0·005 and 0·025 respectively). The maximum rise of serum LH levels after 0·25, 1 and 4 μg of the hormone showed a linear log-dose response (P < 0·01) (Fig. 5).

Serum FSH

A rise in serum FSH was seen in only eight of the nineteen subjects to whom 50 or 100 μg LH–RH were given and the range of response was wider than with LH (Fig. 6). The maximum rise in serum FSH showed no significant difference in individuals receiving both doses of the hormone (P > 0·5). Serum FSH levels reached their height later than LH and were maximal 45 min after the injection. The response in the individual was reproducible but not to the extent found with serum LH (Fig. 7) but the range
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Fig. 3. Changes of serum LH (mean and s.e. mean) in six normal men given 100 μg LH–RH 90 min apart (c). In three, the test was repeated with injections 45 min apart (b) and in another two, 30 min apart (a). The arrows show the time of injections.

Fig. 4. Serum LH levels (mean and s.e. mean) in five normal men injected with 0.25, 1 and 4 μg LH–RH at hourly intervals. The arrows show the time of injections.

was too wide to permit statistical analysis. Paired injections of LH–RH at 90-min intervals elevated serum FSH in two of the five subjects tested (Fig. 8). The rise in serum FSH which followed the second injection in these two subjects was of the same order as that produced by the initial dose.

There was no rise in serum FSH with doses of 0.25, 1 and 4 μg of LH–RH in the five subjects tested. One of these subjects had previously shown a rise of serum FSH with 100 μg of the hormone.

Fig. 5. The maximum rise of serum LH and FSH (mean and s.e. mean) from pre-injection levels in five normal men given 0.25, 1 and 4 μg LH–RH at hourly intervals.

Fig. 6. Serum FSH levels (mean and s.e. mean) in nine normal men after injections of 50 (broken line) and 100 (solid line) μg LH–RH. Subjects who responded and showed no response are shown separately and the figures indicate the number of subjects in each group.
The maximum increment of serum LH in subjects who also showed a FSH response to 50 and 100 μg LH–RH was 3·85 (±1·33 s.d.) μu/ml which was significantly higher (P < 0·001) than the mean maximum increment of 2·2 (±0·87) μu/ml in serum LH levels in those who showed no FSH response (Fig. 9).

**Discussion**

The two- to ten-fold rise in serum LH observed in the present study in response to 50 and 100 μg is similar to previous reports (Schally et al., 1971b; Besser et al., 1972; Yen et al., 1972).

Although the response in individuals proved reproducible, there was considerable variation among different subjects which must be taken into consideration when using LH–RH for clinical purposes. Injections of LH–RH have been shown to produce a less consistent and less marked rise of serum FSH (Kastin et al., 1972; Yen et al., 1972; Besser et al., 1972; Nillius and Wide, 1972; Rebar et al., 1973), and in the present study, only 40% of normal men showed a measurable rise of serum FSH following doses of 50 and 100 μg. In another series, 75% of men similarly tested failed to show a rise of serum FSH beyond the normal range (Besser et al., 1972).

We were interested to find that the subjects showing a rise of serum FSH tended to be those who attained higher levels of serum LH. The explanation for this is uncertain, but may reflect variations in the endogenous LH–RH secretion. Thus, post-menopausal women (Siler and Yen, 1973) and patients with primary hypogonadism (Mortimer et al., 1973; Siler and Yen, 1973) show an exaggerated response to LH–RH.

A significant elevation of serum LH was seen in all the subjects given 1 μg LH–RH (Fig. 4), and a linear log dose-response relationship over the range 0·25–4 μg was observed. There was, however, no rise of serum FSH with these doses of LH–RH. There is conflicting evidence regarding the minimum dose of the hormone required to produce a rise of serum LH. Thus, 1·5 μg (Schally et al., 1971b), 10 μg (Rebar et al., 1973) and 25 μg (Haug and Torjesen, 1973) have previously been reported to cause a significant release of LH. Such differences could perhaps be due...
to variations in the sensitivities of the radioimmunoassays or to the differences in the hormone used. The response to these lower doses of LH–RH may prove more discriminating for testing hypothalamic-pituitary function than the larger doses that have been used to date.

Two successive injections of LH–RH at 90-min intervals produced similar elevations in serum LH. However, when the interval between the injections was reduced to 45 and 30 min, a much reduced LH response to the second injection was observed. This apparent 'refractory period' has been noted by Schneider and Dahlen (1973).

It has been a consistent finding that the increase of serum LH caused by a single injection of LH–RH is greater than that of serum FSH (Kastin et al., 1972; Yen et al., 1972, Besser et al., 1972; Nillius and Wide, 1972; Rebar et al., 1973). This has also been found of biologically active analogues of the molecule (Schally et al., 1973). An enhanced rise of serum FSH has been reported with more prolonged stimulation of the pituitary by LH–RH (Schally et al., 1973). We were, however, unable to show a larger rise of serum FSH with paired injections at intervals of 90 min.

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