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Dose-Response Relationship of Luteinizing Hormone to Luteinizing Hormone–Releasing Hormone in Man

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ABSTRACT In previous clinical studies with highly purified porcine luteinizing hormone–releasing hormone (LH-RH), administration of the somewhat arbitrarily chosen doses of 700–1500 μg resulted in increased serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The present study determined the minimum effective dose as well as the relationship of the response of serum LH and FSH to the dose of LH-RH administered. Three normal men received i.v. injections of 1.1–810 μg of LH-RH. A dose of 10 μg of LH-RH caused a statistically significant elevation in serum LH. 30 μg of LH-RH significantly increased serum FSH levels. A highly significant linear trend was observed in the log dose-response curve. The results indicate that both LH and FSH release occurs in man with doses of LH-RH much lower than previously used and that a linear log dose-response relationship can be obtained.

INTRODUCTION

Three studies have been reported in which highly purified porcine luteinizing hormone–releasing hormone (LH-RH)[1] has been administered to human beings (1–3). The primary purpose of these previous investigations was to demonstrate the effectiveness of this hypothalamic hormone in releasing LH from the pituitary gland of man in a number of clinical and experimental conditions. A single dose of LH-RH was used in these tests. This amount, 700–1500 μg, was calculated on the basis of results obtained from experiments in animals (4–6) and was chosen so as to be reasonably sure of giving an adequate response.

The present study was designed to determine the minimum effective dose of this highly purified preparation of porcine LH-RH in releasing LH in man. At the same time, it was ascertained whether a linear log dose-response relationship of serum LH to the administered LH-RH could be demonstrated.

METHODS

The LH-RH used in this study was the same as that employed in the other clinical studies with porcine LH-RH (1–3). Its preparation from acetic acid extracts of porcine hypothalami was described previously (6, 7) and consisted of gel filtration on Sephadex G-25 columns, followed by concentration with phenol, chromatography, and rechromatography on carboxymethylcellulose columns, free-flow electrophoresis, and counter-current distribution. The fraction utilized for this study stimulated LH release in ovariectomized rats pretreated with estrogen and progesterone at doses of 10 ng; it was 10–15 times less potent than our most highly purified preparation of LH-RH (8).

Three normal men, 30–33 yr old, received a single i.v. injection of LH-RH at intervals of at least 1 wk. The material was dissolved in 0.1 M acetic acid and diluted with saline. The following doses were used: 1.1, 3.3, 10, 30, 90, 270, and 810 μg. No side effects whatsoever were noted. Informed, written consent was obtained from all subjects.

Blood was taken from an indwelling i.v. catheter immediately before injection of LH-RH (time 0), and 8, 16, 32, 64, and 128 min later. After centrifugation, the serum was separated and frozen. Serum LH and follicle-stimulating hormone (FSH) levels were measured by specific radioimmunoassays (9, 10) and expressed as milli-International

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[1] *Abbreviations used in this paper:* FSH, follicle-stimulating hormone; 2nd IRP-HMG, second International Reference Preparation human menopausal gonadotropin; LH-RH, luteinizing hormone–releasing hormone.

Units (mIU) of second International Reference Preparation human menopausal gonadotropin (2nd IRP-HMG) per milliliter serum. 1 mg of LER 907 standard has LH activity equivalent to 210 IU of 2nd IRP-HMG and FSH activity equivalent to 50 IU of 2nd IRP-HMG. LH-RH has no LH or FSH activity in these assays. It has previously been shown that injection of an amount of vasopressin equivalent to that contained in the LH-RH does not significantly affect serum LH and FSH (1, 2).

Statistical significance at the 0.05 level was tested by the analysis of variance followed by Duncan's multiple range test among the treatment means for each dose at each time. Linear, quadratic, and cubic effects were obtained for the log dose and log time effects.

RESULTS

The mean response of serum LH to LH-RH at the doses of 1.1 and 3.3 μg did not differ significantly from the base line (Table I). Injection of 10 μg of LH-RH resulted in a significant increase in serum LH levels beginning 16 min later. The 8 min sample at this dose, however, was not significantly elevated. Using 30 μg of LH-RH, significant increases in serum LH were obtained in all samples, although by 128 min LH had almost returned to the level obtained before injection. The higher doses of LH-RH (90, 270, and 810 μg) significantly elevated serum LH at all the sampling times.

Since the most potent preparations of LH-RH, which appear to be homogeneous, contain FSH-RH activity, FSH levels were also measured (8, 11). No significant increases in serum FSH were obtained with 1.1, 3.3, or 10 μg of LH-RH (Table II). At a dose of 30 μg of LH-RH, a significant increase in serum FSH levels occurred in all samples except the first one (8 min). Administration of 90, 270, or 810 μg of LH-RH caused a significant increase in serum FSH in all the samples as compared with the values obtained before injection.

TABLE I

Mean Serum LH Levels (mIU/ml) after Administration of Porcine LH–RH to Three Normal Men

Dose	Time after injection of LH–RH in minutes					
	0	8	16	32	64	128
μg						
1.1	5.4	5.7	6.5	7.0	7.0	6.7
3.3	6.1	7.1	6.4	6.8	5.2	5.6
10	6.5	10.3	14.5*	12.9*	10.9*	11.7*
30	5.8	14.7*	15.8*	13.3*	10.8*	8.3
90	6.0	18.8*	22.7*	22.2*	17.4*	12.5*
270	7.4	19.5*	24.9*	21.9*	17.5*	12.6*
810	6.8	20.1*	27.6*	24.1*	21.6*	19.4*

* Values indicated by an asterisk are significantly different from those obtained before injection of LH–RH (time 0) at each time.

TABLE II

Mean Serum FSH Levels (mIU/ml) after Administration of Porcine LH–RH to Three Normal Men

Dose	Time after injection of LH–RH in minutes					
	0	8	16	32	64	128
μg						
1.1	8.3	8.6	9.0	8.7	9.2	8.9
3.3	8.3	8.5	8.1	8.3	7.7	8.0
10	12.4	13.3	13.3	13.2	13.3	14.0*
30	11.0	12.2	12.8*	12.8*	13.0*	12.7*
90	10.8	13.5*	14.2*	15.5*	13.2*	15.1*
270	11.6	13.8*	16.7*	16.7*	17.4*	16.8*
810	7.8	11.2*	13.6*	17.6*	17.1*	15.3*

* Values indicated by an asterisk are significantly different from those obtained before injection of LH–RH (time 0) at each time.

A highly significant ($P < 0.01$) linear trend in the log dose-response curve was observed for both LH and FSH after administration of LH-RH. This is illustrated for LH in Fig. 1 at the times of maximum response, which usually occurred at 16 min (Table I).

In addition to there being statistically significant differences in serum LH and FSH at various times and at various doses, there was a significant difference in the responses of the individual subjects (subject-dose interaction, subject-time interaction). One of the men, in particular, showed consistently low responses to each dose of LH-RH. The statistical tests used in these analyses were based upon a pooled estimate of the error term. This estimate is the best measurement of individual variability and is obtained by adjustment of the total variation by all known sources of variation.

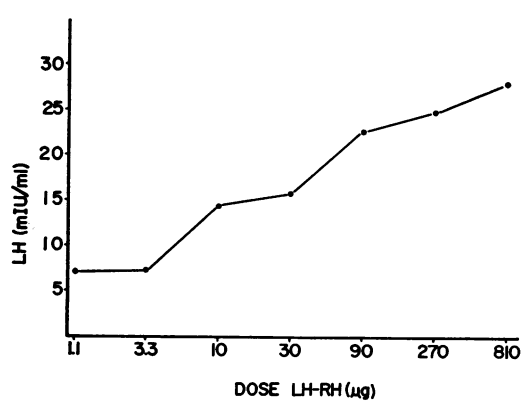

FIGURE 1 Maximum mean responses of plasma LH levels (mIU/ml) in three normal men to porcine LH-RH. The SEM at each point is 1.35 mIU/ml. This was derived from the residual mean square after analysis of variance.

DISCUSSION

Previous studies have demonstrated that administration of 700–1500 μg of porcine LH-RH results in elevation of serum LH values in the human being (1–3). In the present study, the smallest dose of LH-RH which increased serum LH in the three normal men to a level significantly different from that obtained before injection was 10 μg. This is equivalent to less than 1 μg of our most highly purified porcine LH-RH. At this 10 μg dose, however, the 8 min sample did not show a statistically significant elevation. Administration of 30 μg of LH-RH resulted in significant increases of serum LH in all the samples (8–128 min).

A slightly higher dose of LH-RH was required to cause significant increases in serum FSH values. Thus, 30 μg of LH-RH resulted in elevation of FSH in each sample except that obtained at 8 min, and 90 μg significantly increased FSH levels in all the samples. Biochemical and physiological results suggest that the FSH-RH activity of the most highly purified preparations of porcine LH-RH is intrinsic to LH-RH rather than due to contamination with FSH-RH (6, 8, 11, 12). The present study again demonstrates that in humans, also, LH-RH releases FSH as well as LH (1–3). It does not cause consistent changes in the plasma levels of growth hormone, thyrotropin, or cortisol (13).

The linear trend to the log dose-response curve was highly significant ($P < 0.01$). The maximum response of serum LH to each dose of LH-RH has been selected to illustrate this point in Fig. 1. Therefore, in man as in the rat (6–8), LH–RH behaves in a manner similar to other hormones, exhibiting a linear log dose-response curve. Significant LH release can be obtained with doses of LH-RH much smaller than those used previously.

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