

## Two Pools of Luteinizing Hormone in the Human Pituitary: Evidence from Constant Administration of Luteinizing Hormone-Releasing Hormone

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**ABSTRACT.** The pituitary gonadotrophin response to constant intravenous infusions of hypothalamic luteinizing hormone-releasing hormone (LH-RH), 0.2  $\mu\text{g}/\text{min}$  for 4 hr, was studied in 5 normal human men. Serum LH-RH levels were measured by radioimmunoassay to confirm the constancy of the infusions. Serum luteinizing hormone levels in response to the constant LH-RH administration revealed a biphasic pattern of elevation characterized by early and late

peaks. This pattern of release is similar to that of other hormones stored in granules and suggests the existence of two pools of luteinizing hormone in the human pituitary, one requiring longer LH-RH stimulation for release than the other. In contrast, serum follicle-stimulating hormone values revealed a gradual rise during the entire infusion. (*J Clin Endocrinol Metab* 39: 811, 1974)

SEVERAL hormones including insulin (1,2), vasopressin (3,4), and glucagon (5), which are stored in granules in the secretory cells of their glands of origin, have been shown to be released in a biphasic pattern under the constant stimulation of an appropriate agent. Their release is characterized by an initial acute rise, then a stabilization or fall, followed by a second phase of constant or gradually rising secretion rate. Hypotheses to explain the biphasic release have focused on two "pools" of releasable hormone, one pool thought to be synthesized and stored in granules close to the cell membrane and therefore acutely releasable, with the second pool originating in other granules, possibly requiring hormone synthesis or attachment of granules to microtubules before release can occur (6-8). However, the possibility that a portion of the hormone release may be through a mechanism independent of the secretory granules has not been completely excluded (9).

With the isolation and identification of the decapeptide, luteinizing hormone-releasing hormone (LH-RH), from the porcine (10,11) and bovine (12) hypothalamus, and its subsequent synthesis (13), it is now possible to

study gonadotrophin release in response to its stimulus. Since luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are known to be stored in the pituitary in secretory granules (14), it was of interest to examine the pattern of gonadotrophin release in response to constant administration of LH-RH. We report here a biphasic release of LH in response to constant administration of LH-RH and also describe the FSH responses to this stimulus in normal men.

### Materials and Methods

Five normal men between the ages of 20 and 25 yr were selected. Normality was confirmed by medical history, physical examination, complete blood count, urinalysis and blood-chemistry screening, both before and after the experimental protocol. The only significant finding was a history of unilateral cryptorchidism in 1 subject (P.B.). This subject had normal LH, FSH and testosterone levels and a normal sperm count. The subjects were paid, fully informed volunteers.

Synthetic LH-RH<sup>1</sup> was administered by constant intravenous infusion (Harvard pump) through an indwelling needle in one arm. Blood was obtained for hormone measurements from an indwelling needle in the other arm. LH-RH was administered at a rate of 0.2  $\mu\text{g}/\text{min}$  for 4 hr in

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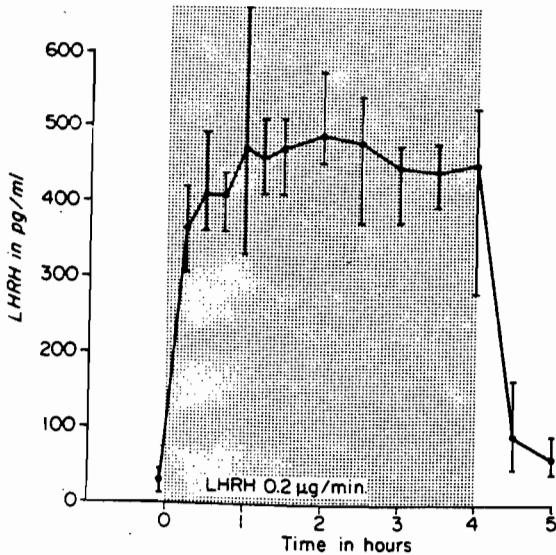


FIG. 1. Means and ranges of serum LH-RH values before, during and after LH-RH infusions in 4 subjects.

each case. All experimental sessions began at 9:00 AM. The subjects were not restricted as to diet and they could move about freely from a chair to a bed during the infusion.

Two base line blood samples, separated by 15 min, were obtained. During the LH-RH infusion, blood was sampled at 15-min intervals for 90 min, then at 30-min intervals until the infusion was stopped. Several samples were obtained from each subject at various intervals following LH-RH administration.

Accuracy of the infusion technique was assessed by radioimmunoassay of serum LH-RH values<sup>2</sup> in 4 subjects. LH and FSH titers<sup>3</sup> were measured in duplicate by double-antibody radioimmunoassay techniques (15,16). All samples from each subject were measured in one assay.

## Results

LH-RH values (Fig. 1), from a base line of 25 pg/ml or less, reached a stable level of

<sup>2</sup> LH-RH assays done courtesy of Dr. David deKretser, Prince Henry's Hospital, Monash University, Melbourne, Australia, using antiserum prepared by Dr. G. Niswender. Assay described by H. A. Jonas, J. K. Findlay, J. R. Goding, H. G. Burger, and D. M. deKretser. *J. Reprod Fertil* 36: 446, 1974.

<sup>3</sup> In our laboratory, 1 mg LER 907 equals 219 IU of 2nd IRP-hMG in terms of LH, and 38 IU of 2nd IRP-hMG in terms of FSH. Normal ranges for adult men: LH, 4-25 mIU 2nd IRP/ml and FSH, 50-450 ng LER 907/ml.

approximately 450 pg/ml by 30-60 min after the infusion had begun and remained there until the end of the infusion, when they fell rapidly. These rapid adjustments of LH-RH levels are consistent with the early component half-life of LH-RH in man, which has been found to be approximately 4.0 min (17-19).

LH and FSH results for each of the 5 subjects are shown in Fig. 2. In response to the constant infusion of LH-RH, serum LH levels rose in a biphasic pattern. After an initial steep rise during the first 30-60 min of the infusion, LH values stabilized or actually decreased until 90-120 min after the onset of the infusion, when they began to increase again, continuing to increase until the infusion was stopped at 4 hr. A fall in LH levels was detectable in each subject by 30 min following cessation of the infusion. LH continued to drop toward base line values over the next 1 to 2 hr.

Serum FSH values (Fig. 2) during constant LH-releasing hormone infusion showed a gradual, progressive rise until the end of the infusion. Maximal levels of FSH obtained (50-100% increase over base line) were markedly less than the levels of LH (300-600% increase over base line).

## Discussion

The present data demonstrate that during constant infusion of LH-RH into normal men, serum LH values reveal a biphasic pattern of elevation. This pattern is characterized by an acute rise during the first 30 min of the infusion, followed by a plateau or fall until approximately 90 min, when a second rise begins that persists until the end of the infusion. Measurement of LH-RH in the serum established that the infusion technique did, in fact, lead to a rapid increase in LH-RH values to a constant level where they remained until the infusion was terminated. Although the high, constant levels of LH-RH were reached by 30 to 60 min, LH values were stable or decreasing at this time. By 90 min to 2 hr, however, with no further changes in LH-RH levels, LH values again began to rise and continued to rise until the end of the infusion. We interpret the two

phases of LH release as evidence for the existence of two functional pools of LH in the human pituitary, one that is acutely releasable and another that requires longer LH-RH stimulation to be released.

The precise mechanisms underlying the biphasic pattern of release are unknown. Studies of the pancreas have shown that chemicals which interfere with protein synthesis, such as puromycin, seem to inhibit selectively the second phase of insulin release (20). This suggests that insulin released during the first phase may be preformed, while that released during the second phase may be at least partially dependent on new synthesis of the hormone or of a protein necessary for the release of the hormone. Recent work has indicated, however, that newly synthesized insulin is responsible for only 17% of the insulin released by 2 hr following initiation of glucose stimulation of perfused islets of the rat pancreas (36). This implies that the second phase of secretion may not require new synthesis of insulin. Other studies (6) have stressed the possible importance of granules close to the cell membrane in the origin of acutely releasable hormone, while later hormone release may be dependent on microtubular transport of other granules to the cell membrane.

Gonzales-Barcena *et al.* (21) recently presented data obtained from normal men in which a biphasic LH response to a 4-hr infusion of LH-RH of approximately 1.0  $\mu\text{g}/\text{min}$  can be seen. Serum LH-RH values were not measured to confirm constancy of the infusion. The authors did not speculate as to the possible mechanism of the biphasic response.

In studies involving frequent blood sampling in the basal state, LH "spikes" (rapid increases and decreases of serum LH values) have been noted in normal males (22-24) and in various hypothalamic, pituitary and gonadal disorders (25,26). In contrast, the LH pattern in the present study during constant LH-RH stimulation did not exhibit

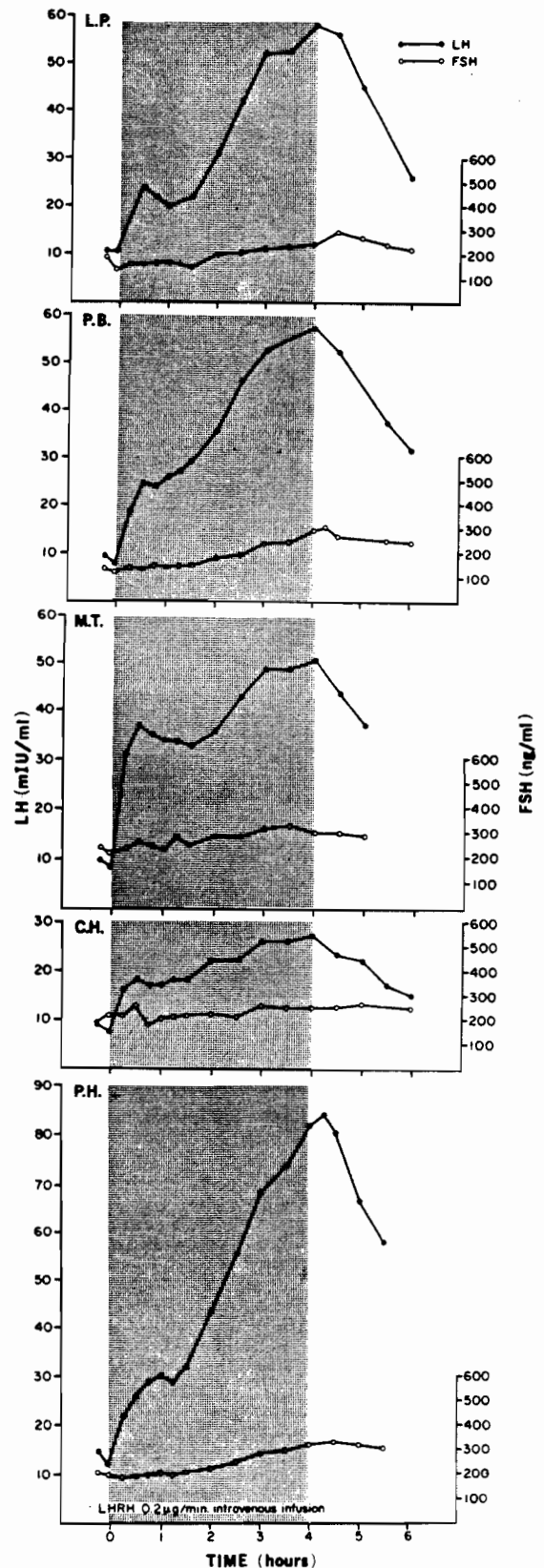


FIG. 2. Serum LH and FSH values before, during and after LH-RH infusions.

spikes although sampling was as frequent as every 15 min. This suggests that the origin of LH spikes may be in the hypothalamus or in some other central nervous system site that affects the hypothalamus and be overridden or inhibited by the exogenous administration of LH-RH.

Serum FSH levels in response to the constant LH-RH infusions did not reveal evidence of a biphasic pattern of release. Instead, there was a gradual, progressive rise until the infusion ended, reaching maximal levels of 50–100% above base line values. The degree of FSH increase noted was similar to that seen with the introduction of an acute intravenous bolus of LH-RH (27,28). This is in contrast to results of experiments on male rats (29) in which 4-hr intravenous administrations of LH-RH yielded much higher levels of FSH than did the same dosage administered as an acute intravenous bolus. It may be that longer infusions of LH-RH are necessary to demonstrate marked FSH rises in humans, since the levels of this hormone were still rising at the end of 4 hr.

It was of interest that the pattern of FSH release was different from that of LH release. An acutely releasable pool of FSH was not identified in the present data. Studies *in vitro* have suggested that early LH release in response to releasing hormones is independent of protein synthesis (30,31). In contrast, early FSH release seemed in most studies (32,33) but not all (34) to require protein synthesis. These data can be interpreted to suggest that there is a preformed pool of LH that is acutely releasable by LH-RH, but no similar, acutely releasable pool of FSH. If this is true, it would provide an explanation for the fact that a biphasic pattern of LH release, but not of FSH release, was seen in the present study. While still controversial at this point (35) an alternative explanation for the lack of a biphasic FSH response to LH-RH may be that there is a more specific FSH-releasing hormone still to be identified that could cause release of an early pool of FSH as well as the later pool. Lastly, it could be that there is, in fact, a small, acutely releas-

able pool of FSH which was not detected in the present study because of dilution of the small amount of hormone released into the total blood volume of the subjects.

The early pool of LH demonstrated in the present study may be the main source of LH released during LH "spikes." More prolonged elevations of LH, as for example in primary gonadal deficiency, presumably require release of hormone from the later pool. The explanation of the physiologic role of each of these pools of pituitary hormone awaits further study.

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