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Follicle-stimulating hormone
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luteinizing hormone
puberty
releasing factor

Characterization of the Hormonal Responses to Luteinizing Hormone-Releasing Hormone (LH-RH) in Prepubertal and Adult Subjects

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Extract

Luteinizing hormone-releasing hormone (LH-RH), purified from porcine hypothalamic tissue, was administered in an intravenous dose of 300 μg to four prepubertal and four adult human subjects. The resulting increases in plasma levels of LH and FSH were statistically significant ($P < 0.01$) in the 16- and 32-min samples, but did not differ with the age or sex of the subject groups. The mean maximum increase in plasma LH values was 290% for men and 425% for women. Injection of LH-RH resulted in a 500% mean maximum increase in LH levels in the plasma of boys and 850% in that of girls. The gonadotropin release induced by injection of LH-RH was sufficient to increase plasma levels of estradiol in some individuals. No significant elevation occurred in the levels in plasma of growth hormone, thyrotropin, or cortisol. It is concluded that LH-RH is a potent and specific hypothalamic releasing hormone for LH and FSH in prepubertal children as well as in normal adults.

Speculation

The increases in gonadotropin levels in plasma of all groups seem to reduce the possibility that a change in pituitary responsiveness to LH-RH occurs at puberty. However, similar studies involving larger numbers of individuals tested at varying doses might reveal differences in responses to LH-RH between age or sex groups.

Introduction

It is now well established that the hypothalamus produces substances which regulate the release of hormones from the adenohypophysis [19, 20, 22]. One of these hypothalamic substances, which controls the release of luteinizing hormone (LH), has been found in hypothalamic tissue from at least seven animal species, including man [17]. Injection of hypothalamic LH-releasing hormone (LH-RH) results in stimulation of the release of LH from the pituitary gland of a number of animals [17, 19, 20, 22].

Although LH-RH prepared from human hypothal-

ami is effective in releasing LH in man [4], all other clinical studies with LH-RH have used a highly purified LH-RH preparation of porcine origin except one [13], in which a crude ovine hypothalamic extract was injected. Administration of porcine LH-RH clearly was shown to increase levels in serum of LH and follicle-stimulating hormone (FSH) in normal men and women [5], even when injected subcutaneously [6]. A linear log dose-response relation has been demonstrated with this material [7]. The increased release of gonadotropins occurred regardless of whether the initial levels of LH and FSH were elevated, as in postmenopausal women [6] and in men [8] pretreated with clomiphene, or whether LH was suppressed by estrogen or an oral contraceptive [5, 6]. The presence of secondary amenorrhea in some women did not prevent the release of LH caused by LH-RH [6]; indeed, administration of LH-RH was associated with the induction of ovulation and subsequent pregnancy in one such subject [9]. LH-RH has also been suggested as a diagnostic test of pituitary function [1, 3].

The mechanisms resulting in initiation of puberty are unknown. Three hypotheses for the latency of reproductive activity in the prepubertal state are (*1*) lack of sensitivity of the gonads to gonadotropins, (*2*) lack of sensitivity of the pituitary to LH-RH, and (*3*) lack of synthesis or release of LH-RH, or both. That the "gonads are able to function in an adult fashion long before puberty" is well known [2]. The third hypothesis cannot be tested at the present time, but an increased sensitivity of the pituitary gland to the gonadotropin-releasing hormone, LH-RH, might occur at puberty. If this happened, children would be expected to be less responsive to the LH- and FSH-releasing effects of LH-RH than adults. This hypothesis was examined in the present study. In addition, the specificity of LH-RH was investigated by measurement of the effects of its administration upon levels in plasma of thyrotropin (TSH), growth hormone (GH), and cortisol as well as LH and FSH. Furthermore, it was determined whether the LH released by LH-RH was sufficient to elevate estradiol in the plasma.

Materials and Methods

Purified LH-RH was prepared by acetic acid extraction of porcine hypothalamic tissue followed by gel filtration on Sephadex, concentration by phenol, chromatography and rechromatography on carboxymethylcellulose, free-flow electrophoresis, and countercurrent distribution, as described previously [20, 21]. This preparation of LH-RH was approximately one-tenth as potent in rat assays as our most highly purified LH-RH preparation [18]. It contained approximately 0.1 pressor unit/mg but was free of anterior pituitary and other hypothalamic releasing hormones.

A dose of 300 μg porcine LH-RH was injected intravenously at time 0 and blood samples were collected 8, 16, 32, 64, 128, and 480 min later. Plasma levels of LH [16], FSH [14], TSH [24], and GH [15] were determined by radioimmunoassay; plasma cortisol [12] and estradiol [10] levels were measured by radioligand assay. One nanogram of LH = 10 milli-international units (mIU) of Second International Reference Preparation human menopausal gonadotropin (2nd IRP-HMG), 1 ng FSH = 4 mIU 2nd IRP-HMG, and 1 ng TSH = 3.2 μU. These assays can detect 0.2 ng/ml of LH and FSH, 1 ng of TSH, 1 ng of GH, and 4 μg/100 ml of cortisol. It was possible in occasional assays to detect less than 6 pg/ml of estradiol by extracting several milliliters of plasma. The LH-RH caused no side effects.

Two subjects were tested in each of four groups. The two boys were 10 and 11 years old, the two girls 8 and 10 years old, the two men 26 and 30 years old, and the two women 19 and 20 years old. None of the children had undergone puberty. Informed written consent was obtained from each adult and from an authorized representative of each child.

A least squares analysis of variance was performed on the six dependent variables: LH, FSH, TSH, GH, cortisol, and estradiol. The experimental design was that of a four-factor, partially nested experiment with repeated measures [23]. The four factors were age, sex, subjects within age-sex group, and time.

Results

Luteinizing Hormone

The mean response of levels of LH in plasma to administration of LH-RH for each group of subjects is illustrated in Figure 1. Although it appears that the prepubertal group might be more responsive than the adults, this was not statistically significant, perhaps because of the small number of subjects. The results of the statistical analyses are summarized in Table I where it is apparent that there was no difference in response to LH-RH by males and females, regardless of age, although there was considerable variability among subjects (Table II). The increased levels of LH in plasma with time were highly significant in the 16-

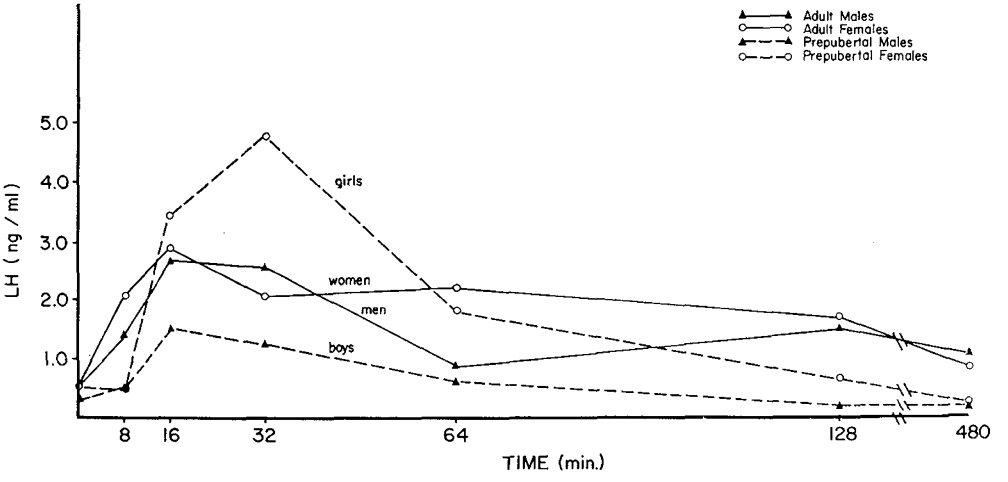

Fig. 1. Mean responses of levels of luteinizing hormone (LH) in plasma to administration of porcine luteinizing hormone-releasing hormone n four groups of subjects (1 ng LH = 10 mIU 2nd IRP-HMG).

and 32-min samples. The mean maximum increases were as follows: men, 290%; women, 465%; boys, 500%; girls, 850%.

Follicle-Stimulating Hormone

Figure 2 illustrates the mean responses of FSH to injection of LH-RH in each of the four age-sex groups. As with LH, the responses of FSH in the subjects were significantly different, and the maximum increases in FSH release were found 16 and 32 min after injection of LH-RH (Tables I and II). In contrast to the release of LH, the responses of FSH to LH-RH tended to be greater in the adults than in the prepubertal groups. The base line levels of FSH were higher in the children. It is not known whether a differential effect of the negative feedback action of the sex steroids is involved.

Thyrotropin

As summarized in Table I, there was no significant effect of LH-RH upon the release of TSH. The subjects did have significant differences in their TSH levels, however, which were not related to the treatment.

Growth Hormone

Luteinizing hormone-releasing hormone caused no statistically significant release of growth hormone (Table I). The levels of GH in women were slightly, but not significantly higher ($P < 0.10$) than in the males.

Table I. Variance of responses to porcine LH-RH[1]

Variant	LH[2]	FSH[3]	TSH[4]	GH[5]	Cortisol	Estradiol
Adult *vs* child	–	–	–	+[6]	–	–
Male *vs* female	–	–	–	–	–	–
Sex × age	–	–	–	–	–	–
Patients/(age × sex)	+[6]	+[6]	+[7]	–	–	+[6]
Time	+[6]	+[7]	–	–	–	+[7]
Time × age	–	–	–	–	–	–
Time × sex	–	–	–	–	–	+[7]
Time × age × sex	–	–	–	–	–	+[6]
0–8 min	–	–	–	–	–	–
0–16 min	+[6]	+[6]	–	–	?	–
0–32 min	+[6]	+[6]	–	–	–	+[6]
0–64 min	–	+[7]	–	–	–	–
0–128 min	–	–	–	–	–	–
0–8 hr	–	–	–	–	–	–

[1] LH-RH: luteinizing hormone-releasing hormone.
[2] LH: luteinizing hormone.
[3] FSH: follicle-stimulating hormone.
[4] TSH: thyrotropin.
[5] GH: growth hormone.
[6] $P < 0.01$.
[7] $P < 0.05$.

Cortisol

Although the analysis (Table I) indicates that the 16-min sample was significantly different from time 0, the lack of significant differences in the subjects and the overall time category make it likely that this is a spurious statistical finding. Thus, LH-RH probably had no significant effect upon the release of cortisol.

Table II. Levels of LH and FSH in plasma after administration of porcine LH-RH[1]

	LH[2]							FSH[3]						
Time, min	0	8	16	32	64	128	480	0	8	16	32	64	128	480
Subjects														
Boy	0.2	0.2	2.1	1.6	0.8	0.2	0.2	1.3	2.3	1.8	1.5	2.6	0.2	2.0
Boy	0.3	0.8	0.9	0.9	0.4	0.2	0.2	0.9	2.5	2.7	2.4	2.0	3.8	1.3
Girl	0.2	0.2	0.3	2.0	0.9	0.2	0.2	0.2	0.2	0.5	1.5	0.7	0.6	0.5
Girl	0.8	0.8	6.6	7.5	2.7	1.1	0.3	2.9	2.3	4.7	5.5	2.5	2.9	2.9
Man	0.7		3.6	3.0	1.0	1.8	1.0	0.4		2.3	3.4	1.3	1.3	1.3
Man	0.6	1.4	1.7	2.1	0.8	1.2	1.2	0.6	1.0	3.2	2.0	2.7	1.1	1.0
Woman	0.7	3.9	5.6	2.9	3.7	1.5	1.2	0.3	6.3	7.0	8.3	4.0	3.2	3.0
Woman	0.4	0.2	0.2	1.2	0.7	0.9	0.5	0.2	1.0	0.2	0.8	1.0	0.8	0.2

[1] LH-RH: luteinizing hormone-releasing hormone.
[2] LH: luteinizing hormone, in ng/ml.
[3] FSH: follicle-stimulating hormone, in ng/ml.

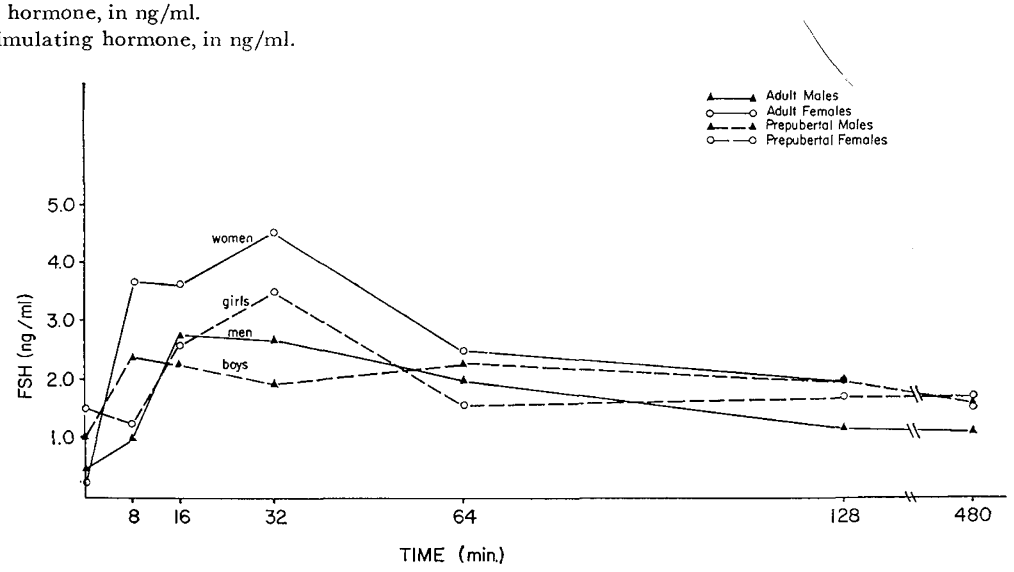

Fig. 2. Mean responses of levels of follicle-stimulating hormone (FSH) in plasma to administration of porcine luteinizing hormone-releasing hormone in four groups of subjects (1 ng FSH = 4 mIU 2nd IRP-HMG).

Table III. Levels of estradiol (picograms per milliliter) in plasma after administration of porcine LH-RH[1]

Subject	Time, min			
	0	32	128	480
Boy	0	5	15	19
Boy	8	17	23	6
Girl	0	8	9	0
Girl	104	118	126	106
Man	20	82	39	19
Man	42	88	20	23
Woman	99	112	125	162
Woman	37	34	40	50

LH-RH: luteinizing hormone-releasing hormone.

Estradiol

Estradiol was determined in samples of plasma obtained at 0, 32, 128, and 480 min. The time × sex interaction was statistically significant, indicating a difference between males and females over time after injection with LH-RH (Table I). In this study, only the males responded to LH-RH with an increased release of estradiol, an increase which was highly significant in the 32-min sample. This might represent increased conversion from testosterone. The data are presented in Table III.

Discussion

Previous studies have shown that adult men and women respond to the administration of highly purified porcine LH-RH with a significant release of LH [5–7]. No significant difference in response between men and women was found [5]. The present investigation confirmed these findings, although the total num-

ber of cases was small. The significant increase in levels of LH in plasma after injection of LH-RH was of the same magnitude in men and women (Fig. 1, Table I).

In every clinical study in which porcine LH-RH has been administered, a significant increase in levels of FSH in plasma accompanied the rise in LH values. Since a homogeneous natural [18] or synthetic [11] preparation of LH-RH releases FSH in the rat, it now appears that the FSH-releasing effect of LH-RH is intrinsic to LH-RH rather than due to contamination with an FSH-releasing hormone. Significant elevation of levels of FSH in plasma was also observed in the present study (Fig. 2, Table I).

The response of the prepubertal pituitary to LH-RH, as measured by release of LH and FSH, was at least as great as that of the adult pituitary. Thus it is unlikely that puberty represents a state of increasing responsiveness to the hypothalamic gonadotropin-releasing hormone. Inasmuch as a change in gonadal sensitivity has already been eliminated as an explanation [2], the hypothalamus or higher central nervous system is probably the site for the initiation of puberty.

Root *et al.* [13] injected "crude ovine hypothalamic extract" into "two human infants with lethal chromosomal anomalies and multiple congenital anomalies and into one child with severe cerebral dysfunction." An increase in plasma LH was observed in all subjects after they received a dose several hundred times greater than that employed in the present study.

Experiments in animals have shown that LH-RH does not release other pituitary hormones [17, 19, 20, 22]. No statistically significant increase in TSH, GH, or cortisol release was found in our human subjects after injection of LH-RH (Table I).

Additional evidence for the relative specificity of LH-RH in the human being is provided by the lack of a significant effect upon LH and FSH release of the administration of 0.1 to 1.0 unit of lysine or arginine vasopressin [4–6]. These doses of vasopressin were approximately equivalent to or exceeded the amounts which the subjects received in the porcine LH-RH preparations.

The increased levels of gonadotropin induced by administration of LH-RH were sufficient to result in increased estradiol levels in the males, but not in the females. The hypothesis that the LH released by administration of LH-RH can stimulate a target gland received additional support from a case in which ovulation was apparently induced by LH-RH [9]. In this instance, pregnancy provided conclusive proof of ovulation.

Summary

Highly purified porcine LH-RH was injected intravenously into two boys, two girls, two men, and two women. A statistically significant increase in levels of LH and FSH in plasma resulted in all groups regardless of age or sex. Levels of GH, TSH, and cortisol in plasma were not significantly changed by the LH-RH. The gonadotropin released by LH-RH was apparently sufficient to increase levels of estradiol in plasma of the males. Thus, investigations with LH-RH might help to elucidate the physiologic mechanisms involved in puberty and in the reproductive cycle.

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