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## SHORT-TERM TREATMENT OF IDIOPATHIC PRECOCIOUS PUBERTY WITH A LONG-ACTING ANALOGUE OF LUTEINIZING HORMONE-RELEASING HORMONE

### A Preliminary Report

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**Abstract** The uncoupling of pituitary stimulation and response observed in adults during administration of the luteinizing hormone-releasing hormone analogue, D-Trp<sup>6</sup>-Pro<sup>9</sup>-NH<sub>2</sub>-LHRH (LHRH<sub>a</sub>) suggested that this drug might be useful in treating precocious puberty. We treated five girls with idiopathic precocious puberty (ages two to eight) for eight weeks with daily subcutaneous injections of LHRH<sub>a</sub>. The patients had Tanner II to IV pubertal development, advanced bone age, an estrogen effect on vaginal smear, measurable basal gonadotropin levels with pulsed nocturnal secretion, and a pubertal gonadotropin response

to LHRH. Irregular vaginal bleeding was present in three patients. LHRH<sub>a</sub> significantly decreased basal ( $P < 0.025$ ) and LHRH-stimulated ( $P < 0.01$ ) gonadotropin levels as well as serum estradiol ( $P < 0.05$ ). The vaginal maturation-index score, which reflects the estrogen effect, fell by 25 per cent. Eight weeks after stopping treatment, all hormonal values and the vaginal maturation index had returned to pretreatment levels. These favorable short-term results will need further study before the benefits and risks of chronic treatment with LHRH<sub>a</sub> can be adequately assessed. (*N Engl J Med.* 1981; 305:1546-50.)

**P**UBERTY is initiated by the pulsed nocturnal secretion of gonadotropins. These gonadotropin pulses, resulting from the episodic release of luteinizing

hormone-releasing hormone (LHRH) by the hypothalamus, stimulate gonadal sex-steroid secretion, which leads to the secondary sexual changes characteristic of puberty.<sup>1</sup> This process begins prematurely in idiopathic precocious puberty. The premature occurrence of pubertal maturation adversely affects subsequent development in several ways. Adult stature may be compromised because of accelerated epiphyseal closure. Adverse psychosocial effects (including libido, the need for contraception, and rejection by peers) may be profound for both the child and the family.

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Supported in part by grants (RR 01066, 1-K08-AM-00573-02, and HD 13527) from the National Institutes of Health, and by the Rockefeller Foundation.

Research in the Peptide Biology Laboratory at the Salk Institute was conducted in part by The Clayton Foundation for Research, California Division. Drs. Vale and Rivier are Clayton Foundation Investigators.

Presented in part at the Joint Plenary Session of the American Society for Clinical Investigation, San Francisco, April 26, 1981 (Abstract 504A).

An adequate treatment for this disorder is not available. Neither antagonizing the effects of sex steroids with antiandrogens or suppressing gonadal secretion with progestins has been fully effective, and both ap-

proaches have been associated with adrenal suppression.<sup>2-4</sup> An alternative approach was suggested by the recent demonstration that continuous administration of LHRH or intermittent administration of potent agonist analogues of LHRH initially stimulated, but subsequently inhibited, the release of luteinizing hormone and follicle-stimulating hormone.<sup>5-8</sup> This phenomenon appears to represent an uncoupling of LHRH-receptor occupation and the pituitary response. Application of this finding to the development of a potential contraceptive has already been described.<sup>7,9</sup> This property of LHRH agonists suggested that these agents might be useful as a treatment for idiopathic precocious puberty. We have previously reported successful early results with such treatment in a single patient.<sup>10</sup>

This report describes our further, but still preliminary, experience with the short-term treatment of idiopathic precocious puberty with an LHRH agonist. These studies have confirmed the favorable hormonal changes previously observed and revealed beneficial clinical effects on the regression of breast and pubic-hair development. Adequate assessment of the risks and benefits of long-term treatment with an LHRH-agonist analogue will require much further study.

## METHODS

### LHRH Analogue

The LHRH analogue D-Trp<sup>6</sup>-Pro<sup>9</sup>-NEt-LHRH (LHRH<sub>a</sub>) was dissolved in normal saline and 10 per cent mannitol.<sup>11</sup> Once dissolved, the compound was stored at -20°C until prescribed. Each batch was bioassayed before its use, and no loss of biologic activity was observed after storage for as long as 12 months. The parents were instructed to keep the preparation frozen until use. LHRH<sub>a</sub> was injected subcutaneously by a parent using an insulin syringe. The frequency of administration of LHRH<sub>a</sub> was based on previous studies demonstrating that daily administration of this analogue would suppress gonadotropin secretion.<sup>6</sup>

### Patients

The clinical features of the five patients with idiopathic precocious puberty are shown in Table 1. The diagnosis of idiopathic precocious puberty was made after excluding a brain, adrenal, or ovarian neoplasm by computerized tomography of the head and abdomen and ultrasonography of the adrenal and pelvis. Plasma 17-hydroxyprogesterone and 11-deoxycortisol were measured to ex-

clude congenital adrenal hyperplasia. Serum levels of human chorionic gonadotropin were measured to rule out a neoplasm producing this hormone.

### Protocol

Patients were admitted to the Clinical Center of the National Institutes of Health (Patients 1, 2, 4, and 5) or the General Clinical Research Center of the Massachusetts General Hospital (Patient 3). Protocols were reviewed by the Clinical Research Committee of the National Institute of Child Health and Human Development and the Scientific Advisory Committee at Massachusetts General Hospital. Informed consent was obtained from either parent and informed consent from older children before therapy. Pretreatment evaluation consisted of basal serum gonadotropin determinations every 20 minutes for four hours during the day (10 a.m. to 2 p.m.) and night (10 p.m. to 2 a.m.). Serum estradiol was measured four times — at the beginning and end of each four-hour period. On Day 2, an LHRH stimulation test was performed. Serum gonadotropins were measured at -30, -15, 0, 15, 30, 45, 60, 90, 120, and 180 minutes, in relation to intravenous administration of 100 µg of LHRH at time zero (Patient 3 received 2.5 µg of LHRH per kilogram of body weight subcutaneously at time zero, with serum gonadotropin measured at zero, 30, 60, 90, 120, and 180 minutes). On Day 3 a vaginal specimen was obtained for determination of the maturation index.<sup>13</sup> The vaginal smears were evaluated by the cytology laboratory without knowledge of the patient's therapy. LHRH<sub>a</sub> (4 µg per kilogram per day) was then given by subcutaneous injection for two months. During the eighth week of therapy, the patients were reevaluated by the same protocol used before therapy. LHRH<sub>a</sub> treatment was then discontinued in Patients 1 to 3. These three patients returned during the eighth week after stopping therapy for a third inpatient evaluation identical to those performed before and during LHRH<sub>a</sub> administration. All patients underwent biweekly outpatient monitoring during the two months of therapy and, in Patients 1 to 3, during the subsequent two-month recovery period. These evaluations consisted of inspection of breasts and pubic hair, and measurement of height, weight, vaginal maturation-index score, and plasma estradiol (three measurements at 20-minute intervals in four of the five patients; no measurements in Patient 3).

### Hormone Assays

Luteinizing hormone, follicle-stimulating hormone, estradiol, and dehydroepiandrosterone sulfate were measured with a modification of previously described methods.<sup>14-18</sup> Delayed addition of trace after three days at 4°C reduced the limit of sensitivity for both luteinizing hormone and follicle-stimulating hormone to 0.5 mIU per milliliter (2d International Reference Preparation of human menopausal gonadotropin).

### Statistical Analysis

Statistical comparisons between groups were made with Student's paired t-test after logarithmic transformation to achieve uniformity of variance. All data are expressed as means ± S.E.M.

Table 1. Clinical Data.

PATIENT No.	AGE yr	HEIGHT * cm	WEIGHT * kg	STAGE OF PUBERTY †		MENSES	BONE AGE yr	VAGINAL MATURATION-INDEX SCORE ‡
				BREAST	PUBIC HAIR			
1 §	2.3	97 (97)	22 (>99)	II	II	+	5	78
2	4.9	124 (>99)	27 (>99)	III	II	+	10	50
3	7.3	131 (85)	33 (96)	III	IV	0	10	60
4	7.8	136 (93)	40 (>99)	III	III	+	10	52
5	5.2	123 (99)	20 (50)	III	II	0	9	53

\*Values in parentheses represent percentiles for age.

†According to the classification of Tanner.<sup>12</sup>

‡An index of estrogen effect on the vaginal mucosa, calculated by adding the percentage of superficial cells multiplied by 1.0 to the percentage of intermediate cells multiplied by 0.5.<sup>13</sup>

§A preliminary account of this case has been reported previously.<sup>10</sup>

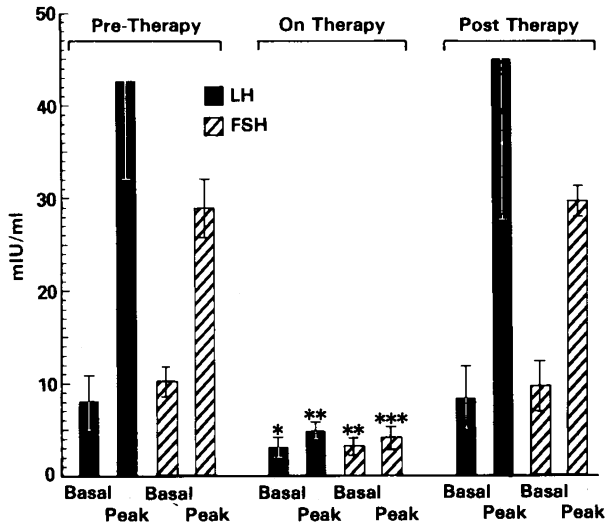


Figure 1. Effect of LHRH Analogue on Basal and Peak (LHRH-Stimulated) Gonadotropin Levels in Five Girls with Idiopathic Precocious Puberty.

The basal LH and FSH values for each patient are means of 26 measurements performed at 20-minute intervals from 10 a.m. to 2 p.m. and from 10 p.m. to 2 a.m. The peak values are the highest LH and FSH levels attained during the standard LHRH stimulation tests performed in each patient. The histograms represent the means  $\pm$ S.E.M. of basal and peak values for each patient. The levels during therapy were measured during the eighth week of treatment. The post-therapy levels were measured in Patients 1 to 3 eight weeks after discontinuation of LHRH<sub>a</sub> treatment. Patients 4 and 5 continued to receive LHRH<sub>a</sub>. The single asterisk denotes  $P < 0.025$ , the double asterisk  $P < 0.01$ , and the triple asterisk  $P < 0.001$ , as compared with pretreatment levels.

RESULTS

All the patients had measurable basal gonadotropin levels and a pubertal response to LHRH stimulation before therapy (Fig. 1). After eight weeks of treatment with LHRH<sub>a</sub>, basal and peak gonadotropin levels fell significantly below pretreatment levels (Fig. 1). Basal luteinizing hormone fell from  $8 \pm 3$  to  $3 \pm 1$  mIU per milliliter ( $P < 0.025$ ) during treatment, and LHRH-stimulated peak luteinizing hormone fell from  $43 \pm 10$  to  $5 \pm 1$  mIU per milliliter ( $P < 0.01$ ). Basal follicle-stimulating hormone declined from  $10 \pm 2$  to  $3 \pm 1$  ( $P < 0.01$ ), and peak follicle-stimulating hormone from  $29 \pm 3$  to  $4 \pm 1$  mIU per milliliter ( $P < 0.001$ ). Basal gonadotropins and the response to the LHRH stimulation test returned to pretreatment levels two months after discontinuation of LHRH<sub>a</sub>. Basal and peak luteinizing hormone were  $8 \pm 4$  and  $45 \pm 17$  mIU per milliliter. Basal follicle-stimulating hormone was  $10 \pm 3$  and peak follicle-stimulating hormone was  $30 \pm 2$  mIU per milliliter. Figure 2 shows the complete LHRH stimulation tests. LHRH<sub>a</sub> completely suppressed the responses of luteinizing hormone and follicle-stimulating hormone to exogenous LHRH. Two months after LHRH<sub>a</sub> was discontinued, both the time course and the magnitude of the response to LHRH were nearly identical to the pubertal pattern observed before therapy.

Plasma estradiol concentrations also fell (from  $28 \pm 5$  to  $16 \pm 2$  pg per milliliter) by the eighth week of LHRH<sub>a</sub> therapy ( $P < 0.05$ ) (Fig. 3). Two weeks after cessation of treatment, the level of plasma estradiol did not differ significantly from the pretreatment level.

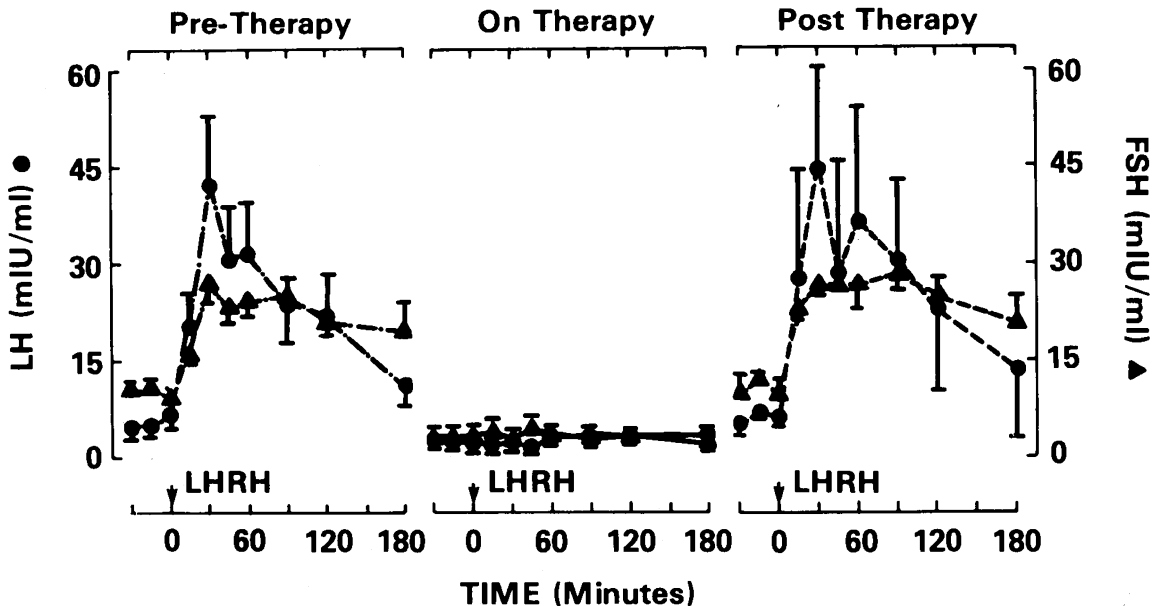


Figure 2. Effect of LHRH Analogue on Gonadotropin Response to Exogenous LHRH in Five Girls with Idiopathic Precocious Puberty.

As in Figure 1, the post-therapy data are from Patients 1 to 3.

The maturation-index score decreased 25 per cent after eight weeks of treatment with LHRH<sub>a</sub> — a change that did not reach statistical significance ( $P = 0.09$ ) (Fig. 4). Two weeks after discontinuing LHRH<sub>a</sub>, the maturation-index score had returned to the pretreatment value.

LHRH<sub>a</sub> was reinstated two months after discontinuation in Patients 1 and 2, and was continued in Patients 4 and 5. After four months of continuous LHRH<sub>a</sub>, mean basal and LHRH-stimulated gonadotropins were less than 5 mIU per milliliter, plasma estradiol was 12 pg per milliliter, and the vaginal maturation-index score was 44. Breast size decreased in Patients 2, 4, and 5 and remained unchanged in Patient 1. Pubic hair decreased (Tanner Stage II to I) in Patients 2 and 5 and was unchanged in the others. No vaginal bleeding occurred during therapy in any of the subjects.

We measured adrenal androgen levels during treatment to determine whether LHRH<sub>a</sub> influenced the

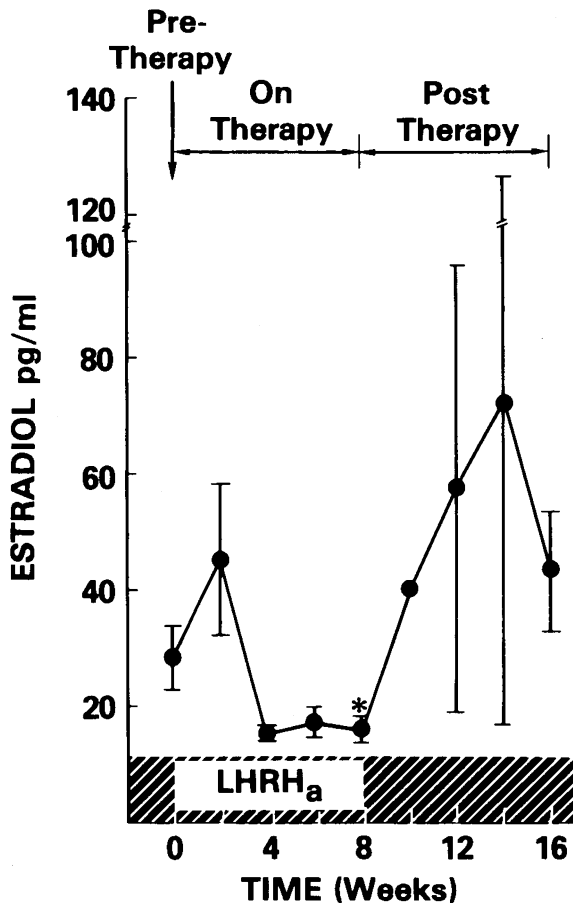


Figure 3. Effect of LHRH Analogue on Plasma Estradiol in Five Girls with Idiopathic Precocious Puberty.

The shaded area represents values below the detection limit of the assay. To convert estradiol values to picomoles per liter, multiply by 3.7. At four weeks, only the data from Patients 1, 2, and 4 were available. Post-therapy data are from Patients 1 to 3. The asterisk denotes  $P < 0.05$  as compared with pretreatment level.

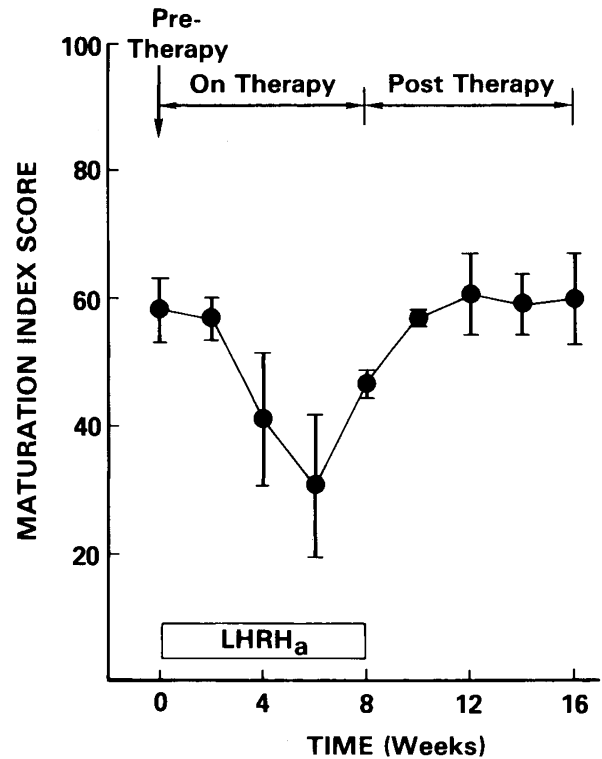


Figure 4. Effect of LHRH Analogue on Vaginal Maturation-Index Score in Five Girls with Idiopathic Precocious Puberty. See third footnote to Table 1 for the method of calculating the maturation-index score. Post-therapy data are from Patients 1 to 3.

adrenarchal component of puberty. The concentration of plasma dehydroepiandrosterone sulfate after two months of LHRH<sub>a</sub> therapy was  $19 \pm 7 \mu\text{g}$  per deciliter ( $510 \pm 190 \text{ nmol}$  per liter) — not significantly different from the concentration before therapy ( $13 \pm 5 \mu\text{g}$  per deciliter [ $350 \pm 140 \text{ nmol}$  per liter]).

## DISCUSSION

The LHRH analogue D-Trp<sup>6</sup>-Pro<sup>9</sup>-NEt-LHRH possesses greater potency and a longer duration of action than the native decapeptide.<sup>19</sup> Continuous administration of native-sequence LHRH or intermittent administration of long-acting LHRH analogues uncouples LHRH stimulation from the pituitary response and decreases pituitary gonadotropin secretion.<sup>5-10,20-28</sup> The pituitary gonadotropes apparently require intermittent periods devoid of stimulation by LHRH or its analogues to maintain sustained gonadotropin release.

Daily administration of LHRH<sub>a</sub> for two months to five girls with idiopathic precocious puberty lowered gonadotropin and estradiol secretion and abolished the gonadotropin response to exogenous LHRH. Reversal of these effects was seen two months after cessation of therapy. Although these data provide convincing evidence that LHRH<sub>a</sub> uncouples the pituitary response to LHRH, an additional direct effect in

which LHRH<sub>a</sub> inhibits steroidogenesis at the gonadal level cannot be excluded. Recent studies in animals have shown that LHRH and its agonist analogues can directly inhibit steroidogenesis by the ovary and testis, in addition to exerting their effects at a pituitary site of action.<sup>29-31</sup>

Both agonist and antagonist analogues of LHRH can inhibit gonadotropin secretion. We chose to use an LHRH agonist rather than an antagonist in this study because only the agonist analogues are sufficiently potent for clinical use. The development of increasingly potent antagonist analogues of LHRH, however, suggests that in the future it may be possible to compare LHRH agonists and antagonists in the therapy of precocious puberty.<sup>32</sup>

This study was confined to girls with idiopathic precocious puberty. The incidence of idiopathic precocious puberty is higher in girls than in boys, and only girls were available for this study. We suspect that similar results of LHRH-analogue therapy would be seen in boys with precocious puberty, but this remains to be documented.

The results of this short-term study of LHRH<sub>a</sub> treatment in idiopathic precocious puberty appear promising, but still must be regarded as preliminary. No serious adverse effects were seen, and full recovery of sex steroids to pretreatment levels was observed in all subjects in whom LHRH<sub>a</sub> administration was discontinued. Regression of breast size and pubic hair was noted in several patients within four months of treatment. Before the merits of this new therapy can be fully assessed, however, investigation of the effects of long-term treatment on pubertal progression and bone maturation will be necessary. Several years of therapy may be required to assess both the benefits and the possible unforeseen consequences of long-term treatment with an LHRH analogue.

We are indebted to the 9W and 12E nursing staff at the Clinical Center at the National Institutes of Health and to the nurses of the Clinical Research Center at the Massachusetts General Hospital for their assistance, to Penny Colbert, Barbara Filmore, Dolores Schwartz, Kathy Kessler Gay, Jody Levy, Carolyn Albers, and Nancy Delaney for their dedication and expertise, and to the referring physicians, associates, and fellows involved in this study for their support.

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