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INDUCTION OF PUBERTY IN MEN BY LONG-TERM PULSATILE ADMINISTRATION OF LOW-DOSE GONADOTROPIN-RELEASING HORMONE

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Abstract Puberty is heralded by the appearance of episodic gonadotropin secretion. Men with idiopathic hypogonadotropic hypogonadism have an abnormality in gonadotropin release and do not undergo normal puberty. Since idiopathic hypogonadotropic hypogonadism is thought to represent a disorder of gonadotropin-releasing-hormone (GnRH) secretion, we used long-term low-dose subcutaneous GnRH, administered in an episodic fashion by a portable infusion pump, in an effort to establish a normal adult pattern of gonadotropin secretion in six men. All subjects noted spontaneous erections, nocturnal emissions, and breast tenderness, which were associated with elevations of serum testosterone levels (77±13 ng per deciliter [mean ±S.E.] before therapy vs. 520±182 ng after one month of treatment; $P<0.001$). Gonadotropin levels rose to normal adult ranges within one week of therapy and to supraphysiologic levels by 14 days. Testis size increased in four patients, and spermatogenesis was achieved in three patients by 43 weeks of therapy. These results suggest that long-term episodic GnRH administration can reverse idiopathic hypogonadotropic hypogonadism. (N Engl J Med. 1982; 307:1237-41.)

THE central event in the initiation of puberty appears to be the episodic discharge of the hypothalamic gonadotropin-releasing hormone, GnRH. In early puberty, GnRH release, as reflected by pulsations of luteinizing hormone, appears to occur in a nocturnal or sleep-entrained program, whereas gonadotropin secretion in adults occurs throughout the day and night.[1,2] Men with idiopathic hypogonadotropic hypogonadism, on the other hand, have not undergone these normal changes at puberty, and prepubertal gonadotropin and testosterone levels persist into adult life.[3,4]

Several lines of evidence suggest that the primary defect in idiopathic hypogonadotropic hypogonadism is faulty hypothalamic secretion of this gonadotropin-releasing factor. GnRH is capable of eliciting variable increases in serum levels of luteinizing hormone and follicle-stimulating hormone in these subjects, especially during repeated administration,[5-15] suggesting that the anterior pituitary of patients with idiopathic hypogonadotropic hypogonadism is capable of synthesizing and releasing gonadotropins. Clomiphene citrate, an antiestrogen that provokes the release of GnRH from the hypothalamus, has been unsuccessful in reversing complete idiopathic hypogonadotropic hypogonadism.[16-18] In conjunction with the noted absence of gross or histologic abnormalities in the hypothalamus and anterior pituitary in this disorder,[19] these observations suggest that idiopathic hypogonadotropic hypogonadism is a GnRH-deficient state that may be amenable to treatment with this peptide. In addition, this condition may also prove to be a suitable model in which to investigate the dynamics of gonadotropin secretion in response to various modes of GnRH delivery.

We conducted a long-term study of six men with idiopathic hypogonadotropic hypogonadism, using episodic administration of GnRH delivered by a portable infusion pump. The clinical and biochemical changes of puberty were induced in all six patients, and the initiation of spermatogenesis was documented in three.

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METHODS

Patient Population

Six men with well-established hypogonadotropic hypogonadism were admitted to the General Clinical Research Center of the Massachusetts General Hospital. Each had failed to undergo puberty spontaneously by the age of 18 and had low circulating levels of gonadotropins and testosterone in the absence of other radiographic and biochemical abnormalities of the hypothalamic–pituitary axis, according to a previously described protocol.[20] Skull roentgenograms and computerized cranial tomograms were normal in each case. Base-line and stimulated levels of growth hormone, cortisol, thyrotropin, and prolactin were normal before and after the intravenous administration of 200 μg of thyrotropin-releasing hormone and 0.15 unit of insulin per kilogram of body weight. Four patients had testicular biopsies before entering the study. The nature of the treatment protocol and alternative modes of therapy with gonadotropins or testosterone were discussed with each patient before informed consent was obtained. All hormonal-replacement therapy was discontinued at least three months before testing. In Patients 1 through 5 the hypogonadotropic state appeared to have a congenital basis, whereas in Patient 6 it was acquired after puberty, according to his history. Patients 1 and 2 had anosmia. Patient 5 had previously been treated with a GnRH analogue for 32 weeks[20] before entering this study.

Protocol

During the first day of the study, gonadotropin levels were determined every 20 minutes for 24 hours. Serum testosterone and estradiol levels were measured in a pool derived from this study. After this base-line evaluation, each patient was instructed in the maintenance of a portable infusion pump (Autosyringe) and the use of aseptic techniques in inserting subcutaneous needles. The patients were then discharged on a regimen of 25 ng of GnRH per kilogram, administered subcutaneously every two hours through the Autosyringe. The patients returned to the Clinical Research Center every week for clinical reassessment and monitoring of four GnRH pulses; gonadotropin was determined after 20-minute intervals for eight hours (for a total of 25 determinations per admission).

Luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol were measured with previously described radioimmunoassays.[20] Serum levels of luteinizing hormone and follicle-stimulating hormone were expressed as milli-International Units per milliliter of LER 907, the pituitary reference preparation. In this system 1 mg of LER 907 contains 60 IU of luteinizing hormone and 20 IU of follicle-stimulating hormone. The normal range for the laboratory values of these hormones had been derived from frequent

Table 1. Clinical Characteristics of Six Men with Idiopathic Hypogonadotropic Hypogonadism (IHH).

Patient No.	Age	Clinical Features	Hormone Levels (Base Line)			Testicular Size (Right/Left)		Findings at Testicular Biopsy
			Luteinizing Hormone	Follicle-Stimulating Hormone	Testosterone	At Base Line	After 3 Mo of Therapy	At Base Line
	yr		*mIU/ml LER 907*		*ng/dl* *	*ml*		
1	28	Anosmia	1.09	2.00	67	2/2	9/8	Hypospermatogenesis
2	18	Anosmia	0.30	1.00	62	†/3	4/5	Right testis — no germ cells; left testis — spermatogonia with early lumen formation
3	19	Microphallus	0.40	1.25	72	3/2	5/4	—
4	19	—	0.84	1.24	110	12/12	20/20	Arrest of spermatogenesis
5	44	Previous treatment with a GnRH analogue	0.64	1.33	36	8/8	8/8	Hypospermatogenesis, arrested maturation, focal tubular hyalinization
6	29	Acquired IHH	0.64	1.00	107	12/12–15	20/20	—
Mean ±S.E.M.			0.65±0.12	1.30±0.15	77±13			
Normal values			0.6–3.1	1.0–3.4	300–1000	15–25		

*To convert to nanomoles per liter, divide by 28.

†Undescended.

sampling of serum from five fertile men.[20] The normal estradiol levels for men are 20 to 50 pg per milliliter (0.074 to 0.184 pmol per liter). Testis size was measured with a Prader orchidometer.

Statistical comparisons were made by means of analysis of variance.

Results

Base-Line Status

The base-line clinical and biochemical features of the patients are shown in Table 1. Before treatment the mean level of luteinizing hormone in this group was in the low-normal range — 0.65±0.12 mIU per milliliter of LER 907 (mean ±S.E.); the level of follicle-stimulating hormone was 1.30±0.15 mIU per milliliter of LER 907; the serum level of testosterone was 77±13 ng per deciliter (0.22±0.04 nmol per liter [Table 1]), and the serum level of estradiol ranged from less than 20 to 29 pg per milliliter (0.074 to 0.107 pmol per liter). Testicular volumes ranged from 2 to 12 ml. Patient 6, who had previously had a normal puberty, had the largest testicular size before treatment. Before the initiation of therapy, none of the patients was able to produce an ejaculate. Biopsies of the testes demonstrated various degrees of testicular immaturity and hypospermatogenesis.

Treatment Outcomes

Short-Term (Three Months)

After treatment with episodic GnRH, the levels of gonadotropins and testosterone rose in each patient (Fig. 1). Serum levels of luteinizing hormone peaked during the fifth week of treatment at 4.80±0.89 mIU per milliliter ($P<0.01$, in comparison with levels before therapy), after which they declined into the high-normal range by the seventh week. Serum levels of follicle-stimulating hormone increased to 4.77±0.81 mIU by Week 2 ($P<0.01$) and remained elevated throughout most of the first three months of treatment. In four of the six patients serum testosterone levels quickly rose into the adult range by the end of the first week of therapy and remained within normal limits for the duration of treatment. In the other two patients serum testosterone levels rose from 62 ng per deciliter (0.179 nmol per liter) to 175 ng (0.504 nmol) by Week 11 (Patient 2), and from 36 ng per deciliter (0.104 nmol per liter) to 296 ng (0.853 nmol) by Week 9 (Patient 5). Testicular size rapidly increased, approximately doubling within four weeks of therapy in Patients 1 through 4.

All patients noted an increase in the frequency of spontaneous erections, the appearance of nocturnal emissions, and androgen-induced skin changes, such as acne, oiliness of the skin, and seborrhea. Somatic growth occurred in Patients 1 through 5, whose epiphyses were unfused before treatment. This change was most marked in Patient 4, whose height increased by 9 cm and whose weight rose by 20 kg, little of which was adipose tissue. Transient mild breast discomfort occurred in all patients, and persistent gynecomastia of a mild degree occurred in three.

Long-Term

Maturation of spermatogenesis was achieved in Patients 1, 4, and 6 after 4, 11, and 43 weeks of therapy, respectively, as determined by analysis of seminal fluid. The maximum sperm counts were 2.3×10^6 per milliliter by Week 57 in Patient 1, 52×10^6 per milliliter by Week 27 in Patient 4, and 4.1×10^6 per milliliter by Week 26 in Patient 6. In Patients 1 and 6 the appearance of mature sperm in the ejaculate was preceded by several seminal-fluid analyses without evidence of mature sperm or spermatogenic precursors. Before treatment, Patient 1 had had a testicular volume of 2 ml and a biopsy specimen containing immature testicular tissue with only rare spermatogonia (Fig. 2). With GnRH therapy, his testicular size increased rapidly, and after three months his testes had a volume of 8 to 9 ml each (Table 1). Subsequently, his dose of GnRH

was lowered from 25 to 10 ng per kilogram (Week 39) and finally to 5 ng per kilogram (Week 51). Despite this sequential tapering of the dose of GnRH, the levels of luteinizing hormone, follicle-stimulating hormone, and testosterone remained within the normal limits throughout the entire 59 weeks of the study (Fig. 3), as the patient underwent the virilizing changes of puberty.

The testicular biopsy in Patient 4 showed arrested spermatogenesis. Patient 6 refused to undergo a pretreatment testicular biopsy; however, after less than six months of GnRH therapy and several seminal-fluid analyses without sperm, sperm were present in his ejaculate, and his wife became pregnant by the 26th week of his continuous therapy. The results of multiple blood typing studies were compatible with paternity in this patient.

Throughout the treatment period the serum estradiol levels remained within the normal adult ranges, even in the patients with gynecomastia.

Discussion

Previous studies employing infrequent administration of large doses of GnRH or its long-acting ana-

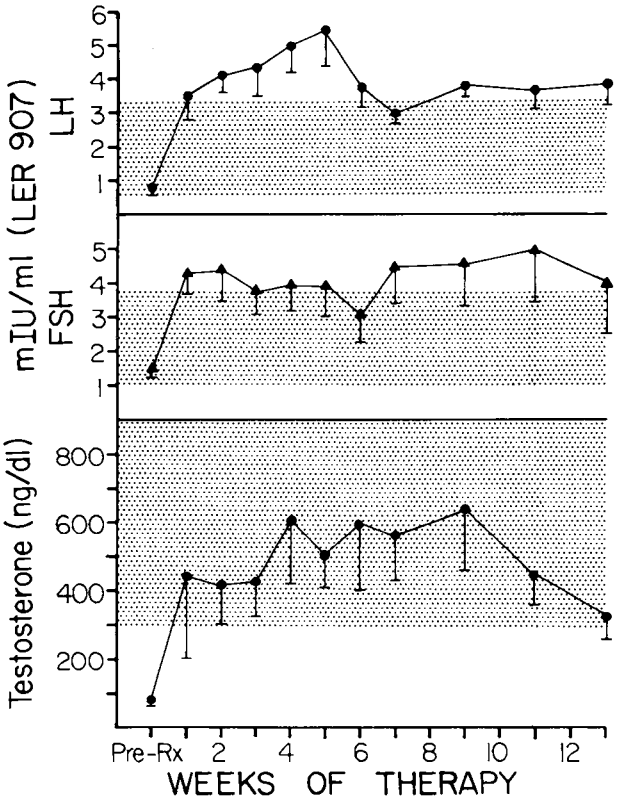

Figure 1. Mean Levels (±S.E.) of Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), and Testosterone in Six Men with Idiopathic Hypogonadotropic Hypogonadism.

The dosage of GnRH during three months of administration was 25 ng per kilogram, given subcutaneously every two hours. The shaded areas represent the range of normal values in men. The values for Weeks 6 and 9 represent those in five of the patients, and the values for Week 13 represent those in four patients.

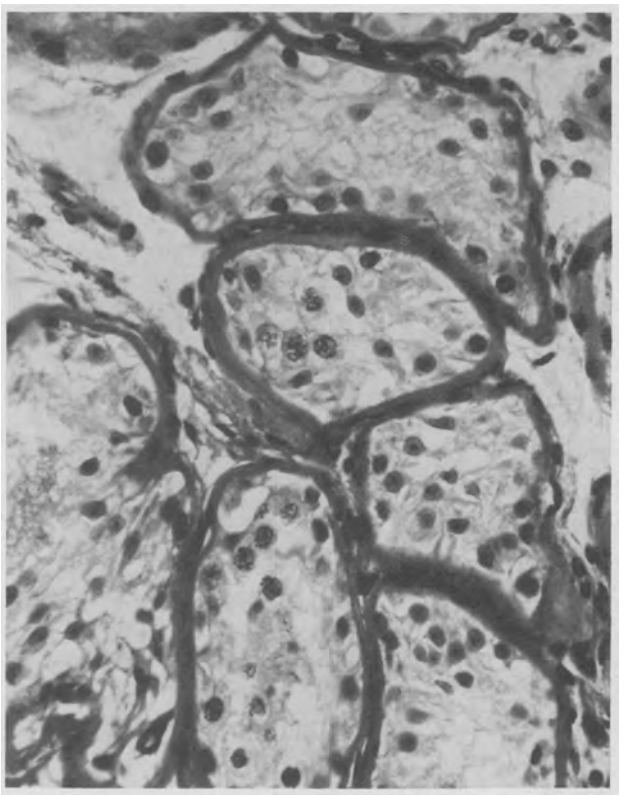

Figure 2. Testicular Biopsy Specimen Obtained from Patient 1 before Therapy (×400).

Very few mature sperm are evident.

logues (or both) to men with idiopathic hypogonadotropic hypogonadism have only partially or temporarily reversed this condition.[5-15,20,22-28] These findings may well be explained by the pioneering set of studies performed in a hypogonadotropic primate model by Knobil and his colleagues, who discovered that GnRH could stimulate appropriate gonadotropin secretion only when the hormone was administered in an episodic mode and at a physiologic frequency.[29,30] Continuous administration was completely ineffective in evoking the desired pattern of gonadotropin discharge. Several subsequent trials using short-term episodic GnRH administration in idiopathic hypogonadotropic hypogonadism have confirmed that an intermittent mode of administration can elicit episodic gonadotropin release, suggesting that longer-term treatment might induce full reproductive maturation in men with this disorder.[14,15,21,31] Using such a regimen of episodic GnRH administration, we were able to induce all the clinical and biochemical changes of normal puberty within three months, through intermittent subcutaneous injections of native GnRH. In an attempt to reproduce the normal secretory dynamics of luteinizing hormone, we treated our subjects with relatively low doses of GnRH designed to mimic the physiologic luteinizing hormone pulse amplitude previously observed by others.[32] We selected an interval of two hours to simulate the normal frequency of episodic

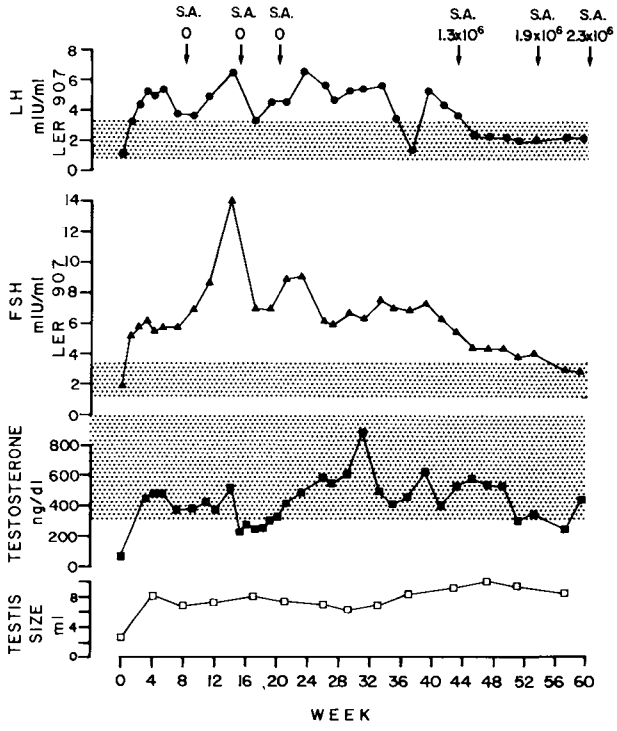

Figure 3. Course of Long-Term Therapy in Patient 1.

GnRH was administered subcutaneously with an Autosyringe. The doses (per kilogram of body weight) were 25 ng (Weeks 1 to 38), 10 ng (Weeks 39 to 50), and 5 ng (Weeks 51 to 59). The drug was delivered every two hours, except during Weeks 23 to 27, when it was administered every hour, and Weeks 28 to 33, when it was administered every 90 minutes.

The results of semen analysis (S.A.) are expressed as the number of sperm per milliliter of seminal fluid. The shaded area represents the range of normal values for adult men. Each measurement of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone represents a value determined for a pool of 25 specimens obtained every 20 minutes over an eight-hour period.

Testis size is expressed as the average value for the patient's testes, as measured by a Prader orchidometer.

release of luteinizing hormone, which occurs approximately 12 times a day in men.[33]

With this regimen, extremely rapid clinical and hormonal changes occurred in all six patients. The clinical changes included a prompt increase in testicular size — the first indication of puberty in normal boys — within two to four weeks of treatment in four of the five patients with congenital idiopathic hypogonadotropic hypogonadism, all of whom reported mild testicular discomfort and tenderness. Breast swelling and tenderness were frequently experienced within the first three months of therapy. The appearance of acne vulgaris and increased sebaceous activity of the skin was also noted by all patients. Within a month of therapy, all commented that they had had early-morning and spontaneous daytime erections. Of particular interest was the fact that all patients voluntarily reported the occurrence of nocturnal emissions — a normal event in spontaneous puberty but a clinical event that in our experience is distinctly unusual in patients with idiopathic hypogonadotropic hypogonadism treated with gonadotropins or testosterone replacement.

After 10 months of therapy, three of our patients had evidence of spermatogenesis, and Patient 6 fathered a normal child. The reason for the persistent azoospermia in the other three subjects may well have been related to the relatively short duration of treatment. It is of interest, however, that in the patient with the smallest gonads and the least mature testicular tissue (Patient 1, who had Kallmann's syndrome), spermatogenesis occurred within 59 weeks of therapy (Fig. 2 and 3). Although the hypogonadotropic state had developed in Patient 6 after puberty, his clinical and biochemical changes during GnRH administration resembled those of all the other subjects, whose disorder was presumed to be congenital. Thus, it appears that all the clinical changes associated with puberty occurred in our patients during the first year of therapy with low-dose episodic GnRH.

The hormonal changes observed in this population were detectable within two weeks of treatment. Serum levels of luteinizing hormone initially rose above the normal range by the fifth week of treatment and then gradually returned toward normal, presumably in response to negative feedback from the persistently normal levels of serum testosterone. The reason for this delay in feedback is unclear, but the time course of delayed feedback on gonadotropin suppression resembles that observed in men with primary hypogonadism in response to testosterone administration.[34] Since the dosage and frequency of GnRH were set by the pump, the only manner in which such a negative feedback could have occurred is through a direct pituitary reduction of gonadotropin secretion. Despite the elevated gonadotropin levels, normal adult levels of serum testosterone were quickly achieved in four of the patients, and were maintained throughout the first three months of the study. Since only physiologic levels of serum testosterone were observed in our patients in the presence of high normal to frankly supraphysiologic levels of luteinizing hormone and follicle-stimulating hormone, these studies suggest that high gonadotropin levels may be required to initiate testosterone secretion in the prepubertal testis. It is also of interest that the serum levels of follicle-stimulating hormone remained above the normal range despite restoration of normal testosterone and estradiol levels (data not shown). The return of the levels of follicle-stimulating hormone to the normal ranges occurred in association with the appearance of sperm in the ejaculate of the patients with spermatogenesis.

In contrast to the accelerated courses of pubertal changes induced by this protocol, normal puberty occurs over a period of approximately 3.5 years.[35] Our patients received GnRH-induced gonadotropin pulses throughout the day and night in a program resembling the natural pulses observed in adults, whereas normal puberty is usually heralded by the appearance of sleep-entrained gonadotropin secretion only. During the

daytime in early puberty, gonadotropin pulses are dampened and serum testosterone levels remain in the prepubertal ranges.[1,2] Only later in puberty do gonadotropin pulsations occur throughout the day and night. Consequently, it appears that GnRH administration every two hours results in persistent stimulation of the pituitary, which in turn may be responsible for the extraordinarily swift pubertal development demonstrated in this study. It is thus of interest that only midpubertal testosterone levels were achieved in another study, in which two boys with Kallmann's syndrome were treated with larger doses of GnRH delivered subcutaneously at hourly intervals during the night only.[14]

In addition to demonstrating the practicability of hypothalamic hormone-replacement therapy, the ability to stimulate secretion of luteinizing hormone and follicle-stimulating hormone with low-dose episodic GnRH in patients with GnRH deficiency should permit investigation into the normal dynamics of the hypothalamic–pituitary interaction. By adjusting the dose or altering the frequency of GnRH administration, investigators may obtain important information regarding pituitary gonadotropin secretion and the attendant gonadal responses.

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